Clin. exp. Immunol. (1990) **82,** 307–312

Adjuvant-induced arthritis in rats. Evidence that autoimmunity to homologous collagens types I, II, IX and XI is not involved in the pathogenesis of arthritis

M. A. CREMER*, A. S. TOWNES† & A. H. KANG* *Veterans Administration Medical Center and Department of Medicine, University of Tennessee, Memphis, TN, and †Veterans Administration Medical Center and Department of Medicine, Vanderbilt University, Nashville, TN, USA

(Accepted for publication 18 May 1990)

SUMMARY

We examined the sera of arthritic outbred Wistar and Sprague–Dawley rats and inbred Fisher 344 and Wistar–Lewis rats for autoantibodies to rat type I, II, IX and XI collagens following the induction of arthritis with mycobacteria (MTB). Although many sera collected over an extended time were assayed in addition to acid eluates of arthritic joints, convincing evidence for autoimmunity to collagen could not be demonstrated. Instead, modest non-specific reactions were observed to collagen, irrelevant proteins, and buffer-treated plastic microtitre wells. In contrast, antibodies to purified protein derivative (PPD) were detected in the sera of rats developing adjuvant-induced arthritis, and antibodies to type II collagen, in the sera and joint eluate of rats developing experimental collagen-induced arthritis. Lastly, delayed-type hypersensitivity responses to collagen could not be detected, nor could adjuvant-induced arthritis be attenuated by soluble collagen injected intravenously before challenge with MTB. We conclude that adjuvant-induced arthritis and experimental collagen-induced arthritis are distinct models of rheumatic disease and that autoimmunity to collagen is neither prevalent in adjuvant-induced arthritis nor necessary for its pathogenesis.

Keywords adjuvant arthritis autoimmunity collagen cartilage

INTRODUCTION

Over 30 years ago Pearson (1956) reported that rats injected intradermally with a suspension of heat-killed MTB and oil developed a chronic erosive polyarthritis. Since his fortuitous observation, adjuvant-induced arthritis (AIA) has become a standard model for the study of experimental arthritis. Despite widespread interest, the precise pathogenesis of AIA remains controversial. Mechanisms proposed include the activation of a latent arthritogenic virus (Chang & Hoffman, 1977; Kapusta, Young-Rodenchuck & Kourounakis, 1979), immunity to disseminated mycobacterial antigen (Pearson, *et al.*, 1963; Quagliata & Phillips-Quagliata, 1973), and autoimmunity to articular antigens (Pearson, 1963; Steffen & Wick, 1971; Berry, Willoughby & Giroud, 1973; Trentham *et al.*, 1980; Thorns & Morris, 1985; Holoshitz, Matitiau & Cohen, 1984; van Eden *et al.*, 1985; van Eden, Holoshitz & Cohen; 1987). Although considerable attention has been focused on the last mechanism, the identity of the tissue factors is still the subject of some debate; however, two families of putative autoimmunogens have been proposed, the collagens (Pearson, 1963; Steffen & Wick, 1971; Trentham *et al.*, 1980; Trentham & Dynesius-Trentham, 1983) and proteoglycans (Van Eden *et al.*, 1985, 1987, 1988). There are currently studies showing that both AIA and streptococcal cell wall-induced arthritis can be abrogated by pretreatment with a recombinant 65-kD mycobacterial heat-shock (stress) protein (DeJoy *et al.*, 1989; van den Broek *et al.*, 1989). The link between this protein and autoimmune arthritis in rats, and possibly in humans, is suggested by the observation that mycobacterial 65-kD heat-shock protein shares a nonapeptide sequence with the core protein molecule of proteoglycan (van Eden *et al.*, 1985, 1987, 1988) and that T cell clones reactive with this epitope transfer adjuvant arthritis to irradiated syngeneic recipients (Holoshitz *et al.*, 1984; Van Eden *et al.*, 1988).

In view of the controversy surrounding the role of autoimmunity to type I and II collagen in the pathogenesis of AIA, the isolation of cartilage-specific type IX (type M) collagen (Shimokomaki, Duance & Bailey, 1980) and the discovery that cartilage-specific type XI collagen (1α, 2α, 3α) is arthritogenic in rats (Morgan *et al.*, 1983; Boissier *et al.*, 1990), and type IX in mice (Boissier *et al.*, 1990) we chose to re-examine AIA to establish whether autoimmunity to rat type I, II, IX and XI

Correspondence: Dr M. Cremer, Research Service (151), Veterans Administration Medical Center, 1030 Jefferson Ave., Memphis, TN 38104, USA.

collagen (RI, RII, RIX and RXI, respectively) is instrumental in the immunopathogenesis of disease. Our studies demonstrate that rats with acute and chronic AIA mount neither humoral nor cellular immune responses to RI, RII, RIX, and RXI. We also show that the clinical course of AIA can not be attenuated when the cartilage-derived collagens are administered in a tolerogenic manner.

MATERIALS AND METHODS

Preparation of collagen

Rat type I and II collagen were solubilized from lathyrtic rat skin and cartilage with 0·5 M acetic acid (Trentham, Townes & Kang, 1977). RII, RXI and RIX were isolated from lathyrtic rat chondrosarcoma tumour by limited pepsin digestion (Burgeson & Hollister, 1979; Shimokomaki *et al.*, 1980; Cremer, 1988). Each collagen was recovered from the supernatant as a precipitate by the step-wise addition of NaCl to 0·86 M, 1·2 M, and 2·0 M, respectively. Type XI and IX collagens were further purified by dissolving the precipitates in 0·5 M acetic acid and dialysing the solution for 48 h in acetic acid containing 0·8 M and 1·2 M NaCl, respectively. After centrifugation at 100 000 ***g*** for 30 min in a Model L5-75 Ultracentrifuge (Beckman, Norcross, GA), RXI and RIX were recovered from the supernatant by dialysis in acetic acid containing 1·2 M or 3·0 M NaCl, respectively. The last three steps were repeated twice to assure the removal of contaminating collagens. Bovine type II, IX and XI collagens (BII, BIX and BXI) were prepared from fetal cartilage using the same protocol. Collagen purity was confirmed by electrophoresis in 7·5% SDS polyacrylamide gel; both bovine and rat type IX collagen contained a mixture of high and low molecular weight fragments.

Animals

Outbred female Wistar and Sprague–Dawley rats and inbred female Wistar–Lewis and Fisher 344 rats weighing 200–250 ***g*** were obtained from Harlan Sprague–Dawley (Indianapolis, IN); DBA/1 and BALB/c female mice weighing 25–30 ***g*** were obtained from Jackson Laboratories (Bar Harbour, ME). All animals were kept in a climate-controlled environment with 12 h light/dark cycles, housed in polystyrene cages containing wood shavings, and fed standard rodent chow and water *ad libitum*. Additional food was scattered on the floor of cages containing arthritic animals and acetaminophen added to the water. Short-term anaesthesia was performed using enflurane. Blood was collected from the external jugular vein of rats at 1–2-week intervals; mice were bled from the tail vein.

Immunization and measurement of arthritis severity

Reference antisera were prepared by immunizing mice with 200 μg of native collagen and 100 μg of MTB (strains C, DT and PN; Ministries of Agriculture, Food and Fisheries, (MAFF) Weybridge, UK) emulsified in 0·1 ml of Freund's incomplete adjuvant (FIA) supplied by Difco Laboratories (Detroit, MI). AIA was induced with 250 μg of agate-ground MTB suspended in 0·05 ml of heavy mineral oil (Squibb & Sons, Princeton, NJ); Experimental collagen-induced arthritis (ECIA) was induced with 200 μg of BII emulsified in FIA (Cremer *et al.*, 1983).

All four paws were scored visually on a scale of 0 to 4. The maximal arthritis index (MAI) was calculated by adding the greatest score recorded for each paw. Rats without arthritis are thus scored 0; those with the mildest arthritis, 1; and those with the worst, 16 (Cremer, 1988). This system best reflects the cumulative severity of arthritis when the onset of arthritis in fore- and hind-paws occurs asynchronously.

Intravenous injection of soluble collagen

Native RI and RII were dissolved overnight in 0·1 M acetic acid (1·2 mg/ml) at 4°C and then dialysed in phosphate-buffered saline (PBS), pH 7·2; BIX and BXI were dissolved in 0·5 M acetic acid and dialysed in 0·2 M NaCl, 0·02 M Tris buffer, pH 7·4. Insoluble collagen was removed from the solutions by high-speed centrifugation at 100 000 ***g*** for 30 min. The collagen content of each solution was determined by measuring hydroxyproline (Bergman & Loxley, 1963). Rats received either 2 mg of RI or RII of 1 mg of BIX or BXI intravenously 7 days before challenge with MTB or BII.

Antibody elution

The hind-limbs of six normal, adjuvant-arthritic and collagen-arthritic rats were excised, stripped of soft tissue, frozen in liquid N2, pulverized, and washed with 0·15 M NaCl, 0·05 M Tris, pH 7·4 containing 0·02% NaN_3. ECIA limbs were washed >60 times before tissue-bound antibody was eluted with glycine–HCl buffer, pH 2·8; limbs from normal and AIA rats were washed six times since antibody was not detected in their sera. The last four buffer washes and the acid eluates of each group were pooled separately and concentrated from 80 ml to 2 ml by positive pressure dialysis after which Tween-20 was added (0·05%). The thoroughness of buffer washing was confirmed by ELISA.

Antibody assays

IgM and IgG anticollagen antibodies were measured by an ELISA as previously described (Cremer *et al.*, 1983). This system is capable of detecting <5 ng/ml of affinity-purified rat anti-RII antibody. Antigens were dissolved at 5 μg/ml in 0·15 M potassium phosphate buffer, pH 7·6, and adsorbed overnight into microtitre plates at 4°C; in some instances, only buffer was added to the wells. PPD was obtained from MAFF; keyhole limpet haemocyanin (KLH) and bovine serum albumin (BSA), were from Sigma Chemical Co. (St Louis, MO). Adjuvant sera were assayed at a 1:10 dilution. Samples twice giving an optical density (OD) greater than the mean value of six normal rats ± 3 s.d. were studied by inhibition assay where soluble collagen was added to a final concentration of 25 μg/ml 2 h before assay.

Delayed-type hypersensitivity (DTH) testing

DTH was performed by injecting 20 μl of antigen dissolved in PBS (500 μg/ml) intradermally into the dorsal surface of the ear with a 30-G needle. The change in thickness was measured 24 h later with an engineer's micrometer. DTH data are expressed as Δ mm; values greater than the mean of six to eight normal rats + 2 s.d. were considered positive.

RESULTS

Specificity of reference sera

Microtitre wells coated with either native RI, RII, RIX, or RXI reacted well with the corresponding reference antisera indicating that the collagens adsorbed to polystyrene and were immunogenic (Table 1). The antibody responses to RI, RIX and

Table 1. Specificity of anticollagen antisera reference produced in mice

Antisera	Strain	Sera dilution	Collagen type RI	RII	RIX	RXI
RI	BALB/c	1/100	1·007	0·094	0·092	0·010
RII	DBA/1	1/4000	0·020	1·088	0·069	0·258
RIX	DBA/1	1/2000	0·018	0·049	0·695	0·021
RXI	DBA/1	1/2000	0·009	0·010	0·009	0·447

Serum pools were prepared from groups of five to six mice immunized 30 day earlier with collagen emulsified in Freund's complete adjuvant. The values represent optical density at 490 nm.

Table 2. Clinical features of adjuvant arthritis in four strains of rats

Rat strain	Incidence of arthritis	Day of onset (mean ± s.e.m.)	Severity (MAI ± s.e.m.)	Blood collection days
Wistar	38/38	11·6 ± 0·2	12·1 ± 3·2	7, 14, 21, 28, 36, 43, 89, 98, 124
Sprague-Dawley	11/12	12·4 ± 1·3	9·5 ± 5·0	8, 15, 32
F344	11/11	15·7 ± 0·8	14·7 ± 2·2	14, 28
Lewis	6/6	10·5 ± 0·7	12·8 ± 1·8	10, 14, 22, 28, 42

Data shown for Wistar rats represent the pooling of data obtained from four separate studies.

MAI, maximum arthritis index (see Materials and Methods, *Immunization and measurement of arthritis severity*).

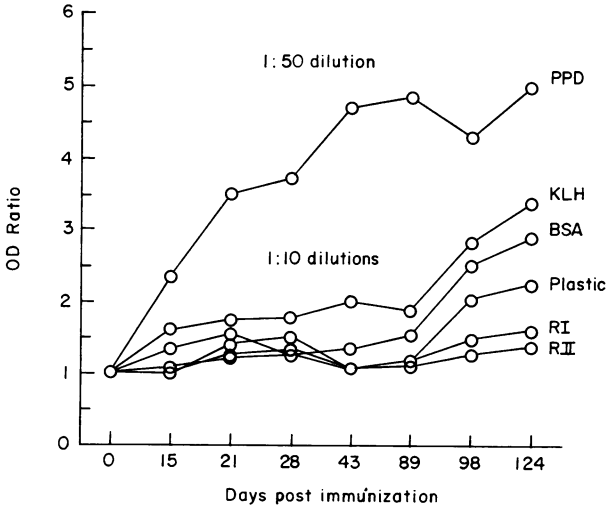

Fig. 1. Reactivity of serum samples with collagen (types I and II—RI and RII), purified protein derivative (PPD) and unrelated proteins. Serially collected serum samples from six arthritic Wistar rats were pooled for each date and assayed by ELISA. Experimental collagen-induced arthritis serum diluted 1/1000 yielded on OD ratio of 600 on assay with RII. OD ratios were calculated by dividing the OD of adjuvant serum by the OD obtained with normal rat serum. KLH, keyhole limpet haemocyanin; BSA, bovine serum albumin.

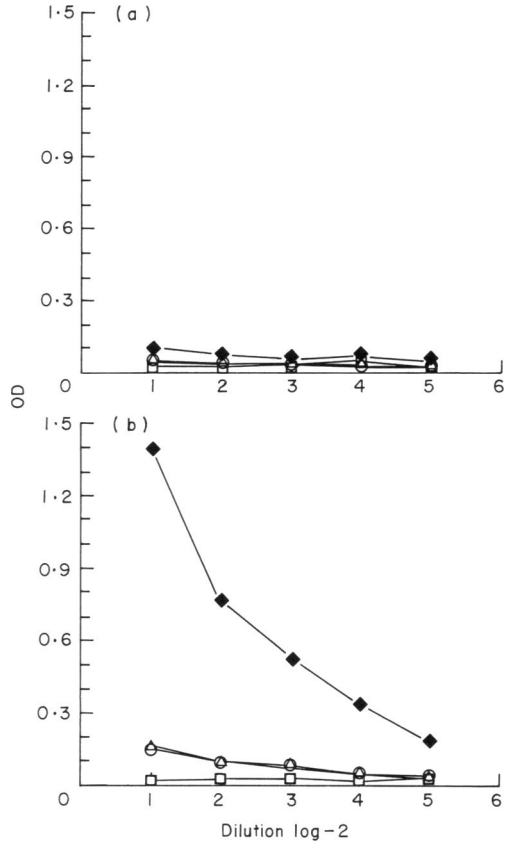

Fig. 2. Joints obtained from arthritic rats immunized 30 d earlier with MTB (a) or BII (b) were treated with acid buffer to elute cartilage bound antibody which was measured by ELISA using native RI (□), RII (◆), RIX (○) and RXI (△) as shown. Results essentially identical to those shown in panel a were obtained when the limbs of normal rats and day 16 adjuvant rats were studied (not shown).

RXI were highly specific; however, anti-RII serum reacted moderately with RXI. This response was seen when individual sera were examined and probably reflects antibodies to the α1 (II) chain of RII cross-reacting with the similar α3 (XI) chain of RXI. Arthritis was noted only in RII-immunized mice.

Studies for anti-collagen antibodies

Table 2 shows the clinical features of rats tested for anti-collagen antibody and the days when sera were collected. The differences shown in the table were not significantly different, except for a 4–5-day delay in the onset of arthritis in F344 rats ($P > 0{\cdot}001$). IgG anti-collagen antibodies were sought in the sera listed in Table 2; IgM antibodies were sought in 12 arthritic Wistar rats bled on day 14. Antibodies to RI, RII, RIX and RXI could not be detected in any of the sera. Occasionally, an OD value 1·5–3 times normal values was noted; however, on closer examination, these responses were frequently confined to a single sample and could not be inhibited with soluble collagen. Figure 1 shows the time-course responses of sera pools prepared from six arthritic Wistar rats. Non-specific IgG reactions to collagen, control antigens and plastic were seen to increase beginning at day 43. Antibodies to PPD rose in a predictable manner, as did anti-RII antibodies in BII-immunized rats.

Table 3. Delayed-type hypersensitivity reactivity of adjuvant- and collagen-injected rats

Immunogen	*n*	Skin test antigens RI	RII	RIX	RXI
None	6–8	0·04±0·02	0·03±0·02	0·06±0·01	0·09±0·01
MTB	8	0·03±0·01	0·04±0·02	0·07±0·01	0·08±0·01
BII	4	ND	0·53±0·02	ND	ND
BIX	6	ND	ND	0·57±0·04	ND
BXI	6	ND	ND	ND	0·82±0·04

The values shown represent the mean Δ mm of ear thickness ± s.e.m. of outbred Sprague–Dawley rats recorded 24 h after intradermal injection of 10 μg of collagen. ND, not done.

Table 4. Intravenously injected collagen does not attenuate the course of adjuvant-induced arthritis

Group	Tolerogen	Antigen	Arthritis incidence	Day of onset	Arthritis severity (MAI)
A	PBS	MTB/oil	10/10	12·0±0·5	11·3±1·1
B	RI	MTB/oil	10/10	12·7±0·7	9·9±0·6
C	RII	MTB/oil	10/10	12·5±0·6	11·7±0·7
D	BIX	MTB/oil	8/8	13·0±0·3	12·3±1·4
E	BXI	MTB/oil	8/8	12·0±0·3	11·6±1·6
F	PBS	BII/FIA	16/20	12·4±0·4	7·6±0·9
G	RII	BII/FIA	2/20	16·5±1·5	4·0±1·0

Intravenous injections of soluble collagen were given 7 days before immunization with MTB or BII; control animals received PBS in place of collagen. The values shown do not differ significantly from one another.

MAI, maximum arthritis index (see Materials and Methods, *Immunization and measurement of arthritis severity*).

Joint elution studies

Being unable to identify circulating autoantibodies, the arthritic joints of MTB- and BII-immunized rats were treated with glycine–HCl to release cartilage bound antibodies. Figure 2 shows that anticollagen antibodies were absent in adjuvant joint eluates but were easily detected in ECIA joint eluates. Eluates of normal joints and adjuvant joints obtained at peak severity (day 16) were also negative for anti-collagen antibodies (data not shown).

DTH studies

Intradermal skin tests were applied to rats with stable AIA lacking ear nodules and the change in ear swelling measured 24 h later. No evidence of DTH to RII, RIX and RXI was detected in adjuvant arthritic rats; however, positive results were noted in animals immunized with collagen (Table 3). Normal non-immunized rats served as controls and showed minimal reaction to the collagens, similar to that seen in rats with AIA.

Studies to attenuate AIA

Earlier, we found that ECIA and immunity to RII could be specifically suppressed by injecting rats intravenously with soluble BII before challenge with BII/FIA, whereas the same procedure had no demonstrable effect on AIA (Cremer *et al.*, 1983). Presently, rats were pretreated intravenously with soluble RI, RII, BIX and BXI before challenge with MTB. Table 4 shows that such treatment did not influence the incidence, onset, or severity of AIA. Equivalent results were noted when group scores were compared at days 10, 14, 21, and 28 (data not shown). In contrast, treatment with RII suppressed both ECIA and immunity (OD values 0·610±0·074 and 0·114±0·028, groups F and G, respectively; $P<0·01$).

DISCUSSION

Pearson (1963) first suggested that autoimmunity to collagen might be involved in the pathogenesis of AIA. The first evidence supporting this hypothesis was provided by Steffen & Wick (1973) who reported weak DTH responses to RI in rats with active AIA. Subsequently, supporting reports (Trentham *et al.*, 1980; Welles & Battisto 1981; Battisto *et al.*, 1982; Trentham *et al.*, 1983; Phadke, Foust & Parrish, 1984) as well as contradictory ones (Cremer *et al.*, 1980; Iizuka & Chang, 1982; Cremer *et al.*, 1983; Trentham & Dynesius-Trentham, 1983; Kiabara *et al.*, 1984; Carlson *et al.*, 1985; Panosian *et al.*, 1986) have appeared, describing the role of collagen autoimmunity in AIA. Data presented here confirm our earlier finding that antibodies to RII are not produced in the course of AIA (Cremer *et al.*, 1983) and extend it by showing that antibodies and DTH to RI, RIX and RXI are also absent.

Of the six studies describing collagen autoimmunity in AIA, only the study of Trentham *et al.* (1980) appears to provide direct evidence. In that report, positive haemagglutination responses to RI and RII were described but only early in course of AIA. We were unable to confirm this result by ELISA, which we and others (Beard, Lea & Ryvar, 1979; Fujii *et al.*, 1989) believe to be a more sensitive and specific assay system. Other investigators (Steffen & Wick, 1971; Trentham & Dynesius-Trentham, 1983; Kaibara *et al.*, 1984; Carlson *et al.*, 1985; Panosian *et al.*, 1986), using a variety of assays, have not been able to demonstrate circulating anti-collagen antibodies in AIA. Phadke *et al.*, (1984), however, did describe 'minute' amounts of antibody to RI and RII. Unfortunately, the specificity of those responses was not tested and may be similar to the non-specific ones we observed.

Indirect evidence for collagen autoimmunity in AIA has been reported by several groups of investigators. These reports describe the attenuation of AIA by techniques previously shown to suppress ECIA and immunity to type II collagen, e.g. the i.v. injection of: (i) antiserum to type II collagen (Welles & Battisto, 1981); (ii) collagen-coated spleen cells (Trentham & Dynesius-Trentham, 1983); and (iii) soluble type II collagen (Phadke *et al.*, 1984). The degree of suppression induced by these techniques was modest and the regimens used to promote suppression were rather vigorous. Furthermore, the specificity of these techniques was not established since all suppressed both AIA and ECIA and none were tested against an unrelated model of immune-mediated disease. Englert *et al.* (1985) attempted to suppress AIA by i.v. pretreatment with affinity-purified anti-BII antibody but were unsuccessful. Our results differ from those of Phadke *et al.* (1984) who described the attenuation of AIA by injecting rats intravenously with soluble collagen. However, their study differs from ours in two important respects. First, the

arthritis we induced was severe, occurred in essentially 100% of rats, and commonly affected three or four limbs. In contrast, the arthritis described in the other report occurred in about one-half of the rats and affected only one hind-paw. Although it can be argued that severe arthritis may be less susceptible to suppression than mild arthritis, it can be argued with equal validity that mild arthritis may be more susceptible to non-specific suppression. Secondly, our protocol called for the removal of insoluble collagen before i.v. injection; this step was not described in the other report. The effect of insoluble collagen on the reticuloendothelial and immune systems is unknown and may be adverse. Lastly, data reported by other investigators militate against collagen autoimmunity contributing to AIA. Iizuka & Chang (1982) demonstrated that pretreatment with a subarthritogenic dose of MTB abrogated AIA but not ECIA. Kaibara *et al.* (1984) showed that rats immunized with BII and treated with cyclosporin A fail to develop immunity to BII and ECIA but remain fully susceptible to AIA, and vice versa. Finally, Arita *et al.* (1987) could suppress ECIA but not AIA with an anti-idiotypic antiserum against anti-type II collagen antibody.

It is not known why non-specific antibody reactions to collagen and other molecules occur in AIA. Clearly, the use of a low serum dilution to seek anti-collagen antibodies was an important factor because OD values in buffer-treated wells decreased as serum dilutions increased. Moreover, high background valuers were not problematic when adjuvant sera were assayed at a high dilution. Other factors that may contribute to non-specific reactions include IgG aggregation, hypergammaglobulinaemia, and the presence of low-affinity polyreactive antibodies. Fujii *et al.* (1989) encountered similar non-specific reactions in the sera of certain normal individuals and some patients with rheumatoid arthritis. To a large extent, these reactions diminished when the sera were diluted in buffer containing heterologous serum obtained from the same species used to produce the second antibody to which enzyme was conjugated.

After examining a number of adjuvant arthritic rats, we were unable to obtain convincing evidence that humoral and delayed-type immunity to rat type I, II, IX and XI collagen occur as demonstrable features of AIA. Moreover, AIA could not be attenuated by the i.v. administration of soluble type II, IX and XI collagen in a manner shown to suppress ECIA. Collectively, these data demonstrate that AIA and ECIA are dissimilar models of arthritis and that collagen autoimmunity plays a minor role, if any, pathogenesis of AIA. In light of these findings and the recent advances in autoimmunity to heat-shock proteins and cartilage proteoglycan in AIA, other avenues of investigation appear more promising in explaining the pathogenesis of AIA.

ACKNOWLEDGMENTS

We would like to acknowledge Ms Janice L. Hyatt and Ms Mary Akin-Deko for their excellent technical assistance. This work was supported in part by the Medical Research Service of the Veterans Administration, the Arthritis Foundation, and National Institutes of Health grant no. 1P50AR39166.

REFERENCES

ARITA, C., KAIBARA, N., JINGUSHI, S., HOTOKEBUCHI, T. & ARAI, K. (1987) Suppression of collagen arthritis in rats by heterologous anti-idiotypic antibodies against anticollagen antibodies. *Clin. Immunol. Immunopathol.* **43,** 374.

BATTISTO, J.R., SMITH, R.N., BECKMAN, K., STERNLICHT, M. & WELLES, W.L. (1982) Susceptibility to adjuvant arthritis in DA and F344 rats. A dominant trait controlled by an autosomal gene locus linked to the major histocompability complex. *Arthritis Rheum.* **25,** 1194.

BEARD, H.K., LEA, D.J. & RYVAR, R. (1979) Anomalous reactions in the hemagglutination assay for anti-collagen antibodies: studies on patients with rheumatoid arthritis and chronic low back pain. *J. immunol. Methods*, **31,** 119.

BERGMAN, I. & LOXLEY, R. (1963) Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. *Anal. Chem.* **35,** 1961.

BERRY, H., WILLOUGHBY, D.A. & GIROUD, J.P. (1973) Evidence for an endogenous antigen in the adjuvant arthritic rat. *J. Pathol.* **111,** 229.

BOSSIER, M.-C., CHIOCCHIA, G., ROZIERE, M.-C, HERBAGE, D. & FOURNIER, C. (1990) Arthritogenicity of minor collagens (type IX and XI) in mice. *Arthritis Rheum.* **33,** 1.

BURGESON, R.E. & HOLLISTER, D.W. (1979) Collagen heterogeneity in human cartilage: identification of several new collagen chains. *Biochem. Biophys. Res. Commun.* **87,** 1124.

CARLSON, R.P., DATKO, L.J., O'NEILL-DAVIS, L., BLAZEK, E.M., DELUSTRO, F., BEIDMAN, R. & LEWIS, A.J. (1985) Comparison of inflammatory changes in established type II collagen- and adjuvant-induced arthritis using outbred Wistar rats. *Int. J. Immunopharmacol.* **7,** 811.

CHANG, Y.-H. & HOFFMAN W. (1977) Adjuvant polyarthritis III. Evidence in support for a viral etiology. *Arthritis Rheum.* **20,** 1507.

CREMER, M.A. (1988) Type II Collagen-Induced Arthritis in Rats. In *Handbook of Animal Models for the Rheumatic Diseases* vol. I (ed. by R.A. Greenwald & H.S. Diamond) p. 17. CRC Press, Boca Raton, FL.

CREMER, M.A., HERNANDEZ, A.D. & TOWNES, A.S. & KANG, A.H. (1983) Collagen-induced arthritis in rats: antigen-specific suppression of arthritis and immunity by intravenously injected native type II collagen. *J. Immunol.* **131,** 2995.

CREMER, M.A., STUART, J.M., TOWNES, A.S. & KANG, A.H. (1980) Collagen-induced arthritis in rats: evaluation of native type II collagen for adjuvant activity. *J. Immunol.* **124,** 2912.

DEJOY, S.Q., FERGUSON, K.M., SAPP, T.M., ZABRISKIE, ORONSKY, A.L. & KERWAR, S.S. (1989) Streptococcal cell wall arthritis. Passive transfer of disease with a T cell line and crossreactivity of streptococcal cell wall antigen with *Mycobacterium tuberculosis. J. exp. Med.* **170,** 369.

ENGLERT, M., MCREYNOLDS, R.A., LANDES, M.J., ORONSKY, A.L. & KERWAR, S.S. (1985) Pretreatment of rats with anticollagen IgG renders them resistant to acute collagen arthritis. *Cell. Immunol.* **90,** 258.

FUJII, K., TSUJI, M., MUROTA, K., TERATO, K., SHIMOZURA, Y. & NAGAI, Y. (1989) An improved enzyme-linked immunosorbent assay of anti-collagen antibodies in human serum. *J. immunol. Methods*, **124,** 63.

HOLOSHITZ, J., MATITIAU, A. & COHEN, I.R. (1984) Arthritis induced in rats with cloned T-lymphocytes reactive with mycobacteria but not collagen type II. *J. clin. Invest.* **73,** 211.

IIZUKA, Y. & CHANG, Y.-H. (1982) Adjuvant polyarthritis VII. The role of type II collagen in the pathogenesis. *Arthritis Rheum.* **25,** 1325.

KALIBARA, N., HOTOKEBUCHI, T., TAKAGISHI, K., KATSUKI, I., MORINAGA, M., ARITA, C. & JINGUSHI, S. (1984) Pathogenic difference between collagen arthritis and adjuvant arthritis. *J. exp. Med.* **159,** 1388.

KAPUSTA, M.A., YOUNG-RODENCHUK, M. & KOUROUNAKIS, L. (1979) Restoration of diminished splenic response to concanavalin A in adjuvant-induced disease by Virazole: possible pathogenic role of a virus and suppressor cells. *J. Rheumatol.* **6,** 507.

MORGAN, K., EVANS, H.B., FIRTH, S.A., SMITH, M.N., AYAD, S., WEISS,

J.B. & HOLT, P.J.L. (1983) 1α,2α,3α collagen is arthritogenic. *Ann. rheum. Dis.* **42,** 680.

PANOSIAN, N., HEYNER, S., COPETOLA, R.J. & ORZECHOWSKI, R.F. (1986) Humoral and cellular immunologic aspects of adjuvant and collagen arthritis in rats. *J. Immunopharmacol.* **8,** 347.

PEARSON, C.M. (1956) Development of arthritis, periarthritis and periostitis in rat given adjuvants. *Proc. Soc. exp. Biol. Med.* **91,** 95.

PEARSON, C.M. (1963) Experimental joint disease. Observations on adjuvant-induced arthritis. *J. chron. Dis.* **16,** 863.

PEARSON, C.M. & WOOD, F.D. (1964) Passive transfer of adjuvant arthritis by lymph node or spleen cells. *J. exp. Med.* **120,** 547.

PHADKE, K., FOUST, R. & PARRISH, J.E. (1984) Collagen-induced and adjuvant-induced arthritis in rats: post-immunization with collagen to suppress or abrogate the arthritic response. *Arthritis Rheum.* **27,** 797.

QUAGLIATA, F. & PHILLIPS-QUAGLIATA J.M. (1972) Competence of thoracic duct cells in the transfer of adjuvant arthritis and delayed hypersensitivity. Evidence that mycobacterial components are required for the successful of the disease. *Cell Immunol.* **3,** 78.

SHIMOKOMAKI, M., DUANCE, V.C. & BAILEY, A.J. (1980) Identification of a new disulfide bonded collagen from cartilage. *FEBS Lett.* **121,** 51.

STEFFEN, C. & WICK, G. (1971) Delayed hypersensitivity reactions to collagen in rats with adjuvant arthritis. *Z. Immunitaets Forsch. Immunobiol.* **141,** 169.

STUART, J.M., CREMER, M.A., TOWNES, A.S. & KANG, A.H. (1982) Type II collagen-induced arthritis in rats: passive transfer with serum and evidence that IgG anticollagen antibodies can cause arthritis. *J. exp. Med.* **155,** 1.

THORNS, C.J. & MORRIS, J.A. (1985) Common epitopes between mycobacterial and certain host tissue antigens. *Clin. exp. Immunol.* **61,** 323.

TRENTHAM, D.E., TOWNES, A.S. & KANG, A.H. (1977) Autoimmunity to collagen: an experimental model of arthritis. *J. exp. Med.* **146,** 857.

TRENTHAM, D.E., DYNESIUS, R. & DAVID, J.R. (1978) Passive transfer by cells of type II collagen-induced arthritis in the rat. *J. clin. Invest.* **62,** 359.

TRENTHAM, D.E., MCCUNE, W.J. SUSMAN, P. & DAVID, J.R. (1980) Autoimmunity to collagen in adjuvant arthritis of rats. *J. clin. Invest.* **66,** 1109.

TRENTHAM, D.E. & DYNESIUS-TRENTHAM, R.A. (1983) Attenuation of an adjuvant arthritis by type II collagen. *J. Immunol.* **130,** 2689.

VAN DEN BROEK, M.F., VAN BRUGGER, M.C., VAN DE PUTTE, H.B., VAN EDEN, W., VAN DER ZEE, R. & VAN DEN BERG, W.B. (1989) Protection against streptococcal cell wall-induced arthritis by pretreatment with 65-kD mycobacterial heat shock protein. *J. exp. Med.* **170,** 449.

VAN EDEN, W., HOLOSHITZ, J. & COHEN, I.R. (1987) Antigenic mimicry between mycobacteria and cartilage proteoglycans: the model of adjuvant arthritis. *Concepts Immunopathol.* **4,** 144.

VAN EDEN, W., HOLOSHITZ, J., NEVO, Z., FRENKEL, A., KLAJMAN, A. & Cohen, I.R. (1985) Arthritis induced by a T-lymphocyte clone that responds to *Mycobacterium tuberculosis* and to cartilage proteoglycans. *Proc. natl Acad. Sci. USA*, **82,** 5117.

VAN EDEN, W.G., THOLE, J.E.R., VANDER ZEE, R., NOORDZJI, A., VAN EMBDEN, J.D.A., HENSEN, E.J. & COHEN, I.R.(1988) Cloning of the mycobacterium epitopes recognized by T lymphocytes in adjuvant arthritis. *Nature*, **331,** 171.

WELLES, W.L. & BATTISTO, J.R. (1981) Suppression of adjuvant arthritis by antibodies specific for collagen type II. *Immunol. Commun.* **10,** 673.