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Long-Term Effects of Azathioprine in Rheumatoid Arthritis

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Azathioprine has been shown to be an effective agent in the treatment of active rheumatoid arthritis (RA); however, potential long-term neoplastic effects remain a serious concern. To clarify this relationship, 36 patients with classic or definite RA being treated with azathioprine (Group 1) were chosen at random and studied by means of clinical, laboratory, and radiologic measures to detect potential early markers of neoplastic disease. Nineteen age matched patients with RA were concurrently similarly studied as controls (Group 2). Group 1 had a longer disease duration (15.3 versus 9.4 years). The mean duration of treatment with azathioprine was 38.3 months, with a mean dose of 71.2 mg/day. Histories of cigarette smoking, hormone treatment, and fertility were comparable. Chest x-rays revealed comparable numbers of inflammatory and fibrotic abnormalities. There was an increased frequency of tumors in first degree relatives in Group 1 (15 versus 2).

Hematologic studies revealed a trend to lower cell counts in Group 1 with a striking increase of marrow megaloblastosis in this group (28 versus 1). There were no differences in iron saturation or B12 levels. Serum folate was decreased in Group 1 (14 versus 2). There were neither liver function test abnormalities in Group 1, nor evidence of malignancy in gastrointestinal x-rays when clinically indicated. No neoplastic lesions were identified on mammography in either group. Four patients in Group 1 and none in Group 2 had atypical or dyskaryotic cells on urine cytology. One patient in Group 1 had an abnormal Pap smear and was subsequently shown to have endometrial carcinoma. On chromosomal analysis, Group 1 showed a greater percent of cells with aberrations, a higher aberration incidence and index, and more patients with hyperdiploidy. Patients in Group 2 had a history of 3 benign neoplasms. Patients in Group 1 had a history of 1 benign neoplasm prior to the initiation of azathioprine; subsequently 7 neoplasms were diagnosed, 4 benign and 3 malignant (2 endometrial carcinomas and carcinoma-in-situ of the cervix).

In summary, Group 1 had an increased number of cellular abnormalities, with cytologic atypia, megaloblastic bone marrows, and increased chromosomal aberrations. Clinically this group had more tumors diagnosed while on treatment, particularly in females, and arising from the genitourinary tract. More long-term studies of patients on cytotoxic agents are clearly warranted.

Correlations of Circulating Immune Complexes (CIC) and Disease Activity in Patients with Systemic Lupus Erythematosus (SLE)

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The occurrence of circulating immune complexes (CIC) in SLE has been documented. This study reports the correlations between serologic determinations and disease activity as well as the predictive capabilities of CIC determinations.

Forty-eight patients with SLE, followed for 6–18 months, had 336 serum samples collected for determination of CIC, antibodies to DNA, C3, and creatinine. Clinical parameters of disease activity and medication dosages were recorded. CIC results were not available to the primary care physicians, thus, they did not influence therapy. Circulating immune complexes were measured by both the fluid phase Clq (FClq) and

solid phase Clq (SClq) radioimmunoassays. C3 was determined by radial immunodiffusion and antibodies to DNA by binding of radiolabeled DNA by the millipore filter technique. These assays were correlated with each other, general presence or absence of symptomatic disease, active renal disease, and active joint disease. To evaluate predictive capabilities, a change in CIC result was correlated with a change in disease activity. Change in disease activity was defined as an SLE related event requiring hospitalization, a change in symptoms requiring an increase in or addition of steroids or immunosuppressive agents, or a marked improvement in symptoms such that drugs were tapered or discontinued.

The FClq results did not correlate with any parameters of disease activity. A fall in C3 or an increase in antibodies to DNA usually signaled a change in disease activity; however, many patients experienced a change in disease activity without any change in C3 or DNA binding. The SClq assay correlated with symptoms of SLE in general ($P < 0.001$), with active renal disease ($P < 0.025$), and with active arthritis ($P < 0.001$). An appropriate change in SClq determination correlated best with a significant change in disease activity ($P < 0.001$).

We conclude that the SClq method of determining CIC correlates best with active clinical manifestations of SLE and is a useful predictor of disease activity.

Significance of Rheumatoid Factors (RFs) Cross Reactive with DNA-Protein (DNP)

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We have recently found that certain rheumatoid factors (RFs) cross react with DNA-protein (DNP). This type of cross reacting RF is frequently present in seropositive rheumatoid arthritis (RA), 21 of 58 patients, and also in order RF positive patients: rheumatoid overlap syndrome 4/10, mixed connective tissue disease (MCTD) 5/7, other diseases 1/10. Thus far RFs reactive with DNP have not been found in systemic lupus erythematosus (SLE) 0/8 or essential mixed cryoglobulinemia 0/7. RFs which react with DNP can give positive antinuclear antibody (ANA) and LE tests which can be blocked by aggregated IgG or DNP. In contrast, positive ANA and LE tests in SLE are not blocked by aggregated IgG. Hence RF induced ANA and LE tests may be considered "false" positives when these tests are used in the diagnosis of SLE. In some sera these RFs could be detected only following isolation with insoluble IgG or DNP, suggesting the presence of RFs complexed with antigen. By use of a monoclonal RF which cross reacts with DNP in a sensitive competitive inhibition radioimmunoassay designed for detection of immune complexes (JCI 59:990, 1977), complexes larger than 19S by ultracentrifugation studies could be detected in several RA sera and synovial fluid and MCTD sera. In some studies DNAse treatment of these complexes resulted in a decrease in size. These studies suggest that complexes consisting of DNP antigen and RF may be present in the circulation in RA and MCTD. The relationship, if any, of these complexes to the immunopathology in these diseases is currently under study. These studies also suggest that not all RFs may be induced by immune aggregated IgG and that a re-exploration of RFs in various diseases for cross reactions with non-IgG antigens is indicated. Such antigen may provide clues to etiologic factors in these diseases.

Characteristics of Rheumatoid Arthritis Associated Nuclear Antigen

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Previous studies demonstrated that patients with rheumatoid arthritis (RA) frequently have an antibody referred to as rheumatoid arthritis precipitin (RAP). This antibody was shown by the indirect immunofluorescent technique to react with a nuclear protein in human, B lymphocytes from continuous cell culture (Wil_2) and the antigen was termed RA associated nuclear antigen (RANA). Infectivity experiments with Epstein-Barr virus (EBV) or other herpes viruses have suggested that RANA may be associated with EBV infection; although RANA shares some characteristics with EB nuclear antigen, they are probably two different antigens.

In this study we have expanded our work on RANA. In addition to its presence in Wil_2 cells, it has now been found in 6 other human, B lymphocyte lines but was not present in 2 T lymphocyte lines. RANA was not found in extracts from normal spleen, kidney, heart, or lungs, or a spleen extract from a patient with Felty's RA. This antigen was not present in normal lymphocytes stimulated with B or T cell mitogens. RANA was not found in peripheral leukocytes from patients with RA. However, it was found in 2 of 3 extracts made from pannus layers and an extract made from nodules taken from patients with seropositive RA but was not present in 2 extracts made from pannus layers taken from seronegative RA patients and synovium from a traumatized joint.

Initial physicobiochemical characterization of RANA demonstrated that it was stable to heat (37°C or 56°C) or cold (4° to −70°C) treatments or at pH values of pH 6.2–8.6. Studies performed by immunoelectrophoresis indicated that

RANA was an anion at pH 7.4–8.6. Wil_2 extracts were separated on a calibrated 10% agarose column and the molecular weight estimated at 120,000 d. In other studies the antigen could not be detected on the surface of Wil_2 cells.

Immunodiffusion studies using Burkitt's lymphoma sera (12) or nasopharyngeal carcinoma sera (12) containing high titers of EBV antibodies to: viral capsid antigen or early antigens D or R do not react with Wil_2 extract, suggesting these EBV antigens are not present in the extract and therefore cannot be RANA.

The data suggest that RANA may be a new, heat stable, acidic, nuclear protein associated with EBV infection which could be important in the pathogenesis of RA.

Diagnostic Arthroscopy for Knee Pain

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After medical workup, arthroscopy was performed on 100 patients (5:2, men:women; ages 14–74) with painful knees of unknown cause. Arthroscopy of the knee was achieved on ambulant patients under sterile technique in an outpatient minor surgery suite utilizing a Needlescope or Watanabe #21 Arthroscope. Biopsies were guided by visualization.

Postarthroscopy diagnoses are reflected in the table. In most cases the procedure allowed adequate visualization of the suprapatellar sac, articular cartilage, menisci, anterior cruciate, and synovial lining. No arthroscopic abnormality was visualized in 12%. Nearly half of the patients had osteoarthritis including 12 patients with chondromalacia patella. The group with osteoarthritis also included 9 patients who had at least one torn meniscus. Septic arthritis was confirmed in 4% by synovial biopsy including one case of tuberculosis. Previous undiagnosed synovitis was confirmed but not further defined in 4 patients. Four biopsies suggested rheumatoid synovitis; the diagnosis was confirmed by subsequent clinical and laboratory course. Results of arthrography (46) were confirmed in 26, contradicted in 12, and provided additional findings in 21. Patients were ambulant after the procedure with minimal morbidity—6 hemarthroses and an acute pseudogout, all treated by arthrocentesis.

The painful knee often presents a diagnostic enigma particularly when degree of disability is a question. Arthroscopy is a safe minor surgery procedure that may reveal the underlying cause of pain and preclude the need for arthrotomy.

Diagnosis by Arthroscopy	
Normal	12
Abnormal Cartilage	
Osteoarthritis	45
Cartilage ulcer	5
Pseudogout	2
Osteochondritis	2
Polychondritis	1
Abnormal Synovium	
Rheumatoid	5
Undiagnosed	4
Septic	4
Osteochondroma	3
Amyloid	1
Internal Derangement	
Meniscus tear	15
Meniscus swollen	2
Cruciate tear	1
Miscellaneous	
Loose body	4
Gout	3

Transaxial Tomography in the Assessment of Back Pain

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Computer assisted tomography of the spine and/or roentgenographic transaxial lumbar tomography were performed on 25 patients (age 69 ± 12 SD years) with back pain and concomitant Paget's disease. Clinical assessment with routine lumbosacral x-rays was performed by a rheumatologist and an orthopedic surgeon. Both tomographic methods are x-ray techniques that transect the spinal canal and the intervertebral foramina producing a computer printout or laminogram along the axial plane of the spine. The following was found:

1. Spinal stenosis: spondylotic osteophytes encroaching on the spinal canal in 14 patients. Three had a history of pseudointermittent claudication. Lumbar x-rays supported a diagnosis of Paget's disease at the involved site in 6.

2. Lateral recess syndrome: spondylotic osteophytes encroaching on the intervertebral foramina in 9 patients. All had sclerotogenous pain (nonspecific pain referred to the buttocks, groin, and/or thighs). Lumbar x-rays supported a diagnosis of Paget's disease at the involved site in 4.

3. Pagetic pain: increased thickness of the laminae, pedicles, or bodies of the vertebrae secondary to Paget's disease in 3 subjects without alteration of the spinal canal.

4. Spondylotic changes of articular facets without stenosis or lateral recess in 6; 5 had adjacent pagetic changes.

In the assessment of back pain, axial tomography proved to be a useful tool in defining anatomic spinal lesions that were not appreciated by routine x-ray techniques. The methods were particularly helpful in outlining the zygapophyseal articulation for definition of radiographic joint space and hypertrophic changes. Neural entrapment by bone due to hypertrophic changes was similarly defined. In several cases the procedure obviated the need for myelography. In Paget's disease, determining the degree of spondylosis could guide therapy since it would predict those that might be expected to respond to suppressive therapy of their Paget's disease.

HLA-C Locus Antigens in HLA-B27 Associated Arthritis

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HLA-B27 is a potent genetic marker for ankylosing spondylitis (AS), Reiter's syndrome (RS), and other spondylitic variants. This cell surface antigen is determined by the HLA-B locus, one of a linear array of genes clustered on the sixth chromosome. Whether HLA-B is the disease-conferring gene or only linked to another nearby gene remains unclear. Studies of HLA-D on one side of HLA-B (1 map unit) have shown no associations with AS or B27. The HLA-C locus lines 0.2 map units to the opposite side of HLA-B. It was our purpose to study C locus antigens in B27 positive and negative patients in order to more precisely localize the disease promoting gene.

Eighty-eight patients (30 AS, 37 RS, 9 sacroiliitis, 9 psoriatic spondylitis, and 3 colitic spondylitis) were HLA typed for A, B, and the C locus antigens, Cw1, Cw2, Cw3, and Cw4. In addition, 88 age, sex, and race matched normal controls and 64 B27 positive normal controls were similarly studied and compared to those with disease. HLA-B27 was present in 68 patients or 78% (AS 83%, RS 81% and other 62%). Results of C locus typing are shown in the table.

Differences between the frequencies of C locus antigens in B27+ and B27− patients were significant: $P < 0.01$ for Cw1, $P < 0.025$ for Cw2, and $P < 0.005$ for either Cw1 and Cw2. Similar significant differences were found when both the B27+ patients and the B27+ controls were compared with the matched controls. There were no significant differences noted between B27+ patients and B27+controls or between B27− patients and matched controls. The occurrence of Cw3 and Cw4 was similar within all groups.

Thus, these data confirm strong linkage disequilibrium between B27, Cw1, and Cw2 which does not relate to disease. Most importantly, the absence of Cw1 and Cw2 in B27− patients provides further evidence that recombination between B and C loci is unlikely, and that disease susceptibility is intimately related to or inseparable from the B locus.

	Disease Groups		Control Groups	
	B27+ (N=68), %	B27− (N=20), %	B27− (N=64), %	Matched (N=88), %
Cw1	47	10	33	8
Cw2	43	10	58	14
Cw1 or Cw2	87	20	88	22

Pathways of Complement Fixation by Nuclear Antigen-Antibody Complexes of Different Specificities

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An immunofluorescent complement fixation (IFCF) test was used to determine if nuclear antigen-antibody complexes of different specificities possessed different capacities to fix complement. Sera from patients with rheumatic diseases were specially selected and demonstrated to contain only antibodies to Sm antigen (5 patients), nuclear RNP (7), SS-B antigen (3), or nuclear histones (7). Column chromatography isolated-IgG from these sera were reacted with sections of mouse liver to form nuclear Ag-Ab complexes of the different specificities described above. Binding of complement components to the nuclear Ag-Ab complexes was determined after washing away excess serum and incubating with 3 different sources of complement: 1) normal human serum (NHS); 2) NHS-EGTA Mg, conditions which are known to block activation of the classic pathway; 3) immunologically Clq-depleted sera in which other complement proteins were demonstrated to be 90% hemolytically active. Fixation of complement was determined by using fluorescein-conjugated antiserum to Clq, C4, properdin, factor B, C3, C6, and C9. All sera with antibodies to Sm antigen, nuclear RNP, and SS-B nuclear antigens showed fixation of all complement proteins when NHS was used as the source of complement. When the source of complement was EGTA-Mg or Clq-depleted serum, Clq and C4 were negative by IFCF. However, complement proteins properdin, factor B, C3, C6, and C9 were positive, showing that these nuclear antigen-antibody complexes were able to fix complement proteins of the alternative pathway, without concomitant fixation of the classic pathway. The 7 sera with antibodies to nuclear histones did not fix any complement component of either the classic or alternative pathways.

These studies demonstrate two important findings. Certain nuclear Ag-Ab complexes are capable of activating the alternative complement pathway, independent of classic pathway activation. Reactions between nuclear histones and antibody, as demonstrated by the immunofluorescent technique, appear not to be complement-fixing reactions or at least not detectable by this technique. The capacity of antinuclear antibodies of certain specificities to activate complement may have relationship to their capacities to cause tissue damage by release of inflammatory peptides mediated by complement activation. Our results may explain why drug-induced lupus erythematosus, characterized by anti-histone Ab, is rarely associated with inflammatory kidney disease.

Immunochemical Characteristics of Antibodies to DNA in Patients with Active Systemic Lupus Erythematosus

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It has been suggested that renal involvement in SLE patients may depend upon qualitative immunochemical characteristics of antibodies to DNA, e.g., precipitating ability, affinity, Ig class or subclass, and complement fixing (CF) capacity. We used the immunofluorescent Crithidia Luciliae method [a-DNA(CL)] to delineate some of these characteristics in sera from 35 patients with active SLE. Using FITC-labeled polyspecific antiserum to IgG, IgA, and IgM, a-DNA(CL) was found in 17 of 18 patients with active lupus nephritis (Gp-I) and 11 of 17 patients with lupus activity without nephritis (Gp-II). Median titer in Gp-I was 1:160 and in Gp-II 1:40; the difference in titers between these groups was significant ($P < 0.005$). CF antibodies to DNA were found in 13 patients in Gp-I and 5 patients in Gp-II using a double sandwich technique to detect C3 binding. When titer of CF activity was compared with a-DNA(CL) titer, a strong correlation was found ($r = 0.73$; $P < 0.001$). IgG, IgA, and IgM a-DNA(CL) were detected with high frequency in both groups.

In addition, we compared titers of a-DNA(CL), which detects antibodies primarily directed against dsDNA determinants, with DNA binding capacity detected by the millipore filter (MF) method of Ginsberg and Keiser, in each of these groups. As antigen we employed ^{125}I-labeled calf thymus DNA (type I, Sigma) passed through an 0.45 μ, type HA millipore filter. In contrast to the CL method, levels of a-DNA(MF) did not differ significantly in Gps I and II. When we compared a-DNA(CL) titer in Gp-I sera with quantitative a-DNA(MF), a highly significant correlation was found ($r = 0.89$, $P < 0.001$), while no correlation between these methods was evident in sera from Gp-II patients.

These findings suggest that: 1) the presence of high titered a-DNA(CL) is strongly correlated with active renal lupus; 2) detection of C-fixing a-DNA(CL) may reflect total amount of antibody; demonstration of C-fixing ability may generally be expected in high titered sera; 3) antibodies to at least 2 sets of constituents of the DNA preparation employed in the MF method can be detected, one occurring in active lupus nephritis and correlated with antibodies detected by CL, and one in patients with active lupus without nephritis, detected minimally or not at all by the CL method.

The Effect of Immune Complexes on Human Monocyte Cytotoxicity: A Comparison with Lymphocytes

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Cell mediated cytotoxicity is an important pathogenic mechanism in certain rheumatic diseases. Recent studies from this laboratory have demonstrated that adherent monocytes are potent killers of antibody coated (ADCC) nonerythroid target cells and also kill uncoated target cells to a lesser extent. Although immune complexes can inhibit lymphocyte cytotoxicity, their effect on monocyte cytotoxicity is unknown. This study compares human monocytes ADCC and natural killer (NK) activities with that of lymphocytes. The effects of heat aggregated IgG (0.02–2 mg per ml) and preformed soluble keyhole limpet hemocyanin (KLH)/anti-KLH complexes were evaluated. Monocytes and lymphocytes were separated by their differential adherence properties in plastic microtiter wells and were at least 95% pure. Cytotoxicity was measured by ^{51}chromium released from antibody coated (ADCC) and nonsensitized (NK) K 562 cells, an established myeloid cell line.

Precipitated KLH/anti-KLH complexes formed at equivalence were the strongest inhibitors of cell mediated cytotoxicity (suppression: monocyte NK 90%, monocyte ADCC 60%, lymphocyte NK 80%, lymphocyte ADCC 30%). Aggregated IgG also strongly inhibited both monocyte and lymphocyte NK (50% and 75% respectively) and also suppressed ADCC to a lesser extent (monocyte 25%, lymphocyte 20%). KLH/anti-KLH in antigen excess had no effect on monocyte NK and suppressed lymphocyte NK by only 25%. Although soluble complexes did not block monocyte NK, they did significantly block monocyte ADCC (45%). By use of serial dilutions of complexes in aggregated IgG, a dose dependent relationship was established between complexes and percent suppression of cytotoxicity.

These studies reveal that both KLH/anti-KLH immune complexes and aggregated IgG can inhibit monocyte cytotoxic activity and that, in general, these effects correlated

with lymphocyte NK and ADCC. Significant differences between soluble immune complexes and aggregated IgG were demonstrated.

Finally, the finding that certain immune complexes inhibit both NK and ADCC would imply that Fc receptors are required for both cytotoxic responses. The relatively greater inhibitory effect on NK would imply that antibody fixed to target cells potentiates attachment by the effector cell and renders it less susceptible to inhibition by immune complexes.

Gouty Arthritis: Prevalence of Chronic Synovitis, Polyarticular Attacks, and Positive Serological Tests for Rheumatoid Factor

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Gout is easily diagnosed from a classic clinical history and the demonstration of urate crystals in synovial fluid and/or tophi; however the diagnosis may not be suspected in patients with an atypical history or physical examination. To ascertain the prevalence of atypical findings we are conducting a prospective study in our gouty population randomly admitted to the Clinical Research Unit.

We report the following findings in the first 30 patients with crystal proved gout: 1) 30% had saturnine gout 2° to moonshine consumption, 37% gave a history of heavy ethanol consumption but did not have lead intoxication, and 53% were obese. All patients were male, 53% black, 40% white and 7% American Indian. The average age at the time of study was 53.2 years with a mean duration of gout of 14 years. Seventy-three percent had evidence of liver disease (elevated SGOT or hepatomegaly), 77% were hypertensive, 47% had elevated triglycerides, and 47% had abnormal glucose tolerance. 2) Twenty-seven percent had a polyarticular onset of gout (>1 joint); 60% subsequently had polyarticular attacks. Seventy-three percent had tophi at the time of the study. Chronic synovitis was present in 63% of patients. In the 30 patients examined the following joints of the upper extremity were involved; 40% MP and/or IP, 20% wrist, 20% elbow, 10% shoulder. In the lower extremity the knee was involved in 40%, MP joints in 27%, and ankles in 13%. 3) A positive latex fixation test was observed in 37% of patients; 6 being >1:40 and 4>1:320. RA latex was present in 36% of patients with tophi and 38% of patients without tophaceous deposits. In the patients with chronic synovitis 37% had a +RA latex, and 36% free of chronic synovitis were also positive. The mean age in patients with a positive test was 53.1 years and 53.3 years for those who were negative. In contrast to these negative associations, evidence of liver disease was correlated with the finding of a +RA latex. Fifty percent of patients with liver disease had a +RA latex whereas it was absent in all 8 without liver disease.

We conclude that chronic synovitis and polyarticular arthritis are common in our gouty patients. In addition, rheumatoid factor is found in a significant proportion of gouty patients and it does not seem to correlate with age, tophi, or chronic synovitis. RA latex seems to correlate best with the presence of liver disease which in most instances was a concomitant of chronic ethanol abuse.

Antigenic Bacterial Polysaccharide from Inflammatory Synovial Effusions

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A polysaccharide identical to a *Proprionibacterium acnes* polysaccharide (PPS) has been isolated from hyaluronidase treated rheumatoid synovial fluids and synovial leukocyte pellets using a phenol-water (Westphal) extraction and Sepharose 4B column chromatography. PPS was identified by counterimmunoelectrophoresis (CIE) by rabbit antibody to sonicated *P acnes* organisms. Sensitivity by this method is approximately 3.5 ng of PPS and identity of synovial and bacterial PPS was shown by CIE. Of common aerobic and anaerobic bacteria tested, only *Ps aeruginosa* contained a similar antigen. PPS is not present in *E coli* or *S typhosa* lipopolysaccharide. PPS could not be identified in synovial fluid without extraction procedures even when a more sensitive radioimmunoassay with ^{125}I labeled rabbit antibody was used. Sixty percent of 35 rheumatoid fluids and 70% of 10 rheumatoid synovial leukocyte pellets contained PPS. Only 1 of 16 (6%) nonrheumatoid fluids was positive. Two of 9 (23%) nonrheumatoid inflammatory synovial leukocyte pellets contained PPS. Antibody to PPS by CIE was found in 33% of rheumatoid sera ($P < 0.01$), 45% of nonrheumatoid inflammatory arthritic sera ($P < 0.05$), and 67% of normal control sera.

Characteristics of PPS (bacterial and synovial) include sensitivity to 50 m*M* periodate oxidation, resistance to RNase, DNase, pronase, and boiling. Uronic acid and hexosamine account for approximately 50% by weight. Less than 0.5% protein is present. It appears polydisperse on Sephadex G200 with a MW of approximately 5×10^4 to $>10^6$. Electrophoresis in 3.5% polyacrylamide showed a single PAS positive band which formed a preciptin line with anti-*P acnes* antibody by crossed CIE. No lipid staining bands were noted.

These findings are consistent with a polysaccharide antigen.

The isolation of a bound antigenic bacterial polysaccharide primarily from rheumatoid effusions associated with diminished antibody suggests a pathogenetic role for PPS in rheumatoid arthritis possibly as an immune complex type.

Spontaneous Anti-DNA Antibody Responses in Vitro in Normal and Autoimmune Mice

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An in vitro system for examining the cellular requirements necessary for initiating and regulating antinucleic acid antibody responses has been developed to facilitate a better understanding of the mechanisms responsible for triggering antibody responses to this group of antigens in murine and human SLE. It has been found that spontaneously appearing anti-ssDNA hemolytic plaque forming cells (PFC) can be detected when spleen cells from normal mice of different H^2 types (CBA, DBA, BDF_1, CFW, C57/B16) or young (4 weeks) autoimmune (NZB × NZW F_1 (B/W), NZB, NZW) strains of mice are cultured under standard Mishell Dutton conditions in the absence of added antigen. Such PFC(200–2600/culture) which peak on day 5 and cannot be detected prior to culture consist entirely of specific IgM anti-ssDNA antibody. The response, which appears to be dependent on active antibody synthesis, can be selectively abolished by specific removal of ssDNA antigen binding cells, by rosette depletion. While low responses can be augmented by pokeweed mitogen, there is no obligatory requirement of serum, thymic (T) cells, or macrophages in the culture system.

This in vitro response can blocked by: 1) adding small quantities of ssDNA (10–50 μg) but not similar concentrations of DNA or RNA directly to culture on day 1; 2) pre-incubation of spleen cells with ssDNA (1 hour) and washing prior to culture. Unblocking can be demonstrated by subsequently treating these cells with trypsin-DNAse, suggesting that this inhibition may be due to a membrane receptor blockade mechanism; 3) the addition to young B/W spleen cells of T cells from young (4 weeks) or old (> 6 months) syngeneic mice similarly abrogates this in vitro anti-ssDNA response without effecting cell viability or the anti-SRBC antibody response. On the other hand syngeneic bone marrow cells which markedly suppress the anti-SRBC antibody response have no effect on the anti-ssDNA response of these spleen cells.

These in vitro studies suggest mechanisms that may be relevant to the normal regulation of anti-nucleic acid antibody and other autoantibody responses in vivo. The ability of old B/W T cells to exhibit inhibitory effects similar to those of young preautoimmune B/W mice on the in vitro antinucleic acid antibody response suggests that the suppressor potential of these old B/W T cells may be blocked in vivo.

The Ehlers-Danlos Syndrome Types I, II, and V: An Analysis of the Structure of the Collagen Fibers of the Skin

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The Ehlers-Danlos syndrome (ED-S) is an uncommon, genetically determined disorder of connective tissue. Of the seven clinically distinct types, a basic molecular defect has been reported in Types IV, VI, and VII, all of which are inherited by a recessive mode, and appear to be related to deficiencies in the activity of specific enzymes involved in postribosomal changes. Types I to III are inherited by a dominant mode and might be expected to have defects at the transcriptional level. Type V is sex-linked and has been reported to result from a cross-link deficiency. We have studied 8 patients with ED-S Type I, 3 patients with Type II, and 3 with the X-linked Type V. The results show that the reducible cross-links are present and undergo the same maturation process to nonreducible cross-links as in normal skin. We were unable to confirm the absence of reducible cross-links in the X-linked type ED-S. The ability of the ED-S skin to produce apparently normal cross-linked collagen was supported by cell and tissue culture studies. Transmission electron microscopy and optical birefringence revealed a normal ultrastructure of the collagen fibrils. At a higher morphological level of organization scanning electron microscopy demonstrated a gradual increase in fiber bundle disorder from the X-linked to the myasthenia gravis, where the fibers making up the large fiber bundles demonstrated a considerable inability to aggregate. This disorder could account for the prolonged lag phase in the stress-strain curves, while the presence of cross-links ensures that once stress is on the individual fibers the elasting modulus is normal and the skin can return to normal after hyperextension. The absence of cross-links would lead to an irreversible elongation of the fiber. The low breaking point of the fiber is therefore due to the lack of integrity of the fiber bundles and suggests that the defect lies at a higher order of organization.

Antibodies to Neuronal Cells in Antisera to SLE Cryoproteins

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The cryoprecipitates (cryos) from many SLE sera contain lymphocytotoxic antibody and, when used to immunize rabbits, induce antibodies to human lymphocytes, suggesting that they contain membrane fragments complexed to antibody. Lymphocyte-reactive antibodies in SLE sera also recognized antigens on neuronal cells. To determine whether the membrane fragments in the cryos have the antigenic determinants shared with neurons, antisera generated against 12 SLE cryos were tested in an antibody-dependent, cell-mediated cytotoxicity assay by use of ^{51}Cr-labeled human neuronal cells, SK-N-SH, as targets and normal human peripheral blood lymphocytes (PBL) as effectors. The anti-cryos produced 9.7 ± 3.8 mean % ^{51}Cr-release. Two of the antisera were strongly positive at 35 and 38% cytotoxicity, and 3 others were modestly cytotoxic in the range of 8.3 to 12.4%. The remaining antisera were not significantly different from the controls; 1.3 ± 0.3% ^{51}Cr-release from 12 normal rabbit sera (NRS), and 1.7 ± 0.5% cytotoxicity from a control group consisting of 4 non-SLE anti-cryos. The antibody activity was not linked to SK-N-SH cells. The same sera reacted with human neuronal line, LA-N-1, and 2 human glial lines, A-172 and U-118MG. Unexpectedly there was no correlation between reactivity against the neuronal cells and antibody activity against normal human PBL. Antibody to PBL was not detected in 4/5 anti-cryos with neuronal antibody, and 2 of the antisera unreactive with SK-N-SH had anti-PBL activity. Absorption of the anti-cryo reactive against both SK-N-SH and PBL with the human lymphoblast line Wl-L2 removed all anti-PBL activity but not the neuronal reactivity. Absorption with SK-N-SH eliminated the neuronal reactivity and also diminished the antibody to PBL. The antineuronal activity was not removed by absorptions with human platelets and human RBC. Passage of the anti-cryos over immunoglobulin containing immunoabsorbents removed the detectable anti-immunoglobulin activity without diminishing the neuronal reactivity. Thus, SLE cryoproteins contain antigenic determinants shared by neuronal and glial cells but not by the major cellular elements in blood. It is not yet clear whether those antigens are present on fragments of neuronal membranes or on antigenic structures eliciting cross reactive antibodies.

The Preferential Reaction of dsDNA Antibodies with ss Regions of Native DNA

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It is generally accepted that antibodies to double stranded (ds) DNA are a feature of active SLE, while antibodies to single stranded (ss) DNA occur in many rheumatic diseases. The natural occurrence of antibodies to dsDNA presumes an interaction with the sugar-phosphate backbone of DNA, the immunogenicity of which is unproved. We have attempted to define the preferential reaction site of dsDNA antibodies on a molecular species of dsDNA containing the multiple ss regions, herewith designated ds/ssDNA.

Calf thymus DNA was sheared to a molecular weight of 1×10^6 daltons. Characterization by hydroxyapatite and benzyolated naphthoylated DEAE cellulose chromatography (J Immunol 118:694, 1977) indicated the DNA was predominantly ds with numerous ss regions. Antibodies to chromatographically pure dsDNA (>80% binding in a standard Farr assay) were partially purified by ammonium sulfate precipitation, iodinated with ^{125}I, and reacted with ds/ssDNA. The resulting complexes were precipitated with 4.5% polyethylene glycol (MW, 6000) and redissolved in 0.1 *M* tris-HCl, pH 8.0, 0.01 *M* $MgCl_2$, 0.15 *M* NaCl (the endonuclease buffer). The solubilized complexes were reacted with ss specific endonuclease from Neurospora crassa (0.5μg enzyme/700μg DNA) and compared to undigested complexes by velocity sedimentation in a 5% to 40% sucrose gradient run at 115,000*g* for 15 hours. Undigested complexes sedimented at a position of ≃ 19S; after endonuclease digestion there was a dramatic decrease in the sedimentation velocity to a ≃ 7S position. These findings could be explained by: 1) a disintegration of the DNA into many smaller ds molecules still attached to labeled antibodies, 2) a liberation of antibodies formerly attached to ss regions of the ds/ssDNA, 3) a displacement, by the endonuclease, of antibodies reactive with ss regions. To test these possibilities the procedure was repeated without labeling of the immunoglobulins, the resulting complexes were reacted with ^{125}I dsDNA in a standard Farr assay. After endonuclease digestion free DNA antibodies were readily detected, as shown by a 20-fold increase in DNA binding, 3% to 63%.

These findings indicate that dsDNA antibodies preferentially react with ss portions of dsDNA molecules. Whether dsDNA antibodies ever react with determinants unique to dsDNA, or are merely a manifestation of high avidity binding to a very short ss region of the dsDNA molecule, remains to be determined.

Unique Capacity of NZB Cytotoxic T Cells to Attack Targets Carrying the Same Major Transplantation Antigens

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T cell cytotoxic function in NZB mice has previously been tested utilizing target cells differing from NZB at the major histocompatibility complex (MHC) of mice, H-2. In the experiments reported here, T cell cytotoxic functions of NZB mice against targets identical to NZB at the MHC were investigated. Normal mice do not generate cytotoxicity under these circumstances. Spleen cell suspensions of NZB mice ($H\text{-}2^d$) were cultivated for 5 days in the presence of irradiated stimulator cells of other $H\text{-}2^d$ strains (B10.D2, BALB/c, DBA/2, HW19). On the fifth day ^{51}Cr labeled target cells identical to the stimulating cells were added and the ^{51}Cr release from these targets was measured after 4 hours. NZB effector cells exerted a highly significant specific lysis in all four of the $H\text{-}2^d$ strains tested; none of these reacted against NZB. In experiments in which NZB cells were sensitized against one $H\text{-}2^d$ strain (BALB/c) target cells from all four $H\text{-}2^d$ strains were lysed, demonstrating crossreactivity of the lytic reaction. The amount of lysis observed was dependent on the ratio of effector to target cells. The action of the effector cells was abolished by treatment with anti-Thy-1 serum, indicating their T cell character. The capacity to respond against BALB/c could be demonstrated in NZB mice as early as 4 weeks and as late as 16 months of age.

The capacity of NZB mice to generate cytotoxic T-cells following primary in vitro sensitization against targets carrying the same major transplantation antigens as NZB establishes a qualitative difference between NZB mice and all normal strains since generation of cytotoxicity is restricted under the applied conditions to targets differing at the H-2 in all normal strains heretofore tested. It is suggested that the observed lack of restriction of the cytotoxic reaction in NZB mice is related to the autoimmune reactivity of this strain.

Immunoperoxidase Staining of the Choroid Plexus in Systemic Lupus Erythematosus

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Immune deposits in the choroid plexus of patients with neuropsychiatric manifestations of systemic lupus erythematosus (SLE) and NZB/NZW F_1 hybrid mice are thought to relate to the pathogenesis of central nervous system (CNS) lupus. Since previous studies did not include in their control groups patients with SLE who had no neuropsychiatric manifestations, we looked for immune deposits in the choroid tissue of patients with and without CNS lupus.

Fixed brain tissue containing choroid plexus was retrieved from 6 SLE patients and 3 controls. Ependymal lining cells, choroid stroma, and cortical vessels were reviewed for deposits of IgG, IgM, IgA, IgD, IgE, and kappa and lambda light chains by an immunoperoxidase technique (rabbit anti-human immunoglobulin and peroxidase-antiperoxidase). Complement components were destroyed by fixation.

Immunoglobulins and light chains were found in the ependymal cells and/or choroid stroma of each SLE patient. The pattern or intensity of immunoglobulin deposition did not distinguish those patients with and without neuropsychiatric manifestations. No staining was seen in tissue from the controls.

Though the choroid plexus serves a filtering function, the finding of immunoglobulin deposits in the choroid plexus cannot be correlated specifically with any of the diverse manifestations of CNS lupus. Other clinicopathologic correlations must be sought.

Subject	Clinical Evidence of CNS Lupus	Immunoperoxidase Staining: Ependymal Cells	Immunoperoxidase Staining: Choroid Stroma	Immunoperoxidase Staining: Vessels
SLE 1	Encephalopathy, seizures	2+	0	0
2	Paranoia, disorientation	1+	2+	0
3	Sensory loss, tremor	4+	4+	0
4	None	2+	0	0
5	None	2+	3+	4+
6	None	3	0	0
Controls	7, 8, 9 (cirrhosis, pulmonary embolus, rheumatic heart disease)	0	0	0

Collagenase Production by Cultures Containing Multinucleated Cells Derived from Synovial Fibroblasts

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Multinucleate cells (heterokaryons) are frequently found in rheumatoid synovium as well as in cultures of isolated, adherent cells from this tissue. Using an experimental system we have explored the possibility that multinucleate cells extend the functional capacity of a cell population to secrete proteinases for extracellular matrix degradation.

Polyethylene glycol (PEG) was used to fuse cells in monolayer cultures of rabbit synovial fibroblasts. Fusion began within 30 minutes of PEG treatment and continued for about 4 hours; approximately 40% of the cells developed 2 or more nuclei. In some experiments, up to 30% of fused cells contained 6 or more nuclei, indicating true giant cell formation. Measurement of intracelluar (86 rubidium) to indicate the presence of a leaky cell membrane showed that immediately after PEG treatment, intracellular (86 Rb^+) dropped to 30% of control levels. It began to approach normal by 5 minutes but did not recover fully for about 4 hours, at completion of fusion. During the first 24 hours after PEG treatment, the treated cultures incorporated 1/5 as much (^3H) thymidine as did control cultures, but incorporation of (^3H) leucine into TCA-precipitable radioactivity was unaffected. Autoradiographic studies using (^3H) thymidine revealed that PEG depressed incorporation of the label into DNA for at least 4 days.

Secretion of proteinases from PEG-treated and control cultures in serum-free Dulbecco's modified Eagle's medium was compared. PEG-treated cultures containing multinucleate cells secreted 3180 U (cumulative) latent collagenase into medium changed every 72 hours over 28 days, compared with 279 U produced by control cells during the same period (1 U collagenase degraded 1 μg collagen/hour at 37°C. Collagenase was activated from its latent precursor form by TPCK trypsin—10 μg/ml, 30 minutes, 25°C, followed by addition of excess soybean trypsin inhibitor.) Neither PEG-treated nor control cells released substantial or significantly different amounts of neutral or acid proteinases into medium during the same period.

Our data show that compared to controls, cultures of multinucleated cells have a decreased rate of cell replication and increased rate of collagenase production. Multinucleate cells in rheumatoid synovium may amplify mechanisms for active collagenolysis.

Double-Blind Prospective Azathioprine Study of Polymyositis

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Fourteen previously untreated polymyositis (PM) patients were treated with prednisone 60 mg/day until CPK values normalized and then with 40 mg/day for a total of 12 weeks. They were also randomly placed on either azathioprine (A) 2 mg/kg/day or placebo (P) in double-blinded manner. Manual muscle testing, total prednisone dose required over 12 weeks, and muscle biopsy changes were used to detect differences in the two groups.

The A group (7 patients) turned out to be weaker at onset (total manual muscle testing score −280) than the P group (7 patients with score of −170) but improved more (44 points to −236) than the P group (6 points to −164). However, the difference in improvement was not statistically significant. CPKs in the two groups normalized at about the same rate and each group therefore received comparable doses of prednisone over the 12 weeks.

All followup muscle biopsies at the end of 12 weeks showed improvement in the majority of the eight microscopic features reviewed and in total numerical score. The trend was toward greater improvement in the A group but not significantly so. Changes consistent with steroid myopathy were noted in 7 of 9 women but only 1 of 5 men (4 in A and 4 in P) and 5 of these 8 patients were weaker at 12 weeks. Of special note is the observation that of 13 patients with normal CPK at 12 weeks, 8 still had significant inflammatory infiltrates.

Therefore, azathioprine plus prednisone is not dramatically better than prednisone alone in polymyositis over the initial 12 weeks of treatment. The development of steroid myopathy in over one half the patients is not influenced by A or P and at the prednisone dosages used complicates the evaluation of changes in muscle strength; nevertheless, the trend is toward greater improvement in the azathioprine group in muscle strength and also follow-up muscle biopsy scores. Surprisingly, a normal CPK does not indicate resolution of inflammation in the muscle and cannot be used reliably to indicate full control of the disease. Followup is continuing.

The Natural History of Reiter's Syndrome (RS) in Academic and Community Settings

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With the appreciation of the relationship between immunogenetics and infection in the pathogenesis of RS, interest in this condition is increasing. In order to determine more precisely the natural history of RS, data are presented from two distinct California communities: University (PA) and Community (SB). To date, 102 consecutive patients have been studied: 77 (PA) and 25 (SB). RS is defined as an asymmetric oligoarthropathy (predominantly lower extremity) accompanied by one or more of the following symptoms: 1) urethritis, 2) diarrhea at onset, 3) inflammatory eye disease, 4) mucocutaneous manifestations consisting of balanitis, buccal ulceration, or keratodermia blennorrhagica. Patients with ankylosing spondylitis, psoriatic arthropathy, or other rheumatic syndromes were excluded.

Nineteen (19%) of the 102 presented with diarrhea and 74 (73%) had at least a triad of the above features. There were

more similarities than differences between SB and PA. For example, females represented 14% (SB) and 12% (PA). The mean duration of disease to date is 76.3 months (SB) and 82.8 months (PA), disease activity continuing (albeit, sometimes, episodically) in 82% of the SB practice and 90% of the PA patients. HLA-B27 was present in 79% (SB) and 82% (PA). Only 7% had a persistent monoarthropathy. Twenty-four percent of patients had a positive family history for an inflammatory polyarthropathy. Other similarities included keratodermia blennorrhagica in 24% (SB) and 25% (PA), tendinitis in 43% (SB) and 38% (PA) and heel disease in 50% (SB) and 40% (PA). Sacroiliitis occurred in 23%, 50% of whom had asymmetric change. Only 1 patient with sacroiliitis was HLA-B27 negative. The ESR (3–118, mean 42) appeared unrelated to disease activity. Aortitis was present in only 3 (PA) patients. Differences between PA and SB patients were minimal; balanitis and uveitis appearing more frequently in the former group. Buccal ulceration was seen in 23% of the PA patients and 12% of the SB group.

From this study we conclude: 1) RS is a major chronic rheumatic disease; at least two-thirds of patients have active disease at an 81 month followup. The prognosis for occupation requiring significant exertion should remain guarded. 2) There are minimal differences between an academic and community population of RS patients. 3) There were no discernible differences between disease severity in HLA-B27 positive and negative patients.

Glomerular Deposition of β1H in Immune Renal Disease

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The activities of the major fragment of the third component of complement, C3b, are controlled by at least two proteins, C3b inactivator and β1H globulin. Using in vitro model systems, we have previously demonstrated that β1H physically binds to C3b and blocks its activity.

In the present report immunofluorescent examination of renal biopsies has been used to demonstrate that similar C3b-β1H interaction occurs in vivo. Twelve biopsies from patients with systemic lupus erythematosus or acute poststreptococcal glomerulonephritis were examined. IgG and complement components were found by immunofluorescence to be deposited in a granular fashion in the glomeruli of these specimens. By electron microscopy, mesangial, subendothelial, intramembranous and/or subepithelial electron dense deposits were found. A single biospy in which only C3 was found to be deposited, consistent with activation via the alternative pathway, was also studied, as was one patient with Goodpasture's syndrome, whose biopsy contained linear deposits of proteins along the glomerular basement membrane. Six specimens from patients with such nonimmune renal diseases as arteriolar nephrosclerosis and acute tubular necrosis were also examined. By use of direct immunofluorescence with anti-β1H specifically shown to be free of any contaminating anti-C3, deposits of β1H were found in every instance (14 of 14 exams) in which C3 deposits were observed. This was true regardless of the underlying disease which resulted in the C3 deposits. The spatial distribution of β1H within the glomerulus was often congruent with the distribution of C3 in the same tissue, suggesting that these two proteins were intimately associated. No deposition of β1H was observed in those with nonimmune renal disease. We conclude that binding of β1H to fragments of C3, presumably C3b, occurs in vivo during immunologic activation of the complement system, just as we had previously demonstrated it to occur in vitro.

Development of Antibodies to DNP and to Hydralazine (Hdz) in Patients Taking Hdz

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Hahn *et al.* (Ann Int Med 76:365, 1972) have shown a relationship between anti-Hdz and anti-DNA in 4 patients with Hdz-SLE, and Yamauchi *et al.* (J Clin Invest 56:958, 1975) have shown immunologic cross reactivity between DNA and Hdz with rabbit antisera to Hdz-albumin conjugates. To find if the development of antibodies to DNA or DNP in patients taking Hdz represents the production of anti-Hdz which cross reacts with DNA/DNP determinants, we followed prospectively 21 hypertensive patients taking this drug (average dose 100 mg/day) for one year. Antibodies to Hdz were measured by passive hemagglutination, to native DNA by

Group	Number	Anti-nDNA	Anti-Hdz	Anti-DNP	ANA
Control (↑BP no Hdz)	52	0	2	2	1
Random (↑BP on Hdz)	52	0	8	0	11
Prospective initial (no Hdz)	21	0	2	0	3
one year (on Hdz)	21	0	16	8	3

millipore filter technique, to DNP by radioimmunoassay, and to ANA by immunofluorescence on rat liver sections.

In the prospective group the major finding was development of increased levels of anti-DNP in 8 patients, 7 of whom also made anti-Hdz. Nine other patients made anti-Hdz without anti-DNP. None made anti-nDNA. One developed a mild Hdz-SLE syndrome with anti-Hdz and anti-DNP. Examination of sera of 4 other patients with Hdz-SLE syndrome by radioimmunoassay failed to show an immunologic cross reaction between Hdz and DNP although such a reaction was found in guinea pig anti-Hdz-BSA serum.

The results show a relationship between production of antibody to Hdz and to DNP in patients taking Hdz but do not support the hypothesis that anti-DNP in such patients is antibody cross reacting with Hdz. They are consistent with the findings of Fritzler and Tan (Clin Res 25:A483, 1977) that anti-DNP in drug-related SLE reacts with the histone moiety. The relationship of the immune response to Hdz to that to DNP remains unexplained.

Idiotypes in Rheumatoid Sera

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Anti-idiotypic antibodies recognize antigenic determinants (idiotypes) uniquely associated with a given antibody molecule, or closely related molecules. The in vivo interaction between idiotypes and anti-idiotypic antibodies has been postulated to play a central role in the regulation of the immune system. The purpose of this study was to characterize the idiotypic antigens on three monoclonal IgM anti-γ-globulins and to determine if cross-reacting idiotypes were present in the sera of patients with rheumatoid arthritis (RA).

Anti-idiotypic antibodies against the purified IgM (κ) anti-γ-globulins Lay and Si, and the IgM (λ) anti-γ-globulin Koh, were raised in rabbits. When analyzed by solid phase radioimmunoassay (RIA), each antibody reacted only with the immunizing antigen, and not with pooled IgG or other IgM paraproteins lacking anti-γ-globulin activity. Studies with recombinant molecules reconstructed from Lay or Si light or heavy chains and light or heavy chains from heterologous proteins demonstrated that the idiotypic antigens were formed from a specific heavy-light chain interaction. The idiotypes were closely related to the antibody combining site, as judged by the inhibition of idiotype–anti-idiotype binding by antigen, i.e., IgG, and of idiotype-antigen binding by anti-idiotype. Idiotypes cross-reacting with the monoclonal IgM (κ) anti-γ-globulin Si, but not with the IgM (λ) anti-γ-globulin Koh, were found in increased concentration in the sera of patients with seropositive RA. The idiotype positive material, as analyzed by gel filtration, was found in the 19S but not the 7S fraction of serum.

From these experiments we conclude: 1) that the idiotypes on IgM anti-γ-globulins are associated with the antibody binding site and are the product of a specific light-heavy chain combination; 2) IgM rheumatoid factors in RA patients, although polyclonal, may have idiotypic antigens cross-reacting with those on monoclonal IgM (κ) anti-γ-globulins; 3) IgG rheumatoid factors probably have different idiotypic antigens than IgM rheumatoid factors and may therefore have different active sites and antibody specificities; 4) anti-idiotypic antibodies may be useful for the selective suppression of anti-γ-globulin production without a general depression of immune responsiveness.

Connective Tissue Activation: Stimulation of Prostaglandin Secretion by Mediators Isolated from Lymphocytes (CTAP-I) and Platelets (CTAP-III)

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Connective tissue activating peptides from lymphocytes (CTAP-I) and platelets (CTAP-III) are known to stimulate glycosaminoglycan synthesis, glycolysis, and mitogenesis in connective tissue cell cultures. Direct evidence suggested that increased accumulation of cyclic AMP was involved in the mechanism of action of these peptide agonists, and increased prostaglandin E synthesis was postulated on the basis of indirect evidence. In the present experiments, CTAP-I and -III were incubated with human and murine cells in culture, and prostaglandin E was measured by radioimmunoassay using antibody directed primarily to prostaglandin E_2.

Both CTAP-I and -III markedly stimulated the elaboration of prostaglandin E_2 into culture medium, the earliest evidence of increased synthesis occurring at 4 hours with maximal concentration found at 24 hours. In a typical experiment, after 24 hours incubation, buffer, CTAP-I and CTAP-III treated cells produced, respectively, 39, 1150, and 580 pg of prostaglandin $E_2/1.5 \times 10^6$ normal human synovial cells. Substantial residual stimulation persisted at least through 48 hours. The lymphocyte factor (CTAP-I) appeared to be more potent than CTAP-III in stimulating prostaglandin synthesis. Indomethacin (13.0 μg/ml) obliterated basal and incremental synthesis of prostaglandin in the presence of mediators. Cycloheximide (8.7 μg/ml) did not affect the stimulation of pros-

taglandin synthesis by CTAP-I and -III. Four nonrheumatoid synovial cell strains showed similar basal levels of prostaglandin E_2 and similar responses to CTAP-I, while a rheumatoid cell strain was unusual in having a very high basal level of prostaglandin in E_2 formation (477 ± 101 pg/1.5 × 10^6 cells) and was markedly stimulated by both mediators. A murine fibroblast cell strain (3T3) showed increased prostaglandin E_2 synthesis on exposure to CTAP-I and the KB tumor cell strain was markedly stimulated by CTAP-III.

These studies substantiate the postulated increase in synthesis of E series prostaglandins by human connective tissue cells on exposure of CTAP-I and -III, and clarify the mechanism of action of these agonists on "activated" target cells. The importance of elevated extracellular concentrations of prostaglandins is uncertain, although they may potentiate the actions of CTAP-I and -III.

Collagen Biosynthesis During Repair of Normal and Injured Articular Cartilage

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In order to examine the repair collagens produced by cells present in injured cartilage, the femoral articular surfaces of three groups of New Zealand white rabbits were scarred in the following manner: superficial and deep lacerations, and drilled holes. Eight weeks after surgery the rabbits were sacrificed and slices of injured articular cartilage harvested. The types of collagen produced at the site of these lesions were identified by labeling the recovered specimens with ^{3}H proline and characterized by SDS gel electrophoresis, CMC chromatography, and CNBr peptide analysis.

In all cases, tissue-specific Type II ($[\alpha_1(11)]_3$) cartilage collagen was synthesized. Histologic examination revealed the chondrocytes bordering the cartilage injured by deep laceration and drill holes responded by increased cellular activity. Grossly, the drilled holes were completely filled with tissue possessing staining and morphologic characteristics similar to that of hyaline cartilage.

These data strongly suggest that in the lacerative type of injury, the surrounding tissue will produce enough matrix for repair only when the subchondral bone is violated. Both repaired and normal cartilage produce tissue-specific Type II collagen.

Activation of B-Lymphocytes in NZB Mice

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We recently demonstrated that, from birth on, NZB splenic B cells spontaneously secrete 40 to 100 times as much pentameric IgM as normal B cells (J Immunol 119:1639, 1977). Spleen cells from 6 to 10 week old mice have been examined in further study of this abnormality. NZB spleen suspensions contain 8 to 10 times as many spontaneous plaques to sheep erythrocytes and to TNP conjugated sheep erythrocytes as normal mice. There is a similar increase in the number of cells secreting IgM as detected by a reverse plaque assay. After cold ethanol-acetic acid fixation, it can be shown that NZB spleen contains 4–6% cells with cytoplasmic IgM compared to 0.2–0.4% in normals. NZB spleen cells were sorted by flow microfluorometry (FMF) into surface Ig negative cells and 4 pools with increasing levels of surface IgM or total Ig. The surface Ig negative fraction produced no IgM but the positive pools each had the same level of IgM production and cytoplasmic staining. When sorted into 4 pools on the basis of size, however, all the IgM production was found in the largest fraction. NZB spleen suspensions contain more large cells than normal spleens by Coulter volume measurements and FMF reveals more large surface Ig positive cells in NZB than normal spleens.

When surface Ig was enzymatically removed before fixation, it was found that the NZB cells with cytoplasmic Ig were 4 to 5 times brighter than those from normal mice. Thus NZB spleen contains approximately 10 times as many cells which synthesize and secrete IgM as normal mice. Each such NZB cell appears to contain and presumably secrete about 5 times as much IgM as do cytoplasmic Ig positive cells in normal mouse spleen.

Because NZB mice spontaneously produce antibody to thymocytes, we utilized FMF at high gain to search for immunoglobulin on peripheral T cells. None could be demonstrated. After fixation, however, immunoglobulin was readily detected in NZB splenic T cells. Whether this immunoglobulin is passively acquired and rapidly pinocytosed and thus not available for staining on the cell surface or endogenously synthesized by the T cell is under investigation.

Increased Ratio of Surface IgM/Surface IgD on Spleen Cells from NZB Mice

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In an effort to define the cellular basis of abnormalities of polyclonal B cell activation previously noted in NZB mice, the surface immunoglobulin (sIg) isotypes of spleen cells from young and old NZB mice were examined. After lactoperoxidase-catalyzed radioiodination, cells were lysed, the immunoglobulins bound to rabbit anti-mouse immunoglobulin and the

immune complexes absorbed to *S aureus*. The complexes were solubilized and the cell surface immunoglobulins analyzed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Cell surface immunoglobulin isotypes were also analyzed by staining spleen cells with fluorescein-conjugated rabbit anti-μ, anti-δ, and anti-Ig sera and examining stained cells by fluorescence microscopy or by automated cytofluorometry on a Becton-Dickinson FACS-III cell sorter.

Spleen cells from both young (6 weeks old) and old (one year old) NZB mice were found to have markedly increased ratios of cell surface IgM/IgD compared to cells from BALB/c control mice. The altered ratio of sIg isotypes was not a consequence of increased proteolytic activity present in NZB cell suspensions since the addition of NZB cells to iodinated BALB/c cells did not alter the BALB/c ratio of sIgM/sIgD. It was not due to the presence of cytophilic antibody or autoantibody since the sIgM was found to have a sedimentation coefficient of 8S and was thus not of serum origin. When examined by fluorescence microscopy or by cytofluorometry, NZB spleen cells were found to have normal numbers of sIgM+ and sIgD+ cells, indicating that the altered sIgM/sIgD ratio observed in the radioiodination experiments was due to abnormal densities of these isotypes on B cells rather than to the absence of an IgD-bearing B cell subset.

The increased sIgM/sIgD ratio may be a consequence of in vivo polyclonal B cell activation, since in vitro polyclonal activation of mouse spleen cells has been shown to alter this ratio in a similar manner. Since the increased ratio was noted as early as 6 weeks, these data provide support for the concept that polyclonal B cell activation precedes the onset of autoimmune disease in NZB mice.

A Comparative Study of Binding of SLE Sera to Poly dAT and a Well Characterized Native DNA Preparation

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Sera from patients with systemic lupus erythematosus (SLE) will bind native DNA (n-DNA). Poly dAT, a synthetic n-DNA, consists of regularly recurring base pairs. This results in a restricted antigenicity but ensures that the molecules do not contain single-stranded (ss) regions. Many laboratory preparations of n-DNA will be contaminated with either ss DNA or structures with ss regions in predominantly duplex molecules. The aim of this study was to determine a relationship between antibodies to poly dAT and native DNA in individual SLE sera.

One hundred sera from 35 patients with SLE were used in this study. Antibodies to poly dAT and n-DNA were measured in duplicate in each serum using a millipore filter technique. Poly dAT was prepared from dATP and ^{3}H labeled dTTP in the presence of *E coli* DNA polymerase. Native ^{3}H labeled DNA was extracted from HAE 70 cells after incubation with ^{3}H thymidine. Optimal concentrations of each antigen were calculated for use in the assay. Each preparation was structurally analyzed by both hydroxyapatite (HAP) elution studies and ethidium bromide fluorescence (EBr) to determine the presence of ss DNA or ss regions within a predominantly duplex molecule. The specificity of antibodies to poly dAT and n-DNA was assessed by passing sera over agarose columns bound to either circular PM2 DNA or poly dAT and measuring both n-DNA and poly dAT antibodies in serum and eluate. Neither preparation contained ss DNA assessed by HAP elution. Poly dAT was entirely duplex as assessed by EBr fluorescence but our n-DNA contained 15% ss regions within the predominantly duplex molecules. Under optimal conditions each serum showed consistently less binding to poly dAT than to n-DNA. A significant correlation was seen between the binding to poly dAT and n-DNA ($r = 0.91$, $P < 0.01$). This correlation was consistent in all sera tested and no serum showed exclusive binding to only one preparation. Binding to both antigens could be removed by passing a serum over an agarose column carrying either poly dAT or n-DNA.

These results suggest that in clinical practice diagnosis and management of SLE will be provided by measurement of antibodies to either poly dAT or n-DNA provided that the n-DNA preparation is well characterized and does not contain ss DNA or significant ss regions. Despite its limited potential antigenic sites, poly dAT provides a useful alternative to n-DNA and does not possess ss regions to which other antibodies may bind with less diagnostic specificity.

PGE_2 Modulates Collagenase Production by Cultured Adherent Rheumatoid Synovial Cells

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Cultured adherent rheumatoid synovial cells (ASC) produce collagenase and prostaglandins, particularly PGE_2. With age in culture and passage, collagenase and PGE_2 production decrease; however, release of these substances can be stimulated up to > one hundredfold by a factor (apparent mol wt of ~ 14,000) released by cultured human peripheral blood mononuclear cells (MCF). MCF stimulation of collagenase production by ASC is enhanced by addition of low concentrations of indomethacin (indo), 1 n*M*; such concentrations of indo block PGE_2 synthesis > 50% even in the presence of

MCF. At higher indo concentrations (1–10 μM) which inhibit PGE_2 synthesis ~ 100%, collagenase stimulation is usually inhibited by ~ 60%. In some ASC stimulated with MCF, this inhibition by indo (10 μM) is reversed by addition of small amounts of PGE_2 (10 ng/ml). Addition of these small amounts of PGE_2 results in augmentation of collagenase stimulation to levels greater than those seen when cells are stimulated with MCF alone. Addition of larger amounts of PGE_2 (1 μg/ml) under these conditions tends to inhibit collagenase. ASC respond to exogenous PGE_2 with increased levels of cAMP. MCF also modulates this cAMP response: preincubation of ASC with either indo or MCF plus indo augments cAMP response to exogenous PGE_2. It is possible that some of the effects of PGE_2 on collagenase in ASC are mediated through adenylate cyclase.

PGE_2 levels thus have profound effects on collagenase production by ASC and modulate collagenase response to stimulation with MCF. Therefore, PGE_2 inhibition with pharmacologic agents in vivo could have beneficial or deleterious effects with regard to connective tissue destruction depending upon the type, sensitivity, and prior environment of the responding cell.

Decreased Levels of T Gamma Cells in Active Systemic Lupus Erythematosus (SLE)

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Tγ cells, a subset of T lymphocytes, bear Fc receptors for IgG and have suppressor activity for antibody production after activation with IgG immune complexes. Levels of Tγ cells as well as total T lymphocytes were measured in 19 patients with systemic lupus erythematosus (SLE), 11 with active and 8 with inactive disease, and in 47 normal subjects. Mononuclear cells were separated on a Ficoll-Hypaque gradient, depleted of adherent and phagocytic cells, and pelleted with neuraminidase-treated sheep erythrocytes. Rosette-forming cells were counted to determine the percentage of total T lymphocytes. Rosettes were then mechanically dissociated and T cells separated from sheep erythrocytes through a Ficoll-Hypaque gradient at 37°C. Purified T cells were pelleted with ox erythrocytes sensitized with rabbit IgG and rosette-forming cells recorded as Tγ cells. A total of 200 cells in each of three replicate assays was counted.

Active SLE patients showed a significant decrease in total T lymphocyte percentages compared to normal subjects ($P < 0.05$), confirming previous reports. In addition, markedly depressed percentages of Tγ cells ($P < 0.002$) were found in patients with active disease. Patients with inactive SLE were not significantly different from normal subjects, both with regard to total T lymphocytes as well as Tγ cells.

	Mean ± Standard Error		
	Total Lymphocytes/mm³	% Total T Lymphocytes	% T_γ Cells
Normal subjects (mean age 38)	2823 ± 227	62 ± 2	11 ± 1
Active SLE (mean age 33)	2410 ± 425	53 ± 4	4 ± 1
Inactive SLE (mean age 38)	1788 ± 465	59 ± 6	14 ± 2

Two acute SLE patients who went into remission showed significant increases in both total T lymphocytes and Tγ cells with clinical and laboratory improvement.

Active SLE patients show decreases both in total T lymphocytes and the Tγ subset of lymphocytes associated with suppressor activity. Low levels of detectable Tγ cells in SLE may reflect the presence of high levels of circulating immune complexes and/or the defect in suppressor activity reported in these patients.

The Natural History of Uric Acid Overproduction in Sickle Cell Anemia

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The 6–8 fold increase in red cell turnover in patients with sickle cell anemia results in uric acid overproduction. Since uric acid overproduction in these patients begins within the first 5 years of life, we studied 91 patients with sickle cell anemia and normal GFR ranging in age from 6 to 48 to determine the natural history of serum uric acid and urate excretion in sickle cell disease.

Hyperuricemia (plasma urate 6.5 mg/dl) was observed in only 2 of 26 patients younger than age 13. However, urinary uric acid/creatinine ratios and 24 hour urinary uric acid levels were elevated in these patients, indicating uric acid overproduction and hyperuricosuria. Thirty-seven percent of adults (24/65) were hyperuricemic (mean plasma urate 8.6 ± 0.5 mg/dl); 41 were normouricemic. Of normouricemic adults studied while on a purine-free diet, 9 of 16 (56%) had 24 hour urine uric acid excretion greater than 600 mg. Urate clearance in these 9 patients was increased (Cur 14.8 ± 0.8 ml/min), indicating that normouricemia was maintained by hyperuricosuria. Urate clearance in the hyperuricemic subjects was decreased (Cur 5.3 ± 0.8 ml/min) as compared to both normal subjects (Cur 8.2 ± 0.6 ml/min) and normouricemic adults with sickle cell disease (Cur 14.8 ± 0.8 ml/min). Urate clear-

ance appears to be the major determinant of serum uric acid concentration even in sickle cell patients with urate overproduction.

Responses of urate clearance to probenecid and pyrazinamide were exaggerated in the normouricemic overexcretors [PZA suppressible urate clearance (PSur):SS, 12.6 ± 0.8 ml/min; control 6.4 ± 0.8 ml/min; probenecid (PB) response; SS, 48.8 ± 9 ml/min; control, 40.4 ± 8.0 ml/min] and were diminished in the hyperuricemic subjects with diminished urate clearance (PSur, 4.9 ± 1.2 ml/min; PB response, 15 ± 2.2 ml/min). Urate clearance was correlated PAH clearance. These results suggest that changes in urate clearance were secondary to changes in tubular secretion in urate.

The initial response to urate overproduction in SS disease is hyperuricosuria with increased urate clearance and maintenance of normal serum urate concentration. Hyperuricemia is found in adults with diminished urate clearance and is associated with other evidence of impaired renal tubular function.

The Glomerulonephritis of Polymyositis

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Nephropathy is a little known complication of polymyositis. Thus, we describe the features of glomerulonephritis in 5 patients with polymyositis.

The patients (4 men and 1 woman) aged 21–41 years each presented with symmetrical limb girdle weakness, widespread polyarthritis, myalgias, recurrent fever, and proteinuria. CPK, LDH, and SGOT levels were elevated in all cases, and the diagnosis of polymyositis was confirmed by electromyography and muscle biopsy.

Although Raynaud's phenomenon and sacroiliitis were each present in one case, no patient had the skin changes of dermatomyositis, and no evidence of other diffuse connective tissue disease or underlying neoplasm. ANA was negative in all patients; cryoglobulins, LE preparations, and ASOT were absent in 4 patients tested. C3 complement level was decreased in one case. Rheumatoid factor was negative in 4 and latex positive 1:160 in one case.

Blood urea nitrogen and serum creatinine were normal in each patient; however, the 24 hour urine protein excretion ranged from 1.7 to 4.4 gm. Three patients had an abnormal urine sediment and one had myoglobinuria. Renal biopsies (4 patients) showed a mild focal segmental mesangial increase. Immunofluorescence was negative in one biopsy and mildly positive in a granular pattern for IgG and IgM in 2 others. Treatment of all patients with high-dose corticosteroids led to rapid clearing of the proteinuria and slower improvement of the polymyositis.

A 10-year review in our center of 70 polymyositis cases showed proteinuria exceeding 300 mg/24 hours in 9 patients and abnormal urinary sediment in 4. None of these latter patients had underlying malignancy.

A focal glomerulonephritis associated with polymyositis may be more common than is generally appreciated. Although the pathogenesis of the renal lesion in these cases is unknown, it may be related to myoglobin.

Lymphocyte Plasma Membrane 5'-Nucleotidase: A Partial Deficiency in Agammaglobulinemia

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Two enzymes of the purine nucleotide degradation pathway, adenosine deaminase and purine nucleoside phosphorylase, are associated with specific immunodeficiency syndromes. A third catabolic enzyme, 5'-nucleotidase, was measured on the external surface of peripheral circulating lymphocytes from patients with immunoglobulin deficiencies. All 8 male patients with congenital agammaglobulinemia demonstrate reduced activity of lymphocyte ecto-5'-nucleotidase to 30 to 48% of the normal value. The mean activity is 5.7 ± 1.0 nM/hr/10^6 cells for congenital agammaglobulinemia as compared to the mean normal value of 15.0 ± 5.6. Patients with common, variable hypogammaglobulinemia or selective IgA deficiency have values within the normal range. Two other lymphocyte plasma membrane ectoenzymes, ATP'ase and nonspecific phosphatase, have similar values in lymphocytes from normal subjects or from subjects with congenital agammaglobulinemia. The activity of 5'-nucleotidase in lymphocyte lysates has similar values in normal and enzyme deficient subjects. Ecto-5'-nucleotidase has similar activity in both T and non-T lymphocytes in all subjects and the deficiency occurs in both these categories of cells in the affected patients. Ecto-5'-nucleotidase from normal and enzyme deficient subjects has a similar pH optimum of 7.5, is similarly inhibited by adenosine-5'-α, β-methylene diphosphonate, has hyperbolic kinetics and a similar Michaelis constant of 25 μM for AMP.

These data suggest that the reduction of lymphocyte 5'-nucleotidase activity is an abnormality localized to the plasma membrane in this X-linked, B-cell immunodeficiency syndrome.

Effect of Immune Complexes on Clearance of Single-Stranded DNA in Mice

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In vivo clearance of exogenous single-stranded DNA (ssDNA) is extremely rapid and occurs primarily through the liver. With higher doses of ssDNA, however, both liver uptake and blood clearance approach a maximum, enabling large molecular weight ssDNA to persist in the circulation (Emlen, Mannik; J Exp Med, March, 1978).

In the present study, we examined the effects of prior saturation of the liver with immune complexes on the clearance kinetics of ssDNA. Heat denatured calf thymus ^{125}I ssDNA was chromatographed on hydroxyapatite and a defined molecular weight was obtained by gel filtration over Sepharose 4B. Immune complexes were prepared at 5-fold antigen excess with human serum albumin and purified rabbit anti-human serum albumin. Female C57BL/6J mice were injected at time 0 with immune complexes containing 5 mg antibodies or with buffer. At 3, 6, 12, 24, or 48 hours, animals were given 50 μg of ^{125}I ssDNA; serial blood samples were assayed for radioactivity and clearance velocities were calculated by linear regression analysis. Clearance of ssDNA was slowed in the mice pretreated with immune complexes. Control mice cleared ssDNA at a rate of 2.63 $\pm$ 0.27 μg/ml/min, while animals pretreated with immune complexes cleared the ssDNA at a rate of 1.70 $\pm$ 0.04 at 3 hours ($P < 0.01$), 1.36 $\pm$ 0.31 at 6 hours ($P < 0.001$) and 1.39 $\pm$ 0.49 at 12 hours ($P < 0.01$). Clearance returned to normal at 24 and 48 hours. The degree of suppression of ssDNA clearance velocity at different times after administration of immune complexes correlated with the previously established values for the amount of immune complexes present in the liver at a given time.

To examine the effects of pretreatment with immune complexes on the saturability of ssDNA clearance mechanisms, 10, 50, or 100 μg of ssDNA were given 6 hours after administration of immune complexes. With the 100 μg dose the clearance velocity approached a maximum, but this value was less than half of the maximum clearance velocity in normal mice. These observations indicate that immune complexes alter ssDNA clearance by decreasing the number of sites in the liver to which DNA can bind, or by decreasing access of DNA to the liver.

By altering clearance kinetics, immune complexes may contribute to the persistence of DNA in the circulation of patients with SLE.

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Surgical Stabilization of the Rheumatoid Wrist

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Rheumatoid arthritis (RA) frequently affects the wrist, leading to pain, loss of function, and destruction of normal anatomy. Surgical treatment by synovectomy alone does not correct carpal deformities and ulnar deviation; fusion of the wrist, although effective in reducing pain, severely limits function.

To prevent loss of upper extremity function while simultaneously correcting deformities and relieving pain, we combined synovectomy with distal ulnar excision, correction of carpal supination-subluxation, and repair of damaged extensor tendons. This surgical stabilization approach was performed 61 times in 50 patients with RA. All had definite or classic RA and had not responded to aggressive physiotherapy, including splints and local and systemic medications. All had synovitis with deformity and instability. The deformity most commonly seen was carpal supination-subluxation with relative prominence of the distal ulna. The primary reasons for operation were frank (23) or potential tendon rupture (3), persistent synovitis with pain at rest (21), and pain with significant deformity (14). The mean age was 48 years (range: 21–76).

The wrist was exposed with preservation of the extensor retinaculum. Following excision of the distal ulna, a synovectomy of the radiocarpal and intercarpal joints was carried out. Any existing carpal dissociations were realigned, and the carpal supination-subluxation deformity was corrected by placing the wrist in pronation and reconstructing the triangular ligament using the volar capsule. Extensor tendon repair and grafting were then carried out. Length of followup was 30 months with a range of 1–8 years.

Results were classified as good if the patient was satisfied, had only occasional pain, a stable wrist, and no recurrence of synovitis. Three patients were lost to followup and of the remaining 58, good results were obtained in 42. An additional 12 had objective improvement but experienced recurrence of synovitis and pain and an extensor lag of up to 15°. Less satisfactory improvement was found in 4 of the 58. The average loss of motion was 47%, leaving patients with a combined palmar and dorsiflexion range of 42°.

Although this stabilization procedure is not considered appropriate for the severely destroyed wrist, the overall 90% improvement (fair and good results) justifies its consideration in preference to either synovectomy or wrist fusion alone.

Synovial Inflammation in Rheumatoid Arthritis: Stimulation of Collagenase Secretion by Type II Cartilage Collagen

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The destructive inflammation observed in synovia of rheumatoid joints may be largely due to autoimmune processes. Antibodies to types I, II, III collagens have previously been detected in joint fluids of rheumatoid patients and cell-associated antibodies which react only with cartilage type II collagen can be locally detected in inflamed rheumatoid synovia. We are interested in finding evidence for cell-mediated immunity to collagen. Immune lymphocytes challenged with antigen produce substances (lymphokines) that can grossly stimulate the secretion of the enzyme collagenase from macrophages. Collagenase, which can degrade cartilage collagen producing cartilage destruction, is also secreted by macrophage-like cells in rheumatoid synovia and its secretion can be stimulated from synovial cells by a factor(s) produced by lymphocytes, unstimulated or stimulated with phytohemagglutinin.

In the present study inflamed synovia were removed at surgery from 9 patients with classic rheumatoid arthritis. Synovial fragments were maintained in a viable state in organ culture for up to 2 weeks. Culture media were changed every 2 days and the collagenase secreted into the culture medium was assayed. To these cultures were added type I or type II collagen in the form of diffusible immunologically active fragments prepared by cyanogen bromide cleavage of native collagen. In 3 of 9 rheumatoid patients, the addition of type II collagen fragments resulted in a significant increase in collagenase secretion compared with explants which had not been stimulated: artificial stimulation of lymphocytes in these explants with phytohemagglutinin also resulted in a similar increase in collagenase secretion. Type I collagen peptides had no stimulatory effect.

These results support the thesis that in some patients rheumatoid synovia contain immune lymphocytes which can respond to type II collagen peptides produced by degradation of hyaline cartilage collagen. When challenged with antigen, these cells may produce factors that stimulate the secretion of collagenase from synovial cells leading to further cartilage destruction.

The Efficacy of Intraarticular Corticosteroid for Osteoarthritis of the Knee

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Although intraarticular corticosteroid injection has been considered a therapeutic adjunct in rheumatology for the last 25 years, scientific proof of efficacy is still lacking. The present study was undertaken, therefore, to determine the effect of intraarticular steroids on pain in the osteoarthritic knee by use of a controlled, double-blind protocol.

Thirty-four patients with symptomatic osteoarthritis of the knee were included in the study. Patients previously injected with local steroids were excluded. Half the patients were treated with 20 mg of triamcinolone hexacetonide injected into the knee, the other half with the same volume of placebo (vehicle without steroid). The two physician-experimenters performed the arthrocentesis and withdrew any synovial fluid, if possible. A nurse-assistant then injected either steroid or placebo through the same needle according to a predetermined, random schedule. Thus, the physicians who conducted the study did not know the nature of the material each patient received. Knee pain was quantified prior to, and at 1, 4, 6, and 8 weeks after injection.

At 1 week the steroid group had significantly reduced knee pain compared with the pretreatment assessment. This reduction persisted through the 8 week evaluation period. The placebo group also exhibited a significant amount of pain relief at 1 week, but this was significantly less than that experienced by the steroid group. From 4 through 8 weeks there was no statistically significant difference between the 2 groups. Whether or not synovial fluid was initially aspirated did not affect results. Postinjection flares occurred as often with placebo as with steroids and did not affect the subsequent course.

It is concluded that intraarticular steroids reduce the pain of osteoarthritis, but such a response cannot be distinguished from a placebo effect by 4 weeks postinjection.

Antibodies to Histone in Patients with Drug-Induced and Idiopathic Lupus Erythematosus

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It has been reported previously that antibodies to histones can be demonstrated by immunofluorescence. When tissue sections are extracted with 0.1 N HCl, histones are eluted from nuclei, and the extracted tissue contains only DNA devoid of nuclear proteins. Histones can be reconstituted to nuclear DNA by incubation of acid-extracted tissues with purified calf thymus histones. Sera containing antibodies to histones show positive antinuclear antibody (ANA) staining

on untreated tissue, become ANA negative on acid-extracted tissue and regain ANA positivity on histone-reconstituted tissue.

Sera from 23 patients with drug-induced lupus erythematosus (procainamide 19, isoniazid 2, nitrofurantoin 2) and 20 patients with idiopathic (not drug-induced) systemic lupus erythematosus (SLE) were studied. All 23 drug-LE patients had positive ANA on control tissues but ANA became completely negative on acid-extracted tissues. On histone-reconstituted tissues, 22/23 again became ANA positive. In contrast, of 20 idiopathic SLE sera which were positive for ANA on control tissues, only 12 became negative and of these, 4 again became ANA positive on histone-reconstituted tissues while the other 8 remained negative. Three other SLE sera showed partial reduction in ANA titer but increased in titer on histone-reconstituted tissue. Thus, 22/23 drug-LE patients (96%) had antibodies to histones compared to 7/20 SLE patients (35%).

To determine the specificity of anti-histone antibodies, the H1, H2A-H2B and H3-H4 fractions of calf thymus histone were prepared by differential salt extraction and Sephadex gel filtration and used in reconstitution experiments. It was shown that in 21/22 of the drug-induced sera antibodies were primarily against H2A-H2B, while in the 5 idiopathic SLE sera studied, antibodies to all classes of histone were found. A further difference was that idiopathic SLE sera had antibodies to native DNA and to the nonhistone proteins Sm and nuclear RNP whereas drug-induced LE sera did not.

The heterogeneity of ANAs in idiopathic SLE and the striking prevalence of anti-histone antibodies in drug-induced LE, as demonstrated by immunofluorescence, have been valuable clinically in differentiating between these two syndromes.

A Syndrome Resembling Progressive Systemic Sclerosis (PSS) Following Bone Marrow Transplantation—A Model for PSS?

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The etiology of PSS is unknown and progress toward its understanding has been hampered by the lack of a model for the disease. It has recently been reported that skin changes similar to those of scleroderma develop in some long-term survivors of bone marrow transplantation (BMT). Five such subjects, who lived an average of 19 months post-BMT, developed sclerodermatous cutaneous involvement and also underwent examination for visceral complications resembling PSS. At present, 2 are alive and 3 have died. We compared their findings with the findings in 49 well-characterized patients with scleroderma to ascertain the similarities and differences between these two groups.

Taut hidebound skin was found in all patients in both populations and selected skin biopsies were also similar. Restrictive lung disease, characterized by decreased vital capacities and diffusing capacities, was found in 88% of the PSS patients and in 4/5 (80%) of the BMT group. Cardiac disease and Raynaud's phenomenon, similar to that found in the PSS group, were noted in one post-BMT patient, and pathologic evidence of PSS-like esophageal involvement was found in another.

In both the BMT and PSS groups, the responses of lymphocytes to suboptimal concentrations of PHA were depressed, while complement levels, screening tests for autoantibodies and circulating levels of IgA and IgM were essentially normal. The frequency of patients with abnormal ANA and DNA-bindings was higher in the PSS group than in the BMT group. The incidence of abnormal levels of immune complexes, IgG levels, anergy, and decreased percentages of B- and T-lymphocytes was higher in the BMT patients than in those with PSS.

Even though only 19 months have elapsed since transplantation, these 5 post-BMT patients have begun to develop signs and symptoms of a PSS-like disease. We suggest that BMT bears closer examination as a model for PSS.

Inhibition of IgM and IgG-Induced Cell-Mediated Cytoxicity with Human Effector Cells by IgM Rheumatoid Factor and Its Fragments

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This study was initiated to further investigate the inhibitory effect of IgM rheumatoid factor (RF) in an antibody-dependent cell-mediated cytotoxicity (ADCC) assay. This assay employs human peripheral blood lymphocytes as effector cells and IgG or IgM sensitized, ^{51}Cr labeled ox erythrocytes as target cells. Serum and synovial fluid were obtained from patients with seropositive classic rheumatoid arthritis (RA) and heat inactivated. RF was prepared by dialyzing the sera and synovial fluid against sodium acetate buffer (pH 4.0), fractionating on Sephadex G-200 with the same buffer and pooling the fractions constituting the leading side of the 19S peak, dialyzing against PBS and further purifying by passage over an IgG-coupled Sepharose 4B column and eluting with acid. This IgM-RF was tryspin digested (enzyme:substrate

ratio = 1:50) at 60°C for 20 minutes and the reaction stopped by adding soybean trypsin inhibitor. The fragments were fractionated on BioGel A5m to separate undigested IgM, $Fc_5\mu$ and Fab. The IgM-RF, $Fc_5\mu$ and Fab fragments were tested for inhibitory capacity in IgM and IgG induced ADCC at both the effector and target cell levels. It was found that IgM and IgG induced ADCC was inhibited by IgM-RF and its tryspin digest fragments. Inhibition at the effector cell level was achieved by RF $Fc_5\mu$ and Fab whereas inhibition of the target cell level was achieved only by RF Fab.

These results indicate that RF may inhibit ADCC at the effector or target cell level and thus modulate an immune response that is of potential importance in immune-complex disease.

Association of Three Interrelated Histocompatibility Determinants with Susceptibility to Rheumatoid Arthritis

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Patients with seropositive rheumatoid arthritis (RA) of adult-onset were compared to control groups by utilizing two primary histocompatibility procedures, B-cell (Ia) alloantigen typing and mixed lymphocyte culture (MLC) reactivity. By use of a two-stage microcytotoxicity assay with a panel of 30 alloantisera, the frequencies of various B-cell alloantigens in patients with RA were compared to those of control populations, including patients with multiple sclerosis. Three B-cell alloantisera were identified that gave particularly high frequencies of reactivity with RA patients. Delineation of the specificity of these 3 alloantisera on a panel of 36 B-cell lines derived from individuals homozygous for MLC alleles revealed that reactions were obtained only with those lines from individuals that were positive for HLA-Dw4, Dw7, or Dw10. Absorption of the alloantisera with B-cell lines from Dw4, Dw7, or Dw10 positive individuals eliminated the seroreactivity with all B-cell lines having any of these three determinants, thus demonstrating that the alloantisera react with a B-cell antigen common to cells with either of the three MLC specificities. MLC testing of the RA patients with a panel of normal homozygous cells defined in the Seventh International Histocompatibility Workshop revealed an increase in the frequencies of HLA-Dw4 and Dw10 as compared to controls (26% versus 14.6% and 13% versus 6.9%, respectively), while the frequency of HLA-Dw7 was not significantly different (23% versus 18.6%). The individuals who were negative for the HLA-D alleles Dw4, Dw7, and Dw10 by conventional MLC testing yet positive for the shared B-cell alloantigenic specificity were of particular interest. The results indicate that a shared antigenic specificity exists among alleles of B-cell alloantigens that is, in turn, partially related to particular MLC alleles. Thus susceptibility to RA may be a function of the inheritance of the molecules bearing this antigenic specificity.

Mechanisms of Cellular Interaction with Monosodium Urate Crystals

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Activation of mediator cells such as platelets and neutrophils plays an important role in the pathogenesis of gout. Monosodium urate crystals (MSU), the probable initiating agents of gouty inflammation, have been shown to stimulate suspensions of washed platelets or neutrophils. When MSU crystals are coated with IgG (as occurs in plasma), stimulation is markedly enhanced. These studies, using MSU induced human platelet serotonin secretion as a model, examined the nature of cellular recognition mechanisms for the IgG-coated MSU crystal and the uncoated crystal. F(ab')2 fragments of specific anti Fc antibody blocked and the lipopolysaccharide of S minnesota R595 enhanced human platelet secretion induced by IgG-coated urate crystals. These agents had little effect on stimulation by uncoated crystals. This indicated that urate crystals stimulate platelets independently of fluid phase IgG. Urate crystals directly stimulated suspensions of washed rabbit platelets which lack Fc receptors. In contrast to human cells, stimulation was blocked by IgG. This again demonstrated IgG-independent cell stimulation by urate crystals. Calcium pyrophosphate dihydrate crystals could trigger human platelet secretion only when coated with IgG. This suggests that when crystals were coated with IgG, the surface-bound IgG alone may be the stimulus to the cell. This was confirmed by the finding that polyvinyl pyridine-N-oxide, a hydrogen acceptor, blocked human platelet stimulation by uncoated but not IgG-coated urate crystals. In contrast to IgG, F(ab')2 fragments, IgM, or IgA did not enhance human platelet stimulation by urate crystals. This indicates that the effect of IgG on urate crystal stimulation of platelets depends on the Fc region of IgG. These data demonstrate that urate crystals (and potentially other surfaces or particles) can stimulate a mediator cell by at least two mechanisms: by direct stimulation without the mediation of absorbed IgG or when coated with IgG, by triggering the cell via Fc receptors.

Serologically Active Clinically Quiescent SLE—A Discordance Between Clinical and Serological Features of SLE

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The correlation of serologic abnormalities, especially low serum complement and high levels of anti-DNA antibody, with disease activity has been established in SLE. The significance of abnormal serologic tests in the absence of active clinical disease is still unclear. This report describes a group of 14 patients seen in the course of a prospective study of SLE in whom a discordance between clinical and serologic features was apparent. These patients had persistently positive LE preps and antinuclear antibody tests, low serum complements, and high levels of DNA binding. The mean lymphocyte response to Con A mitogen was suppressed in these patients as compared to age and sex matched controls done on the same day.

These patients did not differ significantly from other patients with SLE, seen during the same period, in the frequency of skin manifestations, Raynaud's phenomenon, and arthritis; they showed trends toward a lower frequency of photosensitivity, serositis, mucous membrane ulcers, and neuropsychiatric involvement and had a statistically significant lower frequency of alopecia and nephritis. They also resembled the larger group in the presence of most laboratory abnormalities with only hypergammaglobulinemia occurring less frequently. These patients have been followed untreated for a mean of 4¼ years without evidence of clinical exacerbations of disease.

Thus in individual patients with SLE it would seem advisable to determine whether their clinical course and laboratory abnormalities are concordant. Some patients will illustrate this pattern and thus therapeutic manipulation according to changes in laboratory variables may be indicated. Some patients will display clinical-laboratory discordance and could then be treated for clinical exacerbations only, irrespective of laboratory changes. Followup in our 14 patients would indicate that patients with such humoral and cellular immune abnormalities may safely be followed untreated for prolonged periods.

Total	F	M	Mean Age	Mean Duration of Untreated	Mean Number of Criteria	+LE	+ANF	Anti-DNA Ab	↓CH50 or C3
14	13	1	38	4¼ years	5	12	14	13	10

Viral-Like Particles in Cocultivated Rheumatoid Synovial Cells

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Independently and in two separate laboratories, cocultivation techniques have demonstrated the presence of virus-like particles (VLPs) in rheumatoid synovial membrane cells (RSC). One technique consisted of cocultivation of long-term RSC cultures with human lung fibroblasts (WI 38); the other system utilized cocultivation of freshly dissaggregated RSC with fetal rabbit synovial cell cultures. Controls in both laboratories consisted of similar cocultivations using cultures derived from nonrheumatoid membranes. All cocultivations were maintained for 2–3 months without subculture, during which time small foci of heaped-up cells were observed. By electron microscopy, all the rheumatoid cultures (15) but none of the control cultures (7) showed VLPs. These appeared as spherical bodies with a spike-like external array, seen both in sonicated negatively stained (PTA) preparations, and in thin sections of harvested RSC cocultivations. These VLPs were found budding from the cell membranes, and also within cytoplasmic vacuoles. Their morphology resembled that of budding RNA viruses, although coronaviruses and myxoparamyxoviruses may also show these characteristics in certain situations. Similar particles have not been previously demonstrated in RSC. Serial passage of cocultivation extracts containing VLPs onto other cell types resulted in repeated transient cytopathic effects. Extracts of the RSC cocultivations were injected into suckling mouse brains, resulting in a reproducible illness not observed when control extracts were similarly injected. Additional studies are under way to identify the agent and to explain its presence in the RSC cocultivation systems.

Inhibition of Human Marrow-Granulocyte Precursors by Serum from Patients with Felty's Syndrome

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The mechanism responsible for leukopenia in Felty's syndrome (FS) is unknown. Both increased peripheral destruction of leukocytes and decreased marrow production may play a role in this process. The present study was designed to determine whether factors in the sera of patients with FS inhibit the maturation of granulocyte precursors from human bone marrow.

Sera were obtained from 19 patients with FS; 10 patients with rheumatoid arthritis, splenomegaly, and normal white cell counts; 9 patients with active RA, and 10 patients with osteoarthritis. Nucleated cells were isolated from human marrow, and single cell suspensions were cultured in McCoy's medium and fetal calf serum. The serum to be tested, 0.1 ml, was incubated with 0.1 ml of the cell suspension (containing 10×10^6 cells/ml) and 0.1 ml rabbit serum, and the mixture was placed in 3% agar. At the end of 12 days, the number of cells giving rise to granulocyte-macrophage colonies (CFU-C) were counted. Serum from a healthy adult was run simultaneously as a control. Inhibition of maturation was considered to be present if the number of CFU-C in the agar incubated with the test serum was reduced by at least 25% as compared to healthy control serum.

Sera from 8 of 19 patients with FS caused a significant reduction in the number of CFU-C. Seven of the 8 patients with serum inhibitors had undergone splenectomy; in 3 of these subjects the inhibitor was absent prior to splenectomy, but present after splenectomy. None of the sera from the 29 control subjects gave a positive response. These data indicate that the sera of certain patients with FS contain a factor that inhibits human granulocyte precursors in vitro. This factor may play a role in the induction of leukopenia in FS. The presence of a serum inhibitor of granulocyte maturation may also explain the failure of some patients with FS to respond to splenectomy.

Lymphocytotoxic Antibodies in Systemic Lupus Erythematosus (SLE): Evidence for Reactivity with i Antigen

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This study was designed to determine whether lymphocytotoxic antibodies (LCA) in patients with SLE had specificity for the Ii antigen system; the Ii antigens are known to be present on the surface of all formed elements in blood. Sera from 10 patients with SLE were tested for LCA using the standard microdroplet assay. Following determination of the LCA in whole sera, each serum was serially diluted twofold in saline and retested for LCA. The dilution of the serum which gave a 50% decrease in cytotoxicity was used in the following absorption experiments. Each diluted serum was absorbed threefold with either adult (rich in I antigen) or cord (rich in i antigen) red cells at 4°C, and then retested for LCA activity. Eluates were prepared from 2 sera incubated with either adult or cord cells at 4°C, and the eluates assayed for LCA.

The cytotoxicity of the whole sera was 79% or greater in all but one serum. Seven of the diluted sera showed an obvious reduction in cytotoxicity following absorption with cord cells, but minimal or no reduction after absorption with adult erythrocytes. Of the remaining 3 sera, the LCA were equally reduced by adult and cord cells in 1, and were unaffected by absorption in 2. In the 2 sera studied, eluates prepared from cord cells showed greater LCA activity than eluates from adult cells. Statistical analysis confirmed that the mean cytotoxicity values in the sera after absorption with cord cells were significantly different from those obtained after absorption with adult cells ($P < 0.005$). Additional studies showed that there was a strong correlation between the level of LCA and the titers of cold agglutinins against cord red cells.

These data indicate that the LCA in most SLE sera react with i antigen. Cold-reactive antibodies with i specificity have been associated with hemolytic anemia and thrombocytopenia in other disease, thus, the capability of LCA to react with i antigen may explain the observation that LCA were found in higher frequency and greater titer in SLE patients who have hematologic abnormalities.

The Role of Oophorectomy in Experimental Immune Synovitis

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Clinical and experimental observations indicate that estrogens may play a part in rheumatoid arthritis (RA) and in the regulation of immune responses. In order to delineate this relationship, the effect of oophorectomy in an experimental immune synovitis resembling RA was studied.

Nineteen adult female rabbits were sensitized to homologous IgG and then randomly divided into 4 groups. Group I (6) underwent oorphorectomy. Group II (7) had the same but were treated with 1 mg of estradiol intramuscularly every other week. Group III (3) had a sham procedure; Group

IV (3) were unoperated controls. An immune synovitis was then induced in the right knee by the biweekly intraarticular injection of 1 mg of IgG and the animal sacrificed after 8 weeks. Skin testing to IgG was done at 2 week intervals. Serum cortisol levels were performed prior to sacrifice by use of a radioimmunoassay technique.

Gross and histologic assessment of synovial inflammation and cartilage changes was recorded using a "blind" grading previously reported from 0 for no changes to 3+ for severe involvement. Synovial cathepsin D activity was analyzed by the Anson technique.

There were no changes in the initial positive skin tests to IgG in any groups. Cortisol levels in Groups II, III, and IV were 5.06, 3.53, 3.60 μg% without significant differences, while in Group I, the 1.7 was significantly depressed. Synovial inflamation in the oophorectomy rabbits was 1+, compared to the unoperated and sham groups of 2+. The reduced synovitis in Group I was reflected by a significantly lower cathepsin D level of 1.2 μ moles/hour/1 mg of protein compared to 3.15 and 3.06 for the other groups. However, when oophorectomy rabbits were given estrogen replacement, there was an increased synovitis rated 3+ with a significantly elevated cathepsin D level of 5.3. Cartilage changes were rated 2+ and appeared uniform throughout the groups.

The results of this study indicate that in this model of immune synovitis, oophorectomy appeared to inhibit synovial inflammation while estrogen replacement seemed to enhance this response. Whether this inhibition is by direct endocrine mechanisms or through the immune system remains to be studied. Finally, since RA has a significant female predilection, these findings may be important in the pathophysiology of this disease.

Suppressor Cell Function in Patients with Sarcoidosis

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We investigated the role of suppressor cells in the depressed cellular immunity of patients with sarcoidosis. We have recently described a glass adherent prostaglandin producing suppressor cell that inhibits T-cell mitogenesis in phytohemagglutinin (PHA) stimulated cultures of human peripheral blood mononuclear cells (PBMC) by secreting PGE_2 (J Exp Med 146:1719, 1977). Increased activity of this cell is responsible for the hyporesponsiveness to PHA seen in PBMC from patients with Hodgkin's disease (N Engl J Med 297:963, 1977).

The mean response of 14 patients with active sarcoidosis to three concentrations of PHA was significantly ($P < 0.01$) less than controls. Passage of the cells over glass wool resulted in a 116% increase in the mean response to PHA in the sarcoidosis patients and a 39% decrease in controls. The PHA response of the active sarcoidosis patients went from 20% of control before passage over glass wool to 71% of control after. Addition of indomethacin, a prostaglandin synthetase inhibitor, to PHA cultures increased the response of the PBMC from patients with sarcoidosis 192 ± 32% versus a 112 ± 18% increase in controls (mean ± SEM, $P < 0.01$). The sarcoidosis patients had an increased percentage of monocytes in the peripheral blood mononuclear cell preparations (31 ± 5% monocytes for patients, 13 ± 2% for controls, mean ± SEM, $P < 0.01$) and the percent monocytes in a PBMC preparation from patients with active sarcoidosis correlated with the percent increase in PHA response after glass wool passage ($r = 0.62$, $P < 0.05$).

Thus, there appear to be at least three mechanisms operating in the depressed PHA response of PBMC from patients with active sarcoidosis. First, there appears to be increased activity of the prostaglandin producing suppressor cell. Second, the increased proportion of monocytes correlates with increased suppression, either via a diluting effect or perhaps through another active suppressive effect. Blockade of these first two mechanisms by indomethacin and glass wool passage does not result in total restoration of the PHA response of patients with active sarcoidosis, and therefore, a third, as yet unknown, factor must also contribute to the depressed PHA response.

The Hand of the Child with Juvenile Rheumatoid Arthritis

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The rheumatologist is aware of hand deformities in the adult. Very little is written concerning the hand of the child with juvenile rheumatoid arthritis (JRA). Roentgenographic and clinical analysis was done, review of surgery and outline of treatment is given. Five-hundred patients are under treatment.

There are three basic types of onset in JRA. Systemic onset is similar to that described by Still; polyarticular closely resembles the disease in the adult; pauciarticular involves four or less joints. JRA has a much better prognosis than the adult type but tends to produce more stiffness. Examination of 100 successive children showed 10% had radial deviation of the metacarpophalangeal (MCP) joint and 23% had decreased

flexion. Seven percent had boutonniere deformity and 1% had a swan-neck contracture. There was decreased flexion of the PIP in 27% and decreased extension in 10%. The wrist showed 55% decreased extension and only 22% decreased flexion.

A statistical review of the records and x-rays of 200 children showed finger involvement in approximately 50%, wrist involvement in approximately 25%, and very few cases of boutonniere or swan-neck. Ulnar shortening in 93 joints measured on roentgenogram showed a range of 1–10 mm with an average of 4.1 mm. Ulnar wrist deviation was seen in 67 joints with an average of 13 degrees and radial deviation in 12 joints with an average of 11 degrees. Metacarpophalangeal ulnar deviation was seen in 31 with a range of 2 to 25 degrees and an average of 8, and a MCP radial deviation also of 31 between 2 and 15 degrees with an average of 7.4 degrees. There was no statistical correlation between ulnar shortening, ulnar deviation, and metacarpophalangeal radial deviation, as reported in previous literature.

Surgery between 1965 and 1975 was rare and included collateral ligament release in 2 patients, tenolysis on 5 occasions, carpal tunnel release, and reconstruction of thumb MCP. Terminal vasospasm required finger amputation. Synovectomy of 16 MCP and 10 PIP joints was done and studied between 1965 and 1970. No recurrences were seen in late followup, but stiffness precluded continuing this procedure.

The common problem of wrist flexion contracture is best managed by physical therapy and extension orthosis, and the loss of finger flexion by active exercises, occasional injection, and active splinting.

The Influence of Matrix Structure on the Diffusivity of Glucose in Hyaluronic Acid (HA)

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We have previously demonstrated that the translational movement of glucose in pathologic synovial fluids is too rapid to be accounted for by bulk diffusion (Fed Proc 36:1069, 1977). Such enhanced diffusivity is a property of an isolated 2.5% matrix of HA as well, wherein both diffusivity and matrix structure are critically dependent on Ca^{++} concentration (see Semin Arthritis Rheum 7:141–152, 1977 for review). The current study examines translation movement of glucose in a 1% matrix.

Human umbilical HA and agarose were prepared as 1% matrices in phosphate buffered saline at pH 7.0. The bulk diffusion coefficients (D) for glucose and sucrose within each matrix were determined in codiffusion experiments employing a capillary method. The D for both solutes in HA was indistinguishable from that in agarose. If the matrix was prepared in 50% horse serum, glucose diffusivity was enhanced twofold in HA while slightly diminished in agarose. A 50% dialysate of serum as a solvent enhanced glucose diffusivity in HA threefold; the dialyzed protein had little effect. The enhanced glucose diffusivity in the presence of a 50% dialysate was inversely dependent on solute concentration. The diffusivity of sucrose in HA was little altered by these solvent changes.

In order to gain insights into matrix structure that might underlie the enhanced glucose diffusivity, Darcy numbers were determined. Permeability is dependent on HA concentration, has a pH optima of 7.0 in a 1% matrix, and is critically dependent on Ca^{++} concentration rising rapidly below 10 m*M*. Matrix structure is highly dependent on the solvent environment particularly in the physiological range. Constituents in the serum dialysate may induce a matrix configuration that supports the enhanced glucose diffusivity.

The matrix configuration that exists in the presence of the dialysate is likely to be present in synovial fluid as well. This configuration can facilitate the translational movement of a nutrient such as glucose. Facilitated movement may be homeostatic in inflammatory states such as rheumatoid arthritis where low synovial fluid glucose concentrations are well described.

Circulating Immune Complexes in Mixed Connective Tissue Disease (MCTD)

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Sera from 19 patients with MCTD were examined for the presence of immune complexes (IC). The diagnosis was based on serologic and clinical criteria. All patients had antibodies to extractable nuclear antigen by passive hemagglutination. Titers of 1:1,000,000 or greater were found in 17 patients and 1:655,000 and 1:40,960 in the remaining two. Treatment of the antigen with RNAse resulted in a 1,000-fold or greater decrease in titer in 18 patients and a 64-fold decrease in one. Rheumatoid factors were present in 8 patients and antinuclear antibodies in 12. All patients had a mixed rheumatic syndrome. Clinical features included: Raynaud's phenomenon—16, swollen hands and/or sclerodactyly—12, myopathy—11, pleuritis—12, malar or diffuse rash—7, and arthritis—15. One patient had a stable IgA membranous glomerulopathy.

IC were measured in 46 sera by a Raji cell radioassay (RC-RA), monoclonal rheumatoid factor radioimmunoassay (MRF-RIA), and Clq binding assay (Clq-BA). IC were detected by at least one method in 45 (98%) sera. The single negative serum was from a patient with clinically inactive disease. IC were found by the RC-RA in 42 (91%) sera, by MRF-RIA in 22 (48%), and by Clq-BA in 20 (43%). Mean values for each assay compared to normal controls were: 76.8

versus 1.7 μg agg.-IgG equiv/ml for the RC-RA ($P < 0.001$); 12.0 versus 4.4 μg agg.-IgG equiv/ml for the MRF-RIA ($P < 0.001$); 19.2% versus 10.9% ^{125}I-Clq bound for the Clq-BA ($P < 0.001$). IC were detected by all three assays in 9 (20%) sera and by two methods in 21 (46%). Thus, IC were found by two or more methods in 30 (65) sera. Only three sera failed to react in more than one assay. A different pattern of reactivity was found in 89 rheumatoid arthritis sera where IC were detected at a higher frequency by MRF-RIA (71%) and Clq-BA (82%) while the frequency by RC-RA (28%) was lower. Sufficient clinical data were available for 16 of the MCTD patients to permit preliminary evaluation of the relationship of disease activity and IC. Changes in levels of IC appeared to parallel clinical activity in 12 patients by RC-RA, in 9 by MRF-RIA, and in 3 by Clq-BA.

These data indicating a high incidence of IC in MCTD and a different pattern of reactivity from RA emphasize the wide spectrum of immune complex disease.

Identification of Hydroxyapatite Crystals in Synovial Fluid

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Hydroxyapatite (HA) has been found by others in synovial fluid and fluid leukocytes by electron microscopy and electron probe. We have developed a method to quantify HA in synovial fluid which avoids possible EM artifacts. (^{14}C) EHDP, (SA 10.6 μCi/omole) was used in a final concentraion of 16 n*M*/ml in normal serum or phosphate buffered saline (PBS) containing standard HA or to joint fluid samples; serum or joint fluids were processed under oil. After rotation × 3h 4°C, radioactivity/0.1 ml was determined before and after centrifugation (49000 × *g*), and expressed as percent loss of nuclide from the supernatant. Standard HA binding in serum $x \pm$ SEM:10^3μg/ml = 96.6 ± 0.7:10^2μg/ml = 72.1 ± 2.1; 10μg/ml = 13.4 ± 1.8; none = −1.2 ± 0.7.

Monsodium urate or calcium pyrophosphate dihydrate (CPPD) crystals failed to bind in concentrations up to 0.5 mg/ml and 1.0 mg/ml respectively. The centrifuged pellets were washed in distilled water and dried to a spot which showed HA by x-ray diffraction. This method was sensitive to 25–50 μg HA standard.

We confirm the finding of HA crystals in joint fluids, handled physiologically, in amounts equal to 1–15 μg HA standard/ml. Such small amounts and the low WBC in these fluids fail to support a phlogistic role for HA crystals.

	Joint Fluids	Inflammatory (WBC 3000/cmm)	Non-inflammatory (CPPD Crystals Also Found)
% Binding	0	15	29
	1–4	0	7 (1)
	5–9	0	6 (1)
	10	0	6 (5)

A Serial Study of Reticuloendothelial (RES) Fc Receptor Function in Patients with Systemic Lupus Erythematosus

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Patients with systemic lupus erythematosus (SLE) have circulating immune complexes (IC) which are thought to be involved in disease pathogenesis. Using a newly described method for in vivo evaluation of RES IgG Fc membrane receptor function, we previously demonstrated a defect in untreated patients with active SLE and related this RES defect to the presence of circulating immune complexes. A serial study of the correlation of defective RES Fc receptor function, immune complexes, and disease activity was undertaken to further investigate the interaction of these factors. Isologous ^{51}Cr-labeled erythrocytes were sensitized with human anti-Rh(D) and their intravenous clearance determined. Clearance rates (T-1/2) C′ levels and IC levels by ^{125}I-Clq precipitation were determined prior to therapy in 17 SLE patients, 12 of whom had active disease. Fifteen of 17 had markedly prolonged clearance rates (range = 80 to > 1,000 minutes; average clearance T-1/2 in 14 normal volunteers = 40 minutes, range 35 to 50 minutes). The correlations of clinical activity with 1) Clq binding and 2) prolonged clearance were statistically significant ($P < 0.005$ and $P < 0.05$, respectively; Spearman Rank Coefficient). In addition, elevated Clq binding and depressed C4 levels were significantly correlated ($P < 0.05$). At the end of from 1 to 19 months, 10 patients who initially had prolonged clearance were restudied. Patients had been treated with either corticosteroids (7 of 10) or nonsteroidal, anti-inflammatory drugs (3 of 10). Clinically, 7 of 10 were improved; the remaining 3 continued to do well. Although a clearance defect persisted in all 10 (T-1/2 from 88 to 600 minutes), in 7 of 10 T-1/2 was markedly improved, suggesting improved Fc receptor function. In 7 patients, there was an apparent correlation between increased clearance and clinical improvement. There was also a significant correlation between clinical improvement and a decrease in Clq binding ($P < 0.05$). These studies underscore the interrelationships between the presence of circulating immune complexes, defects in membrane Fc receptor function which might lead to continued circulation of these complexes, and disease activity in SLE.

Lyme Arthritis: Immune Complexes Correlate with Stage and Type of Disease

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We have found immune complexes in patients with Lyme arthritis, an apparently tick-transmitted illness that typically begins with the skin lesion, erythema chronicum migrans (ECM). Many patients subsequently develop clinical attacks of synovitis histologically like that of rheumatoid arthritis; some also develop other systemic complications (aseptic meningitis, peripheral neuropathy, or carditis). We measured circulating immune complexes (CIC) serially in 58 patients with ECM, by the ^{125}I-Clq binding assay. The table shows the first test result on each patient's serum in each of the designated categories.

Circulating immune complexes—an antigenic component of which may be related to the causative agent—were almost always present very early in the illness and in patients with systemic complications. In contrast, they were found less often during attacks of arthritis and still less often during remissions. Quantitative levels tended to diminish with time; in individual patients they fluctuated in a dampened hemi-sine-wave pattern in parallel with recurrent bouts of active disease.

Synovial fluid from all of 7 patients contained immune complexes, compared to only 3 of 7 concomitant sera. In the 3 positive sera the concentrations were much less than in the corresponding joint fluids.

We suggest that the causative agent is introduced into the skin, where ECM is the initial clinical manifestation of a systemic, immune-mediated inflammatory disorder that often becomes localized to and propagated in synovium. Thus, Lyme arthritis can be considered a human model for an infectious etiology of rheumatoid arthritis.

Patients	Onset*	Arthritis†	Remission	Systemic Complications
Tested	25	17	13	10
With CIC	21 (84%)	6 (35%)	3 (23%)	9 (90%)

* Within 3 weeks of appearance of ECM.
† More than 4 weeks after appearance of ECM.

Comparative Effectiveness of Five Analgetics for the Pain of Rheumatoid Synovitis

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Little attention has been given to analgesia, divorced from suppression of inflammation, in rheumatoid arthritis (RA), despite its importance to patients. This study was designed to determine the comparative efficacy of five often used analgetics for the pain of active RA synovitis. Single doses of 650 mg aspirin (ASA), 650 mg acetaminophen (Ac), 65 mg codeine (Co), 50 mg pentazocine (Pe), 65 mg propoxyphene (Pro), and placebo (Pl) were given in a randomized double blind fashion, and on a PRN basis, to each of 30 carefully selected hospitalized RA patients with painful synovitis of at least 4 joints. All other drugs and distracting activities were prohibited during the study period and at least 6 hours of observation required between doses of test agents. Subjects recorded time of onset and duration of maximal relief, percent of relief achieved from each agent and all possible side effects, and, after taking all 6 agents, ranked their order of preference, with 1 as most effective. Onset and duration of action of all agents were similar except Ac and Pl, which required longer to act and lasted a shorter time. Side effects occurred significantly more frequently with Pe, somewhat more often with Co, but were otherwise insignificant. Effectiveness was analyzed according to some of all ranks, mean percent relief and percent of subjects achieving 50% or greater relief for each agent.

As expected, by parameters measured in this study and by cost to the patient, ASA is a superior analgetic in RA. However, Ac, by cost and effectiveness, is an acceptable alternative, as codeine would be except for its narcotic properties and cost. Cost, side effects, and limited effectiveness restrict the usefulness of Pe. Pro seems no more effective than Pl and of questionable usefulness in RA, except perhaps as a placebo.

Agent	Sum of All Ranks		Mean % Relief		% with 50% or > Relief	
ASA	87	superior to Pl ($P < 0.05$)	54.8	superior to Pl ($P < 0.05$)	67	superior to Pl ($P < 0.01$)
Ac	99		50.7		57	
Co	100		53.2		57	
Pe	103		43.0		53	
Pro	117		39.5	inferior to ASA ($P < 0.05$)	37	inferior to ASA ($P < 0.01$)
Pl	124		35.8		47	

Activation of C3 by Monosodium Urate, Potassium Urate, and Steroid Crystals

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There is evidence that the inflammatory properties of crystalline material may be modified by their interaction with serum proteins. In this study, the activation of C3 by crystals is quantitated by two dimensional crossed immunoelectrophoresis (TDIEP).

In polypropylene tubes 0.5 ml aliquots of fresh human serum were mixed with 0.1 ml of buffer containing 2.5 mg of washed crystals. The tubes were incubated with agitation of 37°C for 1 hour and then centrifuged at 15,000 *g* for 4 minutes. Aliquots of the supernatants were diluted appropriately in EDTA-containing buffer to inhibit further conversion of C3. By use of standard techniques, TDIEP was performed using rabbit antisera to human C3. A method was devised whereby 6 samples could be compared simultaneously on a single plate. After staining, the combined area under the β_1C and β_1A peaks was measured using planimetry. The numerical results below are percentage points of total area under the β_1A peak in excess of that seen in saline controls. The coefficient of variation of the ratio of the two peaks was 0.008 for a single specimen on the same plate. The areas could be corrected to indicate milligrams of C3 converted.

Sodium urate was highly active in the electrophoretic conversion of C3 with a dose and time related response. Activation was prominent at concentrations of crystals seen in gouty effusions. Heating the crystals at 200° C for 2 hours reduced C3 conversion by nine-tenths, but also altered morphologic properties of the crystal preparations. Conversion was inhibited by increasing concentrations of EDTA and eliminated by 0.005 *M* EDTA. The EDTA inhibition was not seen in the presence of excess calcium. With the conditions described above, potassium urate crystals were much less active (8%) than sodium urate (31%). Eight different commercially available preparations of steroids gave varying but small amounts of C3 activation (−1 to 5%). It is demonstrated that different crystals and altered forms of the same crystals vary in their interaction with serum and their ability to activate complement. It is not known what properties are responsible for these phenomena. The calcium requirement suggests that the interaction of sodium urate with complement occurs at or before C1. It is possible that immunoglobulin, which has a high affinity for sodium urate and is found in sodium urate crystals in vivo, is altered and initiates complement activation by the classic pathway.

Occult Giant Cell Arteritis

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Temporal artery pain, polymyalgia rheumatica, and blindness are well recognized manifestations of giant cell arteritis. However, the disease may often present in a less evident fashion. This is demonstrated by 22 (39%) of our series of patients.

Of 56 patients with biopsy-proved giant cell arteritis, the chief complaint was polymyalgia rheumatica in 17; temporal pain in 15, and loss of vision in 2. In the remaining 22 patients, the predominant complaint that brought them to the physician did not suggest the diagnosis of giant cell arteritis, since headache and polymyalgia were either absent or so minimal as to escape notice.

These complaints included fever of unknown origin in 6 patients; malaise, anorexia, weight loss, and abnormal liver function tests suggesting occult malignancy in 6; and unexplained anemia in 2.

Four patients presented with a neurologic syndrome; 2 had diplopia and 2 acute weakness of one arm. Claudication was the chief complaint of 4 patients, involving the leg in one, the arm in one, and the jaw in 2.

All patients responded well to steroid therapy. The diagnosis of giant cell arteritis in patients such as this who do not present in typical fashion depends on detection of a very rapid erythrocyte sedimentation rate, biopsy of a temporal artery, and the awareness that the disease may manifest itself in a variety of ways.

Prospective Study of Immunologic Effects of Hydralazine in Hypertensive Patients

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The administration of certain drugs induces clinical and laboratory features of systemic lupus erythematosus (SLE) in man but few studies of this human disease model have been undertaken. Hydralazine, a useful antihypertensive agent, is one of these drugs and has acquired a "bad press" because of such observations. A prospective study of 25 hypertensive patients with age, sex, and race matched controls was begun 3 years ago. All subjects had complete clinical examinations; ANF, DNA binding; immunoglobulin and complement levels; skin tests to a battery of antigens and lymphoprolifera-

tive responses to mitogens and antigens obtained prior to starting hydralazine and serially throughout the study. All subjects were ANF negative at the start of treatment. Fifteen of the 25 have shown varying ANF response (in undiluted serum) but only 3 had a titer of 1:10 or above, one becoming positive at 12 months and 2 at 24 months. One of these subjects had clinical symptoms suggestive of SLE and the drug was stopped. Five subjects have shown a proliferative lymphocyte response to hydralazine and/or its metabolites (JCI 56:958, 1975) including the 3 with positive ANF. Acetylation rates were obtained in 22 of the hypertensive patients. Thirteen were slow and 9 fast acetylators. The 3 ANF positive subjects were all slow acetylators as were 4 of the 5 with proliferative lymphocyte responses to hydralazine and its metabolites. This study to date suggests that hydralazine in its present form may be less likely to cause serologic and clinical SLE than previously reported and that screening by acetylation rates may define a susceptible population. This and other lupus related drugs continue to be a rich source of data relevant to lupus in humans.

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Composition and Clinical Significance of the Neutrophil Inclusions Which Form in the Presence of SLE Sera

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Normal neutrophils (PMNs) develop intracytoplasmic inclusions after incubation with SLE sera. The resulting inclusions stain for IgG, IgM, and C3 and are believed to be immune complexes removed from the sera in vitro. Such inclusions have also been identified in the circulating PMNs from SLE patients. The present study examined the relationship between PMN inclusions and clinical and laboratory features of SLE. Sera were carefully collected at 37° from 35 SLE patients. Normal buffy coat cells were incubated in the SLE sera for 90 minutes at 37° and then centrifuged and washed. Slides of the washed cells were prepared in the cytocentrifuge, stained with FITC goat anti-human IgG, IgM, IgA, and C3, and examined under ultraviolet light. The composition of these complexes was also examined with a fluorescent probe for double-stranded polynucleotides with the aid of ethidium bromide (EB). EB was added to sera known to contain high concentrations of anti-DNA antibodies and then incubated with normal PMNs as before. Preparations included 1) no added EB, 2) EB only, 3) FITC anti-human IgG (F1-anti-IgG) or 4) F1-anti-IgG and EB. Inclusions containing both IgG and IgM correlated with clinical activity ($P < 0.001$), depressed serum complement ($P = 0.026$), cryoglobulinemia ($P = 0.014$), and anti-nDNA antibodies ($P < 0.001$). IgG inclusions alone were not significantly correlated with any of the parameters. C3 and IgM appeared to be mutually exclusive, i.e. when one was present, the other was never present. EB staining inclusions (red fluorescence) suggested that polynucleotides were present.

These findings suggest: 1) the PMN inclusions consist of immune complexes which contain double-stranded polynucleotides, 2) these complexes correlate with disease activity when IgG and IgM are both present, and 3) such complexes may contribute to a number of granulocyte disturbances seen in patients with SLE.

Inhibition of Neutral Protease Activity in in Vitro Stored Cartilage by α-Tocopherol

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In previous studies on preservation of articular cartilage performed in our laboratories, it was found that addition of α-tocopherol (200 μg/ml) to the culture media of stored cartilage resulted in improved preservation of its biochemical and biomechanical properties when compared to cartilage stored in absence of the vitamin. Although it has been shown that α-tocopherol stabilizes lysosomal membranes in other systems, its mechanism of action in preservation of articular cartilage in vitro has not been elucidated. To evaluate the possibility that α-tocopherol effects on stored articular cartilage were due to stabilization of lysosomal membranes, we developed a highly sensitive assay for neutral protease activity employing a biosynthetically prepared protein substrate. This was necessary since colorimetric assays for acid phosphatase and β-glucoronidase were not sensitive enough to detect enzymatic activity. The radiolabeled protein substrate was prepared by incubating embryonic chicken livers with ^{3}H-tryphophane in vitro and it was subsequently purified by $(NH_4)_2 SO_4$ precipitation. The very high specific activity of the substrate (324,000 dpm/mg protein) permitted quantitation of the neutral protease activity released into the media of the stored cartilage explants. Employing this assay we found that α-tocopherol resulted in significant inhibition of this activity as shown in the table.

Neutral Protease of Tissue Culture Media at 9 and 13 Days of Culture (Equivalent Trypsin Activity/mg Dry Weight of Explant)

Experiment No.	Day 9		Day 13	
	Control	+ α-tocopherol	Control	+ α-tocopherol
1	3.9	0.11	6.5	0.65
2	4.0	0	2.9	0.42
3	3.6	0	4.8	0.33
4	3.8	0.15	7.6	0.47
5	6.4	0	1.0	0.40
	4.3 ± 1.17	0.05 ± 0.002	4.6 ± 2.1	0.45 ± 0.01

Plasmapheresis in SLE: Correlation of Response with Level of Immune Complexes Measured by Raji-Cell Assay

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Five further patients with SLE have been treated with plasmapheresis. Six to 8 liters of plasma per week were removed, using a Haemonetics Model 30 Hood Cell Separator. From 12 to 32 liters were removed in all. Levels of complement components were measured by immunodiffusion and by a hemolytic assay. DNA antibodies were measured by passive hemagglutination of DNA coated red-cells. Immune complexes were measured in vitro by the Raji-cell assay. Two patients were critically ill. One had seizures, pericarditis, pleurisy, and severe edema, despite treatment with 100 mg prednisone daily for one month. The Raji assay was 750 μg/ml (normal < 40 μg/ml). Following the removal of 14 liters of plasma her condition improved dramatically, complement levels rose to normal, and Raji assay fell to 150–200 μg/ml. The second acutely ill patient had lupus cerebritis, with a level of only 60 μg/ml on Raji assay. After the removal of 18.6 liters of plasma there was no significant improvement in her clinical condition. The remaining three cases were less severely ill and had Raji assays of 350, 257, and 94 μg/ml. They showed a reduction of circulating immune complexes after each plasmapheresis, and an overall, variable fall after 2 to 3 weeks of treatment, but no striking clinical benefit. Those cases with a high Raji-assay also showed increased binding of double-stranded DNA. The Raji assay appears to be of value in predicting the response to plasmapheresis: patients with a very high level may show a massive and sustained reduction, with a good clinical response. In those with lower levels, the response is less predictable. Plasmapheresis appears to be a valuable accessory form of therapy in the severely ill patient with acute SLE.

Specific Endothelial Cell Injury Produced by Scleroderma Serum in Vitro

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To investigate the vascular lesion in scleroderma (SD), the effect on human endothelial cell (EC, umbilical cord) growth of sera from 21 patients with early SD was compared with 10 healthy control sera. EC growth was monitored by tritiated thymidine (^{3}HTdR) uptake and by direct cell counts. ^{3}HTdR uptake of EC was inhibited up to 97% by 11 of 21 SD sera compared to the remaining 10 SD and 10 control sera. Mean ^{3}HTdR uptake was 2747 cpm for control sera (range: 1130–5751), 2448 cpm for the 10 noninhibitory SD sera (989–5162), and 182 cpm for the 11 inhibitory SD sera (58–424). The SD patients with inhibitory serum activity tended toward shorter disease and symptom duration and were more likely to have peripheral edema and hypergammaglobulinemia than SD patients without inhibitory activity. Furthermore, the inhibitory effect was no longer present in one patient after therapy with glucocorticoids which was associated with reduction of peripheral edema and improvement of myositis.

The activity was further characterized in selected sera. SD serum completely inhibited the increase in EC ^{3}HTdR uptake seen with increasing normal serum concentration from 2 to 25%. ^{3}HTdR uptake correlated with reduction in EC number (30% reduction at 20% SD serum concentration versus 100% increase with control serum) and with cytotoxicity (52% cell death versus 6% in control serum). These effects were not observed when the target cells were mouse 3T3 or human fibroblasts, smooth muscle cells, or peripheral monocytes, suggesting specificity of the inhibitory effect of SD sera for EC. The inhibitory effect of SD serum was heat stable (56°C, 30 minutes), nondialyzable, present in both plasma and serum, and unaffected by mixing with control serum. When serum was fractionated on Sephadex G-200 column, the inhibitory activity was found to elute in the position of serum albumin.

Sera from 7 patients with non-SD active rheumatic diseases (RA, SLE, PAN, DM) showed no inhibitory effect on human EC, fibroblasts or smooth muscle cells or mouse 3T3 cells.

These preliminary observations describe an EC-specific cytotoxic serum factor(s) in patients with SD. The in vivo role of this factor(s) in the pathogenesis of the proliferative vascular lesions in scleroderma is unknown.

Metabolism of C4 and Factor B (FB) in Rheumatoid Arthritis (RA): Classical or Alternative Pathway Activation?

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Activation of the complement (C) system in RA has been amply evidenced by a) detection of C and immune complexes in RA synovial leukocyte inclusions; b) depressed CH50, C4, and C2 levels as well as split products of C3, C4, and FB in RA synovial fluids; c) split products of C3 and C4 in RA plasma samples; and d) hypercatabolism of C3 in meta-

bolic turnover studies. We measured the fractional catabolic rates (FCR) of radioactive labeled C4 and FB in RA patients and normal controls to assess the relative importance of classical and alternative pathways of complement activation in RA.

Our results demonstrated: 1) hypercatabolism of C4 in 10 of 15 RA subjects and inverse variation of C4 FCR with plasma C4 levels; 2) hypercatabolism of FC in 5 of 10 RA patients; 3) C4 FCRs disproportionately higher than FB FCRs in 3 of 5 RA subjects undergoing simultaneous studies; 4) correlation of C4 and FB catabolism and homologous IgG catabolism; and 5) occurrence of hypercatabolism of C4 mostly in the extravascular space, a phenomenon previously observed by us also with the IgG hypercatabolism of RA. Patients with high FB FCRs had higher titers for rheumatoid factors. Patients with high C4 FCRs had higher total joint counts and a greater number of manifestations of extra-articular disease. C4 and FB FCRs did not correlate with circulating immune complexes by the Raji cell assay or with the sedimentation rate.

The results suggest that complement activation in RA occurs primarily through the classical pathway and in the extravascular compartment, probably principally by immune complexes not detected by the Raji cell assay, and with participation of the alternative pathway in some of the patients.

The Effect of Virazole on Suppressor Cells in Rat Adjuvant-Induced Disease: The Possible Role of a Virus

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Studies from this laboratory have demonstrated that the PHA and Con-A responses of splenic lymphocytes from rats with adjuvant-induced disease are diminished. These diminished responses return to normal with spontaneous, corticosteroid (CS), or methotrexate (MTX)-induced remissions. The diminished PHA and Con-A responses are due to two types of suppressor cell: one which adheres to plastic or glass, and another which adheres only to glass. The latter cell, which accounts for the bulk of the suppression, is more sensitive to the inhibitory effects of CS and MTX in vitro than are the mitogen-responsive lymphocytes.

Virazole (VZL), a synthetic nucleoside, is a noninterferon inducing antiviral agent which inhibits AID. Four groups of highly inbred Fisher rats were studied as follows: 1) untreated rats; 2) rats treated with 200 mg of VZL given 7 days prior to sacrifice; 3) rats treated with Freund's adjuvant given 14 days prior to sacrifice; 4) rats treated with Freund's adjuvant followed by VZL 7 days later. Whole spleen cell suspensions were studied, as well as suspensions after removal of plastic-adherent cells by incubation in petri dishes or after removal of glass-adherent cells on columns of glass wool. Each of these three types of suspension was also incubated with a range of concentrations of VZL and with either PHA or Con A. ^{3}H-TdR incorporation into nucleic acid was determined at 72 hours.

Spleen cells from untreated rats, rats given 200 mg of VZL 7 days prior to sacrifice, and adjuvant-treated rats given VZL had normal PHA and Con A responses. The spleen cells from rats given adjuvant alone had markedly diminished PHA and Con-A responses due to the two types of suppressor cells, both of which were more sensitive to VZL in vitro than were the mitogen responsive cells. This preferential sensitivity of suppressor cells to VZL was greater than that noted with CS or MTX. Two possible interpretations are: 1) VZL has a more specific immunosuppressive effect on suppressor cells than CS or MTX; 2) suppressor cell function is due to a virus which is inhibited by VZL.

Cerebrospinal Fluid Cyclic-GMP and Other Clinical Markers of Disease Activity in Central Nervous System Lupus Erythematosus

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Twenty-four cerebrospinal fluid (CSF) samples from 16 patients with systemic lupus erythematosus (SLE) were evaluated for cyclic-GMP (C-GMP) concentration by radioimmunoassay. For comparison studies, the patients were divided into three groups based on their clinical status at the time of each lumbar puncture: those with 1) active neurologic and psychologic abnormalities (group I), 2) active neurologic abnormalities only (group II), and 3) psychologic abnormalities only (group III). Groups I and II had mean CSF C-GMP values of 3.2 nM ± 0.64 (SE) and 4.1 nM ± 0.10 respectively, which were both significantly higher than the mean for group III (1.2 nM ± 0.43) ($P < 0.05$) as well as for the mean of a previously studied group of patients with lumbosacral pain syndromes (0.68 nM ± 0.14) ($P < 0.001$). Other CSF findings did not display this close correlation with activity of neurologic disease. The number of samples with abnormal CSF leukocyte counts was significantly greater for group II (6/8) compared with that found for group III (0/11) ($P < 0.01$).However, no significant difference was found between group I and group III, for this abnormality. In 4 SLE patients significantly higher levels of CSF C-GMP were found on serial sampling during times when neurologic abnormaltiies were active. Neither prednisone nor psychoactive drug therapy had any demonstrable effect on CSF C-GMP levels in these pa-

tients. Comparison of clinical markers of SLE disease activity among the three groups revealed hematologic and serologic abnormalities not to be of significant value in distinguishing SLE patients with active neurolgic involvement (groups I and II) from those without (group III). However, fever and, to a lesser degree, rash, proteinuria, and abnormal urinary sediment were present more frequently in groups I and II than in group III.

Thus elevated CSF C-GMP concentration may be a marker of active neurologic disease in SLE. Assay of CSF C-GMP may therefore be helpful in the clinical assessment of SLE patients with neurologic and/or psychologic abnormalities both with regard to their diagnosis and therapy. This study reports the first serial observations of C-GMP in a group of SLE patients with correlation of manifestations of disease activity and extends our previous studies of cyclic nucleotides in SLE.

Effect of Inherited Deficiency of the Fifth Component of Complement on Arthritis Induced in Mice by Mycoplasma Pulmonis (Mp)

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Mp produces a chronic arthritis in mice with histopathologic features resembling rheumatoid arthritis. Since the chronicity of arthritis appears to result in part from persistence of Mp within the joints, elucidation of host mechanisms responsible for elimination of Mp is of considerable importance. The role of complement in the pathogenesis of Mp-induced arthritis was studied in mice congenitally deficient in the fifth component of complement (C5).

In all experiments, at least 5, 5-week-old male mice per strain free of detectable mycoplasmas were inoculated intravenously with 2×10^6 color change units of Mp. Subjective assessment of the arthritis was carried out by scoring ankles, wrists, metacarpal, metatarsal, and digital joints on a scale of 0 to 3.

In a preliminary study C5 deficient DBA/2 mice and 4 strains of normal mice: T.O., C3H, CBA, and C57BL/10 were used. In the normal strains the arthritis peaked in severity within 15 days and declined thereafter. In contrast the arthritis in the DBA/2 mice reached a plateau at 15 days and gradually progressed in severity for as long as 5 months postinoculation.

A second study was carried out with 3 strains of C5 deficient mice, i.e. DBA/2, AKR and A strain and 2 normal strains: C3H and C57BL/10. The arthritis in the C5 deficient strains persisted at a high level 3 months after inoculation despite considerable variability in the severity of the acute arthritis among the strains. The arthritis in the normal strains rapidly declined as before. At 3 months postinoculation, Mp was isolated from the joints, lungs, and spleens of C5 deficient mice more frequently and in larger numbers than from normal mice, consistent with the failure of C5 deficient mice to eliminate Mp. Complement-fixing antibody to Mp was detected in higher titers in C5 deficient strains.

The results demonstrate the importance of C5 in the elimination of Mp from the joints and organs of infected mice. Moreover, the study may have clinical relevance since secondary complement deficiencies exist in a number of rheumatic diseases. It supports the concept that complement deficiency might contribute to the persistence of as yet undefined etiologic agents in human arthritidies.

A Subgroup of Ankylosing Spondylitis Associated with HLA-B7 in American Blacks

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We have studied 34 American Black patients with primary ankylosing spondylitis (AS) unassociated with ulcerative colitis, Crohn's disease, psoriasis, or Reiter's syndrome. All the patients were unrelated and met the Rome criteria for AS. Sixteen (47%) of them possessed HLA-B27. We further studied the remaining 18 HLA-B27-negative patients to see if there was an association with other B locus antigens belonging to the HLA-B7 cross-reactive group (HLA-B7, B27, Bw22 and Bw42). HLA-B7 was present in 10 of these 18 patients (55.6%) compared to 14 of 59 (23.7%) B27-negative Black controls (χ^2 with Yates correction = 5.11, $P < 0.025$, relative risk = 4). Bw22 was found in one patient who also had B7. Bw42 was not tested for. Antisera used to detect B27, B7, and Bw22 were free of noteworthy cross-reactivity.

Duration of disease, age at onset of AS, skeletal manifestations, prevalence of acute anterior uveitis and frequency of a positive family history for AS were compared between the 10 B7-positive (Gp-I) and the 16 B27-positive (Gp-II) patients. Duration of disease at the time of study was not significantly different in the two groups. However, mean age at onset of AS differed significantly: 33.6 years in Gp-I and 22.2 years in Gp-II ($P < 0.005$). Skeletal manifestations of the disease did not differ significantly in these groups. Uveitis occurred in 2 patients (20%) in Gp-I and 8 patients (50%) in Gp-II ($P = 0.158$).

A family history of AS could be obtained in none of Gp-I patients and 6 (37.5%) of the Gp-II patients ($P = 0.034$, using Fisher's exact test).

These data indicate that HLA-B7 may be associated with the development of AS in the B27-negative Black population. The B7-positive Black AS patients are older at onset of their disease than the B27-positive patients and tend to lack obvious familial aggregation of the disease. The findings suggest that American Black AS patients lacking B27 but possessing B7 represent a subgroup of patients with this disease.

Urinary Prostaglandin E and Clinical Status in Systemic Lupus Erythematosus

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Prostaglandin synthesis inhibitors (PGSI) may affect renal function in humans, especially under conditions of renal injury such as lupus nephritis. To explore this preferential effect in systemic lupus erythematosus (SLE), we measured basal excretion of urinary prostaglandin E (iPGE) by radioimmunoassay in 10 female SLE patients and 28 normal women on a constant 109 mEq sodium diet. No patient or normal control received nonsteroidal antiinflammatory drugs during the study period but 7 of 10 patients were on maintenance low dose prednisone ($\leq$ 20 mg/day). Six of 10 patients had biopsy-proved nephritis; 4 had insufficient urinary findings to prompt a diagnostic biopsy. All iPGE values were determined in triplicate on at least three separate 24-hour collections except in one patient with two such collections. SLE patients had a significantly higher mean iPGE excretion than normals (45.8 $\pm$ 5.3 versus 29.6 $\pm$ 2.2 ng/hour; mean $\pm$ SEM; $P < 0.005$). SLE patients with biopsy-proved nephritis had a higher mean excretion than those without biopsy (49.7 $\pm$ 8.6 versus 39.9 $\pm$ 3.2 ng/hour) although the difference was not statistically significant. Urinary sediment and general disease activity did not correlate with urinary iPGE. All 10 patients showed a decrease in creatinine clearance with PGSI administration but the percent change did not correlate with basal urinary iPGE excretion.

Elevated urinary iPGE excretion in SLE may reflect renal inflammation due to immune complex nephritis and may explain the greater sensitivity of renal autoregulation to PGSI in this setting.

Randomized Study of Intravenous Cyclophosphamide (IVCY) and Cyclophosphamide Plus Azathioprine (CY + AZ) in Lupus Nephritis

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The therapy of lupus glomerulonephritis has been evaluated in a randomized study of corticosteroid treated patients comparing bolus IVCy (5.5–26.7 mg/kg, every 3 months), combined oral Cy + Az (50 mg of each, daily), and prednisone alone (Pr).

Five years after the start of the trial, 38 patients have been randomized: IVCy (12), Cy + Az (14), and Pr (12). Renal involvement at study entry has been characterized by urine sediment abnormalities (38), impaired function (CrCl < 100 ml/min) (33), nephrotic proteinuria (10), glomerular proliferation on biopsy (31), and an estimated disease duration of less than one year (25). The assigned drugs have been continued without complications in 31 patients with a median course of 16 months. Toxicity has necessitated discontinuation of the drugs in 4 courses; 3 Cy + Az (pulmonary infection, hemorrhagic cystitis, amenorrhea) and 1 IVCy (hepatitis). Death has occurred in 3 patients; 2 IVCy (epiglottitis, sudden death), and 1 Pr (pulmonary embolism).

The changes of renal function of the 25 patients receiving a minimum of 6 months of continuous therapy have been assessed by evaluating the number of patients demonstrating a reduction of function (CrCl) by at least 10% (Δ10) or 25% (Δ25) from baseline determinations at study entry.

Therapy	Number of Patients	Mean Drug Course (Months)	CrCl (Mean $\pm$ SE)	Δ10	Δ25
Pr	7	30	62 $\pm$ 12	4	2
IVCy	9	25	53 $\pm$ 6	1	1
Cy+Az	9	26	76 $\pm$ 9	1	0

This interim analysis of an ongoing trial is encouraging and suggests that IVCy and Cy + Az may be more effective than Pr in the maintenance of renal function of patients with lupus glomerulonephritis. Extended follow-up will be required to determine the duration of these effects and the development of drug related complications.

Release of a Bone Resorbing Factor(s) by Human Allogeneic Cultures

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The mechanism(s) underlying the destruction of bone observed in chronic inflammatory diseases such as rheumatoid arthritis or osteomyelitis remain(s) to be elucidated. Soluble mediators derived from immunologically activated cells may play an important role in such phenomena.

We have observed that alloantigen-stimulated human mononuclear leukocytes (MNL) release a factor(s) capable of resorbing fetal rat bones in vitro. We have investigated procedures for the large-scale generation and partial characterization of this bone resorbing factor(s). Human MNL obtained by leukophoresis (IBM cell separator) of two nonrelated healthy individuals (yield = 1–2 $\times$ 10^{10} cells/donor) were cultured together. (2.5 $\times$ 10^6 cells/ml, 40 hours, 37°C, Mishell-Dutton atmosphere). Supernatant fluids were removed and assayed for bone resorbing activity by measurement of Ca^{45} release from fetal rat bones in vitro. These supernatants effected significant bone resorption when compared to either control supernatants prepared from a single donor's cells or medium alone. Indomethacin (5×10^{-5} to $1 \times 10^{4}M$) did not inhibit either the production or biological activity of this factor(s). Bone resorbing activity eluted with molecules of 11–18,000 daltons on Sephadex G-75 and appeared in the breakthrough when applied to DEAE cellulose (0.005 *M* phosphate, 0.02*M* NaCl, pH 7.5). Eluate containing bone resorbing activity after Sephadex G-75 chromatography $\times$ 2 and DEAE cellulose chromatography was applied to amionic disc gels (4°C, pH 9.3, Trisglycine). A single peak of bone resorbing activity was observed, although this activity could not be attributed to a stainable protein bond.

The results indicate that alloantigen stimulated human MNL elaborate a low molecular weight bone resorbing factor(s) resembling the previously described osteoclast activating factor (OAF). Methods presented for the large-scale generation and partial purification of this activity should facilitate clarification of the role of this factor in immune tissue destruction.

Suppression of Growth and the Phenotypic Expression of Fibroblasts by Peripheral Blood Mononuclear Cell Supernatants: A Role for Prostaglandins

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Inflammatory cells may play a role in modulating connective tissue growth and function in a number of rheumatic diseases. The interaction of lymphoid and connective tissue cells was studied in vitro by exposing human dermal fibroblasts (FB) to supernatants (SN) of peripheral blood mononuclear cells (PBMC). Proliferation was assessed by ^{3}H-thymidine incorporation and direct cell counts. Protein synthesis was determined by ^{3}H-proline incorporation and collagen synthesis by protease-free collagenase releasable counts.

SN of both unstimulated and PHA-stimulated PBMC suppressed FB growth in a dose-dependent fashion. SN suppressive activity was non-dialyzable, heat-stable, not cytotoxic, and removed by absorption of SN with FB but not with PBMC. There was suppression of protein synthesis by PBMC-SN in direct proportion to the suppression of cell number (41%), but disproportionately greater suppression of collagen synthesis (68%). Culture SN of T-cell depleted PBMC were as active in suppressing FB growth as were SN of unfractionated MC (46–63% suppression, mean 54 $\pm$ 7% for T-depleted versus 41–59%, mean 48 $\pm$ 8% for unfractionated). Supernatants of T-cell enriched populations had decreased activity (8–20%, mean 16 $\pm$ 6% suppression).

The growth suppression seen was due, at least in part, to stimulation of FB prostaglandin (PG) synthesis. Immunoreactive PGE_2 production by FB was increased 70-fold (1.3 versus 90.0 ng/10^5 cells) by PBMC-SN. When inhibitors of PG synthesis (indomethacin, sodium meclofenamate) were added to FB cultures just prior to addition of PBMC-SN, the growth suppressive effect was abrogated (30–100%). Furthermore, exogenously added PGE_2 (final concentration = 50 ng/ml) resulted in suppression of FB growth comparable to that seen with PBMC-SN.

Thus, lymphoid cells produce soluble factors which induce FB to modulate their own growth in vitro and which alter collagen synthesis. The effector of the growth autoregulation appears to be a PG. This system provides an in vitro model for the study of lymphoid and connective tissue cell interaction which may improve the understanding of proliferative and fibrotic human disease.

Kinetics of Pinocytosis and Intracellular Proteolytic Digestion in Monolayer Cultures of Human Synovial Cells

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Although there is compelling evidence to support the view that altered endocytic-lysosomal functions of synovial tissue may play a role in the joint tissue injury seen in rheumatoid arthritis (RA), there are no data which quantitate these functions in human synovial cells (SC). Therefore, we investigated the rates of pinocytosis and intracellular digestion of a

soluble protein, horseradish peroxidase (HRP), in monolayers (4–15 days in primary culture) of SC from RA and non-RA patients. Pinocytosis was linear with increasing HRP concentrations (0.01–10 mg/ml), time (30–120 min), cell numbers ($1–10 \times 10^6$) and cell protein (50–1000 μg; 5×10^5 cells equivalent to 100 μg protein). Specific activity (SA; ng HRP taken up/100 μg cell protein/2 hr) increased in direct proportion to cell concentration. SA of RA cultures (100–200 μg protein) after exposure to 1 mg/ml HRP was 270 ± 182 (mean ± SD; n = 5); an equivalent concentration of non-RA SC had a SA of 142 ± 90 (n = 5). Uptake in both RA and non-RA SC was inhibited most effectively by $10^{-3}M$ potassium fluoride (80%) and 4°C (98%). RA SC digested 50% of cell bound HRP (T½) in 6 hours, whereas T½ for non-RA SC was 13 hours.

Uptake and digestion of immune complexes by RA SC were also studied. Insoluble HRP-anti HRP complexes formed at equivalence were added to SC at 1.25 μg HRP/ml (total protein concentration 12.5 μg/ml). Uptake of complexed HRP was at a rate (4.8% of this load/100 μg cell protein/hr.) approximately 600 times that for soluble HRP (at a load of 1 mg/ml). Only 16% of complexed HRP was digested by 24 hours.

These studies indicate: 1) human SC have a relatively high rate of pinocytosis and RA SC demonstrate the greatest rates of uptake; 2) kinetics of pinocytosis in SC are primarily those of fluid phase uptake; 3) the rate of pinocytosis increases with increased cell density, a relation which may be important in RA synovial tissue; 4) RA SC can readily accumulate immune complexes, but their ability to digest them is much more limited than it is for soluble proteins. Further quantitative studies of the mechanisms whereby SC process joint constituents, soluble antigens and immune complexes should lead to a more precise definition of the contribution of SC endocytic-lysosomal functions to tissue injury in RA.

Gastroscopic Evaluation of the Effects of Motrin, Indocin, Aspirin, Naprosyn, Tolectin, and Placebo on Gastric Mucosa of Normal Volunteers

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This study was designed to evaluate the effects of several nonsteroidal antiinflammatory drugs commonly used in arthritis therapy on gastric mucosa, and to evaluate the effects of 2 agents, Tolectin and Motrin, in subjects with demonstrated clinical intolerance to aspirin. Gastroscopy was carried out before and after all treatment schedules, and gastric mucosa was graded by the endoscopist (FL) on a scale of 0–4+. Photographs were taken and subsequently reviewed in a randomized double-blind manner by both the endoscopist (FL) and an impartial gastroenterologist (RN). Remarkably consistent results were obtained.

Part I. Forty volunteers were randomly divided into 8 groups with 5 subjects in each group and treated for 1 week with either Motrin (2400 mg/day), Motrin (1600 mg/day), Indocin (150 mg/day), Indocin (100 mg/day), Naprosyn (750 mg/day), Naprosyn (500 mg/day), aspirin (3.6 gm/day), and placebo. **Part II.** Five normal volunteers who had developed 4+ hemorrhagic gastritis during a previous study after 1 week of aspirin (3.6 gm/day) were given Motrin (2400 gm/day), Tolectin (2000 mg/day), and placebo for 1 week in a randomized crossover manner with at least a 2-week washout between medications.

RESULTS. Part I. Placebo subjects always showed the least pathology followed by Motrin (1600 mg), Naprosyn (500 mg), Motrin (2400 mg), Indocin (150 mg), Indocin (100 mg), Naprosyn (750 mg), and aspirin (3.6 gm). In 2 patients receiving Naprosyn (500 mg) and Indocin (100 mg) frank gastric ulcer was produced. Differences between Motrin 1600 or 2400 mg and aspirin were highly significant ($P < 0.01$) as were differences between aspirin and placebo ($P < 0.001$). Differences between Motrin (1600 mg) and Indocin (100 mg), and between Motrin (2400 mg) and Naprosyn (750 mg) approached significance ($0.05 < P < 0.10$). **Part II.** Of the 5 subjects taking Tolectin, 2 had hemorrhagic gastritis of a degree comparable to that with aspirin, and 3 had similar but less extensive changes. One subject on Motrin had extensive hemorrhagic gastritis as with aspirin, and 4 had minimal or no changes. In both parts I and II, gastric mucosal biopsies, blood levels, and photographs confirmed endoscopic pathology. This study demonstrated that severe gastric mucosal hemorrhagic and ulcerative changes occur in subjects using nonsteroidal antiinflammatory drugs, and that significant differences exist between various drugs.

Diagnosis of Gonococcal Arthritis by Counterimmunoelectrophoresis: Detection of Antigen and Antibody in Serum and Synovial Fluid

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Seven patients with active gonococcal (GC) arthritis were studied for the presence of GC antigen (AG) and antibody (AB) in serum and synovial fluid by counterimmunoelectrophoresis (CIE). The diagnosis of GC arthritis in all seven patients was made by typical clinical presentation, positive local culture for *N gonorrhoeae* (NG), and response to

treatment. Gram stain and culture of synovial fluid, as well as blood cultures, were negative in all patients. Specimens were run against rabbit antisera to NG (Difco) to detect GC AG and against a turbid solution of fresh isolates of NG to detect GC AB. Glass slides were coated with 3 ml of 0.015 *M* agarose in 0.075 *M* barbital buffer pH 8.6. Pairs of wells 3 mm apart and 3 mm in diameter were cut in the agar and filled with 10 μl of samples—AG in the cathode well and AB in the anode well. Slides were electrophoresed in barbital buffer for 45 minutes at room temperature at 4 mamps/slide, and examined for the presence of precipitin lines. Results are shown in the table.

	Serum		Synovial Fluid	
Patient No.	AG	AB	AG	AB
1	−	−	+	−
2	−	−	+	−
3	−	−	+	−
4	+	−	−	−
5	−	+	−	−
6	−	+	−	+
7	−	+	−	+

GC AG was found in the serum of 1 patient and in the synovial fluid of 3 others. GC AB was detected in the serum of the remaining 3 patients and in simultaneous synovial fluid of 2 of these. GC AB was absent in convalescent serum in patients #6 and #7 at 2 months. Only 1 of 30 patients with initial or recurrent active GC urethritis, cervicitis, or salpingitis had serum GC AB detected by CIE; none had GC AG. These results suggest that the finding of GC AG or AB in synovial fluid or serum by CIE provides evidence for GC arthritis and may be positive when gram stain and culture are negative. CIE therefore appears to be a useful test in the diagnosis of this condition.

Diagnosis and Treatment of Familial Mediterranean Fever (FMF) in Childhood

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FMF frequently presents a diagnostic dilemma in childhood. Its musculoskeletal manifestations may be confused with septic arthritis, juvenile rheumatoid arthritis, ankylosing spondylitis, or other rheumatic disease. Although 80% of patients have symptom onset in childhood, diagnosis is usually delayed. We have followed 31 patients who presented before age 19 years.

There were 16 male and 15 female children. The mean age of onset was 4.4 years (range 1–14); mean age at diagnosis was 9.3 years (range 2–18). There were 23 Armenians, 3 Sephardic Jews, and 5 of other ethnic origins. All had recurrent fever and peritonitis, pleuritis, or arthritis, without evidence of other rheumatic or nonrheumatic disease. All were of Mediterranean ancestry. Presenting complaints included fever in 100%, abdominal pain in 90%, chest pain in 62%, joint pain in 45%, and skin rash in 7%. Musculoskeletal manifestations ranged from arthralgias to arthritis. The knees were involved in 8 children, ankles in 6, shoulders in 2, sacroiliac joint in 2, and wrists in 1. Both children with sacroiliac joint involvement had marked erosive changes and were initially thought to have ankylosing spondylitis. Four patients had splenomegaly. No patient had proteinuria or other evidence of amyloidosis. All of 13 children tested were negative for HLA-B27.

Fifteen children with frequent and severe attacks, mean age 11.6 years (range 3–18), were selected for colchicine therapy. Patients were begun on the nearest whole tablet equivalent of 1.0 mg/meter squared. In nonresponders, the dosage was raised to a maximum of 1.8 mg/day. Six patients were noncompliant, but the remaining 9 children have been followed for 4–52 months (mean 29) on continuous colchicine therapy. The mean number of attacks for the 3 month period prior to initiation of therapy was 5.6 (range 3–12), but only 0.6 (range 0–2) for the 3 most recent months of colchicine therapy ($P < 0.0025$). The only side effect was transient diarrhea, which usually resolved without reduction in dosage. Although the long term safety of colchicine in childhood has not been established, no deleterious side effects have been observed. It appears that children tolerate colchicine well. Colchicine therapy may allow children incapacitated by severe attacks of FMF to assume a nearly normal life.

Vitamin K Dependent Synthesis of the Calcium Binding Amino Acid, γ-Carboxyglutamate, in Bone Microsomes

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A new amino acid, γ-carboxyglutamic acid (Gla), originally discovered in prothrombin where Gla residues are sites for specific binding of Ca^{++} ions, more recently has been identified in osteocalcin, a non-collagenous protein from bone matrix. Based on amino acid composition and sequence of bovine bone Gla-protein, osteocalcin is distinct from all other

vitamin K dependent clotting proteins (factors II, VII, IX, and X) synthesized in the liver. The posttranslational formation of Gla, studied extensively in liver microsomes from warfarin treated rats, occurs in a vitamin K dependent enzymatic oxidative carboxylation of selected glutamic acid residues in prothrombin requiring bicarbonate ion and NADH or chemical reducing agent. To show that Gla synthesis occurs in bone tissue and that the carboxylase enzyme has similar requirements in bone, microsomes were prepared from the long bones of 17 day old chick embryos. The eggs were injected at 12, 14, and 16 days with sodium warfarin, an inhibitor of Gla synthesis, to allow for the accumulation of endogenous substrate available for specific labeling with $NaH^{14}CO_3$. The microsomes separated from bone homogenates by differential centrifugation incorporated ^{14}C into Gla only in the presence of vitamin K_1. ^{14}C-Gla was identified after alkaline hydrolysis of the microsomes and separation on an amino acid analyzer. Further confirmation of ^{14}C-Gla was obtained by acid hydrolysis of the putative Gla component resulting in decarboxylation to glutamic acid and recovery of approximately 50% of the incorporated radioactivity in glutamic acid. The posttranslational enzymatic generation of specific calcium binding sites in bone suggests that the vitamin K dependent carboxylation reaction may have a possible role in regulating mineral deposition in calcified tissues. Since the formation of a specific calcium binding amino acid in bone is vitamin K dependent, anomalies might be expected to occur in mineralized tissues under conditions of vitamin K deficiency or anticoagulant therapy. Of significance is the finding of Gla-containing proteins in ectopic calcifications (subcutaneous scleroderma and dermatomyositis plaques, atheromatous plaque) which suggests Gla may be important in the formation and resorption of bone in a wide variety of disorders.

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The Ability of Serologic Tests to Predict Changes in SLE Renal Disease Activity

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A retrospective study was instituted on 10 patients in the UCLA lupus nephritis clinic in an attempt to determine the ability of three serologic indicators—specifically immune complexes (IC), anti-DNA antibodies (DNA-ab), and C3—to predict the activity of SLE renal disease as indicated by changes in 24 hour proteinuria, serum creatinine, and creatinine clearance. We chose not to assess urinary sediment because of difficulty in quantitating terms such as "rare," "occasional," and "full field" as used by different observers. Patients' records and serum samples from the period 1975–1977 were employed. The mean number of matched clinical samples per patient was 20 with a range of 9–28. Similarly, the mean number of matched serologic samples per patient was 16 with a range of 8–24.

"Normal" daily or short term variation for each clinical and serologic parameter was assessed from paired samples taken within 1–7 days of each other. These "normal" values were then used as a baseline, variations from which could be tested for abnormality by statistical techniques.

By use of simple regression analysis it was noted that IC as determined by 4% polyethylene glycol precipitation correlated better than either DNA or C3 with clinical parameters. Intriguingly, IC correlated with neither DNA-ab or C3.

More importantly, when attempts were made to predict deterioration of clinical parameters 3–5 weeks after either a single abnormal serologic determination or after paired determinations of serologic parameters demonstrating significant deterioration, no test or combination of tests produced less than a 50% false positive rate. However, all tests were capable of predicting stability or improvement of clinical parameters with a true negative rate that averaged 92.3% for IC, 91.4% for C3, 90.8% for DNA-ab. No combination of tests improved these rates.

Septic Discitis

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Pyogenic infection originating within the intervertebral disc space, once thought to be uncommon, is becoming increasingly recognized. We report the clinical findings in 15 adult patients with non-tuberculous pyogenic infection of the intervertebral disc occurring in two teaching hospitals over an 18 month period. Chills, rigors, and low grade fever were common prodromal complaints (7:15). The acute onset of back and neck pain (14:15) with point tenderness over the involved spinous process (8:15) most accurately indicated the diagnosis. Referred abdominal pain or sciatica were less commonly seen. Acute paraparesis or quadaparesis occurred in 2 patients with epidural abscess. The sedimentation rate reflected disease activity in all patients. Plain radiographs were positive in only 7 patients. Tomography was helpful in another 3. ^{99m}Tc pyrophosphate bone scan was positive in 11:12 cases and responsible for diagnosis in 4 of 5 patients with normal radiographs. Overall the bone scan was positive in 11 of 12 cases studied. Responsible organisms were cultured usually

from the involved disc space (7:9). *Staphlococcus aureus* (4) and *E. coli* (3) were the most common pathogens, although other microorganisms were responsible. No pathogen was isolated in 6 cases.

Delay in diagnosis averaged 14 weeks. Previous spinal roentgen abnormalities (9:14), disc surgery (3:14), another site of active infection (3:14), and alcoholism (2:14) were predisposing factors. Intensive intravenous antibiotics followed by two months oral antibiotic therapy proved curative. Ten of 15 patients could be followed one year after therapy and were asymptomatic except for 2 patients with pre-existing mechanial low back pain. None had evidence of active septic discitis. One patient died of pulmonary embolism post-discectomy. Epidural abscess was the usual indication for surgery. The sedimentation rate returned to normal in all survivors.

Septic discitis is a common disease, but delay in diagnosis is usual despite a characteristic clinical syndrome. The sedimentation rate and bone scan are the most useful adjuncts to diagnosis. Aspiration of the suspected disc space for responsible organisms is advocated. Most patients are afforded excellent prognosis following judicious rest and prolonged antibiotic administration.

Joint Effusions after Renal Transplantation

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Musculoskeletal complaints of uncertain etiology have been noted in up to 30% of patients receiving a kidney transplant. We have studied prospectively 35 consecutive renal transplant recipients. Joint pain, stiffness, and swelling were assessed 5–6 days in every week of the postoperative period. Six patients experienced joint pain affecting chiefly knees but also shoulder, ankle, and temporomandibular joints. Arthralgias were variable in severity and duration, and in time of onset after transplantation. In 2 patients pain coincided with reduction of steroid dosage and was abolished by modifying the treatment regimen. A third patient, who developed transient joint pain 17 days after appearance of a painless effusion, had hyperparathyroidism.

Joint effusions, confirmed by arthrocentesis, appeared in 18 knees of 11 patients. Mean duration from transplantation to first joint aspiration was 17 days. Effusions were generally unaccompanied by pain, except on extreme flexion, and persisted for 24 hours to 6 weeks. Twenty-five synovial fluid samples were obtained from the 18 knees. The mean synovial fluid volume was 15 ml (range, 3–65 ml) and the mean cell count was 28/mm^3 (range 0–150/mm^3). In every case viscosity and mucin test were normal and crystals were not found on polarization microscopy of the centrifuged fluids. Joint x-rays showed only soft tissue swelling. Electron microscopy of three fluids revealed no hydroxyapatite-like material. Synovial biopsies in 2 patients were normal by light microscopy. No cases of aseptic necrosis have been identified. Tests for serum ANA, immune complexes, and rheumatoid factors were all negative. Neither the arthralgias nor effusions bore any apparent relationship to the source of the donor kidney, renal function, fever, or use of anti-thymocyte globulin. No major transplant rejection episodes occurred in any of the patients with effusions or arthralgias, whereas no joint problems arose in any of 9 patients who rejected donor kidneys in the postoperative period. Thus, benign effusions are common after renal transplantation. They appear unrelated to immunologic factors or crystal deposition and may be due to transudation associated with high-dose steroid treatment.

Serologic Search for Infectious Agent Associations with Early-Diagnosed Arthritis: A Controlled Study Including *Yersinia Enterocolitica* Titrations and HLA Typing

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To investigate the hypothesis that infection may precipitate certain arthritis syndromes, we performed a battery of infectious agent serologic titrations on 130 early-diagnosed arthritis patients and 60 normal controls. Forty-five antigens were employed: 8 for common bacteria, 6 for mycoplasma, 9 for respiratory viruses, 5 for other viruses, and 17 for *Y enterocolitica*. The arthritis patients included 25 with SLE, 48 with RA, 14 with JRA, and 43 with arthritis of undetermined diagnosis (AUD). Data were analyzed by diagnostic groups and B27 status.

No significant difference was found among the arthritis and control groups in antibody titers to any of the common bacterial antigens or the mycoplasma species, 8 of the respiratory viruses, and 2 of the other viruses. In JRA patients, adenovirus (group) and herpes simplex (types 1 and 2) virus titers were somewhat low, and in the RA and AUD groups cytomegalovirus titers were low. In SLE, mumps virus titers were slightly higher than in the other groups. However, in contrast to the occasional difference in serologic titrations with previously mentioned antigens, highly significant differences in antibody titers to all 13 domestic *Y enterocolitica* antigens (7 serotypes) were found among the groups. Arthritis patients had consistently lower median levels than normal controls with the lowest titers found in SLE, followed by JRA, AUD, and finally the RA group. The 4 Finnish *Y enterocolitica* antigens (serotypes 3 and 9) showed no significant difference

among groups but yielded the lowest median titers of all yersinia antigens. Evidence of yersinia reactive arthritis was not found in this patient sample, and median antibody titers of the 11 B27 positive and 76 B27 negative arthritis patients without either rheumatoid factor positivity or the diagnosis of SLE were highly comparable.

The results provide no serologic evidence of infectious agent association with early-diagnosed arthritis but reveal impressive hyporeactivity to domestic *Y enterocolitica* serotypes which suggests decreased natural IgM agglutinating antibodies to gram negative organisms as previously reported in SLE patients (Baum J, Ziff M: J Clin Invest 48:758, 1969).

Prevalence of Scleroderma-Type Capillary Abnormalities in Connective Tissue Diseases

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Among the various microvascular abnormalities present in the skin of patients with connective tissue diseases and demonstrable by in vivo microscopy, the most characteristic pattern of capillary abnormalities is found in patients with scleroderma (SD) (Maricq HR, Spencer-Green G, LeRoy EC: Skin capillary abnormalities as indicators of organ involvement in scleroderma (systemic sclerosis), Raynaud's syndrome, and dermatomyositis. Amer J Med 61:862–870, 1976).

The prevalence of this SD pattern in a larger sample of patients with SD and related connective tissue diseases was studied in three separate Arthritis Centers and in 147 patients with the following diagnoses: SF (50), systemic lupus erythematosus (SLE, 60), mixed connective tissue disease (MCTD, 26), and Raynaud disease (RD, 11).

Results from widefield microscopic observations and photomicrography show SD-type capillary abnormalities present in the following numbers of patients in each diagnostic category: SD, 41 (82%); SLE, 1 (2%); MCTD, 14 (54%); and RD, 1 (9%). Tortuous capillaries were noticed in 25 patients with SLE (42%), 3 with SD (6%), 3 with MCTD (12%), and 4 with RD (36%). Only 2 patients (with MCTD) had both a SD pattern and tortuous capillaries (8% of MCTD or 1% of all patients). Non-specific microvascular changes were present in 17 patients with SLE (28%), 4 with SD (8%), 3 with MCTD (12%), and 2 with RD (18%). Many SLE (17 patients, 28%) and RD (4 patients, 36%) patients showed no capillary abnormalities, as did 6 patients with MCTD (23%) but only 2 with SD (4%).

These results confirm the high frequency of SD-type capillary pattern in SD patients and separate them from patients with SLE, who do not show a pathognomonic pattern by the present techniques. Tortuous capillaries, frequent in SLE, occur in other diseases and in normal subjects, although to a lesser degree.

It is interesting to note that patients with MCTD, an overlap syndrome having clinical features of both SD and SLE, exhibited no specific pattern of capillary abnormalities. About half of the patients showed SD pattern, and the other half demonstrated tortuous capillaries, other non-specific changes, or no observable microvascular abnormalities.

Clinical Criteria for Early Diagnosed Systemic Sclerosis: Preliminary Results of the ARA Multicenter Cooperative Study

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Clinical criteria for classification of systemic sclerosis (SS) were derived from prospectively entered data on 799 early-diagnosed rheumatic patients contributed by 29 centers. Cases included patients with definite or probable SS, or scleroderma in overlap. Controls were patients with systemic lupus erythematosus (SLE), polydermatomyositis, or Raynaud's phenomenon alone. Computer assisted and multivariate analysis techniques were used to construct criteria with minimal redundancy.

Careful definition of cutaneous involvement contributed the most powerful criteria. Sclerodermatous involvement proximal to the metacarpal phalangeal or metatarsal phalangeal joints (ie, "proximal" scleroderma), whether in an acrosclerotic distribution (acrosclerosis), on the face or neck (scleroderma facies), or on the trunk or abdomen, was present in 91% of definite SS cases and in only 1% of combined controls. If the major criterion of proximal scleroderma was absent, SS cases could be distinguished by having at least 2 of 4 minor criteria, ie, sclerodactyly, digital pitting scars, pulmonary fibrosis, or large bowel sacculations. Employing the major with minor criteria yielded 98% sensitivity in definite SS patients and 97% specificity in total controls, without using exclusions.

When applied to an independent rheumatic patient

Preliminary Clinical Criteria	Definite n = 261	Probable n = 36	Overlap n = 80	SLE n = 175	PM/DM n = 121	Raynaud's n = 126
Major: Proximal scleroderma	91%	44%	58%	1%	2%	0%
Minor: Sclerodactyly	96%	69%	73%	1%	5%	10%
Digital pitting scars	49%	42%	40%	9%	7%	15%
Pulmonary fibrosis	33%	6%	28%	10%	22%	4%
Colonic sacculations	9%	0%	1%	0%	0%	0%

databank available in ARAMIS, the criteria yielded 92% sensitivity and 96% specificity. Other prominent features of SS, for example Raynaud's phenomenon, esophageal dysmotility, and low pulmonary diffusing capacity did not improve discrimination in combination with proposed criteria. These criteria require further assessment in probable SS and overlap groups, and possibly supplementation by specialized serologic or histologic testing.

Temporal Patterns of Articular Involvement in Early Adult Rheumatoid Arthritis (RA)

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The course of articular involvement in early rheumatoid arthritis (RA) is variable and has not been quantitatively characterized. In a prospective study of 50 younger adult, early RA patients (negative for HLA-B27), detailed articular, systemic, laboratory, and therapeutic data were collected twice yearly for a mean followup of over 5 years. Based upon the number of joints involved during followup, each patient was assigned to separate pain/tender (P/T) and swelling (SW) articular course patterns. Monocyclic pattern was defined as one cycle of documented arthritis with articular remission observed for at least 1 year; intermittent as two or more arthritis cycles, each separated by joint remission of at least 6 months; continuing as continuous joint involvement but without persistent progression; and progressive as continued progressive involvement. The table shows numbers of cases in each pattern, mean numbers of joints involved at entry, and the mean annual change (Δ) in numbers of joints involved during followup, determined by regression analysis.

Monocyclic groups included significantly more males (P/T, $P < 0.05$; SW, $P < 0.001$), and seronegative cases (P/T, $P < 0.05$; SW, $P < 0.01$). Only one woman experienced complete articular remission while progressive swelling occurred in only 2 cases. Entry joint involvement correlated with subsequent P/T and SW articular patterns ($P < 0.01$ and $P < 0.05$, respectively). Cross-sectional analysis of the numbers of joints involved at each protocol exam and longitudinal analysis of regression slopes of individual patient joint involvement both indicated trimodal distributions: monocyclic; chronic (ie, combined intermittent and continuing groups), and progressive patterns, which were independent of therapy. The data provide quantitative evidence for monocyclic, chronic, and progressive articular course patterns in early RA, with identification of significant sex and clinical correlates.

	Painful or Tender Joints					Swollen Joints				
Articular Patterns	Cases	M	F	Entry Mean	Annual Δ	Cases	M	F	Entry Mean	Annual Δ
Monocyclic	4	3	1	7.0	−4.7	18	5	13	2.8	−3.4
Intermittent	14	2	12	8.2	−0.6	17	1	16	4.7	−0.3
Continuing	26	2	24	13.5	−0.8	13	1	12	6.8	−0.1
Progressive	6	0	6	26.0	+2.3	2	0	2	12.0	+1.5
All Patterns	50	7	43	13.0	−0.6	50	7	43	4.9	−1.1

Type C RNA Virus Antibody in Lupus Diffuse Proliferative Glomerulonephritis

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In some postmortem cases of systemic lupus erythematosus (SLE) associated with diffuse proliferative glomerulonephritis (DPGN), an antigen related to mammalian type C RNA viral core (p30) protein is deposited in the glomerular lesions in an immune complex pattern (Proc Natl Acad Sci 73:233, 1976). Now an attempt is made to support this finding

by examining the glomerular immune deposits in SLE-DPGN for evidence of type C virus antibody. Human immunoglobulins (Igs) were eluted from the glomerular immune deposits in two fractions by sequential treatment with DNase to elute anti-DNA antibodies, followed by acid buffer to elute remaining antibodies. The eluted Igs were then assayed by a sensitive, specific, and quantitative enzymoimmunoassay, which compares favorably with radioimmunoassay, for the detection and measurement of anti-p30 antibody against purified p30 proteins of the four chief groups of mammalian type C viruses: murine, feline, endogenous feline RD-114/endogenous primate, and simian sarcoma virus type 1 (SSV-1)/infectious primate virus groups. Human Igs which showed specific anti-p30 antibody activity, particularly against p30 antigen of the RD-114 virus group and to lesser extent against p30 antigen of the murine and SSV-1 virus groups, was eluted by acid buffer from the glomerular immune deposits in two cases of SLE-DPGN that were known from previous study to contain deposits of viral p30-related antigen in the same tissue lesions. These findings support the hypothesis, stemming from studies of the New Zealand mouse model of SLE, that subinfectious antigenic expression of a type C virus is involved in the pathogenesis of DPGN associated with SLE.

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Appearance of a Suppressor Lymphocyte Associated with Immunodeficiency in Aged NZB/NZW F_1 (B/W) Mice

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Male B/W mice generally die after one year of age with lupus nephritis and/or lymphoid malignancy. These mice gradually develop a severe depression of cell-mediated immunity which may contribute to the subsequent fatal autoimmune and lymphoproliferative disease. We studied T lymphocyte function in 8 young (3–4 month) and 9 old (15–20 month) male B/W mice in separate paired experiments. The spleen cell response to phytohemagglutinin was 62,087 cpm (geometric mean) for young mice compared with 490 cpm for old mice ($P < 0.0001$). When spleen cells from young and old mice were mixed together, there was significant suppression of young cells by old splenocytes in all experiments (57–98% suppression). This suppression is mediated by a radio-resistant, nonadherent, mononuclear leukocyte, probably a small lymphocyte. Although this suppressor cell is eluted in the "T cell fraction" of a nylon wool column, it cannot be identified as a T cell because it is resistant to anti-θ and complement treatment.

In a typical experiment, unfractionated old spleen cells incorporated 291 cpm compared to 42,825 cpm for young spleen cells. A mixture of old and young cells incorporated 6,683 cpm, whereas the predicted incorporation for this 50/50 mixture was 21,558 cpm (69% suppression). Old spleen cells from the T cell fraction of the nylon wool column were enriched for suppressor cells (95% suppression in the mixing experiment). In contrast, the B cell fraction was completely depleted of suppressor activity (actually 23% greater than predicted incorporation). Anti-θ and complement treatment of spleen cells from an 18 month old B/W mouse demonstrated 80% suppression by the untreated cells and 98% suppression by the preparation depleted of θ-bearing cells. These results have been consistently reproduced. There is no evidence of a suppressor cell in old mice of four normal strains (DBA/2, C57Bl/6, Balb/c, and NZW).

We conclude that a mononuclear suppressor cell, probably a lymphocyte, contributes significantly to the severely depressed T lymphocyte function of aging B/W mice. This suppressor mechanism may participate in the pathogenesis of the lymphoproliferative and autoimmune disorder characteristic of the strain.

Immunofluorescence of the Urinary Sediment: A New and Reliable Method for the Study of Renal Lupus

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None of the clinical and laboratory parameters presently used for the assessment of SLE kidney disease is completely reliable. Immunofluorescence of the urinary sediment (IFUS) has been recently reported to accurately predict the rejection of renal transplants, and the purpose of this study was to evaluate its usefulness in renal SLE.

Thirty consecutive patients who met the ARA Preliminary Criteria for SLE were included in the study. All had recent kidney biopsies and were treated with prednisone and immunosuppressors at usual doses with urinalysis, urinary light chains, C3, anti-DNA binding, NPN, and creatinine as parameters of activity. Random urine specimens were collected weekly for 6 months and the urinary sediment was studied by direct immunofluorescence with antisera anti IgA, IgG, IgM, and fibrinogen degradation products. Clinical grading and fluorescence readings were done by independent observers.

Nine patients had normal kidney biopsies and IFUS

was always negative. Nine patients had abnormal biopsies, were classified as inactive, and IFUS was always negative. In 12 patients with abnormal biopsies and active renal disease, IFUS was always positive, even before the appearence of proteinuria, hemoglobinuria, casts, and light chains. IFUS was positive 1 to 3 months before C3 dropped in 4 patients and became negative 1 to 4 months before C3 returned to normal in 7 patients. In the presence of residual proteinuria, IFUS became negative in 5 patients. There was no correlation between the histological type and the immunofluorescence findings in the kidney biopsy with the type of immunoglobulin found in the urine.

This procedure is easy to perform, not costly, and the results are available within 1 hour. Our data suggest that it reflects the changes in renal status months before the other parameters and therefore it may be a valuable method for guiding a more flexible treatment of patients with SLE kidney disease.

Complement-Fixing Hidden Rheumatoid Factor in Juvenile Rheumatoid Arthritis

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Fifteen percent or less of patients with juvenile rheumatoid arthritis (JRA) have positive latex fixation tests (LFT); whereas, approximately 45% have hidden rheumatoid factor (RF) (19S IgM RF in the peripheral blood probably bound with 7S IgG and, therefore, not detectable by the standard LFT). In this study, a complement-dependent hemolytic assay was used to determine the presence of hidden RF.

Sera of 59 children with seronegative JRA, 2 with seropositive JRA, 7 children with connective tissue diseases, 3 with leukemia, and 12 normal children were separated by gel filtration at pH 4.0 to obtain the IgM-containing fraction. These IgM fractions were subjected to the complement-dependent hemolytic assay in which sheep erythrocytes (SRBC) are coated with reduced, alkylated, and acid-treated rabbit IgG anti-SRBC antibody and are hemolyzed by guinea pig complement in the presence of 19S IgM RF.

Thirty-seven of 61 patients with JRA, of which 23 of 33 polyarticular JRA, 12 of 21 pauciarticular JRA, and 2 of 7 systemic JRA, had titers >1:16. None of 12 normal controls and only 1 of 10 disease controls had titers >1:16. The median titer of all JRA patients was 1:42 and healthy and disease controls, 1:7. Estimates of the significance of the differences between the median titers of JRA patients and of the controls were obtained by Mann-Whitney analysis. They were significant at $P < 0.001$. When data from patients with active disease were analyzed separately, the median titers for polyarticular JRA were 1:97, pauciarticular 1:91, and systemic 1:23. Patients with inactive disease did not have significantly different titers from controls. The active polyarticular and pauciarticular values were significant at $P < 0.001$ and $P < 0.005$.

These results demonstrate: (1) 59% of seronegative JRA patients have hidden RF by this procedure; (2) the hemolytic assay is more sensitive than the LFT or sheep cell agglutination tests (SCAT) in determining the presence of hidden RF in JRA; and (3) activity of disease correlated with higher titers in the hemolytic assay, and the assay was superior to the LFT or SCAT as an indicator of disease activity.

Analysis of Proteoglycans from Femoral Condyles of Partial-Meniscectomized Rabbits

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This study was devised to answer in relation to articular cartilage proteoglycans (PGs) whether degradation products or products of incomplete synthesis are detectable in the earliest pathological lesions of this rabbit model of osteoarthritis. An ultramicro transport method for obtaining S value polydisperse profiles for PG aggregates (PGagg) and subunits (PGS), and miniaturized extraction, dialysis and CsCl density gradient ultracentrifugation techniques have allowed study of these PGs and various PG products within the S value range of 1–200 from histologically defined sites.

Thirty New Zealand white rabbits (weighing 2–2.5 kg) were subjected to partial medial meniscectomy and killed 10–12 weeks postoperatively under Nembutal anesthesia. Microdissected medial femoral condyle samples included 1) ulceration, 2) 1 mm rim surrounding the ulcer often with fibrillation, and 3) cartilage from the medial and lateral femoral condyle which occasionally revealed fibrillation but usually appeared to be normal. Amounts of cartilage equivalent to 3) above were obtained from the contralateral (left) knee for use as a primary control and from sham operated (right) knees for use as a second control.

The cartilage from 4 rabbits was required to obtain a pool of 10–20 mg wet cartilage for each zone 1–3 above. These cartilages were diced and PGs extracted, dialysed, and partitioned. For PG extracted with 4.0 *M* GuCl from left femoral articular cartilage of control knees, the profile shows a subunit

peak with weight average sediment coefficient S_{ω}^{20} of about 16 and an aggregate peak extending from 40 up to 120 S with an average of 62. Similar profile data on the tibial cartilage provided an average sediment coefficient of 59. About 1/3 of PG was in aggregated form and the rest PGS. Interestingly, the undissociated PGagg revealed an S value range of 40–180. PGs extracted without dissociation in 0.5 *M* GuCl gave similar data.

In regard to the profile of S values of PGs from small ulcers, the weight average sediment coefficent S_{ω}^{20} of the peak was 16. Peaks indicative of PG products with lesser values were found in 3 pooled samples with large ulcerations. A similar study run after isolation of PGS in a dissociative CsCl gradient confirmed this result. In conclusion, the major abnormality detected early was a disturbance in PG aggregation, and later PGS degradation.

Immune Complex Glomerulonephritis and Circulating Immune Complexes in Patients with Sicca Syndrome

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We recently observed the development of glomerulonephritis in 3 patients with sicca syndrome (SS) who did not fulfill the diagnostic criteria of systemic lupus erythematosus. These patients developed SS 5 to 17 years prior to the onset of glomerulonephritis. Two were diagnosed as having membranoproliferative glomerulonephritis by light and electron microscopy, and 1 was found to have membranous glomerulonephritis. Although immunofluorescent studies were not available, all clearly had microscopic evidence of immune complex (IC) deposition. Moreover C'3 levels were decreased in all three at the time of onset of renal disease, and all had high levels of circulating IC as determined by the ^{125}I Clq precipitation test. These findings raised the possibility that IC plays a role in the development of certain aspects of tissue injury in SS.

For these reasons 55 patients with SS were studied for the presence of circulating immune complexes with the ^{125}I Clq precipitation test. Increased ^{125}I Clq binding activity (ClqBA) (10%) was found in 47 (86%) patients (mean ClqBA = 48%, range 2–98%). Thirty-five patients (64%) had rheumatoid factor (RF) present in their serum. Analysis by Spearman rank correlation revealed a positive association between the ClqBA and the titer of RF as determined by the bentonite flocculation test (BFT), (rho = 0.551, $P < 0.0005$). It was found that the highest positive correlation between ClqBA and BFT titer existed for those patients with SS alone (rho = 0.687, $P < 0.005$), and then decreased for those patients with SS plus extraglandular disease (rho = 0.462, $P < 0.05$) and even more for patients with SS plus rheumatoid arthritis (rho = 0.429, $0.05 < P < 0.10$). There was no apparent correlation between ClqBA and BFT titer in patients with SS plus another connective tissue disease. Pretreatment of 10 sera with 0.01 *M* 2-mercaptoethanol to dissociate IgM into monomers resulted in the eradication of rheumatoid factor as measured by BFT but caused only slight to moderate reductions in ClqBA. Thus circulating immune complexes exist in many patients with SS, are distinct from rheumatoid factor, and may contribute to certain aspects of tissue damage in this disease.

B-Lymphocyte Antigens in Sicca Syndrome

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We have recently described a high (69%), but not absolute, association of sicca syndrome (SS) with the HLA-Dw3 allelle, which is in linkage disequilibrium with HLA-B8 (NEJM 296:895, 1977). In this study we determined the B-lymphocyte antigens of 24 patients (21 females and 3 males, ages 26 to 73) and 184 normal controls. In addition to SS, 3 of the patients have rheumatoid arthritis and 3 have systemic lupus erythematosus. Immunoglobulin-bearing lymphocytes were separated from heparinized peripheral blood on goat anti-human IgG (Fab) coated plastic plates and tested with 60 antisera in complement dependent cytotoxicity tests. The antisera used were obtained from multiparous women and absorbed with pooled platelets to remove HLA-A, -B, and -C antibodies. Two antisera, 172 and AGS, reacted with the B-lymphocytes from all the SS patients compared to 37 and 24% of the normal controls, respectively. Four additional antisera (35, 350, 590, and 715) reacted more frequently (67, 63, 58, and 54%) with the patients' B-cells than with those of controls (17, 21, 24, and 14%). The remainder of the antisera tested had the same frequency of reactivity in this disease as in normals. To determine if these antisera recognized the same or different antigens, their reactions with the lymphocytes from the normal controls were compared by the χ^2 test. Antisera 172 correlated with both 35 and 350 but not with AGS or 715. Antisera AGS correlated only with 715. Similar statistical analysis was done for the patients. The antisera 172 and AGS were excluded because of their 100% prevalance in SS. In the SS patient group antisera 35, 350, and 590 were associated with each

other but not with 715. This association can be due to either cross-reactivity or linkage disequilibrium. Family studies are in progress to determine whether SS patients can be heterozygous for 172 and AGS and, if so, whether they can be present in *trans* position. Assuming that the B-lymphocyte antigens in humans are coded by loci of an Ir region, our results suggest that two immune response genes may be involved in the pathogenesis of SS. In addition, the absolute association of 172 and AGS antigens with these patients should provide an additional aid in diagnosis of SS.

Glucocorticoid-Induced Osteopenia in Rabbits

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Glucocorticoid administration is associated with induction of osteopenia in man and in some laboratory animals. There is debate about dose and duration required, and whether bone loss is primarily trabecular or cortical. Accordingly, 18 adult female rabbits were divided into 3 groups: 6 received saline, 6 received 1.5 mg cortisol/kg body weight (wt), and 6 received 2.5 mg cortisol/kg wt. All received equal volumes subcutaneously once daily early in the morning. Xylenol orange (50 mg/kg 13 days before kill) and tetracycline (15 mg/kg 2 days before kill) were injected as bone markers. Rabbits were given food and water ad libitum, and weights were measured every 5–6 days. Bone mineral content (BMC) and width (W) were measured by photon absorptiometry before treatment and every 2 weeks. Measurement site was the humerus 4 cm proximal to the bent elbow (all cortical bone). Results are shown in the table.

Histopathology. The amount of bone was reduced in cortisol-treated rabbits. In the humerus this was localized to the endosteal surface. In the lumbar vertebrae the endosteal side of the ventral cortex was primarily affected with some thinning of trabeculae. Calculation of rates of formation (F) and resorption (R) showed F was essentially *nil* in the cortisol groups. R was identical in the saline and low-dose cortisol rabbits, while R was accelerated in the 2.5 mg/kg treated rabbits. Analysis of BMC and cross sections from the same site of the humerus for bone area showed a good correlation (r = 0.94).

Summary. Rabbits treated with cortisol in the doses used developed generalized bone loss. This loss appears to involve more cortical than trabecular bone. Bone marker studies show that the main effect is interference with F, and, with the larger dose, acceleration of R. In vivo measurement of BMC accurately reflects the progression of the bone loss.

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Percent Change from Baseline to 8 Weeks

	N	Weight	BMC	W	Density BMC/W
Saline	6	0%	+5.4	+2%	+2.4
Cortisol 1.5 mg	6	−2%	−6.9*	+4%	−13.5*
Cortisol 2.5 mg	6	−16%	−10.2*	+4%	−14.8*

* $P < 0.01$

Detection of Intermediate Complexes by Evaluation of the Difference between the Electrophoretically Determined γ-Globulin Concentration and the IgG Concentration Determined by Radial Immunodiffusion

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Intermediate complexes are polymers of IgG-rheumatoid factor which sediment between the 6.6S and 19S components of normal serum. They are best identified by analytical ultracentrifugation. Their presence in serum can be inferred by a double "gullwing" precipitin line of the IgG on immunoelectrophoresis due to accumulation of the molecular aggregates of IgG around the well. In this study we have shown they can also be detected by evaluation of the difference between the electrophoretically determined γ-globulin concentration and the IgG concentration determined by radial immunodiffusion (RID). Molecular aggregates of IgG are quantified by serum protein electrophoresis, but they are underestimated by RID because their effective or molar concentration is low relative to the monomeric IgG standard. Therefore, when molecular aggregates of IgG are present, the difference between the electrophoretically determined γ-globulin and the IgG measured by RID is abnormally high.

Sixty-one consecutive blood donor sera and 46 hypergammaglobulinemic sera from patients with diseases known not to be associated with the presence of intermediate complexes or in which the presence of intermediate complexes was excluded by analytical ultracentrifugation made up the reference population. Eleven sera with known intermediate complexes were examined. The mean and 95% tolerance intervals (covering 99% of the population with 95% confidence) of the reference population for the difference between the electrophoretically determined γ-globulin concentration and the IgG as measured by RID was 0.31 ± 0.73 g/dl. Eight of 11 patients' sera with known intermediate complexes fell outside the upper limit. All 3 sera which fell within the 95% intervals

had concentrations of intermediate complexes less than 1.8 g/dl. In addition, the degree of deviation from the reference mean showed a direct linear correlation with the level of intermediate complexes present. If the electrophoretically determined γ-globulin concentration minus the IgG concentration by RID is greater than 1.0 g/dl, intermediate complexes should be presumed to be present in concentrations greater than 1.8 g/dl.

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Anti-DNA Synthesis by Peripheral Blood Lymphocytes in SLE

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Synthesis of antibodies to DNA (anti-DNA) in systemic lupus erythematosus (SLE) is postulated to result from a lack of control mechanisms which normally suppress autoantibody production. We investigated these mechanisms by measuring anti-DNA synthesis by normal and SLE peripheral blood lymphocyte (PBL) populations stimulated with pokeweed mitogen (PWM).

PBL isolated from 5 patients with SLE and 5 controls were further separated into T- and B-cell enriched fractions by density sedimentation of spontaneous rosettes formed by AET treated sheep red blood cells and T lymphocytes. A portion of the T-cell fraction was irradiated with 3000 rads to inactivate suppressor T cells. Normal-T or SLE-T cells (± irradiation) were cultured with normal or SLE-B cells (0.4×10^6) at ratios of 1:1, 4:1, and 10:1 in the presence of PWM (Saxon, Stevens, Ashman, J Immunol 118:1872, 1977). After 9 days of culture anti-DNA was measured in the supernatants by a solid phase radioimmunoassay.

Cultures from 3 patients demonstrated anti-DNA synthesis (see table). In each case anti-DNA synthesis by SLE-B cells was greater in co-culture with SLE-T compared to normal-T cells. Irradiation of normal-T or SLE-T cells markedly enhanced anti-DNA synthesis by the SLE-B cells. Normal B cells did not synthesize anti-DNA in any co-culture situation.

These studies show that SLE-B cells from some patients are capable of synthesizing anti-DNA which is best demonstrated in co-culture with suppressor inactivated, irradiated T cells. Normal-T cells suppress the response, whereas suppression exerted by SLE-T cells is variable from patient to patient.

SLE/Normal Co-Culture Experiment #3

Co-Culture		Anti-DNA
B cells	T cells	ngm/culture
Normal	Any	<2.5
SLE	Normal	6.3
SLE	SLE	24.3
SLE	Normal*	25.2
SLE	SLE*	18.8

* Irradiation – 3000 rads

Studies of Lymphoblastoid Cell Lines Derived from Rheumatoid Arthritis Synovial Membrane Lymphocytes

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The antibody produced by rheumatoid arthritis (RA) synovial lymphocytes may be directed at a unique joint antigen inciting the inflammation, and thus may be useful to identify the antigen. We have made permanent RA synovial lymphoblastoid cell lines capable of producing unlimited quantities of antibody. RA synovial membranes were obtained surgically, finely minced in medium (RPMI 1640, 20% fetal calf serum, glutamine, antibiotics), placed in 60mm Linbro plates, infected with Epstein-Barr virus, incubated (37°C, air + 5% CO_2) and fed weekly. Typical lymphoblastoid cell lines developed in 13 of 43 (32%) cultures after a mean 33 days, range 18–53. Once established, the cell lines were put in large flasks and medium changed completely 3 times a week. Eight of the 13 permanent lines were lost after a mean duration of 54 days (range 7–120) due to bacterial contamination or poor viability. The latter was due to suboptimal growth support by specific lots of RPMI or serum; subsequently these were pretested. The 5 remaining lymphoblastoid lines now have a mean duration of 159 days (range 120–210), during which their mean volume was 207 ml (range 98–332). Maximum volume was 1.4 liters, but could be expanded indefinitely in 2 liters or larger flasks on shaker platforms at low speed. Mean cell doubling time was 34 hours, range 24–48. IgG concentration in harvested supernatant medium was measured sequentially by a radioimmunoassay described previously. Mean IgG production by each line was 55, 246, 420, 426, and 1405 μg IgG/day, decreasing slowly with time. Adjusted for cell concentration, mean production was 0.9, 3.9, 4.6, 5.8, and 9.7 μg IgG/10^6 cells/day. Total production to date was 2.418, 29.061, 36.684, 50.768, and 59.0 milligrams. The culture producing the lowest amount may be

producing a predominance of another immunoglobulin type. In 3 of 5 lines IgG production tended to be significantly higher when cell concentration was lower ($r = 0.465 - 0.673$; $P < 0.001$). These RA lines produced more IgG than previously reported for lymphoblastoid lines derived from other human tissues (1–3 $\mu g/10^6$ cells/day). They also produced 5–10 times more IgG than our previous batch organ cultures of RA synovia. Their increased IgG production may be due to improved culture conditions and/or intrinsic immunologic hyperactivity of RA lymphocytes. These cultures may provide an excellent source of antibody specific for the antigen(s) inciting RA.

Rapid Onset and Reversal of Defective Matrix Organization in Cartilage of an Immobilized Joint

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Recently we reported that partial disuse of a joint produced a defect in proteoglycan (PG) aggregation in human articular cartilage indistinguishable from that seen in osteoarthritis. The present study examines the rapidity with which defective PG organization develops after total immobilization of a limb, and the reversibility of the defect.

The right hind limb of dogs was immobilized in a cast for 5 days to 8 weeks, at which time the animals were killed. During this period the dogs ambulated freely on 3 legs but bore no weight on the immobilized limb. In some cases the cast was removed after 6 weeks and the dogs then ambulated fully for up to 4 weeks prior to sacrifice. Cartilage from the distal femur of the immobilized and the contralateral control knees was cultured for 24 hours in Ham's F-12 nutrient mixture containing 10% fetal calf serum and $^{35}SO_4$. PGs were extracted with 4 *M* guanidinium chloride (GuHCl) and purified by successive cesium chloride density gradient centrifugations in 0.4 *M* and 4.0 *M* GuHCl, ie, under conditions favoring formation of PG aggregates and disaggregation of PGs, respectively.

After only 5 days of immobilization $^{35}SO_4$ incorporation into PGs was suppressed by 50%, and this diminution in PG synthesis persisted through 8 weeks of immobilization. After 3 weeks of immobilization no evidence of PG aggregation could be found. At that time PGs from the second gradient were the same size as those from the first gradient (Sepharose 2B $K_{av} = 0.63$) and showed no shift in their Sepharose 2B elution profile after incubation with hyaluronic acid (HA) in vitro, indicating that PG-HA interaction had not occurred. However, 1 week after removal of the cast, aggregates had again formed in the cartilage and were as large in hydrodynamic size as those in control cartilage. These results emphasize the importance of joint motion in maintenance of the normal organization of cartilage PGs. The PG aggregation defect which occurs with immobilization alters hydraulics of the cartilage, especially with impact loading, and may thus predispose to chondrocyte injury.

A Specific Inhibitor of Complement (C5)-Derived Chemotactic Activity in Serum from Patients with Active Systemic Lupus Erythematosus

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In the course of examining host defenses against infection in patients with systemic lupus erythematosus (SLE), we have found a previously undescribed serum inhibitor of complement (C5)-derived chemotactic activity (CTxA). Serum from 6 of 23 patients, when activated with zymosan, failed to attract polymorphonuclear leukocytes (PMN) comparably to normal zymosan-treated serum (ZTS) (measured by the "leading front" method of Zigmond and Hirsch). Incubation of normal PMN with these sera did not affect their random motility or subsequent chemotactic response to normal ZTS. Whereas levels of C3 and C4 (measured immunochemically) were modestly low in these sera, no gross abnormalities involving alternative complement pathway activation could be detected. When preincubated with normal ZTS (1:1) at 37° for 30 minutes, these sera caused significant inhibition (30–100%) of CTxA. They also inhibited the CTxA of column-purified C5-derived peptides (from normal ZTS), but had no effect on the CTxA of either the synthetic peptide, N-formyl-met-leu-phe or the bacterial chemotactic factor from *E coli*. The inhibitor in these patients' serum was heat-stable (56°C for 30 minutes) and acted specifically on C5-derived CTxA (not on PMN). Mixing (without preincubation) of patient serum with normal ZTS failed to cause inhibition of CTxA. The inhibitor also acted reversibly; molecular sieve chromatography dissociated heat-stable inhibitory activity (a single peak with an apparent molecular weight of 50–60,000 daltons) from normal amounts of C5-derived CTxA in patients ZTS and in mixtures of normal ZTS incubated with patient serum. Further characterization of the inhibitor has revealed it to be a basic protein (pI between 9 and 10) which can be inactivated completely by treatment with pronase. Despite its effect on C5-derived CTxA, the inhibitor did not influence two other C5-derived biologic activities in ZTS: PMN lysosomal enzyme releasing activity and PMN aggregating activity. This heat-stable inhibitor, uniquely specific for C5-derived CTxA in serum from some patients with active SLE, may account, in part, for increased susceptibility to infections caused by pyogenic microorganisms.

Multiple Detection Methods for Type C Oncornaviruses in Systemic Lupus Erythematosus

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The evidence that type C viruses are involved in systemic lupus erythematosus (SLE) is conflicting. We tried to detect type C expression in a total of 34 SLE patients during various collaborative studies over the past four years. Forty tissues (17 placenta or gestational products, 10 spleen, 6 kidney, 7 other) and/or cell cultures derived from them were tested a total of 110 times using 10 different methods. Seventy-five percent of the tissues were tested by 2 or more methods and 33% by 4 or more. Type C virus isolation was attempted using four distinct protocols: culture with sedimentation of ^{3}H-uridine-labeled virions, cocultivation with viral RNA-dependent DNA polymerase assay, cocultivation with focus formation assay for helper virus rescue of the defective murine sarcoma virus genome (*A Albino*), and triple cell fusion with viral polymerase assay. Thirty-four tissues from 31 patients were tested a total of 55 times with negative results except for one type C isolate in a recent experiment. Detection of both type C interspecies and primate species antigens was attempted using 3 different radioimmunoassays in 2 separate laboratories (G. J. Todaro, H. P. Charman), and indirect immunofluorescence (R. C. Mellors). Twenty-two tissues from 18 patients were tested a total of 37 times with negative results. Electronmicroscopy of gestational products from 9 patients revealed type C-like particles in 4 placentas, but also in 2 of 3 normal controls (M. Imamura). Type C-related sequences were not found in cellular DNA from 9 patients using hybridization to a murine type C cDNA probe (G. S. Aulakh). Various false-positive results were also encountered in most of the studies. The virus isolate-positive patient has not yet been tested by other methods, but the 4 patients with type C-like particles were each tested by 3 to 5 other methods with negative results. Thus 5 of the total 110 tests were positive on 5 of the 40 tissues from 34 patients. These combined collaborative studies are the most comprehensive yet done in SLE. If type C expression is enhanced in SLE, it is not regularly demonstrable using current methods.

Autoimmune Exocrinopathy: A New Definition of Sjögren's Syndrome Confirmed by Labial Salivary Gland Biopsy

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A prospective clinical, serologic and histopathologic study was performed on 267 consecutive patients suspect for Sjögren's syndrome (SS) referred to an interdepartmental university clinic during the years 1972–1977. One hundred female and 25 male patients met at least two of the following three criteria: 1) lymphocyte focus score greater than one (FS > 1) on labial salivary gland (LSG) biopsy, 2) keratoconjunctivitis sicca (KCS), 3) associated extraglandular connective tissue or lymphoproliferative disorder. Of the 125 SS patients, LSG biopsy FS > 1 occurred in 96%, definite KCS in 55%, and associated extraglandular disease in 85%. Rheumatoid arthritis was present in 27%, scleroderma in 7%, systemic lupus erythematosus in 6%, and polymyositis in 2%. Lymphoproliferative disease or connective tissue abnormalities not fulfilling criteria for a coexisting connective tissue disease (CTD) were present in 43% of patients. Patients with extraglandular disease and KCS almost always had a FS > 1 (33:35 patients), whereas patients with extraglandular disease and a FS > 1 did not necessarily also have KCS (only 38:77 patients).

We conclude that LSG biopsy is more sensitive than KCS for detection of SS in patients with an underlying connective tissue or lymphoproliferative disorder, and may help establish a diagnosis in patients with clinically undiagnosed autoimmune or lymphoproliferative disease. Moreover, LSG biopsy is far superior to clinical symptoms or signs of salivary gland dysfunction which are not specific for SS. In fact, only 50% of patients with any symptoms suggestive of SS actually had the disease confirmed. Local glandular disorders, anxiety-depressive syndromes, and parasympatholytic drugs were common causes of oral and/or ocular complaints. Our study suggests a new definition of SS as an autoimmune exocrinopathy based on the utility and diagnostic value of LSG biopsy. In view of the HLA-B8 association and unique precipitating antibodies to nuclear antigens (Ha, SS-A, SS-B) found in SS, autoimmune exocrinopathy might be considered a genetically and serologically distinct connective tissue disease related to but separable from other connective tissue diseases. Rheumatoid arthritis or a coexisting connective tissue disease occurs in only a minority of patients with autoimmune exocrinopathy and should not be a requirement for diagnosis.

Chemotactic Attraction of Human Monocytes to Homologous Type I, II, and III Collagens and Collagen Degradation Peptides

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Degradation of collagen at sites of tissue injury and inflammation is effected by the dual action of collagenase and nonspecific proteases present in neutrophils, macrophages, and other cells. Monocytes eventually accumulate at such sites and as macrophages perform important phagocytic functions. Mechanisms whereby monocytes are attracted to areas of in-

flammatory reactions are incompletely understood, although several different chemotactic factors have been described.

We have measured the chemotactic response of normal human peripheral blood monocytes to different types of human collagens and collagen degradation peptides by a modified Boyden technique. Chemotactic activity (CTX) expressed as monocytes per oil immersion field for various preparations tested were as follows: type I collagen (1.8 μM) 74 ± 4, type II collagen (1.6 μM) 62 ± 6, type III collagen (1.5 μM) 84 ± 7, α1(I) (10 μM) 69 ± 3, α2(I) (9.3 μM) 67 ± 3, α1(II) (10 μM) 49 ± 4, α1 (III) (8.7 μM) 47 ± 3, and buffer 8 ± 1. Small peptides obtained by degrading these collagens with bacterial collagenase retained chemotactic activity. Additional studies were undertaken with synthetic tri- and dipeptides containing amino acids common to the three different collagens. Peptides containing proline or hydroxyproline (for example, Gly-Pro, Gly-Hyp, Pro-Hyp, Gly-Pro-Hyp, and Gly-Pro-Ala) were chemotactic for monocytes at concentrations ranging between 10^{-4} and $10^{-6}M$.

These data suggest that peptides generated as a result of degradation of collagen by collagenase and other proteases might function to chemotactically attract monocytes to sites of tissue damage and inflammation in vivo.

The Prevalance of Sjögren's Syndrome in Non-Hodgkin's Lymphoma

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Although it is known that a small percentage of patients with Sjögren's syndrome (SS) may develop malignant lymphoma, the frequency of SS in lymphoma has not been established. Therefore, 50 consecutive untreated patients with biopsy-proven non-Hodgkin's lymphoma were screened to establish the prevalence of SS. Patients were defined as having SS if they exhibited objective evidence of keratoconjunctivitis sicca and xerostomia. All were assessed by history, physical examination, Schirmer test, Rose Bengal staining of the cornea and bulbar conjunctiva, and if possible a serial salivary scintiscan, serologic studies, and a lip biopsy.

Of the 50 patients seen, 13 were identified as having SS. Of these, all 13 had a history of xerostomia and keratoconjunctivitis sicca, while 12 had a positive Schirmer test, 5 had positive staining with Rose Bengal, and 3 had a markedly abnormal salivary gland scan. Three had a positive anti-nuclear antibody (ANA) and 1 anti-salivary duct antibody. Of the 13 with SS, 6 had adequate lip biopsies. Of these, 3 were normal, and 2 showed mild, non-specific lymphocytic infiltration, while 1 was highly suggestive of SS with lymphocytic infiltration and salivary gland atrophy. Five of the 13 had musculoskeletal complaints, 4 had classic or atypical Raynaud's phenomenon, and 2 had a concurrent diffuse connective tissue disease (1 had scleroderma and 1 had probable rheumatoid arthritis).

In summary, of 50 patients screened, 13 had SS as defined above. Of these, 12 had positive Schirmer tests, 5 had positive Rose Bengal staining, and 3 had abnormal salivary scans. Three had a positive ANA, 1 had anti-salivary duct antibody, and 3 had abnormal lip biopsies. Five had musculoskeletal complaints, 4 had Raynaud's phenomenon, and 2 had a concurrent diffuse connective tissue disease. Thus, SS in patients with non-Hodgkin's lymphoma appears to be more common than is generally appreciated.

Experimental Hydroxyapatite Articular Calcification

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Hydroxyapatite crystals have been recently suggested as a cause of crystal induced synovitis in humans. We have performed the following studies in an attempt to develop an experimental model to further study the relation of hydroxyapatite to inflammation.

Articular calcification was induced in six-week-old NZW rabbits with techniques similar to those described by Selye in other tissues. Eight rabbits were given a single dose of 500,000 U oral vitamin D, and the next day left knees were injected with 1 mg $FeCl_2$. Right knees were injected with saline. Four rabbits received intraarticular $FeCl_2$ without vitamin D. Cartilage and synovial membrane were studied by light and electron microscopy at 5, 15, and 45 days. Synovianalysis, roentgenograms, and microradiography were also done. Six other six-week-old rabbits were given vitamin D orally 500,000 U for 90 days without any intraarticular injection. Six controls were followed without vitamin D and all were killed at 90 days for studies as above.

Mild synovial inflammation was seen in $FeCl_2$ injected joints without vitamin D. In rabbits given vitamin D there was tissue necrosis in the $FeCl_2$ injected joint with synovial calcification visible by light microscopy by the fifth day. Calcifica-

tions were all characteristic of hydroxyapatite by electron microscopy and could be seen in interstitium, occasionally on collagen fibers, and in vacuoles of synovial cells. Small amounts of iron were seen in phagocytes without relation to the calcification. Calcification increased over 45 days. Cartilage was not calcified except for a small surface deposit at 45 days in one rabbit. Synovial fluids had only very low leukocyte counts with predominance of mononuclear cells. Joints not injected with iron were normal.

The rabbits given vitamin D for 90 days all developed round, mid-zone articular cartilage calcifications similar to those spontaneously occurring in older rabbits. There was no synovial calcification, inflammation, or joint effusion. By electronmicroscopy all calcium deposits were in the interstitium and were hydroxyapatite-like needles. Many chondrocytes showed degenerative changes. Thus, different patterns of articular calcification can be produced in rabbits' knees with techniques described. Acute crystal associated inflammation was not demonstrated. Crystals formed and sequestered in synovial or cartilage tissue appear to be tolerated without inflammation.

Genetic Factors in Systemic Lupus Erythematosus: B-Lymphocyte Alloantigens

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Studies of systemic lupus erythematosus (SLE) families and populations suggest that a gene(s) linked to the major histocompatibility complex (MHC) influences SLE. We have examined the MHC in SLE families, patients, and controls by determining the cytotoxicity of antisera which detect MHC-determined antigens expressed selectively on B lymphocytes.

B lymphocytes from 41 SLE patients and 184 controls were tested against a panel of 47 pregnancy sera, and reaction frequencies of individual sera were compared. Twenty-eight of the SLE patients were also typed with a panel of HLA-D-related sera from the 7th International Histocompatibility Workshop, HLA-DRw types assigned, and compared with 490 workshop controls. The HLA-DRw types and individual serum reactivities which were increased in the SLE population are shown.

Serum Ia 715 is not strongly correlated with either HLA-DRw2 or -3 in normal controls and may identify a distinct disease-associated B-cell alloantigen.

Analysis of HLA types and B-cell alloantigens in 6 SLE families shows that one HLA haplotype is usually shared among those individuals with SLE and other autoimmune abnormalities in a given family. Exceptions exist, however, indicating that the haplotype itself is neither necessary nor sufficient for the expression of autoimmunity.

This study demonstrates that certain MHC-related B-cell alloantigens, possibly products of immune response genes, are increased in SLE. Family study indicates that the requirements for SLE development are not limited to the MHC, however, and probably involve additional genetic and/or environmental factors.

	SLE Tested	% Positive	NI Tested	% Positive	*P* (Uncorrected)
HLA-DRw2	28	57	490	26	<0.005
HLA-DRw3	28	46	490	22	<0.01
HLA-DRw2 or 3	28	89	490	(48)	<0.0001
Serum Ia 715	41	76	184	14	<0.0001

Effects of Sex Hormones on Experimentally Induced Osteoarthritis and Cartilage Metabolism

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Clinical observations of increased osteoarthritis (OA) with menopause and studies of OA in experimental animals suggest that androgens worsen and estrogens ameliorate OA. Further studies have demonstrated that estrogens suppress and androgens increase $^{35}SO_4$ incorporation into nonarticular cartilage.

Using a rabbit partial meniscectomy model of experimentally induced OA, we compared nontreatment (18 rabbits), estradiol valerate 1.6 mg/kg intramuscularly every 2 weeks (20 rabbits), and testosterone cypionate 5 mg/kg intramuscularly every 2 weeks (20 rabbits) on the development of OA and on proteoglycan (PG) synthesis by cartilage. Animals were killed 12 weeks post partial meniscectomy, and osteoarthritic lesions were noted. Knee sections processed for histologic examination were stained with H and E and safranin-O with fast green counterstain. Cartilage metabolism in the 3 groups was examined by in vitro measurement of $^{35}SO_4$ incorporation into articular cartilage after 2 hour incubation in Dulbecco's modified Eagle medium. Medial and lateral components of femoral and tibial knee joint surfaces were studied separately. Normal unoperated knees served as additional controls.

Frequency and severity of osteoarthritic lesions were the same in all 3 groups. Osteoarthritic and normal articular

cartilage from estradiol-treated animals revealed statistically significant reduction in $^{35}SO_4$ incorporation as compared to untreated animals. Androgens had no significant effect on $^{35}SO_4$ incorporation. Femoral $^{35}SO_4$ incorporation was uniformly greater than tibial $^{35}SO_4$ incorporation in all groups ($P < 0.05$).

Estradiol did not ameliorate nor testosterone worsen OA. Both normal and osteoarthritic articular cartilage were susceptible to estradiol suppression of proteoglycan synthesis. The poor correlation between severity of OA and rate of PG synthesis may require reevaluation of the role of the latter in the OA disease process. Variations in cartilage metabolism from different surfaces (femoral versus tibial) may relate to known differences in susceptibility of joints to development of OA.

Immune Reactivity of Personnel Working in Systemic Lupus Erythematosus and Other Laboratories

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We have been analyzing antibody activities in sera from individuals working in various laboratories and in sera from a normal population. Compared to the normal sample, the prevalence of elevated anti-DNA activity was significantly greater in samples of sera from over 40 personnel in systemic lupus erythematosus (SLE) laboratories ($P < 0.001$), from laboratories involved with nucleic acids ($P < 0.001$), and from routine hospital laboratory personnel ($P < 0.01$). Evidence that this anti-DNA activity was due to gammaglobulin was obtained by the presence of ^{125}I-DNA bound to IgG in a radioautograph. In addition, differences were found in the prevalence of DNA reactivity between the normal sample and the samples from the three laboratory groups when anti-human gammaglobulin was used as the precipitating agent. Also, we have isolated the material from a serum containing high DNA reactivity by a DNA-cellulose affinity technique, and the fraction with DNA binding activity contained only IgG.

Lymphocytotoxic activity was also increased in the sera from SLE laboratory personnel compared to the other laboratories and to the normal sample ($P < 0.0005$), as previously reported (XIV International Congress of Rheumatology, Abstract p. 131, 1977).

Immunoglobulin levels were analyzed by immunofluorescence. There was no significant difference in the IgG and IgM levels, but the mean IgA level of the SLE laboratory personnel was 2.27 ± 0.96 mg/ml compared to 1.42 ± 0.76 mg/ml in the normal sample ($P < 0.001$).

When sera from 17 of the laboratory personnel with the highest anti-DNA activities were compared to 17 normal sera, additional abnormalities were found. Not only was there a significant difference in the anti-DNA activity ($P < 0.001$) and the IgA levels ($P < 0.001$), but also the mean IgM level in the laboratory personnel (1.93 ± 0.58 mg/ml) was significantly greater than in the normal sera (1.01 ± 0.75 mg/ml)($P < 0.001$). No difference in the mean IgG levels was found. Of 5 additional specific antibody activities quantitated by radioimmunoassay, one of these (anti-bovine gammaglobulin) was elevated more frequently in the laboratory personnel than in the normals ($P < 0.02$).

The data suggest that laboratory personnel tend to have an increased immune reactivity, particularly those working with SLE sera. This condition might be due to laboratory exposure to a stimulus, causing an immune response that includes autoantibody production.

Amyloid Arthropathy in the Absence of Dysproteinemia: A Possible Association with Chondrocalcinosis

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In most cases, amyloid arthropathy (AA) has been associated with multiple myeloma or demonstrable paraproteinemia. Various modes of presentation and laboratory features have been described. We have analyzed clinical, laboratory, and radiographic features of 5 patients with AA seen over a 2-year period. None of these patients had identifiable diseases known to cause secondary amyloidosis. Presenting symptoms were those of carpal tunnel syndrome (3:5) and/or swollen hands with stiffness (4:5). Periarticular tenderness of the hands and thickened palmar tendons were noted. Pitting edema of the hands, at times massive, was noted in 4 of 5. Knee and elbow effusions were present in 2 patients. Sedimentation rates, rheumatoid factor, and antinuclear antibodies were normal or negative. Serum and urine protein electrophoresis and immunoelectrophoresis failed to detect any paraprotein. joint fluids (4) were noninflammatory except for one aspirated during an acute attack of pseudogout. Radiographs showed degenerative changes (3:5) and chondrocalcinosis (4:5). Synovium obtained by open biopsy (5:5) revealed deposits of material which displayed metachromasia after crystal violet staining. In 3 cases, electron microscopy was performed and revealed typical fibrils in synovium. Deposits were localized to perivascular and subsynovial locations.

Attention is called to the presentation of AA as edema

of the hands with or without median nerve compression. Edematous hands and seronegative arthritis should raise the possibility of AA. These patients are also unusual in that AA was not associated with dysproteinemia and absence of a paraprotein should not discourage invasive measures to document AA. The associated chondrocalcinosis has been previously recognized in only one case report. Chondrocalcinosis in 4 of 5 patients may be a chance occurrence in an elderly population (age range 67–90) or may cause AA by chronic local inflammation.

Ophthalmologic Safety of Long-Term Hydroxychloroquine Treatment

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Antimalarial therapy for connective tissue disease has been limited by its potential retinal toxicity. The present study was undertaken to assess visual problems in patients treated with long-term hydroxychloroquine given in standard dosage. All patients who received both treatment and ophthalmologic evaluation at Albany Medical College were included and consisted of 99 patients treated for at least 1 year. Each patient was examined at baseline and then every 6 months for visual acuity, central fields using a red test object, funduscopic abnormalities, accommodation, corneal deposits using a slit lamp, and keratoconjunctivitis by Shirmer test. Electro-oculograms (EOG) were performed in 14 patients who had received high total doses or had abnormal central fields suggesting toxicity. Almost all patients received hydroxychloroquine in the maximal daily dose of 400 mg. Total dose ranged from 146 to 927 g (median 365) and duration of treatment from 13 to 68 months (median 33). Diseases treated were rheumatoid arthritis in 58 patients, systemic lupus erythematosus in 31, juvenile rheumatoid arthritis in 4, mixed connective tissue disease in 2, and other in 4.

Ophthalmologic toxicity was minimal. No patients were precluded from taking hydroxychloroquine at baseline evaluation. No corneal deposits or accommodation defects were found. Three patients had abnormalities in central fields: paracentral scotomata and/or minor field restrictions. All 3 had rheumatoid arthritis. Toxicity occurred after total doses of 73, 206, and 316 g. Visual changes were completely reversible in the first patient who was the only one in the series with funduscopic changes. She has now received a total dose of 535 g and has normal fundi, central fields, and EOG. The second patient continues to have central field restriction but has had resolution of paracentral scotomata, a normal EOG, and no visual complaints. The third had reversal of central field defects but had an abnormal EOG when subsequently tested.

We conclude that hydroxychloroquine in a dosage of 400 mg/day is safe from significant ophthalmologic toxicity if followed by appropriate testing, and we find no evidence from an association of increased toxicity with higher total dose or with the diagnosis of systemic lupus erythematosus.

Multiple Forms of the Neutral Proteoglycanase from Human Articular Cartilage

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The metal-dependent neutral proteoglycanase, extracted from 1,200 grams of human articular cartilage, occurred in four electrophoretic forms. These were purified 2,000-fold and produced single proteolytically active bands on disk electrophoresis. These forms were separated by preparative flat-bed isoelectric focusing and had approximate isoelectric points of 8.7, 8.3, 7.6, and 7.1. Gel filtration and SDS gel electrophoresis showed that they have an apparent molecular weight of 25–27,000 and are composed of subunits of 13–14,000. Gel filtration and dialysis indicated their tendency to disaggregate and reaggregate into monomers and dimers. Their proteoglycanase activity passed through Visking tubing and the passage continued on repeated dialysis with fresh buffer. This was prevented to a large extent by dialysis against zinc or cobalt ions.

All the forms degraded the protein core of proteoglycan subunit optimally at pH 7.25 and were inhibited almost completely by addition of o-phenanthroline or passage through the chelating resin, Chelex. However, they differed in their inhibition by EDTA, the most cationic forms being the least inhibited. Also, the 3 most cationic forms had no activity on casein, histone, and the link proteins, indicating a relative specificity for degrading proteoglycan.

These enzyme forms which are active at the pH in cartilage matrix may have an important role in proteoglycan degradation in osteoarthritis.

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Early Appearance of Autoimmunity and Antibodies to DNA in Male BXSB Mice

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BXSB mice (developed by Dr. E. D. Murphy, Jackson Laboratory) spontaneously evolve a lupus-like disorder similar to the disease of NZB/NZW (B/W) mice. However, in contrast to B/W mice, the BXSB disease is more severe in males who die of immune complex nephritis at a mean age of 5 months. We have compared the formation of antibodies to DNA in BXSB and B/W mice. The early appearance of autoimmunity in male BXSB mice is associated with a premature switch from IgM to IgG serum antibodies to DNA. This is analogous to the early switch from IgM to IgG antibodies in female B/W mice which is associated with accelerated disease and impending death. We have also measured the spontaneous synthesis of antibodies to DNA by spleen cells cultured for 96 hours. Culture supernatants contain immunoglobulin which binds DNA specifically as determined by radioimmunoprecipitation. Two month old male BXSB mice produce more antibody to DNA in culture than do age-matched female mice of BXSB, B/W, Balb/c, or C57 Bl/6 strains.

Anti-DNA production in culture increases with age in B/W mice. Older female B/W mice subjected to prepubertal castration and treated with androgen produce significantly less anti-DNA in culture as compared to sham or estrogen-treated controls ($P < 0.017$). The effect of hormone treatment on BXSB mice is currently under investigation. These results suggest that the male-dominant disease of BXSB mice is reflected in early synthesis of antibodies to DNA which is apparent both in vivo and in vitro.

Strain (2 months old)	Sex	Antibodies to DNA (ng DNA Bound/10^6 Spleen Cells)
BXSB	Male	163
BXSB	Female	68
B/W	Female	69
Balb/c	Female	40
C57 Bl/6	Female	43

The Rheumatoid Nodule: Clinical Significance

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Although the subcutaneous nodule (SCN) is the extra-articular hallmark of rheumatoid arthritis (RA), its clinical significance has not been established. It was our thesis that the presence of SCN would serve to identify the subset(s) of rheumatoid patients with systemic and highly immunoreactive disease.

All patients with definite (46) and classic (165) rheumatoid arthritis who had an initial unit admission during the interval January 1974 through June 1976 were selected for study. Primary reasons for admission included active synovitis (57%), orthopedic surgery (17%), and a miscellany (26%) of therapeutic complications and intercurrent illness. Of the 211 patients, 91 (43%) had SCN on admission or by documented history.

Medical record analysis per protocol revealed patients with and without SCN were comparable in terms of demography, duration, and activity of RA. Similarly, systemic features did not differ significantly between two groups, as shown in the table.

With regard to sero-reactivity, patients with SCN and the anodular group were also alike in terms of the presence of rheumatoid factors (97% versus 85%), antinuclear factors (61% versus 42%), and hypocomplementemia (13% versus 10%). There was a tendency only for those with SCN to have higher Latex test titers.

Thus, contrary to expectations, subcutaneous nodules cannot be relied upon to screen out those patients with rheumatoid variants and systemic disease.

Subcutaneous Nodule	No. Patients	Sjögren's	Vasculitis	Felty's	Serositis	"RA" Lung
+	91	8 (9%)	7 (8%)	2 (2%)	2 (2%)	4 (4%)
−	120	7 (6%)	5 (4%)	4 (3%)	2 (2%)	7 (6%)
Total	211	15 (7%)	12 (6%)	6 (3%)	4 (2%)	11 (5%)

A Double-Blind Controlled Study of Levamisole in Rheumatoid Arthritis

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Twenty-four patients (18 women, 6 men, mean age 54 years) with definite rheumatoid arthritis were entered into a double-blind study using the immunomodulator levamisole, 150 mg daily, versus placebo for 6 months, while continuing baseline non-steroidal antiinflammatory therapy and/or prednisone up to 10 mg/day. Assessment criteria included articular

index, grip strength, 50 feet walking time, duration of morning stiffness, subjective pain relief, sedimentation rate, and latex fixation titers.

To date, 21 patients have completed the initial phase. Eight of 9 on levamisole improved while the remaining patient, who did not appear to respond, flared after drug discontinuation. Two patients on levamisole were excluded because of skin rash. Four of 10 on placebo appeared to improve but not to the degree seen with levamisole.

The mean changes in articular index, number of swollen joints, and duration of morning stiffness were statistically significant at 6 months for levamisole treated patients but not for placebo ($P < 0.02$). Subjective pain scale responses were significantly better among levamisole versus placebo, but no differences were observed between the groups in grip strength or 50 feet walking time. Five levamisole treated patients converted to seronegativity after 6 months. Sedimentation rates, however, remained stable in both groups: skin rashes developed in 5 patients on levamisole, and 2 were discontinued; in 2, rash was unrelated, and 1 continued on a lower dose. Of interest, 3 of 10 on placebo had rashes.

Preliminary investigations of delayed skin hypersensitivity, lymphocyte responsiveness, phagocytic function, and cutaneous inflammatory responses failed to show any correlation with clinical responsiveness.

Levamisole is a potentially therapeutic agent in rheumatoid arthritis, but its mode of action is yet to be determined.

Electron Microscopic Study of Synovial Fluid Cells in Systemic Lupus Erythematosus

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Since synovial fluid cells in patients with systemic lupus erythematosus (SLE) have not previously been examined by electron microscope, we have studied 17 SLE joint effusions with emphasis on the occurrence of LE cells and tubuloreticular structures (TRS) and on correlations with clinical and light microscopic findings. All patients fulfilled at least 4 ARA preliminary criteria for SLE and had antinuclear antibodies in their serum. Three patients had drug-induced SLE. Synovial fluid volumes varied from 0.75–25 cc; leukocyte counts ranged from 875–58,000 but only 3 were over 15,000. Polymorphonuclear leukocytes predominated in 3 effusions including the one with 58,000 cells. Cultures were all negative. Monocytes and large Sudan positive macrophages were prominent. Joint fluid LE cells were found in 8 patients while 12 had extracellular hematoxylin bodies, and 4 had a variety of other smaller eosinophilic, hematoxyphilic, or cellular inclusions.

LE cells were identified by electron microscope in 10 patients. The major and often sole visible constituent of the inclusion was a clump of short filaments about 200 nm in diameter. These lay in vacuoles which also contained acid phosphatase. These filaments which appear to be products of nuclear chromatin also were seen extracellularly surrounding some cells and in smaller vacuoles. Small acid phosphatase positive vacuoles also contained cell cytoplasmic debris, intact nucleii, erythrocyte fragments, and large amounts of finely granular protein-like material. The finely granular material was shown to contain IgG by immunoperoxidase electron microscope staining. TRS were found in 8 synovial fluids and were predominantly in mononuclear cells with dense bodies and rough endoplasmic reticulum. They were infrequent in small lymphocytes or transformed lymphocytes with polyribosomes. Two LE cells had TRS. Buffy coat blood cells contained TRS in the 2 cases studied. Synovial fluid cell TRS were seen in 2 of 130 inflammatory and noninflammatory synovial fluid controls but no control joint fluids had the filamentous LE inclusions seen by electron microscope. No correlation of any synovial fluid finding with therapy, effusion duration, or disease severity has been found.

Synovial fluids in SLE contain TRS, many phagocytic cells, and can have higher leukocyte counts and PMN percentages than often appreciated. LE inclusions in joint fluid are composed predominantly of filaments derived from nuclear chromatin.

Anti-SS-A Antibody and Other Antinuclear Antibodies in Systemic Lupus Erythematosus

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The sera of 81 patients fulfilling the ARA criteria for systemic lupus erythematosus (SLE) were studied by immunodiffusion and the modified Farr technique for the presence of antibodies to the following antigens: SS-A, SS-B, RANA (RAP antigen), RNP, Sm, Scl-1, and DS-DNA. The incidence of antibodies to SS-B, RNP, Sm, and DS-DNA was similar to those previously reported. The incidence of anti-Scl-1 antibody was 0%, a finding which has not been reported previously. The incidence of RAP (23%) was higher than the 7% previously reported.

The incidence of anti-SS-A antibody was 26%. This antibody was found in high frequency in primary Sjögren's syndrome (70%) in the past but was not found in the small number of SLE patients studied at that time. In this study where a large number of SLE patients were studied and anti-SS-A antibody was present, only 48% had any evidence of

keratoconjunctivitis sicca based on Schirmer's tests. This indicates that not only is anti-SS-A an antibody "marker" for primary Sjögren's syndrome, but it is relatively common in SLE as well and furthermore, can exist in SLE in the absence of keratoconjunctivitis sicca.

Serial studies were also performed on some patients. Titers of anti-SS-A antibody were determined. These varied from neat to 2048 and correlated with disease activity. Anti-SS-A often paralleled anti-DS-DNA levels; however, anti-SS-A in some cases was an earlier predictor of clinical flares. Thus, anti-SS-A antibody may be helpful not only in the management of SLE patients, but with further investigation, aid in determining the pathogenesis of this disease.

Anti-DNA Antibody Binding as Measured by the Inhibition of Ethidium Bromide Fluorescence

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The phenanthridine dye ethidium bromide (EB) intercalates with native double-stranded DNA (dsDNA) resulting in an enhancement of fluorescence when assayed fluorometrically. Contamination of dsDNA preparations by single-stranded DNA (ssDNA) does not affect the assay because there is no fluorescence enhancement when ssDNA is added to EB. IgG purified by DEAE column chromatography from the sera of 10 normal volunteers: 19 systemic lupus erythematosus (SLE) sera with DNA binding activity as measured by the Millipore filter (MPF) radioimmunoassay, and 5 SLE sera without DNA binding activity were tested for their ability to compete with EB for binding to dsDNA. In 18 of 19 of the SLE with MPF-DNA binding activity, there was greater than 20% inhibition of the fluorescence normally shown when EB binds dsDNA. In contrast, 0 of 10 control IgG preparations and 0 of 5 of the SLE IgG from non-DNA binders had any such effect. Furthermore, the decrease in fluorescence due to the inhibition of EB binding to dsDNA was linear with increasing amounts of IgG, thus allowing direct quantitation of anti-DNA activity.

Since the binding constant of EB is known to be $>2 \times 10^{-6} M$, this assay measures high avidity antibodies and is not affected by non-specific, low-avidity DNA binding molecules. The specificity of anti-DNA antibodies in the EB assay was shown when absorption with excess nucleoside monophosphates did not alter the inhibition of EB binding in unabsorbed samples. However, the synthetic polynucleotides poly dA, poly dT, poly dC, poly dA:dT, and poly dG:dC were able to absorb DNA binding activity. Therefore, the EB assay has demonstrated several features which make it an important adjunct in studying the specificity and binding characteristics of anti-DNA antibodies. In addition, the assay is inexpensive, is easily and quickly performed, and it does not require the use of radiolabeled substrates making it practical for further development in investigative and clinical use.

The Effect of Cross-Linking in the Collagen Fibril on Degradation by Rheumatoid Synovial Collagenase

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Degradation of collagen in tendon, bone, and cartilage by collagenase is a significant factor contributing to the morbidity of the inflammatory arthritides. Although considerable information is available as to the effect of drugs and mediators of inflammation on collagenase production and activity, the mechanisms during aging in the collagen fibril causing increased resistance to collagenase degradation are poorly understood. In this study, the effect of collagen cross-linking on collagenolysis was measured with an in vitro model system consisting of purified chick calvarium collagen fibrils and lysyl oxidase, the cross-linking enzyme. Chick calvaria collagen fibrils were cross-linked by incubation with lysyl oxidase for varying time. Controls consisted of collagen incubated without enzyme or with lysyl oxidase plus β-aminopropionitrile, an irreversible inhibitor. The fibrils were subsequently measured for nascent cross-link content or incubated with purified rheumatoid synovial collagenase, and the rate of collagenolysis was measured. After synthesis of approximately 0.1 Schiff base cross-links per collagen molecule, a ten-fold resistance to collagenase digestion was observed. Inhibition of collagenase by EDTA prevented digestion. Synthesis of collagen fibrils with higher cross-link content resulted in further resistance. These results demonstrate that the increased resistance of collagen to collagenolysis observed during fibril maturation in vivo is due to synthesis of native, unreduced Schiff base cross-links. The cross-link content at which resistance to degradation develops is significantly less than that which affects fibril tensile strength in vivo. This suggests that resistance to collagenase is the earliest physiological effect of cross-linking in vivo. The rate of cross-link synthesis may be a significant factor regulating the rate of net collagen deposition in normal and pathologic states.

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Coagulation Abnormalities and Possible Relationship to Renal Disease in Progressive Systemic Sclerosis

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Vascular abnormalities have been shown to play an important pathogenic role in progressive systemic sclerosis (PSS), especially in the group of patients who develop rapidly progressive kidney disease. As part of a study of the kidney in PSS, we have also performed comprehensive investigations of coagulation parameters in order to evaluate the possibilities that: 1) coagulopathy occurs in these patients; and 2) coagulation abnormalities may be related to the development of renal disease in PSS.

Seven patients with PSS and normal renal function, as judged by normal blood pressure, creatinine clearance, and absence of proteinuria, were enrolled in the study. Coagulation parameters studied included prothrombin and partial thromboplastin times, fibrin split products, fibrin monomer, Sonoclot® (which reflects fibrin monomer formation in recalcified whole blood), thromboelastograph (which reflects fibrin polymer formation), and platelet aggregometry. In addition, each patient underwent percutaneous renal biopsy. Patients with PSS showed platelet hyperreactivity to collagen but not to other agents (91% aggregation versus control of 80.6%, $P < 0.01$). The following abnormalities, indicative of a hypercoagulable state, were found: increased rate of clotting by thromboelastograph in 5 patients; increased rate of clotting by Sonoclot in 4 patients; and premature onset of clotting by thromboelastograph in 3 patients. The patient with the most hypercoagulable profile has subsequently developed mild proteinuria. Five patients had renal biopsy findings on light microscopy suggestive of PSS kidney disease, including vascular intimal fibrosis, sclerosis, and fibrinoid necrosis. Four of these 5 were hypercoagulable by one or more tests; both patients with normal biopsies had normal coagulation profiles.

These data suggest that: 1) platelet-collagen interactions may be abnormal in PSS; 2) certain patients with PSS manifest laboratory evidence of hypercoagulability; and 3) hypercoagulability appears to correlate with renal biopsy abnormalities in PSS patients with normal renal function.

Inhibition of Natural Killing of a Tumor Cell Line by Systemic Lupus Erythematosus Sera

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Normal human mononuclear cells spontaneously lyse in vitro targets derived from cell lines of tumor origin. The natural killing of K-562, a tumor cell line derived from a patient with chronic myelogenous leukemia, varies in patients with systemic lupus erythematosus (SLE) (27% ± 21%) and controls (35% ± 20%). To evaluate the possible role of serum factors, normal peripheral blood mononuclear cells were incubated with 12 SLE sera, 10 normal control sera, or media alone in the natural killing assay.

0.5×10^6 Ficoll-Hypaque purified mononuclear cells after pretreatment with sera or media were incubated for 4 hours with 1×10^4 ^{51}chromium (^{51}Cr) labeled K-562 cells. Cytotoxicity was measured by the release of ^{51}Cr into the supernatant. The mean ^{51}Cr release in the presence of normal human sera was 96% ± 4% of that with media alone. In the presence of SLE sera, mean ^{51}Cr release was 36% ± 24% of that with media alone.

The magnitude of natural killing suppression by SLE sera did not correlate with complement levels, ANA titer, or DNA binding. The natural killing suppression by an individual SLE serum did not correlate with its inhibition of the antibody dependent cell mediated cytotoxicity of chicken red cells. The factors in SLE sera responsible for suppression of natural killings are non-dialyzable, precipitable with 50% $(NH_4)_2SO_4$, and removable by absorption with anti-Ig antibody. The factors are excluded by a G-200 column. The major portion of the suppressive activity was found in fractions of IgM or greater density on sucrose gradients.

These findings are compatible with factors in SLE sera, most likely immune complexes or antibodies, capable of inhibiting the natural killing of tumor cell lines. These factors may prevent cell mediated cytotoxic destruction of malignant cells in vivo and may explain the increased association of SLE and malignancy noted by Canoso *et al.* (Arthritis Rheum 17:383, 1974).

Uric Acid Excretion: Assessment from Spot Mid-Morning Serum and Urine Samples

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Full evaluation of any hyperuricemic patient requires assessment of uric acid excretion, but the standard 24-hour urine method presents many problems. Ill-timed and incomplete collections may combine with bacterial contamination and crystal precipitation to cause significant errors. These problems can be largely avoided by assessing uric acid excretion per 100 ml of glomerular filtrate. This simple, physiologically sound parameter is obtainable from single, untimed urine specimens by multiplying urinary uric acid by plasma creatinine and dividing by urinary creatinine (all in mg/100

ml). To minimize possible diurnal effects on uric acid excretion, all samples were taken in the morning.

To evaluate the precision of the method, 15 normal men collected two 24-hour urines. Spot urine specimens with concurrent serum samples were also obtained on two separate mornings. Uric acid was measured spectrophotometrically, and creatinine was measured by standard autoanalyzer technique. The coefficient of variation ($SD/\bar{\chi}$) was 31% for paired, quantitative 24-hour urine uric acid determinations, while spot, mid-morning assessments of uric acid excretion per 100 ml of glomerular filtrate had a more satisfactory coefficient of 21%. Since most laboratories do not employ the enzymatic spectrophotometric method, urine uric acid determinations were also performed by a colorimetric, autoanalyzer technique. These values correlated well ($r = 0.90$) with the more specific spectrophotometric findings, but were an average of 7% higher.

Samples from 23 normal, adult men, the mean urinary excretion of uric acid in mid-morning was 0.405 ± 0.098 mg/100 ml (SD), while 19 gouty men (studied between attacks and off hypouricemic drugs) had a mean of 0.679 ± 0.423 mg/100 ml. Included in the latter group were 5 significant overexcretors with values of 0.821, 0.905, 0.982, 1.45, and 2.01 mg of uric acid per 100 ml of glomerular filtrate. We believe that this assessment of mid-morning serum and urine samples effectively identifies overexcretors of uric acid. In addition, it is more convenient, more physiological, and more precise than the conventional 24-hour method.

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Histologic Evaluation of Mixed Connective Tissue Disease in Children and Adults

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In mixed connective tissue disease (MCTD), a newly described rheumatic syndrome, no comprehensive histopathologic descriptions exist. We have followed 16 MCTD children over a range of 1–11 years, (mean 6 years); 4 have died. Three autopsies and 6 renal and 3 muscle biopsies were reviewed. Among adults with MCTD, tissues from 2 autopsies, and 18 kidney, 21 muscle, and 4 lung biopsies were reviewed.

In children, proliferative vascular lesions, with intimal and medial thickening and luminal narrowing but without fibrinoid or inflammatory change, affected large vessels (aorta, coronary, renal), and small arterioles of many organs. Inflammatory infiltration, predominantly plasmacytic, was marked in skeletal muscle 6:6, liver 3:3, salivary glands 2:2, intestine 3:3, and heart 3:3. Distinctive lesions of esophagus (atrophy of inner muscle layer), thymus (hyperplasia), kidney (membranous change), liver, lung, and salivary glands differed significantly from those expected in childhood systemic lupus erythematosus, polyarteritis, or scleroderma. Many of these organs were not clinically involved during life. Histologic estimates of numbers of T and B lymphocytes in spleen and lymph nodes, and degree of plasmacytosis (with hyperglobulinemia), differ from systemic lupus erythematosus and juvenile rheumatoid arthritis.

In adults with MCTD, muscle changes included: diffuse inflammatory infiltrate 15:21 (peri- or endomesial, and peri- or intravascular). By ATP-ase, 5:11 had type I fiber predominance. On muscle immunofluorescence, 8:10 had vascular, sarcolemmal basement membrane, or granular fiber staining, with IgG or IgM. In 20 kidneys there were included: mesangial proliferation 5, focal-local change 5, membranous 3, membrano-proliferative 1, proliferative vessels 1, normal 5. Lung tissue (6) revealed: vascular proliferation 2, vascular medial hypertrophy 2, and interstitial fibrosis 3.

In MCTD, children and adults have similar lesions: inflammatory lesions may predominate early, but can occur late. Immunofluorescent data suggest an immune basis for injury; the late predominance of proliferative vascular lesions suggests that vascular sclerosis is a serious complication. The findings of many significant histologic lesions, without clinical signs or symptoms, suggest that features of this multi-system disease evolve slowly, and that the full spectrum of MCTD is not yet known.

In Vitro Comparison of Peripheral Blood Lymphocytes from Normal Subjects and Patients with Rheumatoid Arthritis after Infection with Epstein-Barr Virus

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Recent experiments have shown that patients with rheumatoid arthritis (RA) frequently have precipitating antibodies reacting with Epstein-Barr virus (EBV) infected human B-cell lines (Alspaugh, MA, *et al.*, J Exp Med, in press). To further delineate the possible role of EBV infection in RA, we studied the effects of EBV on lymphocytes from 11 patients

with RA and 10 normal subjects. After isolation of the lymphocytes by isopycnic sedimentation, the cells were placed in tissue culture and half were infected with EBV. Every 6 days for 1 month the supernatant fluid was removed from each culture and fresh medium was added. Total IgM and IgM-RF secreted into the supernatant were measured by solid phase radioimmunoassay. Independently the cells were examined for transformation and numerically graded. All infected cultures produced IgM-RF, which correlated with a high grade of transformation. The amount of rheumatoid factor (RF) produced by EBV infected normal, but not rheumatoid, lymphocytes correlated with levels of RF in the plasma. Nine of 11 uninfected cell cultures from RA patients, but none from control subjects, also made RF. Furthermore, 6 of 11 cultures from the RA patients transformed in the absence of EBV to become continuous RF-producing cell lines. On the contrary, only 1 of 10 cultures from normal subjects spontaneously transformed. From these experiments we conclude: 1) EBV infection can induce the production of autoantibodies in both normal subjects and patients with RA; 2) PBL from patients with RA have a high rate of transformation in culture in the absence of superinfection with EBV.

Evidence for Microtubule Control of PMN Chemotaxis

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The incubation of PMNs with a chemotactic factor (CF), in the absence of a gradient, prevents the cells from responding with directional migration when, after washing, they are challenged with a gradient of the same or a different chemotactic factor (deactivation and cross-deactivation). Structurally unrelated CFs, some shown to bind to distinct cell receptors, can cross-deactivate, which suggests that deactivation is not due to receptor blockade. We propose that deactivation is the result of generalized microtubule assembly induced by CF incubated with the cells in the absence of a gradient, thus rendering the cells incapable of responding to a CF gradient with distinctive localized assembly, a proposed requirement of normal chemotaxis. If this scheme is correct, the simultaneous preincubation of PMNs with suitable concentrations of colchicine, a microtubule disrupting agent, and a CF should protect the cell against deactivation and colchicine-induced suppression of chemotaxis. Human neutrophils were preincubated, 20′ at 37°C, with colchicine (10^{-3} to $10^{-6}M$); the chemotactic factors Gly-His-Gly (10μg) or crystal-induced chemotactic factor (CCF) 25μg alone; colchicine and either chemotactis factor; or Hanks. Cells were washed and tested for chemotactic response against the CF using a radioassay that utilizes ^{51}Cr labeled neutrophils. Preincubation of cells with either chemotactic factor or colchicine alone resulted in a dose-dependent inhibition of chemotaxis. When cells were preincubated with both chemotactic factor (Gly-His-Gly 10μg or CCF 20μg) and suitable concentrations of colchicine (10^{-5} or 10^{-6}), a reversal of the inhibition of chemotaxis was noted. Deactivation reappeared when the balanced ratio between colchicine and CF was altered.

Preincubation of CCF with colchicine had no direct effect on its chemotactic activity, and colchicine ($10^{-5}M$) did not alter the specific binding of radiolabeled CCF to neutrophils. Additionally, both CCF and Gly-His-Gly induced microtubule assembly by electron microscopy.

Functional evidence is presented with two distinct chemotactic factors, which suggests that the basis for deactivation is overpolymerization of microtubules that prevents the PMNs from responding to a chemotactic gradient with directional migration.

Fever in Systemic Lupus Erythematosus

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Fever is a common occurrence and frequent reason for hospitalization of patients with systemic lupus erythematosus (SLE). To assess the frequency, causes, and clinical and laboratory characteristics of febrile episodes in hospitalized patients with SLE, the medical records of 568 admissions of 160 SLE patients during the 5 year interval 1973 to 1977 were reviewed.

Eighty-three febrile episodes, defined as an oral temperature ≥38°C and one blood culture drawn for evaluation of fever, occurred in 63 patients. The febrile episodes were classified: Group A—clinically active SLE only (50), Group B—documented infection (19), and Group C—miscellaneous (14). Clinically active SLE accompanied 6 episodes in B and 2 in C. Treatment at the time fever developed included steroids in 64, 84, and 100% and cytotoxic agents in 16, 32, and 50% of episodes in A, B, and C, respectively. Infectious causes of fever were bacterial septicemia (8), localized bacterial infections (7), herpes zoster (3), and miliary tuberculosis (1). Miscellaneous causes were procedure-related fever (4), drug fever (3), Addisonian crisis (1), myocardial infarction (1), and unidentified (5). The initial clinical impression was correct in 45 of 50 infectious episodes. Two episodes of bacterial septicemia were unexpected; one occurred in a patient with concommitantly active SLE. The clinical impression was correct in 47 of 51 episodes in A; infection (3), acute appendicitis (1), and drug fever (1) were suspected in the others. Comparing A and B,

patients' age, disease duration, and fever patterns were similar. Shaking chills were more frequent with infection ($P < 0.001$) but were present in 28% of A. Laboratory studies helpful in identifying patients with infection were leukocytosis $>12 \times 10^3$/mm ($P < 0.001$), neutrophilia $>8 \times 10^3$/mm ($P < 0.001$) and normal DNA binding ($P < 0.001$). Four deaths occurred despite appropriate therapy: 1 in A with necrotizing lupus pneumonitis, and 3 in B with gram negative sepsis, 2 of whom had active SLE.

In summary, infection accounted for 23% of febrile episodes in hospitalized patients with SLE. Bacterial septicemia was found in 11% and was associated with a high mortality. The initial clinical impression was usually correct and the WBC count, absolute neutrophil count, and DNA binding were helpful in distinguishing between infection and active SLE.

The Kidney in Progressive Systemic Sclerosis: A Prospective Study

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Renal involvement in progressive systemic sclerosis (PSS) is the most devastating form of the disease with one year mortality close to 100% unless hemodialysis or transplantation is successful. At present, there are no reliable predictive factors which allow us to separate those who will eventually develop renal disease from those who will not. Because of this, we have studied 9 patients with PSS who were normotensive and had normal renal function (mean serum creatinine 0.89 mg%, mean creatinine clearance 88 cc/min, mean protein excretion 55 mg/24 hours) and normal urinalysis. Studies included percutaneous renal biopsies, evaluation of the renin-angiotensin system, and a cold pressor test to try to determine renal vascular reactivity.

Five of 9 renal biopsies demonstrated distinct vascular lesions on light microscopy with intimal fibrosis in 4, hyaline intimal sclerosis in 3, and arteriolar fibrinoid necrosis in 1. Electron microscopy of the vessels showed changes similar to those on light microscopy. In addition, wrinkling of the glomerular basement membrane (GBM) with expansion of the mesangium by GBM-like material was seen in 8 of 9 biopsies. Although nonspecific, these changes are suggestive of ischemia in the kidney and are compatible with the vascular changes of PSS. Immunofluorescence studies revealed C3 in vessels in all 9 biopsies.

Plasma renin activity (PPA) was performed in 8 of 9 patients. Of the 5 patients with abnormal biopsies, 4 had elevated plasma renins (9.31 ng/ml/hr) at the time of biopsy and the fifth patient has subsequently developed significant elevation. The 3 patients with normal biopsies had normal plasma renins (2.38 ng/ml/hr). Cold pressor testing with PRA determination resulted in a mean maximal increase of 6.52 ng/ml/hr in those with abnormal biopsies, 0.4 ng/ml/hr in those with normal biopsies, and 0.19 ng/ml/hr in 6 control subjects.

These findings indicate that renal histologic vascular involvement may precede the onset of clinical renal disease in PSS and correlates well with PRA elevations and increased responsiveness of the renal vasculature. Followup of these patients will determine the clinical significance of these findings.

Increased Tear Lysozyme in Rheumatoid Arthritis

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Ninety-six patients with classic or definite rheumatoid arthritis were consecutively screened for the presence of early ocular manifestation of disease. Each patient was followed for an average of 18.7 months and received an average of 4 ophthalmologic exams at an average of 6 month intervals. None of the patients demonstrated ocular lesions definitely attributable to rheumatoid arthritis. Eighteen patients developed new ocular pathology consisting of chronic blepharitis, chronic iritis, corneal subepithelial defects, keratitic precipitates, keratitis, corneal abrasion, and abnormal tear film. Twenty-one patients of this group and 15 normal adult controls were screened for keratoconjunctivitis sicca. Slit lamp examination, rose bengal staining, Shirmer's testing, and tear lysozyme concentration were measured in each patient. Tear lysozyme concentration was indirectly determined spectrophotometrically by measuring lysis of the dried cell walls of *Micrococcus lysodeikticus*. Patients with rheumatoid arthritis demonstrated abnormal rose bengal staining (rheumatoid arthritis, 5 patients; control, none), significant decreased tear production (rheumatoid arthritis, 14.5 ± 1.9 mm wetting Shirmer strip; control, 24.0 ± 2.6 mm wetting Schirmer strip) $P < 0.01$, and markedly increased tear lysozyme concentration (rheumatoid arthritis, 41.0 ± 4.4 μg/ml; controls 16.9 ± 3.8 μg/ml) $P < 0.01$. Thus, unlike patients with severe Sjögrens syndrome who have low tear lysozyme concentrations, patients with rheumatoid arthritis demonstrate significant increases in tear lysozyme levels. This new finding, as yet unexplained, may represent the earliest lesion in the development of keratoconjunctivitis sicca in patients with rheumatoid arthritis.

Effect of Immunosuppressive Regimen on Oncogenesis in NZB/NZW Autoimmune Disease

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NZB/NZW mice develop autoimmune disease similar to that of humans with lupus systemic erythematosus. Treatment with cyclophosphamide (Cp) protects against the disease but often is associated with a high incidence of tumors. Tumors resulting from direct chemical carcinogenic activity increase with rising cumulative dose and time after exposure and are of diverse types. Immunosuppression itself is reported to facilitate tumor growth or, perhaps, oncogene expression and has been primarily associated with sustained immunosuppression, predominantly with tumors of reticuloendothelial (RE) origin. We compared tumor development in NZB/NZW mice given different Cp regimens (see table).

Reticuloendothelial tumors increased with daily dose but did not correlate with cumulative dose or duration of treatment. Smaller numbers of other (non-RE) types of neoplasms occurred in all groups. Our results 1) suggest that RE tumor development in autoimmune disease is more dependent on continuous, high doses of cytotoxic drug than on cumulative dose or duration of treatment and 2) are compatible with the possibility that RE tumors result from immunosuppression or oncogene expression.

Treatment Group	Cp Dose	No. Animals	No. RE Tumors	Mean Cumulative Dose of Cp	Mean Duration of Treatment	*P*
I	1.5 mg/kg/day	12	2 (17%)	332 mg/kg	32 weeks	<0.01
II	3.5 mg/kg/day	30	12 (40%)	4022 mg/kg	72 weeks	<0.01
III	12.0 mg/kg/day	19	17 (89%)	2754 mg/kg	33 weeks	
IV	Saline only	13	0	—	35 weeks	

Intravenous Colchicine in the Treatment of Acute Pseudogout

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The success of colchicine in the treatment of acute gouty arthritis is well established. However, the usefulness of this drug in the therapy of the pseudogout syndrome has been less enthusiastically regarded. In general, assessments of such therapy refer to lower success rates and somewhat inconsistent results. Nevertheless the use of other antiinflammatory drugs to treat acute pseudogout is sometimes militated against by the presence of associated conditions commonly seen in the age group of these patients, including congestive heart failure, gastrointestinal disease, or neurological deficits.

We have treated 7 consecutive patients with the acute arthritis of pseudogout with a standard regimen of colchicine by the intravenous (IV) route. There were 4 males and 3 females, ages 56 to 88, and all presented with acute mono- or oligoarticular arthritis. In all cases typical calcium pyrophosphate dihydrate (CPPD) crystals were identified by red-compensated polarized light microscopy of the synovial fluid. None of the patients had elevated serum uric acid, except 1 which was on diuretics, and synovial urate crystals were absent. Synovial fluid white counts varied from 2600 to 54000/mm^3 with 70–88% polymorphonuclear leukocytes; 4 of the 7 had radiologic evidence of chondrocalcinosis.

All patients were treated within 54 hours of the onset of the acute arthritis. Colchicine was usually given at a dose of 2.0 mgm IV over a period of 20 minutes followed by 0.5 mg IV every 6 hours for the next 24–48 hours. Total IV colchicine dosage varied from 4 to 7.5 mgm. An excellent response occurred in 24–36 hours in 5 of 7 patients and in 37–48 hours in the other 2, with complete resolution of inflammation in all 7.

We conclude that 1) IV colchicine may provide effective and consistent therapy for the acute arthritis of pseudogout; and 2) because of this, prompt response of an acute arthritis to IV colchicine remains an insufficient clinical criterion for the diagnosis of gout.

Histologic Assessment of Lymphokine Mediated Suppression of Chondrocyte Glycosaminoglycan Synthesis

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Irreparable degradation of articular cartilage is the major consequence of inflammatory and degenerative forms of articular disease. Responsible mechanisms beyond direct enzyme mediation are not well understood. Though indirect evidence has incriminated cell mediated immunologic events in pathogenesis, few studies have investigated potential operative mechanisms. We have previously shown: a) the capacity of mitogen-induced lymphokines (LK), via monocyte interaction, to be capable of inducing cartilage proteoglycan degradation (Arthritis Rheum 20:922, 1977) and b) a non-monocyte

dependent, LK-induced inhibition of glycosaminoglycan (GAG) synthesis by chondrocytes in explant cultures as gauged by radio-labeled sulfate incorporation (Clin Res 25:615, 1977). In the current study, an experimental model has been developed to assess the effect of LK on chondrocyte GAG synthesis at a histologic level. LK, its presence documented by MIF detection employing rabbit alveolar macrophages, was generated in 72 hour cultures by pulse PHA-P stimulation of rabbit splenocytes substantially depleted of monocytes by surface adherence. Controls comprised media or cells cultured alone and mitogen pulsed cells maintained at 4°C. Supernatants were subsequently dialyzed, lyophilyzed, and reconstituted to original volume with fresh F-12 media supplemented with 15% FCS. Test and control supernatants were incubated for varying time periods with rabbit auricular cartilage explants that had been enzymatically depleted of GAG content by limited tryptic digestion. Analogous media was replaced each 48–72 hours. Explants were subsequently stained with H & E, toluidine blue, and safranin O and processed for electron microscopy. Results indicated that explant exposure to LK significantly diminished chondrocyte GAG regenerative capacity. This inhibitory effect was reversible in that synthetic activity could be restored if LK supernatants were replaced with control media following as long as 12 days of LK exposure. Histologic results thus corroborate earlier biochemical observations of the modulatory capacity of LK on chondrocyte synthetic function and suggest their potential significance in the pathogenesis of cartilage degradation.

Further Observations on Cartilage Phosphatases in Osteoarthritis and Chondrocalcinosis

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The high pyrophosphate (PPi) concentrations observed in osteoarthritis (OA) and chondrocalcinosis (CC) synovial fluid (Arthritis Rheum 16:171, 1973) suggest that PPi may be a precursory ion involved in human articular mineral formation, whether in midzone sites in CC—CaPPi deposition—or in "tidemark" cartilage in OA. Why predominantly hydroxyapatite and often some CaPPi (Bjelle, personal communication) are seen in OA, and CaPPi is deposited in CC, might hypothetically depend in part on moderate and severe PPi hydrolase(s) deficiencies. Such deficiencies are postulated to be associated with the calcifying subcellular apparatus in OA and CC articular cartilage, respectively. This postulated PPi deficiency would be relative to normal calcifying growth cartilage in which PPi in mineral is present in only trace amounts (Wuthier *et al.*, Calc Tiss Res 10:198, 1972).

To examine this hypothesis, articular cartilage from 8 patients with OA (4 male, 4 female, age 49–74), 6 with CC (4 male, 2 female, age 60–79), and 4 normal controls was assessed for various phosphohydrolase activities. Cartilage was sliced in a cryostat, homogenized, and extracted in Triton X-100. The extract was centrifuged and the supernatant, once dialyzed, was passed through a DE-52 column and enzyme fractions were eluted according to previously described techniques.

Although total protein and DNA content were about equal from all cartilage samples, alkaline phosphatase activity was found in the following ratios: normal:CC:OA = 1:6:60. In all samples, 2 peaks of alkaline phosphatase activity were eluted similar to the findings of Arsenis *et al.* in calf growth cartilage (Calc Tiss Res 20:159, 1976). Uniquely, with CC some of the total activity (14%) was found in the void volume of both peaks.

Characterization of alkaline phosphatase using various metals, inhibitors, and substrates was similar for all samples tested. However, analysis of the ratios of PPiase:alkaline phosphatase for growth cartilage (Arsenis, above) versus OA and CC samples ranged from 6:1 to 3:1 respectively. In conclusion, the high PPiase to alkaline phosphatase ratio in growth cartilage compared to this ratio in CC and OA supports the view that reduced PPiase activity might play a role in 1) the probable elaboration of PPi into synovial fluid in OA and CC; 2) the in vitro elaboration from articular cartilage of PPi into Eagle's medium in patients with OA and CC (J Clin Invest 56:1473, 1975); and 3) provision of PPi ion for variable CaPPi crystal deposition.

Detection of Cerebral Involvement in Systemic Lupus Erythematosus by Using 15-Oxygen Brain Scanning

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Despite the prominence of neuropsychiatric features in systemic lupus erythematosus (SLE), no satisfactory method exists for the diagnosis and monitoring of central nervous system (CNS) involvement.

This study describes the application of a new technique for studying both cerebral metabolism and blood flow in SLE patients by using inhaled molecular 15-oxygen and 15-oxygen labeled carbon dioxide.

Tracer amounts of 15-oxygen are inhaled, and after a period of equilibration, the brain is scanned with a gamma-camera. The image produced represents cerebral metabolism. The procedure is repeated using carbon 15-dioxide, and the resultant image represents cerebral blood flow. Twenty-eight scans were performed on 24 SLE patients who had been classified as having clinically definite CNS disease (13), clinically probable CNS disease (7), or no clinical evidence of CNS disease (8). The scans, which were reported blind, were classified as normal (showing a full pattern of both cerebral blood flow and metabolism), as showing a major abnormality, or as showing a minor abnormality. The results are shown in the table.

	Definite CNS Disease (13)	Probable CNS Disease (7)	No Clinical CNS Disease (8)
Major scan abnormality	11	6	1
Minor scan abnormality	1	1	5
Normal scan	1	0	2

Scan abnormalities were seen in 25 of the 28 studies, usually afecting several cortical areas. In 6 patients in whom multiple recordings were made, improved 15-oxygen scan appearances correlated with clinical improvement. 15-oxygen brain scanning appears to offer a highly sensitive non-invasive technique for the identification and study of cerebral involvement in SLE.

Antiimmunoglobulin Effects on Functional Responses of Human Peripheral Blood Neutrophils

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Neutrophils stimulated at sites of inflammation release lysosomal enzymes. Experimentally, a variety of agents are capable of stimulating neutrophils to release their lysosomal enzymes. Neutrophil membranes contain Fc receptors for IgG, and thereby possess surface bound IgG. In these studies we examined the hypothesis that specific antibodies could combine with surface bound IgG, perturb the cell membrane, and provoke inflammatory responses of neutrophils. Antisera were raised to immunoglobulins G, M, A, D, E, and isolated γ chains, Fc and $F(ab')_2$ of IgG. These were rendered monospecific by solid phase immunoabsorbents. Isolated normal human peripheral blood neutrophils were incubated with specific antisera and the release of lysosomal β-glucuronidase, α-mannosidase, and lysozyme were measured. Non-lethal release of lysosomal enzymes was observed with antisera to whole IgG, IgA and to γ and Fc of IgG. The IgG fraction of anti-IgG antiserum also stimulated non-lethal release, thereby suggesting that the response was not complement-mediated. Specific IgG and IgA receptors were detected on neutrophils using immunofluorescent labeled antibodies. No such receptors were detected for IgM and IgD. These results indicate that inflammatory responses of neutrophils can be triggered directly by anti-immunoglobulins, and suggest one mechanism for initiation of the inflammatory response in patients with diseases characterized by the presence of antibodies to IgG and other immunoglobulins.

Interaction of Polymorphonuclear Leukocytes with Immune Complexes Sequestered in Joint Collagenous Tissues

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Incubation of polymorphonuclear leukocytes (PMN) with immune complexes trapped in micropore or collagen membranes results in the phenomenon of "frustrated phagocytosis," that is, enhanced release of lysosomal hydrolases. We have previously shown immune complexes irreversibly trapped in joint collagenous tissues in antigen-induced rabbit experimental arthritis and in rheumatoid arthritis. The present experiments were designed to investigate in vitro interactions between PMN and joint collagenous tissues obtained from rabbit joints with experimental arthritis or control tissues from saline or monosodium urate crystal-injected joints. Experimental and control articular cartilage samples and menisci obtained from such animals were incubated for 1 hour with normal PMN isolated from rabbit peritoneal exudates or blood. After fixation, the tissues were examined by electron microscopy. Fresh cartilage and menisci from arthritic joints showed only few damaged PMN near the articular surface. After incubation with PMN, large numbers of PMN attached to the articular surface were seen. In areas of superficial erosion, the PMN invaded the tissue several cell diameters below the lamina splendens. Degranulated PMN were observed with immunoelectron microscopy in scattered areas to phagocytose amorphous material containing rabbit Ig. Following addition of PMN to control tissues, only a few PMN became attached to the articular surface. In monosodium urate injected joints, after incubation with PMN, these cells were found attached to the surface in moderate numbers, but no invasion into the tissues was seen. These studies indicate that immune complexes trapped in joint collagenous tissues may induce the phenomenon of "frustrated phagocytosis" leading to enhanced release of lysosomal hydrolases. Similar studies using rheumatoid joint tissues are in progress.

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Clinical and Immunologic Study of the Post-Intestinal Bypass Arthritis-Dermatitis Syndrome

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Intestinal bypass surgery has become a successful means of effecting weight reduction in morbid obesity. As a complication of surgery, a fraction of patients develop an arthritis and tenosynovitis which may be associated with circulating cryoproteins. We have had the opportunity to study 6 patients with post-jejunoileostomy arthritis, all of whom had, as a striking feature of the illness, a widespread dermatitis.

The patients were all female. The arthritis developed from 12 to 62 months after surgery and predilected both large and small joints of the upper and lower extremities. Associated with the arthritis were erythematous macules, ranging in size from 2 to 12 mm in diameter. These lesions developed into papules over 2 days, subsequently became pustular-vesicular, and predilected arms, legs, trunk, and face. Lesions were found in different stages of evolution.

Cryoglobulins consisting of IgG, IgM, and C3, C4 were found in 3 of 4 patients. Immune complexes (Raji cell) were found in 3 of 3 patients, including 1 patient with absent cryoglobulins. Synovial fluid analysis in 3 patients revealed WBC ranging from 1200 to 5800 with from 20 to 75% PMN. Immune complexes (Raji cell) were found in 3 of 3 synovial fluids.

Excisional biopsies of the skin lesions were performed in 3 patients. There were no areas of fat necrosis. Small capillaries and venules were infiltrated with PMN in all cases. Immunofluorescent stains revealed deposits of IgG and C3-C4 in vessel walls in 1 of 3 patients. Fluorescein conjugated antisera against 5 bacterial pathogens revealed no staining in 1 patient. Treatment with oral antibiotics was initiated in all patients but was not invariably successful in decreasing the dermatitis or arthritis. In 2 patients, intravenous antibiotics were also unsuccessful, but oral prednisone was promptly followed by decreased dermatitis-arthritis. In responsive patients, the serum cryoprotein titer decreased; the kinetics of this decrease and the reappearence of the cryoprotein after stopping antibiotic-prednisone treatment was studied.

Post-intestinal bypass surgery may cause a systemic immune complex disease primarily involving joints and skin.

Frentizole Therapy of Active Systemic Lupus Erythematosus

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Frentizole, 1-(6-methoxy-2-benzothiazolyl)-3-phenyl urea (Eli Lilly Co.) inhibits humoral and cellular immune responses in animals and prolongs survival of NZB/NZW mice. To date 6 systemic lupus erythematosus (SLE) patients have received Frentizole (2,3,4, or 6 mg/kg for 21–42 days). Each met at least 4 SLE classification criteria, had 2 clinical criteria of disease activity, and had elevated serum DNA binding (30–93% by Farr assay). Three had low serum hemolytic complement levels (62–89 U). Their drug regimen, including prednisone (20–45 mg/day), remained stable for 21 days prior to, during, and for 28 days after Frentizole therapy. Rash (4 of 6), synovitis (4 of 6), mucosal ulcers (3 of 3), and pleurisy (1 of 1) improved during Frentizole therapy. Synovitis worsened in 1 case. The only toxicity was transient SGOT and SGPT elevation in 2 cases. Bone marrow and skin tests were not suppressed by the drug. Frentizole had no effect on hemoglobin concentration, creatinine clearance, or urinary protein excretion, and did not consistently alter lymphocytic mitogenic response. Other laboratory findings (mean ± SEM) are summarized in the table.

These results suggest that Frentizole is an active, relatively non-toxic immunoregulatory drug in SLE patients. Current long-term studies should define its role in the therapy of SLE and other autoimmune diseases.

Test	Pre-Frentizole	End of Frentizole	4 Weeks off Frentizole
DNA Binding (%)	46.0 ± 10.0	30.0 ± 5.0*	28.0 ± 4.0
Complement (units)	105.0 ± 13.0	128.0 ± 11.0	128.0 ± 8.0
IgG (mg/ml)	10.2 ± 1.1	9.2 ± 1.4	9.2 ± 1.5
WBC × 10^3	7.9 ± 1.3	7.7 ± 1.0	7.7 ± 1.4
Lymphocytes × 10^3	2.3 ± 0.3	1.5 ± 0.2	1.7 ± 0.4
T Lymphocytes × 10^2	17.0 ± 3.1	9.7 ± 1.3†	13.1 ± 3.0
B Lymphocytes × 10^2	1.9 ± 0.4	1.1 ± 0.2	1.0 ± 0.3

* = $P < 0.05$

Prospective Study of Childhood Systemic Lupus Erythematosus

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Clinical and laboratory findings of 45 children with systemic lupus erythematosus (SLE) prospectively followed since 1962 have been summarized. Mean disease duration was 5.3 years (range 2 months to 16 years, median 5 years). There were 34 girls and 11 boys, less of a female preponderance than that of most adult series. Youngest age for onset of systemic complaints was 4 years. Ten of 11 boys had disease onset prior to sexual maturation, whereas disease began after menarche in

half of the girls. Intervals between onset of symptoms and diagnosis ranged between 1 month and 10 years (median 3 months). Twenty-eight children were white, 5 black, 6 native American, 4 Asian, and 2 of other races.

Most frequent presenting findings were fever and malaise, musculoskeletal complaints, skin rash, and renal disease. Unusual presentations included cholecystitis, isolated nephrotic syndrome, and chorea. Three patients presented with isolated thrombocytopenia. Serious pulmonary manifestations occurred in 5 patients; 2 died within 2 months of disease onset. Clinical evidence of nephritis occurred in 9 of 11 boys and 25 of 34 girls. Thirty-six patients had 1 or more renal biopsies. Three patients with normal urinalyses had histologic nephritis by biopsy.

Therapy consisted of prednisone (41 of 45 patients) and either azathioprine or chlorambucil (21 of 45 patients). Since 1969 therapy has been geared to normalizing serum hemolytic complement values as well as controlling clinical manifestations of disease. Chronic renal failure developed in 4 patients; only 1 had had adequate therapy early in disease. One renal failure patient received a renal transplant, and 2 are on hemodialysis.

Six girls and 3 boys have died (20%). Seven patients had active lupus at time of death with severe uncontrolled multisystem lupus (3), pulmonary lupus (2), central nervous system lupus (1), and renal failure (1). One patient with active SLE also had a myocardial infarction (age 7) as a contributing cause of death. Two patients died while in clinical remission, 1 of a myocardial infarction (age 20) and 1 of clostridial sepsis with hemolysis. Infection played a major role in 4 deaths (fungal brain abscess, acute staphylococcal endocarditis, disseminated herpes infection, clostridial sepsis). Thirty-three patients continue to have active SLE requiring treatment; 2 are in remission and are not on medications; 1 patient was unavailable for followup in 1978.

Suppressor Macrophages in Systemic Lupus Erythematosus

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In patients with systemic lupus erythematosus (SLE), abnormal unfractionated lymphocyte responses to phytohemagglutinin (PHA) can be corrected by removal of adherent mononuclear (M) cells (Arthritis Rheum 20:127, 1977). The present study demonstrates that this abnormal response occurs when 1) autologous adherent mononuclear cells or 2) cell-free supernatant fluids from autologous and allogeneic adherent cells are added to T-cell cultures, and that 3) suppression does not occur when adherent cells are pre-treated with indomethacin.

Ficoll-Hypaque (FH) separated peripheral blood mononuclear cells from normals and from patients with SLE were further fractionated into T cells by passage over an anti-human $F(ab)_2$ column (97% E-rosettes) and M cells by plating on glass (88% peroxidase-staining). Cell number was constant in all experiments. Supernatants from normal controls and from SLE patients were obtained after 48 hour culture of FH, T, and M cells in RPMI containing AB serum. Normal and SLE T cells were tested for PHA response in the presence and absence of autologous and allogeneic M cells or cell-free supernatants from FH, T, or M-cells.

Autologous but not allogeneic M cells suppress T cell responses. Cell-free supernatants derived from M cells of 14 consecutive experiments with SLE patients caused an average 44% suppression of normal T-cell response to PHA (mean peak CPM relative to control). Suppression was not due to media exhaustion. Supernatants from normal M cells caused an average 35% suppression. Supernatants from both normal and SLE T cells did not suppress either autologous or allogeneic T-cell responses. When M cells of patients with SLE were cultured in the presence of 1 μg indomethacin/ml, the obtained supernatant was not inhibitory.

The data indicate that poor in vitro unfractionated lymphocyte response to PHA in patients with SLE is mediated by a soluble indomethacin-sensitive product derived from the adherent mononuclear cells, possibly prostaglandin. Since supernatants from normal adherent cells were also inhibitory when M-cell concentration was held constant, it is likely that the difference between SLE and normal lymphocyte responses is quantitative rather than qualitative.

Survival with Medical Management after Scleroderma Renal Crisis

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Renal crisis in scleroderma, with malignant hypertension and rapidly progressive renal failure, is perhaps the most ominous of clinical syndromes in the rheumatic diseases. Survival with medical management has not been reported, although a few patients have survived with renal dialysis or transplantation. We report 3 patients surviving scleroderma

kidney with medical management alone for periods of 3, 4, and 10 years.

Patient 1 with malignant hypertension in the fourth year of his classic systemic sclerosis was hospitalized with blood pressure of 220/140, generalized seizures, visual acuity decrease to virtual blindness, and striking grade 4 hypertensive retinopathy. Creatinine rose to a high of 8.0. Extraordinarily vigorous antihypertensive treatment with massive doses of 5 drugs reduced blood pressure to low normal ranges. Renal function 4 years later is stable with a creatinine of 2.2 mg %.

Patient 2 developed her malignant hypertension after 3 years of classic scleroderma, with blood pressure 200/130, grade 3 hypertensive retinopathy, generalized seizures, a creatinine of 2.9, and urine protein of 2 gms per 24 hours. Plasma renin was 4400 ng/dl. Aggressive antihypertensive treatment with 5 drugs reduced blood pressure to the low normal range, and over the following 3 years renal function has improved to a creatinine of 1.5.

Patient 3 developed malignant hypertension after several years of classic scleroderma. Blood pressure was 250/150, creatinie rose to 2.8, hypertensive grade 4 eye changes were noted, and renal biopsy confirmed afferent arteriolar necrosis. Hemorrhage following renal biopsy resulted in transient hypotension, with subsequent control of blood pressure in the low normal range with combination antihypertensive drug therapy. Her renal function remains stable with a creatinine of 1.5 mg% after 10 years.

Skin lesions improved following hypertensive control in 2 of these 3 patients. The common denominator in these 3 successfully managed patients appeared to be aggressive and determined reduction of blood pressure to low normal ranges, with later reliance upon propranolol in 2 of the 3 patients. The percentage of patients who will respond to such management is unknown, but aggressive early treatment of hypertension appears indicated.

Treatment Decisions in Systemic Lupus Erythematosus

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The multi-system involvement and varied clinical presentation of systemic lupus erythematous (SLE) make its treatment difficult and often controversial. To determine present clinical practices, 200 rheumatologists and nephrologists, selected randomly from the Directory of Medical Specialists, were surveyed for their management practices for 8 hypothetical SLE patients. Responding physicians follow over 1900 patients with SLE.

Patients were managed as follows: (1) *Joint and skin involvement only:* Ninety-four percent of respondents chose ASA (12–16 tabs/day) with 9% also using hydroxycholoroquin. (2) *Early clinical nephritis:* Seventy-eight percent would initially treat with prednisone, usually at about 1 mg/kg/day but with 25% choosing 0.5 mg/kg. (3) *Active nephritis with rising creatinine:* Ninety percent would initially employ prednisone, at doses approximating 1 mg/kg, and 21% would also use immunosuppressives. (4) *Serological flare in an asymptomatic patient:* Forty-six percent would treat with prednisone and 51% would withhold treatment. (Three percent demanded a renal biopsy). (5) *Central nervous system SLE:* Uniformly treated with prednisone, at least 1 mg/kg, with 23% also employing immunosuppressants. (6) *Late stage azotemic inactive SLE:* Fifty-nine percent elected to treat with prednisone. (7) *Prednisone tapering practices:* From a starting dose of 60 mg/day, physicians tapered to a mean of 33 mg/day after 3 months and 17 mg/day after 6 months. Thirty-three percent used a divided dose initially, and 57% used alternate day schedules later. (8) *Flare while tapering:* All physicians increased medication, with 87% increasing prednisone dose, 13% adding an immunosuppressant, and many doing both.

Rheumatologists and nephrologists were similar in treatment of most problems. However, nephrologists used immunosuppressive agents twice as frequently in early disease but treated late stage nephritis less vigorously than did rheumatologists. Nephrologists also used fewer divided daily doses of prednisone and used alternate day schedule sooner and more frequently. Treatment was highly case specific. Substantive disagreement occurred when there was serological disease without clinical disease, and in treatment of end-stage nephritis. Central nervous system episodes and early progressive nephritis were uniformly treated aggressively. Immunosuppressive agents were employed only by a minority of respondents.

Monocyte Antibody-Dependent Cell-Mediated Cytotoxicity in Rheumatoid Arthritis

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Antibody-dependent cell-mediated cytotoxicity (ADCC) was studied in adult patients with rheumatoid arthritis (RA) and in healthy controls. Three effector cell populations from the peripheral blood were studied. These included a mixed mononuclear population (8–12% latex positive), a monocyte-depleted fraction (less than 1% latex positive), and a monocyte enriched fraction (65–80% latex positive). The target cells were chicken erythrocytes coated with rabbit anti-chicken erythrocyte antibody (IgG fraction); multiple effector:target ratios were studied. There was no significant difference in

	ADCC (% Cytotoxicity)			
	10:1	1:1	1:5	1:10
Mixed Mononuclear Cells				
Normal (10)	39.2 ± 1.6	12.8 ± 1.1	2.3 ± 0.8	2.4 ± 0.9
RA (10)	37.7 ± 4.4	15.2 ± 3.4	3.2 ± 1.8	2.2 ± 1.0
P-value	NS	NS	NS	NS
Monocyte Depleted Population				
Normal (10)	35.7 ± 4.6	8.1 ± 1.7	−1.0 ± 2.4	−2.7 ± 1.8
RA (10)	25.9 ± 6.0	6.7 ± 1.5	0.1 ± 0.9	−0.2 ± 0.4
P-value	NS	NS	NS	NS
Monocyte Enriched Population				
Normal (10)	45.7 ± 2.0	27.4 ± 1.9	7.4 ± 0.7	2.7 ± 0.4
RA (10)	46.4 ± 3.3	38.5 ± 2.8	15.0 ± 2.5	9.1 ± 1.4
P-value	NS	<0.005	<0.01	<0.01

* Effector: Target Ratio

ADCC activity between patient cells and control cells when either the mixed mononuclear population or monocyte-depleted population were studied as effectors. The monocyte-enriched fraction from patients with RA, however, mediated a significantly increased degree of cytotoxicity (Table). Enhanced cytotoxicity was more evident at low effector:target ratios and was independent of phagocytosis. ADCC may be important in RA since it reflects both humoral and cell-mediated immune mechanisms. The enhanced effector function of the peripheral blood monocyte in this system may be an indication that mononuclear phagocytes are "activated" in patients with RA.

Anti-Native DNA Antibodies and Serum C3 Levels: Candidates for the ARA Preliminary Criteria for the Classification of Systemic Lupus Erythematosus

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The ARA Preliminary Criteria for the Classification of systemic lupus erythematosus (SLE) were developed before the widespread use of tests for anti-DNA antibodies and serum C3 levels. Analysis of our data suggests that these tests should be included in the ARA Criteria and could replace 2 of the present 14 manifestations which are less specific and less sensitive than the serum C3 level and circulating anti-native DNA antibody level (a-DNA).

Three thousand three hundred thirty-four sera from 98 SLE patients were studied. Only patients observed by us and whose sera were tested both during periods of remission and disease activity were included in this study. An average of 34 serum samples per patient was studied. The average period of observation was 38.4 months. Patients had at least 4 of the 14 manifestations of the ARA Criteria. C3 levels and a-DNA were studied from 59 normal individuals, 118 patients with rheumatoid arthritis followed by us, and from patients with other diseases.

Two of the non-SLE patients (1%) had a-DNA level (expressed as % DNA bound) of more than 41% (46%,52%). Thus, a a-DNA of >41% binding had a specificity for SLE of 99%. Eighty-two percent of the SLE patients had a-DNA of >41% binding as their highest value—a sensitivity of 82%. None of the non-SLE patients had a C3 of <71 mg%—a specificity of 100%. Fifty-five percent of the SLE patients had a C3 level of <71 mg% as their lowest C3—a sensitivity of 54%.

These data were analyzed using the method employed to select the 14 manifestations of the ARA Criteria. The average rate of correct classification (the arithmetic mean of the sensitivity and specificity) (ARCC) for a-DNA of >41% binding is 90.5 and for C3 of <71 mg% is 77. The ARCC for the 22 items which comprise the 14 manifestations of the ARA Criteria varied from 52.0 to 94.9. Of these, only the presence of LE cells had a ARCC greater than that for a-DNA. If C3 and a-DNA data had been available at the time the ARA Criteria were developed, they would have been included. The ARCC for the mean of the 2 items combined for the CNS manifestations and the ARCC for the mean of the hematologic items were lower than those for either C3 or a-DNA. Thus, the C3 and the a-DNA could replace these 2 manifestations. The data suggest that the ARA Criteria should be revised.

Lymphocytotoxicity of Cerebrospinal Fluid from Patients with Systemic Lupus Erythematosus

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Lymphocytotoxic antibody is found in the serum of patients with systemic lupus erythematosus (SLE). The antibody is defined by its reaction with lymphocytes, but it also reacts with antigens on human brain tissue. The existence of an antibody in SLE serum recognizing both brain and lymphocyte antigens prompted a search for a similar substance in the cerebrospinal fluid (CSF) of SLE patients, with and without central nervous system (CNS) disease.

CSF was obtained from 17 patients with SLE in the course of evaluations for fever, headache, or manifestations of CNS dysfunction. An independent clinical analysis of the SLE patients and their hospital records revealed that 10 of the 17 had CNS dysfunction attributable to SLE. Criteria for CNS disease were one or more of the following: seizures, transverse myelitis, chorea, ataxia, hemiparesis, psychosis, or hallucinations. Multiple abnormalities were present in 5 of the 10 patients, with 1 patient having 3, and 4 patients having 2 of the above abnormalities. A population of patients without rheumatic disease who were being evaluated for a variety of neurological abnormalities served as controls.

The CSF was tested in a dye-exclusion microcytotoxicity assay using peripheral blood lymphocytes from 3 normal donors as targets. Cytotoxicity was defined as greater than 15% killing of lymphocytes from 1 or more of the normal donors. Nine of the 17 SLE patients had lymphocytotoxic activity in their CSF compared to 1 of 31 controls. When lymphocytotoxicity of CSF was compared with presence or absence of lupus CNS disease, a positive correlation was found ($\chi^2 = 3.82$, $P < 0.05$). The mean % lymphocytotoxicity in the CNFs did not correlate with the serum titer of lymphocytotoxic antibody ($r = 0.12$).

Cytotoxic activity to human lymphocytes has been identified in the CSF of patients with CNS manifestations of SLE. If the cytotoxicity is cross-reactive with neuronal antigens, as is the serum antibody, it may have a pathogenetic role in CNS lupus.

Transfer of Amyloid Resistance by Bone Marrow Allografts in Mice

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The role of bone marrow derived cells in determining genetic variation in susceptibility to experimentally induced amyloidosis in mice was investigated.

Mice of the amyloid susceptible CBA strain were lethally irradiated and repopulated with marrow cells from the highly resistant A strain. Lethally irradiated CBA mice reconstituted with syngeneic marrow served as controls. To determine the effects of irradiation per se on amyloid production, normal CBA mice, as well as A strain mice that were irradiated and reconstituted with A marrow, were also studied. Following 4 weeks of daily subcutaneous casein injections, spleen sections stained with Congo red were scored for the degree of amyloidosis by independent blind observers using the following 4 point scale: grade 1—a rim of amyloid around 1 or more splenic follicles; grade 2—a rim of amyloid in more than 50% of splenic follicles; grade 3—a rim of amyloid in all splenic follicles; grade 4—diffuse splenic amyloid with bridging between almost all follicles and some distortion of the splenic architecture.

Thirty percent of CBA mice given A marrow failed to develop amyloid, with an average score of 1.1 for this entire group. In contrast, control mice given syngeneic marrow all developed significantly more amyloid, with an average score of 3.4. Non-irradiated CBA mice developed an intermediate severity of lesions. Irradiated A mice remained amyloid free.

The progression of amyloidosis in CBA mice was significantly retarded by grafting of marrow from the resistant A strain, even though irradiation seemed to accelerate amyloid production in CBA mice. The resistance of the A strain to amyloidosis was not affected by lethal irradiation. These data demonstrate that bone marrow derived cells are an important factor in the pathogenesis of amyloidosis and that the genetic difference between susceptible and resistant mouse strains lies, at least partially, in their hematopoietic tissues.

Biochemical Heterogeneity in Purine Nucleoside Phosphorylase Deficiency

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The biochemical features of 2 families with purine nucleoside phosphorylase (PNP) deficiency and T-cell immunodeficiency disease were compared. The erythrocyte enzyme from 2 brothers in family 1 had 0.45% normal PNP activity. Plasma urate values in these patients are 2.6 mg/dl and plasma inosine levels are 41.2 and 45.2 μM. Urine samples contain uric acid 454 and 527 mg/gm creatinine, inosine 5.1 and 4.7 millimoles/gm creatinine, and guanosine 1.4 and 1.8 milli-

moles/gm creatinine. When compared to normal, their enzymes showed a) a 10-fold increase in the apparent Km for inosine, b) a diminution of the isoelectric pH from normal values of 5.4 to 5.8 to 5.08 to 5.26, c) an inability of inosine to protect the mutant enzyme against thermal inactivation as compared to the protection of the normal enzyme, d) a loss of the normal near optimum activity at pH 7.4, and 3) a normal value for the stokes radius.

The enzyme from a patient in family 2 has 0.07% of normal activity. This patient has a plasma urate of 1.8 mg/dl and a plasma inosine of 38 μM. Urine samples contain uric acid 73 mg/gm creatinine, inosine 14.8 millimoles/gm creatinine, and guanosine 4.8 millimoles/gm creatinine. The enzyme protein had a) a 3 to 4-fold increase in the apparent Km for inosine, and b) only minor changes in enzyme activity over a pH range compared to normal.

These observations suggest that a) the degree of abnormality in uric acid and nucleoside concentrations in the plasma and urine reflect the severity of the enzymatic deficiency, b) structural alterations of the mutant PNP proteins result from structural gene mutations and genetic heterogeneity in the disease PNP deficiency. Since PNP activity was found in every human tissue assayed, the systemic involvement of PNP deficiency can be accounted for.

Disease Pattern of Patients with PM-1 Antibody

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Recently we reported the finding of an antibody, PM-1, directed toward one of the nuclear acidic protein antigens (NAPA), in sera of patients with polymyositis. This study was undertaken to investigate the relationship between PM-1 antibody, polymyositis syndromes, and other rheumatic diseases. Sera were tested against nuclear extracts by immunodiffusion and counterimmunoelectrophoresis for the presence of antibodies to PM-1 and other NAPA. Clinical data were then obtained and analyzed. Fifty-two patients were identified who had polymyositis syndromes as defined by significant muscle weakness, elevated serum muscle enzymes, myopathic electromyogram, and muscle biopsy typical of polymyositis. Of these, 24 had polymyositis, 18 had polymyositis-scleroderma overlap, and 10 had dermatomyositis.

PM-1 antibody was found in 50% of polymyositis, 73% of polymyositis-scleroderma, and 20% of dermatomyositis. The PM-1 antibody was found rarely (<1%) in 1000 patients with other connective tissue diseases and was not found in other muscular disorders such as myasthenia gravis, muscular dystrophy, and polymyalgia rheumatica. Among patients with polymyositis syndromes, a difference in the prevalence of the clinical characteristics was noted as shown in the table.

Blinded serum exchanges of 58 sera between 3 medical centers revealed the prevalence of the PM-1 antibody to be 25% in childhood dermatomyositis (4 of 16), 17% in adult dermatomyositis (3 of 18), and 38% in polymyositis (3 of 8). Patients with other rheumatic diseases were negative for PM-1 antibody (0 of 16).

We conclude that the PM-1 antibody has a high specificity for polymyositis and that it may identify a subset of polymyositis patients who have other connective tissue disease manifestations.

Clinical Characteristic	PM-1 Positive n = 27	PM-1 Negative n = 25
Raynaud's phenomenon	52%	16%
Arthritis	48%	28%
Sclerodactyly	41%	28%
Pulmonary disease	64%	24%

Acute Induction of Joint Inflammation in Rats by Poly I:C and Interferon

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An association between viral infection and joint inflammation has repeatedly been reported. Our recent studies showed that interferon inducer, double stranded polyinosinate-polycytidylate (Poly I:C), as well as human interferon, stimulated both prostaglandin E (PGE) and hyaluronic acid production by human cultured synovial fibroblasts. The present study was performed in order to evaluate further the role of interferon and Poly I:C in joint inflammation.

Polynucleotides, mouse interferon, or phosphate buffered saline were injected intraarticularly in 10-week-old Wistar derived male rats by methods previously described. Animals were killed 24 hours later and the synovia were removed for histological examination and determination of PGE.

Injection of Poly I:C or mouse interferon induced an inflammatory response. Poly A:U was only slightly active in this respect. By contrast, PBS or single stranded polyinosinate

(Poly (I)) or polycytidylate (Poly (C)) did not induce any significant inflammatory response. Rat knee joints injected with either Poly I:C (50, 100, or 250 μg/joint) or interferon (500 or 1000 μ/joint), appeared macroscopically swollen and edematous and contained an increased amount of viscous fluid. Microscopically inflamed synovium contained variable amounts of inflammatory cells, most of them polymorphonuclear. Synovial PGE levels were increased by Poly I:C, Poly A:U, and interferon but not by Poly (I) or Poly (C). Mouse interferon also induced an inflammatory response when injected in mouse knee joint.

We suggest that interferon may be a mediator in the initiation of inflammation by viruses.

Clinical and Immunological Features in Patients with Post-Intestinal Bypass Arthritis

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Nineteen patients with musculoskeletal complaints after jejuno-ileal bypass for morbid obesity were reviewed for evidence of connective tissue disease and immunologic abnormalities. Presence of arthritis or arthralgias and extraarticular connective tissue symptoms, elevated Westergren sedimentation rate (ESR), and absence of other significant forms of arthritis were criteria for diagnosis of postintestinal bypass arthritis. Eleven of the 19 patients met these criteria. Seven were females. Six of the 11 had joint swelling and 5 had only arthralgias. All had moderately elevated ESR. Extraarticular manifestations include erythema nodosum (3 patients), pleural effusion (2 patients), and carpal tunnel syndrome (1 patient). Synovial fluid analysis in 5 showed mild to moderate inflammation. Serum rheumatoid factor was present in 1 patient in low titer while 3 patients had positive ANA in titers ranging from 1:128 to 1:1024. In 2 patients the ANA reverted to negative with therapy, and in the third patient the ANA became negative after the bypass was reversed. Four had hypercomplementemia, 6 had elevated serum IgG, and 3 had elevated serum IgA. None of the 11 patients demonstrated cryoglobulins, but 1 had soluble immune complexes by complement inhibition technique. Synovial biopsy in 1 case showed chronic inflammation and IgA and C′ deposition by immunofluorescence. HLA typing of 6 of the 11 patients with post bypass arthritis showed no trend.

In 1 patient the length of bypassed bowel was reduced, and in another the bypass was reversed. In both cases the arthritis remitted. Two patients required low dose prednisone therapy for the arthritis; the others were treated effectively with nonsteroidal antiinflammatory agents.

These 11 patients had an inflammatory arthropathy often accompanied by features suggestive of connective tissue disease, such as erythema nodosum, pleural effusions, and circulating ANA. Although none had cryoglobulins, which were previously reported by others, 1 had circulating immune complex. This suggests that immune mechanisms are involved in the pathogenesis of arthritis in some, if not all, of these patients.

Significance of Serum C-Reactive Protein Elevations in Patients with Systematic Lupus Erythematosus

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Honig *et al* (Arthritis and Rheum 20:1065, 1977 recently reported that patients with active systemic lupus erythematosus (SLE) usually do not have markedly increased serum concentrations (>200 μg/ml) of C-reactive protein (CRP) and that such a finding suggests the presence of superimposed infection rather than active lupus. Since a semi-quantitative capillary precipitin technique was employed in that study, in which only very high CRP concentrations were regarded as positive, we investigated the significance of lesser increases in serum CRP concentration in patients with SLE, using a quantitative radial immunodiffusion method sensitive to 1.5 μg/ml. We retrospectively determined CRP concentrations in 141 serum samples collected from a group of 17 patients with SLE over a mean period of 19 months. Thirty-two episodes of significant increase in serum CRP concentration were detected. Careful review of clinical findings associated with each CRP peak revealed that these episodes were associated with active lupus without infection in 20 instances (median CRP concentration 21 μg/ml, mean 43.7, range 7.8–197.2) In 9 instances, elevations of serum CRP concentration were attributed to proven or suspected superimposed infection or bone fracture (median 45 μg/ml, mean 52.6, range 16.3–118.2). Three CRP elevations of 7.3, 8.2 and 39.4 μg/ml occurred at a time when there was clinical evidence of neither lupus activation nor infection.

There were 8 instances of onset or exacerbation of lupus activity at times when serum samples did not show elevated CRP levels. In 2 of these, very active disease was present; CRP elevations were noted in the next samples obtained, 13 and 33 days later. In a third patient with both infection and lupus activity, CRP levels were found elevated 8 days later.

These data indicate that moderate to marked increases in serum CRP concentration may occur in the course of SLE in association with activation of disease, as well as in association with infection or other cause of tissue necrosis. Rarely no obvious clinical cause can be found. Occurrence of serum CRP elevation in patients with SLE does not differentiate between lupus activity and infection.

Medical Versus Surgical Management of Ischemic Necrosis of Bone in Systemic Lupus Erythemotosus

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The significance of ischemic necrosis of bone (INB) in SLE and the cause of the bony lesion are not established. In this study, 36 patients with SLE and INB have been compared to 124 patients with SLE alone; and, in those with INB, the cause of the osseous lesion has been analyzed in relation to medical versus surgical management.

Those clinical features of SLE significantly correlated with INB include Raynaud's phenomenon (61% versus 13%, $P < 0.001$), myositis (25% versus 7%, $P < 0.01$), and vasculitis (50% versus 26%, $P < 0.025$). The course of 73 INB sites in the 36 patients initially grouped into medical (20 patients; 41 sites) and surgical (16 patients; 32 sites) is shown in the table.

The initial orthopedic procedure was uniformly core decompression. All patients were receiving corticosteroids. The failure of medical management alone is shown by the persistence of symptoms (39 or 95%), x-ray progression (35 or 85%), and need for reconstructive surgery (22 or 53%). In stages II and III of the surgical group, only 7 sites (25%) were symptomatic, 5 sites (17%) progressed on x-ray, and only 3 (10%) required further surgery. None in stage I advanced. Differences in followup intervals would not appear to explain these statistically significant differences.

Thus, in the steroid-treated SLE patient, especially with Raynaud's, myositis, and/or vasculitis, the risk of INB is major and the need for early diagnosis essential if progression of the bony lesion is to be retarded by orthopedic intervention.

Stage INB per Treatment Group	No. INB Sites	Duration of Follow (Mos)		Progression INB (No. Sites)		
		Mean	Range	By Symptom	By X-ray	To Prosthesis
Medical:						
II	30	31	3–74	28	25	15
III	11	28	3–71	11	10	7
Surgical:						
I	4	20	6–45	0	0	0
II	8	11	4–25	1	1	1
III	20	12	4–20	6	4	2

Abdominal Syndromes in Systemic Lupus Erythematosus and Polyarteritis: Predisposing Factors

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Acute abdominal syndromes have been increasingly recognized as major life-threatening events in patients with rheumatic disease. Early diagnosis is critical to the institution of appropriate medical/surgical management and may be impeded by the masking effects of antiinflammatory, especially steroidal, therapy. It is our purpose here to report those features, clinical and laboratory, which appear to relate specifically to intraabdominal arteritis, our major cause of an acute abdomen in those with systemic lupus erythematosus (SLE) and polyarteritis (PA).

During the past 7 years, 15 of 140 patients admitted to the Unit with SLE and 4 of 8 patients with PA developed an acute abdomen. In 11 (73%) of those with SLE and all 4 with PA, the abdominal event was secondary to mesenteric arteritis. The remaining 4 patients with SLE had polyserositis (2), pancreatitis (1) and, in the other patient, an undiagnosed recurrent syndrome which responded each time to increased corticosteroids alone. When patients with SLE and an acute abdomen were compared to the 125 patients without abdominal syndromes, the significant discriminating features in the abdominal group were increased peripheral vasculitis (57% versus 25%, $P < 0.025$), thrombocytopenia (57% versus 19%, $P < 0.0005$), and presence of rheumatoid factor by the Latex test system (92% versus 45%, $P < 0.0005$). Of the 4 patients with PA, peripheral neuropathy was present in all, thrombocytopenia developed concomitant with the abdominal syndrome in all, and rheumatoid factor was uniformly present in the 3 patients tested (1:1280, 1:1280, 1:10,240). Eight of those with SLE and the 4 with PA died from the abdominal catastrophe. Six of the 7 surviving SLE patients represent those most recently seen.

Thus, in the patients with SLE and PA, the presence of thrombocytopenia and circulating rheumatoid factor are poor prognostic signs and may pathogenetically predispose the patient to intraabdominal arteritis. Outcome can only be favorably altered by early recognition and prompt institution of appropriate medical and/or surgical management.