Thymus-dependent and -independent Regulation of Ia Antigen Expression In Situ by Cells in the Synovium of Rats with Streptococcal Cell Wall-induced Arthritis

Differences in Site and Intensity of Expression in Euthymic, Athymic, and Cyclosporin A-treated LEW and F344 Rats

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Abstract

Euthymic LEW rats, when injected with streptococcal cell walls, exhibited rapid onset development of acute exudative arthritis coincident with enhanced synovial expression of Ia antigen. By 21 d after injection, the expression of Ia was markedly increased compared with basal conditions and paralleled the severity of the later developing proliferative and erosive disease. Immunodeficient athymic and cyclosporin A-treated LEW rats developed only the early phase arthritis, which was again paralleled by synovial Ia expression. Chronic expression of high levels of Ia antigen was not observed. Histocompatible F344 rats, both athymic and euthymic, developed minimal, if any, clinically significant arthritis and did not exhibit the enhanced Ia expression demonstrated in the LEW rats. Our results indicate that enhanced synovial Ia expression parallels clinical disease severity and varies by rat strain, and that the rapid onset enhanced synovial Ia expression is thymus independent, whereas the markedly enhanced chronic phase Ia expression is thymus dependent.

Introduction

Chronic proliferative and erosive synovitis, resembling rheumatoid arthritis in humans, can be induced in LEW/N female rats by intraperitoneal injection of an aqueous suspension of cell wall fragments from group A streptococci and various other bacteria (1-6). The course of the experimentally induced disease is biphasic. Within 24 h of cell wall injection, acute erythema and swelling develop in peripheral joints coincident with the deposition of cell wall fragments in synovial blood vessels and subchondral bone marrow of involved joints (4, 6-8). This initial phase of disease reaches maximum severity at 3 d and then slowly recedes. Histologically, it is characterized by low grade hyperplasia of the synovial lining cell layer, prominent fibrin deposits, and diffuse infiltration of the synovium and joint space with cells of monocytic and granulocytic lineage (9). This phase appears to be complement dependent because it, but not the proliferative and erosive chronic disease discussed below, is inhibited by treating the rats with cobra venom (10).

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About 14-28 d after cell wall injection, an exacerbation of swelling develops that waxes and wanes in severity but persists for months. This later developing chronic phase is dependent upon the continued presence of cell wall fragments in the inflammatory site (4, 9). It is characterized by more exuberant synovial lining cell hyperplasia, villus formation, infiltration of the sublining spaces with macrophages and T-lymphocytes (predominantly the T helper/inducer subset), proliferation of the fibroblast-like cells in the sublining stroma, and the erosive destruction/invasion of marginal bone and cartilage (9, 11, 12).

The onset of the chronic phase 14-28 d after cell wall injection coincident with the infiltration of large numbers of T-helper/inducer lymphocytes into the synovium suggest that these cells play an important role. Additional support for this view is provided by the observations that the severe chronic proliferative and erosive phase disease does not develop in immunodeficient congenitally athymic NIH-rnu/rnu or cyclosporin A-treated LEW/N rats despite persistence of cell wall antigens in the joints (9, 12). Moreover, reconstitution of athymic rats with T-lymphocytes permits development of the chronic disease (13). Considered together, these data provide cogent evidence that the chronic proliferative and destructive aspects of the disease process, but not the acute exudative processes, are dependent upon the thymus and thymic-derived lymphocytes.

In striking contrast to the euthymic LEW/N female rat, cell wall administration to euthymic F344/N female rats induces no, or at most, mild transient, clinically observable arthritis (2, 4). The mechanisms underlying this difference in disease susceptibility are not understood. After intraperitoneal injection, cell wall fragments disseminate to and persist in synovial blood vessels, subchrondral bone marrows, livers, and spleens of both rat strains but, as stated above, at most only very mild, transient inflammatory joint disease develops in the F344/N rats (4, 7, 8).

Evidence of a response, however, does develop in the F344/N rat. The spleen mildly enlarges and is infiltrated by increased numbers of macrophages. The splenic hypertrophy is more prominent, however, in the LEW/N rat. Functionally, mononuclear cells from the spleens of both rat strains are abnormal after cell wall injection. These cells proliferate poorly in response to mitogens, such as concanavalin A and phytohemagglutinin, but they spontaneously release cytokines such as lymphocyte-derived monocyte chemotactic factor, fibroblast-activating factors, and prostaglandin E_2 (PGE_2). Interestingly, the level of spontaneous in vitro production of these mediators, particularly PGE_2, is consistently higher in cell wall-injected F344/N than in the LEW/N rats (4).

In addition to the changes observed after intraperitoneal injection, subcutaneous injection of cell wall fragments elicits an

acute inflammatory reaction in both rat strains. Histologically, the infiltrate consists of cells of both granulocytic and monocytic lineage. The response, however, subsides more rapidly in the F344/N rats (14). Because the F344 rat strain clearly can respond to the cell walls in some anatomical locations but generally fails to develop significant joint disease, we have proposed that factors peculiar to the joint regulate the development of joint disease (8). As a step in pursuit of this hypothesis, we analyzed the expression of nonpolymorphic class II major histocompatibility complex antigens (Ia antigens) in situ by cells in the synovium of LEW/N and F344/N female rats before and at various time points after administration of cell walls. This issue needs clarification because T helper/inducer lymphocytes interact with antigen presenting cells in context of Ia antigen expression as part of the developmental sequence of a cell-mediated immune response. This issue is also relevant to our understanding the relevance and mechanisms driving the intense expression of Ia antigen by cells in synovium of inflammatory joint diseases in humans, such as rheumatoid arthritis (15–17).

To facilitate analysis of regulatory mechanisms, particularly the role of T-lymphocytes, we also developed and studied congenitally athymic LEW and F344 rats, as well as cyclosporin A–treated LEW/N and F344/N. We hve demonstrated that enhanced Ia antigen expression by cells in synovial tissues from cell wall–injected LEW/N rats develops rapidly and parallels the severity of the joint disease. We also have demonstrated similarly enhanced Ia expression during the acute phase disease in the T-cell immunodeficient athymic LEW.rnu/rnu and cyclosporin A–treated LEW/N rats, but after day 3, both the disease process and Ia antigen expression slowly subsided. Severe proliferative and erosive chronic disease did not develop in these rats. In contrast to the LEW rats, neither euthymic F344/N nor athymic F344.rnu/rnu rats developed clinically significant acute or chronic disease, despite the fact that the cell walls disseminated to the joints. Synovial Ia expression was significantly less than that observed in the LEW rats, although LEW and F344 rats are compatible at the major histocompatibility complex.

Methods

Animals. Female, specific pathogen-free defined flora euthymic inbred LEW/N and F344/N, and congenitally athymic nude LEW.rnu/rnu and F344.rnu/rnu congenic rats were obtained from the Small Animal Section, National Institutes of Health. The rats weighed ~ 100 g at the initiation of each experiment.

The development of the athymic nude congenic rats was as follows and was predicated on the fact that the nude (*rnu*) locus is autosomal recessive. The original stock carrying this gene was obtained from the Medical Research Council Laboratory Animal Center (Carshallton, U. K.) in 1979. Animals carrying this gene locus were established in the Small Animal Section's barrier facility via hysterectomy and foster nursing onto germfree foster mothers. Once established, this gene was then transferred to the N:NIH outbred stock by repeated outcrossing and incrossing. Next, this gene locus was introduced to the inbred F344/N and LEW/N strains by repeated outcrossing and test matings. That is, the first outcross consisted of mating a F344/N or LEW/N female to a N:NIH homozygous nude male. All of the progeny from this mating were heterozygotes (*rnu*/+). The second outcross involved mating a heterozygous female from the above mating to either a F344/N or LEW/N male. Half of the progeny from this mating were heterozygotes and remainder were homozygous normal. The females were mated to a homozygous nude male to identify the heterozygotes. The female progeny that produced at least one homozygous offspring from the test mating were retained and mated to either a F344/N or LEW/N male for the third outcross. The cycles of outcrossing and testmatings were repeated until a sufficient number had been reached to reasonably assure that all the foreign genes introduced by the first outcross were eliminated in the F344/N and LEW/N backgrounds. The animals used in this study were produced by mating heterozygous females to homozygous males.

Congenitally athymic rats carrying the *rnu* locus lack functional T-lymphocytes as indicated by failure to reject skin allografts, the absence of a delayed hypersensitivity reaction to tuberculin, the inability to generate immunoglobulin G in response to thymus-dependent antigens, the lack of OX-19–bearing lymphocytes (a thymus-dependent lymphocyte surface antigen [18]), and unresponsiveness of spleen cells after stimulation with T-lymphocyte mitogens as indicated by failure to proliferate or produce interleukin-2 (reference 9 and 19, Wilder, R. L., unpublished data).

The rats were maintained together under specific pathogen-free conditions. Throughout the course of experimental observation, the animals were housed in cages with filter tops (Lab Products, Inc., Maywood, NJ) that were opened only in a laminar flow biological safety cabinet. These animals were tested serologically (Microbiological Associates, Bethesda, MD) at the completion of the experiments for mycoplasma, and the following viruses: reo type 3, pneumonia virus of mice, encephalomyelitis, Sendai, mouse adenovirus, Toolan H-1, Kilham rat virus, lymphocytic choriomeningitis, and rat coronavirus/sialodacryo-adenitis virus. Serologic evidence of infection was never detected in the experimental animals nor was the presence of pathogens found in sentinel animals also maintained in the animal holding areas. As an additional monitor on the health of the animals, gross examination of the lower respiratory tract was performed.

Induction and evaluation of arthritis. The preparation of cell wall fragments from group A streptococci and the induction and scoring of the polyarthritis were done as previously described in detail (2). Briefly, a sterile suspension of sonicated streptococcal cell walls in phosphate-buffered saline (PBS) (pH 7.4) was injected intraperitoneally into the rats at a dose equivalent to 20 μg of cell wall rhamnose per gram of body weight (20). This dose consistently induces severe acute and chronic arthritis in LEW/N female rats. Extremities distal to the elbow or knee were graded on a scale of 0 to 4, based on the number of joints involved, the degree of erythema and swelling, and the degree of distortion of normal joints contours. The scores for each extremity were summed. Thus, the maximum joint score was 16 for each rat. Control rats were injected with an equal volume of PBS.

Cyclosporin A treatment. Cyclosporin A, a kind gift of Sandoz Pharmaceutical Div., Sandoz, Inc. (E. Hanover, NJ) was dissolved initially in absolute ethanol and then diluted in sterile olive oil in a boiling water bath to final concentration. The final concentration of ethanol was always 5% or less. Stock cyclosporin A was freshly made every 4 d, stored at room temperature, and protected from light. It was administered intramuscularly in the thigh at a dose of 25 mg/kg per d in a volume of 0.1 ml. This dose has been previously shown to consistently suppress the development of clinically evident chronic arthritis without effects on the acute phase (12). Control animals received intramuscular olive oil with the appropriate concentration of ethanol.

Histologic evaluation. Rats were sacrificed 3, 21, and 63 d after cell wall injection. The extremities were removed and fixed in 10% formalin, decalcified in 10% EDTA, and sections stained with hematoxylin and eosin and in certain situations with Wright-Giemsa stain. The slides were coded and analyzed for evidence of an inflammatory reaction or other abnormalities.

Immunoperoxidase staining of the joints for Ia antigen expression. The distal extremities were removed skinned, trimmed, and then snap frozen in a glycerol base embedding medium (Tissue-Tek, O.C.T. compound; Miles Laboratories, Lab-Tek Div., Naperville, IL) by immersion in a mixture of dry ice and acetone. The frozen blocks were stored at −20°C in sealed containers until sectioned for staining. 8-μm sections were cut on a cryostat at −20°C (model Ct; International Equipment Co., Damon Corp., Needham Heights, MA) and placed on glass slides. The slides were dipped into a 60°C solution of 0.5% wt/vol gelatin, 0.025% wt/vol chromium potassium phosphate, and 0.015% wt/vol thymol in distilled water, and allowed to air dry before tissue was applied. The sections were fixed in room temperature acetone for 5 min, washed,

and then preincubated for 10 min with a 1:200 dilution of horse serum. Ia bearing cells were identified by the use of the mouse monoclonal anti-rat nonpolymorphic Ia antigen, (OX-6 [21], Accurate Chemical & Scientific Corp., Westbury, NY) in combination with a sensitive immunoperoxidase-staining technique (ABC Vectastain Kit, Burlingam, CA). Specifically, the sections were incubated, in a humid chamber, for 60 min with saturating amounts of anti–Ia antibody (~ 1 μg), washed for 5 min in buffer (0.05 M Tris/0.15 M NaCl [pH 7.4]), and incubated for 30 min with biotinylated horse anti-mouse IgG (ABC Vectastain Kit) (22, 23). The slides were then again washed. Endogenous peroxidase activity was exhausted by incubating the sections in 1.5% H_2O_2 in methanol for 10 min. The slides were washed in two changes of buffer and incubated for 45 min with reagent (ABC Vectastain Kit), which consists of an avidin and biotinylated horseradish peroxidase complex. The slides were washed and color was developed by immersion in a solution of 0.05% wt/vol 3,3, diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO), 0.04% wt/vol nickel chloride, and 0.003% hydrogen peroxide in buffer. Sections were counterstained with 2% methyl green in methanol, dehydrated in three changes of absolute ethanol, cleared in xylene, and mounted in Permount (Fisher Scientific Co., Pittsburgh, PA) under a glass coverslip. Control sections were stained with no primary antibody or irrelevant monoclonal antibody.

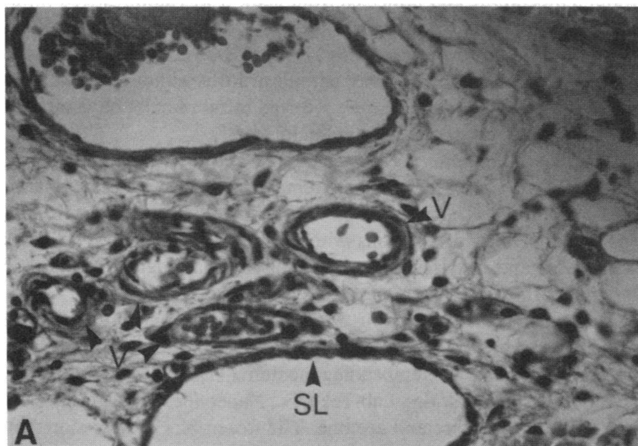
The sections were coded and analyzed for the location, frequency, and intensity of positively staining cells in the joint.

Identification of streptococcal cell wall antigens in synovial tissues by an immunoperoxidase technique. Formalin-fixed joint specimens were embedded in paraffin, sectioned, and stained for group A streptococcal cell wall antigens using the immunoperoxidase technique (ABC VectaStain Kit), as previously described in detail (9). The sections were coded and analyzed for location and intensity of staining. Control stains included sections from saline-injected rats, as well as sections from cell wall–injected rats stained with normal rabbit globulin. The anti–group A streptococci globulin and the normal rabbit globulin were absorbed with rat liver powder (Sigma Chemical Co.) and were used at the same protein concentration (0.01 μg). Specificity was also verified by absorption of the rabbit anti–group A streptococci globulin on group A streptococcal cell walls. This procedure completely eliminated positive staining.

Results

Ia antigen expression in synovium of control rats. Histologically, the synovial membrane from joints of non–cell wall–injected LEW/N, F344/N, LEW.rnu/rnu, and F344.rnu/rnu rats consisted of one or two layers of cells, overlying, deep to this layer, a highly vascular or fibrous connective tissue. The cells in the lining cell layer were typically elongated or spindle shaped (Fig. 1 *A*). Nondecalcified, frozen sections of hind feet from the various rat strains were stained with saturating amounts of monoclonal anti–Ia antibody using the immunoperoxidase technique and multiple fields (> 20) were examined for Ia antigen expression. Fig. 1 *B*, from a LEW/N female, shows a typical section. The synovial lining layer and occasional fibroblast-like cells in the sublining synovial stroma expressed equivocal or very low intensity Ia antigen (grade +/− −1+ on a scale of 0–4+). Vascular endothelial cells, in general, did not stain. No differences, either in histology or extent and intensity of Ia expression, between the various rat strains were noted. Tables I–III summarize these findings.

Ia antigen expression on cells in synovium from cell wall–injected euthymic LEW/N and F344/N rats. As in previous reports, intraperitoneal injection of cell walls into LEW/N female rats induced rapid onset acute, clinically observable, arthritis followed by the development, at ~ 3 wk after injection, of a second or chronic phase arthritis (Fig. 2). Cell wall antigens were readily demonstrated in the cytoplasm of capillaries and venules throughout the synovium (Table IV) (2, 4, 11, 12). As Table I summarizes, synovial tissues from this rat strain obtained during the early acute phase experimental disease (days 1–3) exhibited changes consistent with diffuse microvascular injury. Although the intensity of inflammatory abnormalities varied markedly from one site to another in the affected joints, in general, most fields showed some degree of change in the blood vessels. The lumens of many vessels were obliterated, the endothelium thickened, and a perivascular mononuclear and polymorphonuclear infiltrate present. Other vessels were congested or contained thrombi. The interstitial spaces were edematous and contained prominent fibrin deposits and variable numbers of acute inflammatory cells. The synovial lining cell layer frequently showed evidence of low grade proliferation. The joint cavity was filled with fibrin and predominantly polymorphonuclear leukocytes (Fig. 3, *A* and *B*). In areas of synovium that were heavily infil-



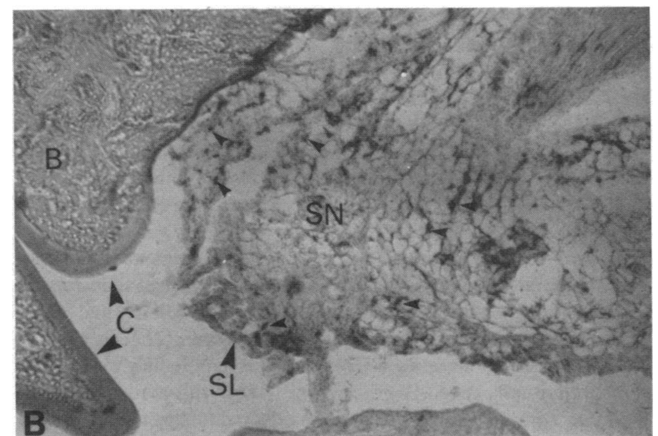


Figure 1. Representative hematoxylin and eosin-stained histologic and Ia immunoperoxidase–stained sections of synovium from control, non–cell wall–injected rats. *A*, a hematoxylin and eosin-stained section of synovium from a tarsal joint from a LEW/N female rat demonstrating the normal thin lining cell layer and the highly vascular sublining stroma. The lining cell layer and a blood vessel are marked *SL* and *V*, respectively (135 × on original photography). *B*, an immunoperoxidase stain for Ia antigen and demonstrates only equivocal or the low grade expression (*arrows*) on scattered cells in the sublining stroma. The labels denote cartilage (*C*), subchondral bone (*B*), synovial lining (*SL*), and subsynovial tissue (*SN*) (25 × on original photograph).

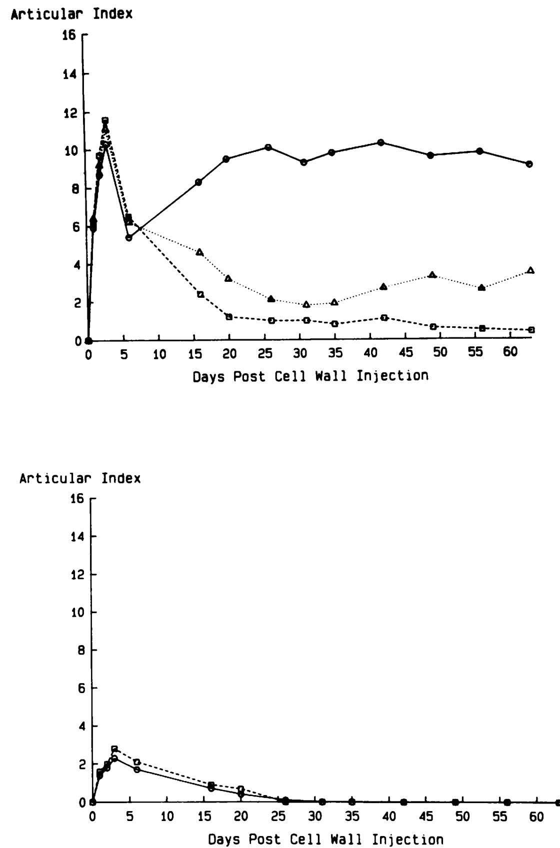


Figure 2. Articular Indices (0–16) of various rat strains at various times after cell wall injection. *A,* euthymic LEW/N (*open circles*), athymic LEW.rnu/rnu (*open squares*), and cyclosporin A-treated LEW/N (*open triangles*). *B,* euthymic F344/N (*open circles*) and athymic F344.rnu/rnu (*open squares*).

trated with cells, the vascular endothelial cells, particularly post capillary venules, faintly expressed Ia antigens (grade 1+ on a scale of 0–4+). Endothelial cells in areas with lesser degrees of cellular infiltration expressed Ia antigen more equivocally. In addition to the endothelial cells, ~ 40–60% of the infiltrating inflammatory cells also expressed Ia antigen (grade 2–3+ on a scale of 0–4+) (Fig. 4, *A* and *B*). The synovial lining cell layer exhibited little or no enhanced Ia expression compared with basal or control level expression. Table III summarizes these results.

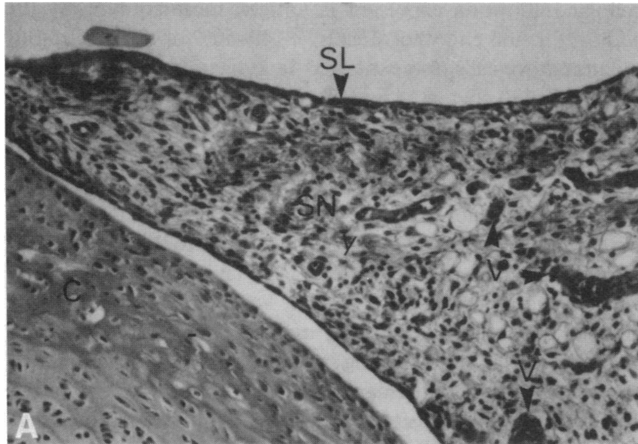
Synovial tissues obtained during the chronic phases of the experimental disease (weeks 3–9), in contrast to the acute phase, exhibited exuberant synovial lining cell layer proliferation with villus formation, diffuse and nodular lymphoid infiltration (particularly around blood vessels), marked proliferation of fibroblast-like cells in the sublining stroma, and destructive invasion of cartilage and periarticular bone (Fig. 5, *A–C,* and Table II). The pattern and intensity of Ia antigen exhibited differed significantly from that observed in the synovium in the basal state or during the acute phase disease (Fig. 6). In areas of major interstitial inflammatory cell infiltration, the lining cells, the endothelial cells, and many of the fibroblast-like cells in the synovial stroma expressed Ia antigen in high intensity (grade 2–4+ on a scale of 4+). Ia expression was also noted on many of the infiltrating cells, particularly in the nodular lymphocytic aggregates (Fig. 7, *A–D*). The intensity of staining, however, varied from cell to cell.

As in previous reports (2, 4, 7, 8), cell wall injection induced, at most, very mild rapid onset arthritis and no chronic, clinically observable, arthritis in F344/N female rats (Fig. 2 *B*), although cell wall antigens were readily detected in the synovial blood vessels (Table IV). Histologically, sections of joints obtained during the acute phase disease showed changes similar in type to those observed in the LEW/N rats but were markedly less severe (Tables I and II). In particular, the vascular abnormalities and intensity of cellular infiltration were significantly less. As Table III summarizes, enhanced synovial Ia expression was limited principally to the infiltrating inflammatory cells. These cells expressed Ia antigen in lower frequency and intensity (10–20% positive with a grade of 1–2+ on a scale of 0–4+). Endothelial cell and synovial lining cell layer Ia antigen expression was not

*Table I. Articular Histopathologic Changes in the Joints 3 d after Injection of Streptococcal Cell Walls**

Abnormality	Nonstreptococcal cell wall–injected control rats	Euthymic LEW/N	Athymic LEW.rnu/rnu	LEW/N treated with cyclosporin A	Euthymic F344/N	Athymic F344.rnu/rnu
Synovial lining cell hyperplasia	0	0–1+	0–1+	0–1+	+/−	+/−
Villus formation	0	+/−	+/−	+/−	0	0
Fibrin deposition						
Joint space	0	2–3+	2–3+	2–3+	0–1+	0–1+
Sublining	0	2–3+	2–3+	2–3+	0–1+	0–1+
Vascular changes						
Thrombi	0	2–3+	2–3+	2–3+	0–1+	0–1+
Congestion	0	2–3+	2–3+	2–3+	0–1+	0–1+
Endothelial cell swelling	0	1–3+	1–3+	1–3+	0–+/−	0–+/−
Inflammatory cell infiltration						
Joint Space	0	3–4+	3–4+	3–4+	0–1+	0–1+
Perivascular	0	3–4+	3–4+	3–4+	0–1+	0–1+
Sublining	0	2–3+	2–3+	2–3+	0–1+	0–1+
Pannus	0	0	0	0	0	0
Erosions	0	0	0	0	0	0
Cartilage destruction	0	0	0	0	0	0

* Graded 0–4+ on coded slides by blinded observers. 0, normal; 4+, maximal reading. See Methods section for further details.



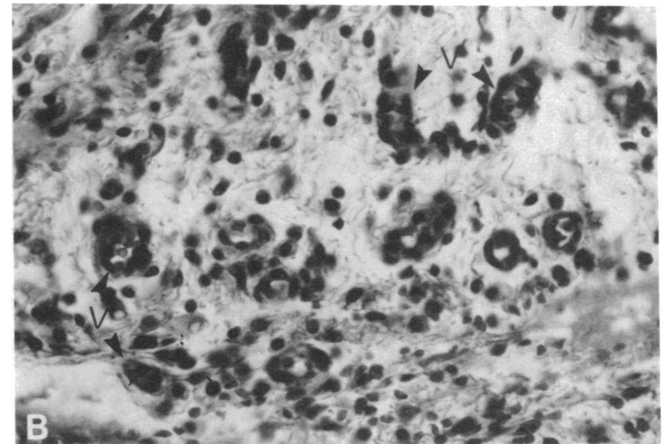


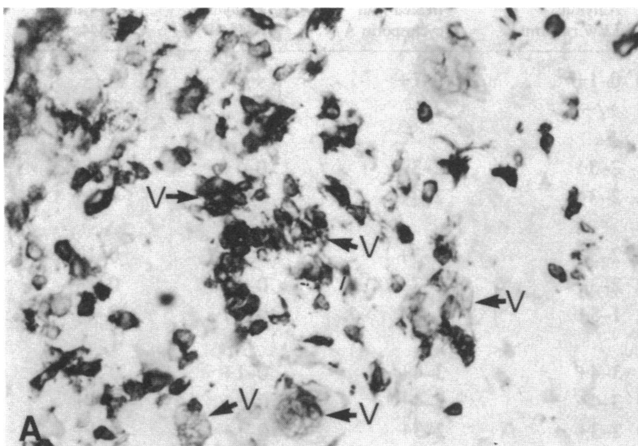
Figure 3. Representative hematoxylin and eosin-stained histologic sections from a hind limb joint of a LEW/N female rat 3 d after cell wall injection. *A*, a low power view of synovium (50 × on original photograph) demonstrating edema, vascular congestion, endothelial cell swelling, and perivascular inflammatory infiltrates. The labels denote cartilage (*C*), synovial lining (*SL*), subsynovial tissue (*SN*) and several blood vessels (*V*). *B*, a higher magnification (135 × on original photograph) demonstrating the marked vascular abnormalities and the mixed inflammatory cell infiltrate. Similar findings were observed in LEW.rnu/rnu and cyclosporin A-treated LEW/N rats.

reproducibly different from that in non–cell wall–injected control rats.

F344/N joint specimens obtained during the chronic phase disease (weeks 3–9) were either entirely normal or exhibited low grade, persistent synovial lining cell layer and capillary proliferation, as well as scattered fibrin deposits. Intense mononuclear cell infiltration, exuberant proliferation of the cells in the synovial lining, and sublining stroma and erosive destruction of cartilage and bone were not observed. In striking contrast to the LEW/N, enhanced Ia expression was limited principally to the sparse residual infiltrating inflammatory cells, although equivocally increased Ia expression (grade +/−) was also apparent on the vascular endothelium. The synovial lining cell layer did not show enhanced Ia expression. Tables I–III summarize our observations.

Ia antigen expression on cells in the synovium from cell wall–injected, congenitally athymic LEW.rnu/rnu and F344.rnu/rnu rats. We have previously demonstrated that cell wall–injected, congenitally athymic NIH-rnu/rnu homozygous female rats develop acute arthritis but do not develop the chronic proliferative and erosive disease (9). In the present study, we observed similar responses in congenitally athymic LEW.rnu/rnu congenic rats (Fig. 2 *A*). These rats developed severe acute arthritis that slowly subsided, but they did not develop the characteristic exacerbation of joint swelling at ∼ 14–28 d after cell wall injection. The acute joint lesions, histologically, were indistinguishable from the acute lesions in the female euthymic LEW/N rat, as described above (Fig. 3, *A* and *B*) and summarized in Table I. Histology of joints obtained 3–9 wk after injection showed low grade inflammatory cell infiltration and prominent fibrin deposits (Table II, Fig. 8, *A* and *B*).

When the joints of these animals were studied for Ia antigen expression, we noted, as in the LEW/N, enhanced expression coincident with the most intense cellular infiltration at day 3 (Table III). 40–60% of the infiltrating cells expressed Ia antigens and in areas where cellular infiltration was intense, weakly en-



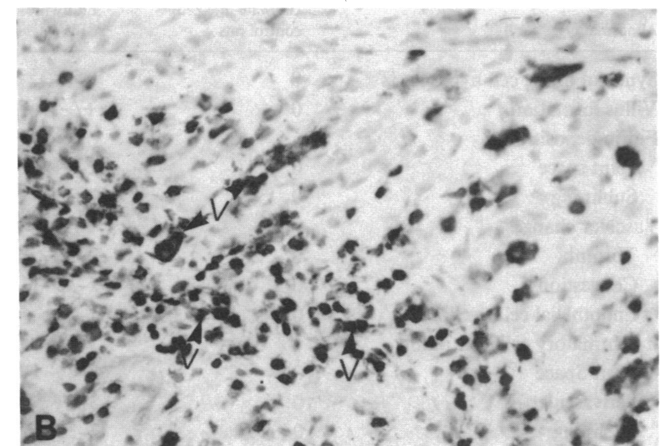


Figure 4. Demonstration of Ia antigen expression in situ in the synovium during the acute phase disease (Day 3). *A*, a section from a LEW/N female and shows Ia expression on perivascular inflammatory cells (presumably macrophages), as well as low grade Ia expression on swollen vascular endothelium, greatest in areas of intense cellular infiltration (135 × on original photograph). *B*, another microscopic field demonstrating variable degrees of Ia expression by the infiltrating inflammatory cells. Several blood vessels are marked (*V*). (135 × on original photograph). Similar findings were noted in LEW.rnu/rnu and cyclosporin A-treated LEW/N rats.

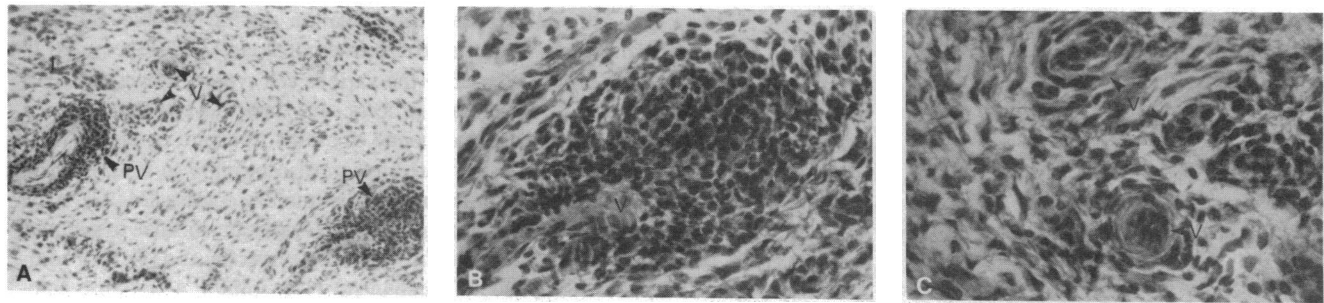


Figure 5. Representative hematoxylin and eosin-stained histologic sections from a hind limb joint of a LEW/N female rat 3–9 wk after cell wall injection. *A*, a low power view of synovium (50 × on original photograph) demonstrating diffuse and nodular perivascular inflammatory cell infiltration (*PV*), swollen endothelial cells and proliferation of sublining stromal cells. *B* and *C*, respectively, a higher magnification (135 × on original photograph) of the perivascular mononuclear cell infiltrates and swollen endothelial cells producing obliterated vascular lumens. Several blood vessels are marked (*V*).

hanced expression of Ia (grade of 1+) was also noted on vascular endothelial cells. The lumens of vessels in these areas were frequently obliterated. The expression of Ia decreased thereafter, in striking contrast to the euthymic LEW/N.

Congenitally athymic F344.rnu/rnu female rats, like the euthymic F344/N and in striking contrast to the LEW.rnu/rnu rats, failed to exhibit significant arthritis (Fig. 2 *B*). Histologically, the synovium was either normal or showed low grade infiltration with granulocytes and macrophages, and fibrin deposits (Table I and II). The intensity of cellular infiltration decreased from day 3 on. Chronically, the synovium was only remarkable for scattered areas of low grade fibrin deposits. Ia antigen expression in these tissues was either similar to synovium from noncell wall–injected rats, or, in areas of synovium with low grade cellular infiltration Ia antigen expression was minimally enhanced, i.e., 10–20% of the infiltrating cells bore low intensity Ia antigens (Table III). Thus, by these criteria, F344.rnu/rnu female rats, like the euthymic F344/N, responded to the cell walls with only very mild transient synovitis that was paralleled by low grade expression of Ia antigen by the infiltrating cells. As is the case for LEW/N and F344/N rats, the differences in disease severity between the LEW.rnu/rnu and F344.rnu/rnu were not explained by failure of the cell walls to localize to the joints (Fig. 9 and Table IV).

Ia antigen expression on cells in the synovium of cell wall–injected rats treated with cyclosporin A. The experiments in the congenitally athymic LEW.rnu/rnu and euthymic LEW/N rats suggested that the rapid onset acute phase arthritis and enhanced Ia expression were thymus independent, whereas the exacerbation and further amplification of Ia expression and the disease beginning 14–28 d after cell wall injection were thymus dependent. For additional confirmation of this point, we also examined the effects of cyclosporin A, a drug that primarily inhibits T helper/inducer lymphocyte activation, on Ia antigen expression by cells in the synovium. We have previously shown that cyclosporin A administration to LEW/N female rats induces defective T lymphocyte function and markedly suppresses development of the chronic proliferative and erosive arthritis. The treatment has little or no effect on the incidence or severity of the acute disease (Fig. 2 *A*) (12).

In parallel with the observations in the LEW.rnu/rnu rats, cyclosporin A–treated LEW/N female rats developed acute phase disease that was not demonstrably different from that observed in the LEW.rnu/rnu or LEW/N rats (Fig. 2 *A* and Tables I and II). Again, enhanced Ia expression was noted on 40–60% of the infiltrating inflammatory cells and weakly on the endothelial cells, particularly in areas with intense cellular infiltration. The intensity of cellular infiltration and enhanced Ia expression slowly

*Table II. Articular Histopathologic Changes in the Joints 3–9 wk after Injection of Streptococcal Cell Walls**

Abnormality	Nonstreptococcal cell wall–injected control rats	Euthymic LEW/N	Athymic LEW.rnu/rnu	LEW/N treated with cyclosporin A	Euthymic F344/N	Athymic F344.rnu/rnu
Synovial lining cell hyperplasia	0	3–4+	1+	1+	+/−	+/−
Villus formation	0	3–4+	+/−–1+	1+	0	0
Fibrin deposition						
Joint space	0	1+	1+	1+	0	0
Sublining	0	1–2+	3–4+	2–3+	0–1+	0–+/−
Vascular changes						
Endothelial cell swelling	0	3–4+	2–3+	2–3+	0–+/−	0
Inflammatory cell infiltration	0	3–4+	1+	1–2+	0–+/−	0
Sublining synovial cell hyperplasia	0	3–4+	+/−	+/−	0	0
Erosions, marginal	0	4+	0	+/−	0	0
Cartilage destruction	0	4+	0	0	0	0

* Graded 0–4+ on coded sections by blinded observers. 0, normal; 4+, maximal reading. See Methods section for further details.

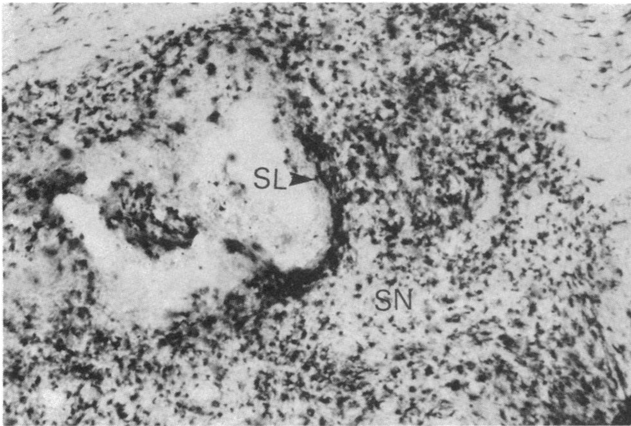


Figure 6. Demonstration of high intensity, diffuse Ia antigen expression in situ in the synovium during the chronic phase disease (weeks 3–9) in the LEW/N rat. A low power (35 × on original photograph) view is shown that demonstrates Ia expression in synovial lining layer and diffusely on cells throughout the sublining synovial tissue. The intensity of Ia expression paralleled the intensity of cellular infiltration. Compare to the Fig. 1 *B*, which shows basal Ia expression. The synovial lining cell layer (*SL*) and sublining tissue (*SN*) are marked.

subsided over the period of observation, but did not resolve completely. The chronic lesions were characterized by low grade inflammatory cell infiltrates and prominent interstitial fibrin deposits (Tables I and II). Low grade Ia expression persisted on endothelial cells (Table III). Of significance, however, proliferation of the fibroblast-like cells in the sublining stroma was mild (compared with the untreated LEW/N), and erosive destruction of bone and cartilage was equivocal. We have previously shown that cyclosporin A treatment does not accelerate the clearance of cell wall antigens from the synovium (12).

Cyclosporin A administration to cell wall–injected F344/N rats resulted in a high rate of toxicity (lethargy, failure to gain weight), and thus this rat strain was not extensively studied. It was clear, however, that cyclosporin A treatment did not permit disease to develop in this strain. Most importantly, the data provide strong evidence that the rapid onset acute disease is regulated independently of the thymus, whereas the chronic disease requires the thymus or thymic-related factors for its fullest expression. The data show that the severity of disease, at all stages, is paralleled by the extent and intensity of Ia expression by cells in the synovium.

Discussion

Bacterial cell wall–induced chronic proliferative and erosive arthritis in rats provides a useful model system for defining pathogenic mechanisms of inflammatory joint disease. In the group A streptococcal cell wall polyarthritis model, the antigen is defined and can be readily demonstrated in tissue, the time of initiation of the response is a known variable, and the sequence of events can be closely monitored. A role for various humoral mediators and cellular elements has been demonstrated (9, 10). Furthermore, the ability to pharmacologically modify the disease and to study the responses of rat strains that are either susceptible or resistant to various stages of the disease has provided deeper insight into mechanisms regulating inflammatory disease in general (2, 4, 11, 12).

Although further investigation (using double-antibody staining, immunoelectron microscopy, etc.) will be required to more rigorously define the various cell types/lineages that participate in the disease process, our data, nevertheless, suggest that the following sequence of events transpire in the LEW/N female rat, a strain noted for its susceptibility to several forms of experimentally induced inflammatory disease (24–27). After intraperitoneal injection, cell wall fragments localize to synovial blood vessels and subchrondral bone marrow and (9), by a complement-dependent mechanism (10), initiate a response associated with interstitial edema, fibrin deposition, and infiltration of these tissues with phagocytic cells of granulocytic and monocytic lineage. Low grade proliferation of the synovial lining cell layer is also observed. A pivotal role in this response, however, appears to be played by the vascular endothelial cells, particularly endothelial cells in postcapillary venules. These cells, apparently stimulated by phagocytized cell walls, appear to swell or enlarge, resulting in vascular congestion and ultimately obliteration of the vessel lumens. These cells coincidentally express cell-surface Ia antigen, albeit, initially in low intensity. Inflammatory cell emigration into the tissues appears to occur in the areas of these vascular changes because the vascular abnormalities are most impressive in areas with intense cellular infiltration. In addition, many of the infiltrating inflammatory cells also express cell surface Ia antigen.

In LEW rats with intact T-lymphocyte function, a secondary phase of disease begins 14–28 d after cell wall injection that is clinically evidenced by a marked exacerbation of joint swelling that, although it waxes and wanes, persists for months. Histologically and radiologically, it differs from the acute phase and ultimately results in bone and cartilage destruction (11, 12). Ia antigen expression by endothelial cells, particularly the postcapillary venules, increases markedly, and diffuse and nodular perivascular lymphocytic aggregates appear in the synovium. The nodular aggregates are composed primarily of W3/13+, OX-19+, and W3/25+ T-helper/inducer lymphocytes (9). Many of the cells in the perivascular cellular aggregates also express Ia antigens. In addition to lymphocytic infiltration, synovial lining cell hyperplasia and villus formation increase significantly, and the expression of Ia by these cells is noted. Moreover, Ia expression by fibroblast-like cells throughout the sublining synovial stroma develops.

This course of events is consistent with the view that the rapid onset, acute phase disease is thymus independent, whereas the chronic proliferative and erosive aspects of the experimental disease process are thymus dependent. The ectopic expression of Ia antigens by endothelial cells and infiltrating phagocytic cells during the acute phase presumably provides a component of the induction signal necessary to recruit T-lymphocytes to the inflammatory site and to induce their activation. A similar regulatory role for Ia expressed on endothelial cells in the central nervous system, has been proposed in experimental allergic encephalomyelitis in guinea pigs, a T-cell–dependent autoimmune process (28, 29).

More cogent evidence that T-lymphocytes regulate the development of the chronic proliferative and erosive phase disease is provided by our observations in the athymic LEW.rnu/rnu and the cyclosporin A–treated LEW/N female rats. These rats developed acute phase disease similar to the euthymic, T-cell–competent LEW/N female rats. Over the first 3 d, they exhibited enhanced endothelial Ia expression and diffuse synovial infiltration with cells of granulocytic and monocytic lineage, many of

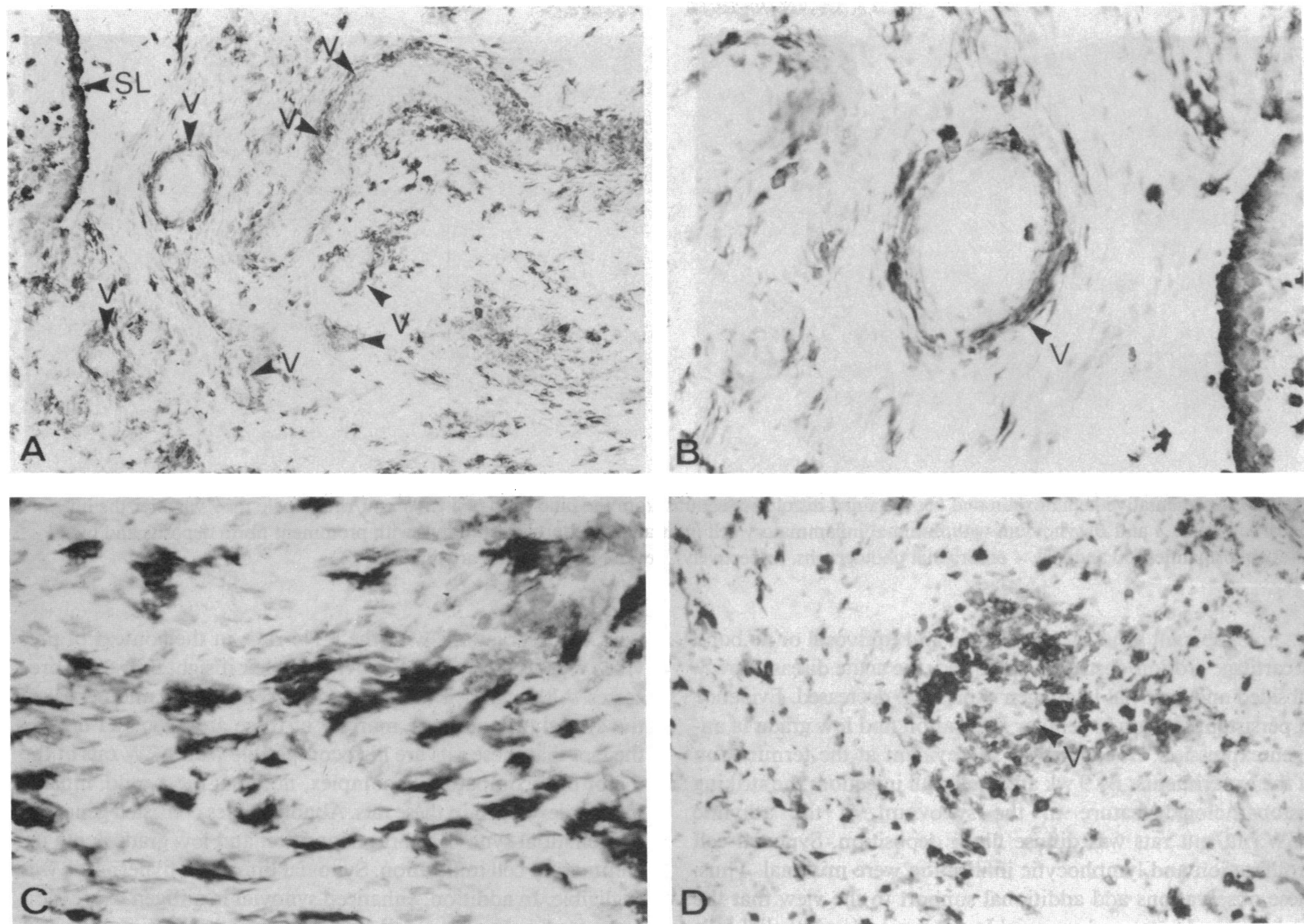


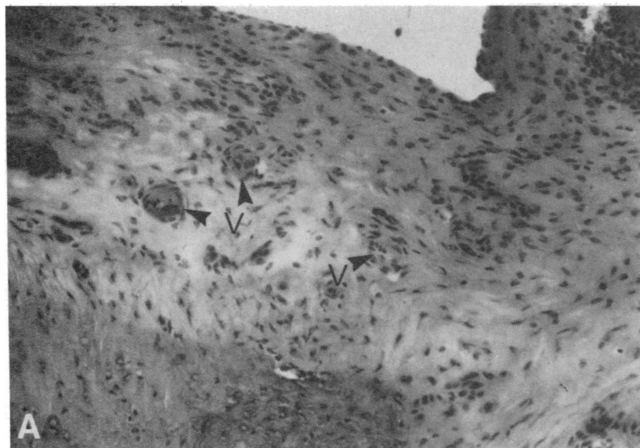
Figure 7. Demonstration of Ia antigen expression in situ on endothelium, sublining fibroblast-like cells and perivascular inflammatory cells in the sublining synovial tissue from a LEW/N rat during the chronic phase disease. *A,* a low power view (35 × on original photograph) of several blood vessels in an area with low intensity cellular infiltration. Note that the endothelial cells express Ia antigen. *B,* a high power view (220 × on original photograph) of blood vessel shown in *A.* Again note that endothelial cells express Ia antigen. *C,* Ia expression by fibroblast-like cells in the synovial stroma (135 × on original photograph) *D,* the variable degree of Ia expression by perivascular inflammatory cells (80 × on original photograph). Blood vessels are marked (*V*) and the synovial lining cell layer is marked (*SL*).

which expressed Ia antigen. The intensity of cellular infiltration and expression of Ia antigens during the acute phase disease was indistinguishable from the T-cell–competent LEW/N. In sharp contrast to the progressive disease in the T-cell–competent LEW/N rat, these rats, which have defective T-cell function, did not develop the severe proliferative and destructive chronic disease,

*Table III. Articular Expression of Ia Antigen 3 d and 3–9 wk after Injection of Streptococcal Cell Walls**

Anatomical structure	Nonstreptococcal cell wall–injected control rats	Euthymic LEW/N	Athymic LEW.rnu/rnu	LEW/N treated with cyclosporin A	Euthymic F344/N	Athymic F344.rnu/rnu
Acute phase (day 3)						
Synovial lining cell layer	0	+/−	+/−	+/−	0	0
Vascular endothelium	0	+/−–1+	+/−–1+	+/−–1+	0	0
Inflammatory cells	0	2–3+	2–3+	2–3+	1–2+	1–2+
Fibroblast-like cells in synovial stroma	+/−–1+	+/−	+/−–1+	+/−–1+	+/−–1+	+/−–1+
Chronic phase (weeks 3–9)						
Synovial lining cell layer	0	3–4+	+/−	+/−–1+	0	0
Vascular endothelium	0	3–4+	+/−–1+	1–2+	+/−	+/−
Inflammatory cells	0	3–4+	0–1+	1+	+/−	+/−
Fibroblast-like cells in synovial stroma	+/−–1+	2–3+	+/−–1+	1+	+/−–1+	+/−–1+

* Graded 0–4+ on coded slides by a blinded observer. 0 = no staining, 4+ = maximal intensity. See Methods for further details.



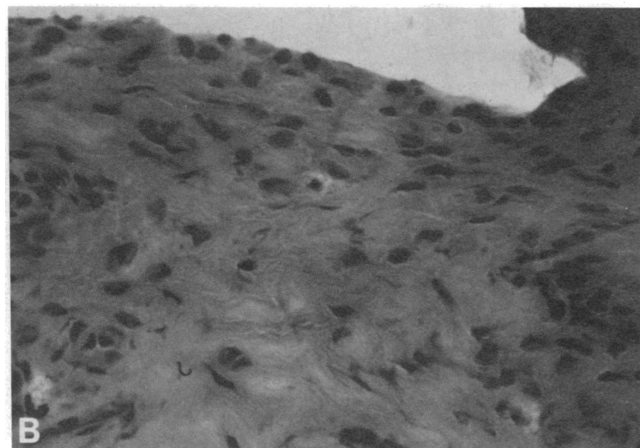
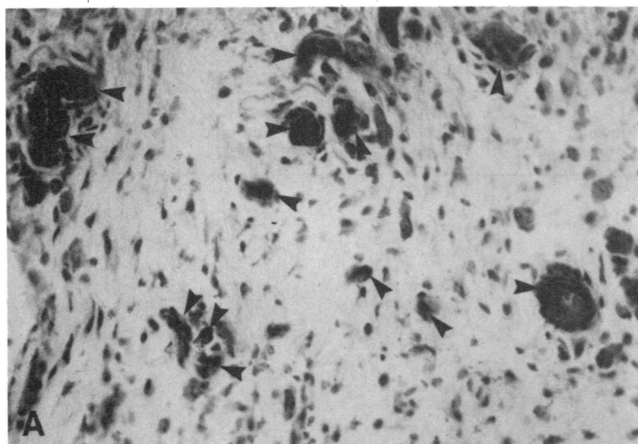


Figure 8. Representative hematoxylin and eosin-stained histologic sections from the hind limb of a LEW.rnu/rnu female rat 9 wk after the injection of cell walls. *A* and *B*, synovium with minimal inflammatory cell infiltration and proliferation but with prominent fibrin deposits and blood vessel abnormalities (50 and 135 × on original photographs, respectively). Several blood vessels are marked (*V*).

i.e., no exuberant synovial hyperplasia and equivocal or no bone or cartilage erosion/destruction. Instead, the acute disease slowly subsided and synovial Ia antigen expression decreased. Evidence of persistent low grade cellular infiltration and low grade Ia antigen expression, however, was still evident at the termination of the experiments. By 9 wk after cell wall injection, the striking histopathologic feature in the synovium of the athymic LEW.rnu/rnu rats was diffuse fibrin deposition. Synovial cell proliferation and lymphocytic infiltration were minimal. Thus, these observations add additional support to the view that the acute phase disease and synovial Ia expression (endothelial cells and infiltrating phagocytic cells) are thymus independent, whereas the development of chronic proliferative and erosive disease and the maintenance and further enhancement of synovial Ia expression requires the thymus and/or thymic-dependent lymphocytes. In the absence of functional T-lymphocytes, the chronic response appears to reflect an unresolved foreign body type of reaction that is driven by persistence of the cell walls in the synovial blood vessels.

Our observations with the F344 rats, in the context of previous reports (2, 4–8, 14), provide further insight into these processes. Although cell wall antigens disseminate to and persist in the synovium and bone marrow of these animals, and despite the fact that these rats are histocompatible with LEW rats at the major histocompatibility complex, no, or at most mild, inflammation developed in these rats. Abnormalities consisted generally of interstitial synovial fibrin deposition and low grade acute inflammatory cell infiltration. Synovial lining cell hyperplasia was negligible. In addition, enhanced synovial Ia antigen expression was noted only on a small fraction of the infiltrating cells and equivocally on the vascular endothelium. The intensity and severity of these changes were unquestionably less than those observed in the LEW/N rats.

Our data also provide evidence that not only is the development of the thymus-dependent chronic disease genetically regulated, but the development of the thymus-independent acute phase disease is also genetically regulated. Whereas severe acute phase disease, along with enhanced Ia expression, developed in



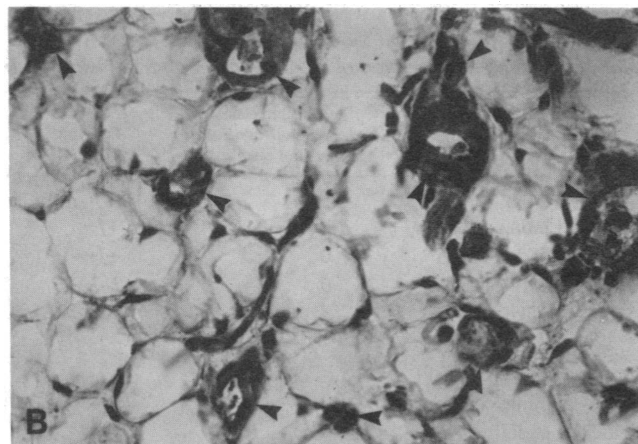


Figure 9. Demonstration of streptococcal cell wall antigens in vascular endothelial cells of LEW.rnu/rnu and F344.rnu/rnu rats 3 d after cell wall injection. *A*, positive immunoperoxidase staining in several vessels and the associated severe inflammatory abnormalities in a LEW.rnu/rnu. *B*, a similar preparation from a F344.rnu/rnu rat with unequivocal positive staining for cell wall antigens in the blood vessels but without an associated inflammatory response (both 80 × on original photograph). Positively stained blood vessels are marked by arrows.

*Table IV. Localization of Streptococcal Cell Wall Antigens to the Synovium**

Phase of disease	Nonstreptococcal cell wall–injected control rats	Euthymic LEW/N	Athymic LEW.rnu/rnu	LEW/N treated with cyclosporin A	Euthymic F344/N	Athymic F344.rnu/rnu
Acute (day 3)	0	2–3+	2–3+	2–3+	2–3+	2–3+
Chronic (wk 3–9)	0	0–1+	0–1+	0–1+	0–1+	0–1+

* Extent and intensity of immunoperoxidase staining in synovium. Graded 0–4+ on coded slides by a blinded observer. 0, no staining; 4+, maximum reading. See Methods section for further details.

the athymic LEW.rnu/rnu and cyclosporin A–treated LEW/N rats, minimal or no acute disease developed in the athymic F344.rnu/rnu and cyclosporin A–treated F344/N rats and Ia antigen expression was not enhanced. Therefore, the evidence suggests at least two major levels of regulatory control exist in the streptococcal cell wall–induced arthritis model. As indicated in the previous discussion, the first level is thymus independent and involves regulating the development of the complement-dependent exudative component of the disease process. Its intensity is paralleled by the extent and intensity of Ia antigen expression on synovial endothelial cells and infiltrating phagocytic cells. The second level is thymus dependent and involves regulating the participation of T-lymphocytes in the development of the proliferative and erosive aspects of the disease and again is paralleled by Ia antigen expression on various synovial cells, particularly the endothelial cells in postcapillary venules, the fibroblast-like cells in the sublining stroma, and infiltrating mononuclear cells. Thus, the thymus-independent phase, as suggested above, probably provides triggering or activating signals for T-lymphocytes. The T-lymphocytes appear to mediate the development of disease in chronic phase, in part, by modulating (enhancing) the expression of Ia on cells throughout the synovium.

The mechanisms by which T-lymphocytes regulate Ia antigen expression on macrophages has been the subject of considerable research. Much less work, however, has been done on T lymphocyte regulation of Ia expression on other cell types. T-lymphocytes produce a cytokine(s) that induces Ia antigen expression on macrophages, endothelial cells, and even human rheumatoid synovial fibroblast-like cells (17, 30–32). Most data suggest that the primary cytokine involved is gamma interferon. Indeed, systemic administration of gamma interferon to mice induces enhanced expression of cell surface Ia antigens in a variety of tissues (32). We, therefore, suspect that T-cell–derived gamma interferon produced locally in the synovium plays an important role in regulating the intense expression of Ia on cells in the synovium during the chronic phases of the streptococcal cell wall–induced arthritis model. Work is in progress in an attempt to demonstrate T-cell production of gamma interferon by synovial T-lymphocytes in this model. It is likely, however, that multiple T-lymphocyte–derived cytokines, other than gamma interferon, will play important roles in driving the chronic proliferative and erosive disease process.

Thymus- and T-lymphocyte–independent regulation of Ia antigen expression in situ by cells in synovium has not, to our knowledge, been previously described or studied. Studies in mice have shown that adjuvant active substances can upregulate Ia expression on peritoneal macrophages in vivo in the absence of a thymus or T-lymphocytes (33). Our data appear to confirm these observations with regard to the synovium and suggest that synovial endothelial cells and infiltrating macrophages can respond similarly. Streptococcal cell walls are adjuvant active and, as shown here in our study, induced Ia expression in both T-cell–immunodeficient athymic LEW.rnu/rnu and cyclosporin A–treated LEW/N rats on infiltrating macrophages and synovial vascular endothelial cells. These observations further add to the growing body of literature that suggesting that endothelial cells play a role in activating T-helper/inducer lymphocytes in vivo and initiating the inflammatory cascade. Recent in vitro data have shown that endothelial cells, like macrophages, can produce interleukin 1 and present antigens. They also, when injured, express procoagulant activity and increased adhesiveness for leukocytes (34–37). All of these processes are probably involved in the streptococcal cell wall arthritis model.

With regard to thymus-independent negative or inhibitory mechanisms regulating Ia expression in the synovium, we have speculated that E class prostaglandins may be important. These substances act directly on macrophages and are known to inhibit or downregulate Ia expression both in vivo and in culture. Although their effects on Ia expression by other cell types is less well characterized, it is conceivable that they also inhibit Ia expression by endothelial and synovial fibroblast-like cells. Consistent with this concept is the observation that these agents also suppress inflammation in various disease models in animals when given systemically in pharmacological doses (38–41).

Our previously reported observation that splenic mononuclear cells from cell wall–injected arthritis resistant F344/N rats spontaneously secrete substantially more PGE_2 during in vitro culture than do cells from cell wall–injected LEW/N rats is also consistent with a possible important inhibitory role for PGE_2 (4). Stated differently, E class prostaglandins, produced locally in the synovium, may play a role in the disease process by negatively or downregulating synovial Ia antigen expression and the associated cellular activation.

A complicating aspect of this hypothesis, however, is our observation that synovial explants from cell wall–injected LEW/N rats produce substantial quantities of PGE_2 (11). Moreover, drugs such as retinoids (11), corticosteroids, and cyclooxygenase inhibitors inhibit production of PGE_2 and decrease the severity of the inflammation. Regulatory mechanisms are, therefore, complex. It seems likely that T-cell–independent mechanisms regulating synovial Ia expression may involve the synergistic action of various mediators, i.e., prostaglandins, leukotrienes, non-T-cell–derived cytokines and other substances. Clearly, much more work is necessary.

In any event, our study further clarifies but also raises a multitude of new questions regarding the pathogenesis of streptococcal cell wall–induced arthritis in the rat. More importantly,

it is clear that the model provides a highly useful tool for defining pathogenetic mechanisms in acute and chronic inflammation. Considering the fact that the model closely mimics many features of human rheumatoid arthritis, our findings may be particularly relevant to our evolving concepts of this disease process.

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