Agents and Actions, vol. 14, 5/6 (1984)

0065-4299/84/060673-07$2.90

The absence of antibodies to type II collagen in established adjuvant arthritis in rats

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Abstract

Utilizing an adjuvant arthritis model in rats, we examined humoral immunity to collagen and inflammation in animals with active disease and during drug therapy. Humoral immunity to types I or II collagen was not detected in the sera of rats with advanced adjuvant arthritis; this was in marked contrast to rats with type II collagen-induced arthritis which possessed serum antibodies to native and denatured type II collagen. Hind paw edema and bone pathology were monitored as parameters of inflammation. A new investigational drug, Wy-41,770, was most effective in reducing all of these aspects of inflammatory disease while indomethacin, methylprednisolone, and D-penicillamine caused a less significant diminution of only some of these parameters of inflammation. Antibodies to collagen were not detected in the sera of rats treated with the drugs under study. These data demonstrate that adjuvant arthritis can occur in rats in the absence of antibodies to types I or II collagen.

Introduction

The induction of polyarthritis in rats was first demonstrated by STOERK et al. [1] and has become one of the most commonly used models of inflammation and arthritis. While adjuvant arthritis has been thoroughly reviewed [2–5], its mechanism of pathogenesis remains unclear. It is thought to result from a cell-mediated immune response to disseminated mycobacterial antigen, although a humoral [6, 7] and viral [8] involvement have also been suggested. Recently, TRENTHAM et al. [9] found both cell-mediated and humoral immunity to type II collagen in rats with adjuvant arthritis. These data appear to link the adjuvant model to type II collagen-induced arthritis in rats [4, 10]. Furthermore among type II collagen-sensitized rats, the highest titers of antibodies to collagen were associated with the development of arthritis [11].

In this report, we have examined the inflammatory response, bone pathology, and humoral immunity to collagen in rats with established adjuvant arthritis. We have also evaluated the effects of a number of antiarthritic drugs on these parameters. For comparative purposes, we have also measured the humoral immune response to collagen in rats with type II collagen-induced arthritis. The results are discussed with respect to the previously suggested role of humoral immunity in rats with adjuvant arthritis.

Materials and methods

Animals

Male outbred Wistar rats (Charles River, Wilmington, MA) initially weighing 160–170 g were fed Purina laboratory chow and supplied water *ad libitum*.

Adjuvant arthritis

The disease was induced with a single subcutaneous injection of 0.5 mg *Mycobacterium butyricum* suspended in 0.1 ml light mineral oil into the right hind paw. Normal rats were untreated or injected with light mineral oil alone. The volumes (ml) of the left hind paws (measured at the hairline) were determined just prior to injection of adjuvant (day 0), and at 21 and 49 days later using a mercury plethysmograph (Buxco Electronic, Inc., Sharon, CT). At the start of drug therapy on day 21, rats with adjuvant arthritis were randomized to ensure that all groups had similar mean paw volumes. Drugs were administered in 0.5% methylcellulose (400 centipoise) orally (subcutaneously in the case of gold sodium thiomalate), and administration was continued daily for 28 days (Fig. 1).

Enzyme-linked immunosorbent assay (ELISA)

Rats were bled on day 47 from the retroorbital plexus (Fig. 1) and the blood was allowed to clot at 4°C. After centrifugation, sera were removed and stored at −70°C until use. Antibodies to types I and II collagen were detected using the ELISA as described previously [12, 13]. Type I

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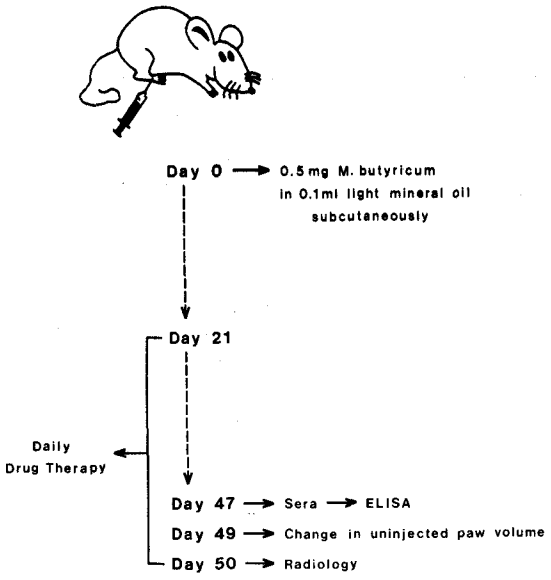

Figure 1
Schematic representation of the initiation of arthritis and the treatment of rats with adjuvant arthritis.

(1.25 μg/100 μl) and II (1 μg/100 μl) collagens were added to microtiter wells (Flow Laboratories, McLean, VA) in 0.02 *M* carbonate buffer (pH 9.6) with 0.02% sodium azide. After 1 h at 37°C, the plates were sealed and stored at 4°C. Alternatively, native type II collagen was plated at 4°C and stored until use at 4°C (Fig. 3A, D). Type II collagen (10 μg/ml) was denatured by heating at 60°C for 1 h immediately before adding to microtiter plates which were stored at 50°C until use (Fig. 3C, F). The plates were extensively washed with Dulbecco's phosphate-buffered saline (pH 7.8) containing 0.05% Tween 20 (PBS-Tween) before use. Rat sera were diluted in PBS-Tween containing 1% bovine serum albumin (PBS-Tween-BSA) and 100 μl per well was added in duplicate to collagen-coated plates. After 45 min incubation at room temperature with rocking, the plates were again washed and 100 μl of peroxidase conjugated goat anti-rat immunoglobulin (IgG, IgM, and IgA) antibodies (1:250 in PBS-Tween-BSA, N.L. Cappel Laboratories, Cochranville, PA) was added to each well. The use of a multivalent conjugate permits simultaneous screening for antibodies of several distinct classes in the ELISA. The plates were incubated for an additional 45 min and then washed. 100 μl 0.03% 2,2'-azino-di-(3-ethylbenzthiazoline-6-sulfonate) (ABTS, Sigma Chemical Co., St Louis, MO) was added per well in 0.1 *M* citrate buffer (pH 4.0) with 0.05% H_2O_2 and, after incubation for 1 h with rocking, absorbance was determined at 414 nm on a Multiskan (Flow Laboratories) which was zeroed on wells incubated with PBS-Tween-BSA in the absence of rat serum. Results are expressed as mean absorbance units ± SE.

Radiography

All radiographs were taken with Kodak Occlusal Film DF-49 using a General Electric 1000 dental x-ray machine. The settings for the radiographs were: 15 mA, 52 KVp and 0.5 sec exposure. The film was placed 20 in below the x-ray source and radiographs of both dorsal and lateral projections of the left hind paws were taken on day 50 (Fig. 1). The following parameters were evaluated using the dorsal projection without knowledge of treatment: osteoporosis, measured as a decreased density of the bone recognized as increased radiolucency; erosion, a destruction of bony structure resulting in irregular bone surface; periosteal reaction, a fine ossified line paralleling normal bone producing bone thickening; and increase in soft tissue which was manifested as an increase in width of the soft tissue. These parameters were scored using a subjective system as follows: 0, no signs; 1, mild; 2, moderate; and 3, severe.

Preparation of collagen

Type I collagen was obtained from rat tail tendon in native form as previously described [12–14]. Tendons were treated with 0.05 *M* Tris-HCl (pH 7.4) containing 1 *M* NaCl. Collagen in the precipitate was repeatedly dissolved in 0.5 *M* acetic acid and reprecipitated with 7% NaCl. The final collagen precipitate was solubilized, dialyzed against 0.05% acetic acid, and lyophilized. Type II collagen for sensitization and the ELISA was obtained from Dr J.M. Stuart (Scripps Institute, La Jolla, CA) and was isolated from fetal bovine articular cartilage. The native type II collagen was extracted by pepsin treatment of pulverized cartilage shavings as previously described [15].

Sensitization of rats

Bovine type II collagen sensitization was accomplished by two separate subcutaneous injections of 200 μg collagen and 25 μg muramyl dipeptide (MDP, Calbiochem-Behring Corp., La Jolla, CA) contained in 0.1 ml of emulsion (1:1; 0.1 *M* acetic acid in incomplete Freund's adjuvant, Difco Lab. Detroit, MI). The first injection was given into the right hind foot pad (day 0), and the second was given 7 days later into the distal third of the tail. Control rats were similarly injected at days 0 and 7 with acetic acid and MDP, and acetic acid in emulsion. Normal rats were left uninjected.

Drugs

Rats were treated with gold sodium thiomalate (GST; Merck, Sharp, and Dohme, West Point, PA) at 5 mg Au/kg, chloroquine $(PO_4)_2$ (Sigma Chemical Co., St Louis, MO) at 25 mg/kg, levamisole HCl (Aldrich Chemical Co., Milwaukee, WI) at 25 mg/kg, D-penicillamine HCl (Sigma Chemical Co.) at 100 mg/kg and 200 mg/kg, methyl prednisolone sodium succinate (Upjohn, Kalamazoo, MI) at 10 mg/kg, indomethacin (Merck, Sharp, and Dohme, West Point, PA) at 1 mg/kg, or Wy-41,770 (5*H*-dibenzo-[*a*,*d*]cycloheptene-5-ylidene)acetic acid (Wyeth Laboratories, Inc., Philadelphia, PA) at 30 mg/kg.

Statistics

The unpaired Student's *t*-test was used to determine statistical significance. A one-way analysis of variance with Dunnett's comparisons to control ($\alpha = 0.05$) was used to analyze the weight and radiographic data in Table 1.

Results

Antibody titers

Individual sera from rats with type II collagen-induced arthritis and adjuvant arthritis

Table 1
Radiographic evaluation of the extent of disease in rats with adjuvant arthritis following drug therapy.

Group	ELISA[a] (mean ± SE)	Weight[b] gain	Uninjected paw involvement[c] (Mean ± SE)			
			Osteoporosis	Erosion	Periosteal reaction	Increase in soft tissue
Adjuvant Arthritis [AA][d]	0.039 ± 0.007	87 ± 5	2.68 ± 0.19	2.69 ± 0.16	2.69 ± 0.13	2.31 ± 0.23
AA + GST (5)[e]	0.036 ± 0.008	63 ± 10	2.31 ± 0.33	2.44 ± 0.39	2.31 ± 0.33	2.12 ± 0.40
AA + Chloroquine (25)	0.034 ± 0.009	107 ± 7	2.21 ± 0.26	2.35 ± 0.26	2.00 ± 0.25	1.71 ± 0.29
AA + Levamisole (25)	0.047 ± 0.012	106 ± 8	1.75 ± 0.20	2.13 ± 0.25	2.00 ± 0.27	1.44 ± 0.33
AA + D-Penicillamine (100)	0.028 ± 0.008	101 ± 11	1.94 ± 0.32	1.81 ± 0.31	2.13 ± 0.31	1.94 ± 0.31
AA + D-Penicillamine (200)	0.024 ± 0.003	109 ± 10	1.87 ± 0.40	1.94 ± 0.39	2.00 ± 0.33	1.25 ± 0.40*
AA + Methyl prednisolone (10)	0.032 ± 0.004	95 ± 9	2.29 ± 0.16	2.50 ± 0.23	2.50 ± 0.13	2.19 ± 0.21
AA + Indomethacin (1)	0.019 ± 0.005	136 ± 13*	1.75 ± 0.16	2.06 ± 0.23	1.88 ± 0.16	1.68 ± 0.31
AA + Wy 41770 (30)	0.034 ± 0.005	129 ± 9*	1.63 ± 0.16*	2.07 ± 0.17	1.15 ± 0.10**	1.18 ± 0.09*

[a] Individual sera from 6 rats per group were examined for antibodies to native bovine type II collagen in the ELISA at a 1:10 dilution. (Normal sera = 0.024 ± 0.005.)
[b] Rats were individually weighed on day 21 and again on day 49; the increase in body weight in grams is expressed for each group as the mean ± SE.
[c] Data were scored blind by two investigators at day 50 on 8 rats per group: 0 = no signs, 1 = mild changes, 2 = moderate changes, 3 = severe changes.
[d] Normal and mineral oil injected rats show no bony changes in uninjected paws.
[e] Doses (mg/kg) are indicated in parentheses.
* $p \leq 0.05$. ** $p \leq 0.01$.

were examined for antibodies (IgG, IgM, and IgA) to types I and II collagen using the ELISA as described in the Materials and Methods. Type II collagen-sensitized rats ($n = 13$) demonstrated high levels of humoral immunity to type II collagen (Fig. 2A) and only slight reactivity to type I collagen (Fig. 2B) at 42 days after the initiation of immunization. Control sera from rats ($n = 8$) immunized with acetic acid and MDP (in the absence of type II collagen) showed minimal binding to type II (Fig. 2A) and type I (Fig. 2B) collagen. However, sera from rats with adjuvant arthritis at day 47 ($n = 10$) did not display significant antibody binding to type II (Fig. 2C) or type I (Fig. 2D) collagen at dilutions of 1:10. This time period is consistent with the highest reported incidence of anti-collagen antibodies in adjuvant arthritis [9]. Similar negative antibody results were found using sera obtained at day 18 in this adjuvant arthritis model (data not shown); however, at this stage in the disease, a low incidence of antibodies had previously been reported [9]. There was no significant difference between the binding of normal rat sera and sera from rats with adjuvant arthritis (Fig. 2C and D). Sera from rats immunized with type II collagen reacted with both native and denatured type II

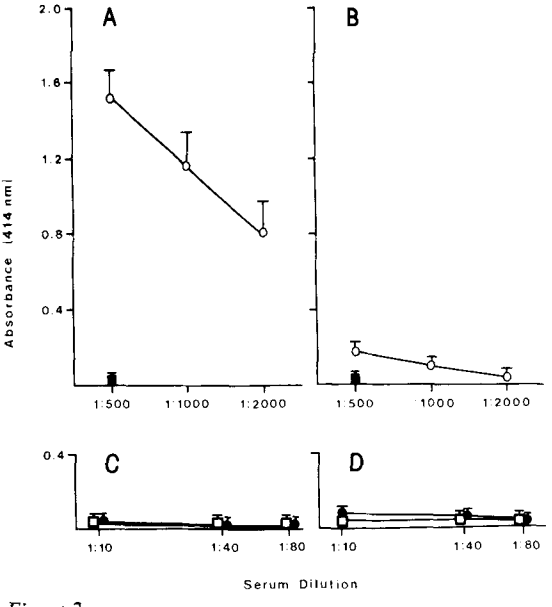

Figure 2
Antibodies to types II (A, C) and I (B, D) collagen were determined using the ELISA. Individual sera from rats with type II collagen-induced arthritis (○) and acetic acid injected controls (■) were assayed at dilutions of 1:500–1:2000 (A, B), while sera from rats with adjuvant arthritis (●) and normal controls (□) were examined at 1:10–1:80 (C, D). Results are expressed as the mean absorbance ± SE.

collagen (Fig. 3A–C) at a 1:2000 dilution, while antibodies to type II collagen in either state were not found in day 47 sera (1:10 dilution) from rats with adjuvant arthritis (Fig. 3D–F). The antibodies to type II collagen in the sera of type II collagen-sensitized rats proved to be predominantly IgG as determined through the use of IgM and IgG specific conjugates in the ELISA (data not shown). Sera from individual rats were obtained from each group indicated in Table 1 at day 47. No serum from any adjuvant arthritic or drug-treated adjuvant arthritic rat demonstrated significant reactivity to type II collagen in the ELISA (Table 1) at a 1:10 dilution compared to sera from normal controls. Despite the diminution of inflammation with drug treatment, humoral immunity to type II collagen (native or denatured) was not detected in rats with adjuvant arthritis and does not appear to be an essential feature of the adjuvant arthritis model.

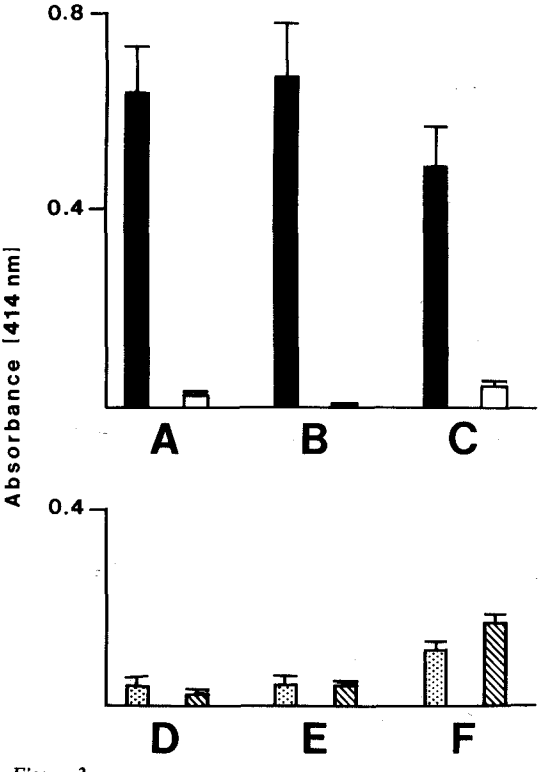

Figure 3
Humoral immunity to native and denatured type II collagen was assessed in the ELISA. As detailed in Materials and Methods, native type II collagen was coated to microtiter wells at 4°C (A, D) and 37°C (B, E), and heat-denatured type II collagen was plated at 50°C (C, F). Sera from rats with type II collagen-induced arthritis (■) and controls (□) were tested at a dilution of 1:2000 (A–C), while sera from rats with adjuvant arthritis (▦) and normal controls (▧) were assayed at 1:10 (D–F). Each group consisted of 12–15 rats and the results are expressed as the mean absorbance ± SE.

Hind paw inflammation and bone radiography

In the adjuvant arthritis model, the presence of active inflammatory disease and joint erosion was monitored in the uninjected hind paw by volume changes and radiographic analysis. At day 49, there was a 4-fold increase in the change in uninjected paw volume in arthritic versus normal rats. No differences were seen between untreated rats and those injected with mineral oil alone, in the absence of *M. butyricum* (Fig. 4). In evaluating the radiographs of uninjected hind paws (Table 1), there were significant increases in osteoporosis, erosion, periosteal inflammation, and soft tissue deposition in rats with adjuvant arthritis. Wy-41,770 was found to be a very effective drug, reducing paw edema by 55% and significantly reducing the bony changes, especially periosteal reaction and the increase in soft tissue. Indomethacin, methylprednisolone, and D-penicillamine (200 mg/kg) also significantly

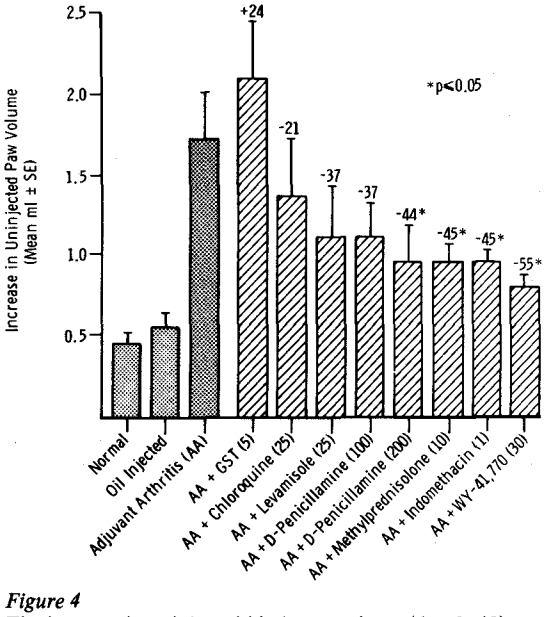

Figure 4
The increase in uninjected hind paw volume (day 0–49) was determined for control, adjuvant arthritis, and drug-treated adjuvant arthritis groups of rats as indicated. The numbers above each bar represent the percentage change from the control adjuvant group. Results are expressed as the mean ± SE and statistical comparisons were made to the adjuvant group. Each group consisted of 8–10 rats.

inhibited paw inflammation by aproximately 45% (Fig. 4). None of the other drug treatments effectively reduced this inflammation. D-Penicillamine (200 mg/kg) reduced bone erosion and the increase in soft tissue while indomethacin was less effective in reducing these bony parameters (Table 1). Surprisingly, methylprednisolone inhibited paw swelling by 50%, but had no effect on bony changes as assessed by radiographic evaluation. Levamisole and chloroquine did not significantly influence paw edema; furthermore, they did not reduce osteoporosis, periosteal reactions, and increases in soft tissue. GST did not affect either paw inflammation or bony changes, but the site of injection became necrotic during the 28 days of therapy. Rats were weighed at the start of drug therapy (day 21) and again at the close of the experiment (day 49), and the weight gain for each experimental group is included in Table 1. The data indicate that only rats given GST showed a reduced weight gain when compared to adjuvant arthritis controls. This diminished ability to thrive was statistically insignificant but correlated with the necrotic changes and ineffectiveness seen with GST in this regimen. Furthermore, rats treated with Wy-41,770 or indomethacin showed a statistically significant increase in weight when compared to the control group (Table 1). Under the conditions of this study, Wy-41,770 proved highly effective in reducing the inflammation associated with adjuvant arthritis while not showing any signs of general toxicity.

Discussion

The arthritis produced by the injection of bacterial agents in mineral oil into rats has been extensively used as a model for inflammation [1–5] but the exact mechanism of pathogenesis remains unclear. It was postulated that the adjuvant arthritis in rats is due to cell-mediated immunity to disseminated antigens expressed on the mycobacteria [16], and the ability to adoptively transfer adjuvant arthritis with lymph node or spleen cells (but not serum) from sensitized rats supports the role of cellular immunity in this disease [17, 18]. More recently, TRENTHAM et al. [9, 10] have demonstrated both humoral and cell-mediated immunity to type II collagen in both adjuvant arthritis and type II collagen-induced arthritis in rats. These data have strengthened the suggestion that these two models of arthritis have a similar etiology [4, 9]. A primary role for antibodies against type II collagen has been implicated as the major contributing factor in type II collagen-induced immunity [19], and humoral immunity against collagen has been proposed as a contributing factor in adjuvant arthritis [4, 9]. However, recent data from HOLOSHITZ et al. [20] indicate that adjuvant arthritis can be adoptively transferred to irradiated rats by a T-cell line selected for reactivity with *M. tuberculosis*; these authors conclude that it is unlikely that humoral immunity could have contributed to the arthritis in these heavily irradiated recipients.

The data presented here indicate that humoral immunity to type II collagen is not necessary for adjuvant arthritis in rats and cannot be the major etiological event. Using the ELISA, antibodies to types I or II collagen were not found early in the disease process (day 18) or later (day 47) when inflammation and joint erosion were clearly evident. No antibodies could be detected to either native or denatured type II collagen in day 47 sera from rats with adjuvant arthritis. Furthermore, treatment of rats with adjuvant arthritis with the new investigational anti-inflammatory drug, Wy-41,770 [21, 22], indomethacin, D-penicillamine, and methylprednisolone resulted in significant anti-inflammatory effects as measured by reduction in uninjected paw volume and improvement in radiologic assessment of joint disease. This sequence reflects the rank order of overall anti-inflammatory activity in this study. The therapeutic effectiveness of D-penicillamine is of interest since this drug is not generally thought to be inhibitory in adjuvant arthritis [23, 24]. It is possible that the extended duration of administration in this study may have contributed to the beneficial effects of this drug at the highest test dose. Methylprednisolone and similar steroids have previously been shown to be effective in the rat adjuvant arthritis model [23, 24]. However, the failure of GST to influence adjuvant arthritis is not altogether surprising since equivocal results have been reported for this drug in the past [25]. The anti-inflammatory results obtained with these drugs in the type II collagen-induced arthritis model in rats is significantly different from the data in the adjuvant arthritis model (R. Carlson et al., in preparation). Antibodies to collagen could not be detected in the sera of rats during the disease process or after significant reduction in inflammation through drug therapy (Table 1).

These results indicate that antibodies to type II collagen are not required for adjuvant arthritis in rats, and anti-inflammatory drug therapy can result in effective amelioration of joint disease without the detection of anti-collagen antibodies. Differences in the presence of anti-collagen antibodies and the effectiveness of drug therapy distinguish adjuvant arthritis from type II collagen-induced arthritis in rats.

The disagreement between the findings of the present study and those of TRENTHAM et al. [9] may in part be due to differences in the models employed. The ELISA in our study affords a sensitive detection of collagen-specific antibodies of IgG and IgM classes without the interference of other serum proteins [12, 13, 26], while the hemagglutination assay used by TRENTHAM et al. [9] more readily detects IgM and is considered to be less sensitive [26, 27]. It has been demonstrated that IgG and not IgM fractions of sera from rats with type II collagen-induced arthritis could passively transfer disease to normal recipients [19]. Furthermore, the Wistar rats utilized in this study were males, while TRENTHAM et al. [9] used females and employed type II collagen from lathyritic rats. The use of bovine type II collagen in our ELISA is supported by the interspecies similarities demonstrated by previous investigators [12, 28] and especially in light of the extensive cross-reactivity reported by STUART et al. [19] and CREMER et al. [29] between bovine and rat type II collagen. Immunization of rabbits [30, 31] and rats [11, 31] with heterologous native type II collagen has been shown to induce a humoral immune response directed against both native and denatured type II collagen. Similarly antibodies were found against either native or denatured type II collagen in the present studies using sera from rats with native, bovine type II collagen-induced arthritis. It was found that treatment of type II collagen-coated wells with trypsin prior to the ELISA completely abolished the binding of these antibodies to denatured type II collagen, while only partially reducing the reactivity to collagen plated at 4°C or 37°C (unpublished observations). Furthermore in competitive inhibition assays, soluble (4°C) type II collagen completely blocked rat antibody binding to plates coated at 4°C, while heat-denatured type II collagen caused only a partial reduction in activity (data not shown). However while antibodies to both native and heat-denatured types I and II collagen have been reported in the sera of rats with advanced adjuvant arthritis [9], we found no detectable antibodies against type I or type II collagen in the native or denatured state in the sera of rats at day 47 after the initiation of adjuvant arthritis (Fig. 3). Also, TRENTHAM et al. [9] utilized *M. tuberculosis* for the induction of adjuvant arthritis while *M. butyricum* was used in our studies. It is possible, though doubtful, that the differences in the antigenicity of these strains of mycobacteria may also contribute to the conflicting results. Nonetheless, antibodies to type II collagen were not found in sera of rats with *M. butyricum*-induced adjuvant arthritis using the ELISA, indicating they are not essential in this system.

Humoral involvement of anti-mycobacterial antibody has been described [6], and the delayed development of adjuvant arthritis after treatment with cobra venom factor [32] also suggests a role for humoral immunity in this model. However, WALZ et al. [33] reported that decomplementation with cobra venom factor had no effect on the onset of inflammation in adjuvant arthritis. Furthermore, MACKENZIE et al. [7] have suggested that the initial lesions of adjuvant arthritis may result from soluble immune complexes formed in antigen excess. According to these authors cell-mediated immune responses then prevail in the further development of the disease.

In conclusion, while we cannot assume from these results that antibodies against type II collagen are never formed or, under other circumstances, do not contribute to the joint inflammation evidenced in adjuvant arthritis, humoral immunity against type II collagen is not necessary for active inflammation and polyarthritis in this model.

Acknowledgments

The authors would like to thank Drs M.B. Kahaleh, M. Swartz, and E. Huffstutter for suggestions, and Judy Anderson, Carol Cook, and Kay Marley for secretarial assistance. In addition, the technical assistance of L. O'Neill-Davis and E.M. Blazek was greatly appreciated. Some of the authors (F.D., B.D.) were supported by funds from NIH AM30431, the R.G.K. Foundation, and the Charlotte and Sidney Lifschultz Foundation.

Received 29 January 1983.

References

[1] H.C. STOERK, T.C. BIELINSKI and T. BUDZILOVICH, *Chronic polyarthritis in rats injected with spleen adjuvants*, Am. J. Path. *30*, 616 (1954).

[2] A.S. WATNICK, The adjuvant arthritic rat: A tool for

discovering anti-arthritic drugs. In *Immunopharmacology*, pp. 155–171 (Eds M.E. ROSENTHALE and H.C. MANSMANN). Spectrum, New York 1975.

[3] C.G. VAN ARMAN, *Pathway to adjuvant arthritis*, Fedn Proc. *35*, 2442–2446 (1976).

[4] M.E. ROSENTHALE and R.J. CAPETOLA, *Adjuvant arthritis: immunopathological and hyperalgesic features*, Fedn Proc. *41*, 2577–2582 (1982).

[5] M.E.J. BILLINGHAM and G.E. DAVIES, Experimental models of arthritis in animals as screening tests for drugs to treat arthritis in man. In *Anti-inflammatory Drugs*, pp. 108–144 (Eds J.R. VANE and S.H. FERREIRA). Springer-Verlag, Berlin 1979.

[6] R.J. PERPER, A.L. ORONSKY and V. BLANCUZZI, *Antibody to mycobacterial antigens in adjuvant arthritis (A.A.). I. Relationship to disease and delayed hypersensitivity*, Arthritis Rheum. *15*, 283–292 (1972).

[7] A.R. MACKENZIE, C.R. PICK, P.R. SIBLEY and B.P. WHITE, *Suppression of rat adjuvant disease by cyclophosphamide pretreatment: evidence for an antibody mediated component in the pathogenesis of the disease*, Clin. exp. Immunol. *32*, 86–96 (1978).

[8] Y.-H. CHANG, *Adjuvant polyarthritis III. Evidence in support of viral etiology*, Arthritis Rheum. *20*, 1507–1513 (1977).

[9] D.E. TRENTHAM, W.J. MCCUNE, P. SUSMAN and J.R. DAVID, *Autoimmunity to collagen in adjuvant arthritis of rats*, J. clin. Invest. *66*, 1109–1117 (1980).

[10] D.E. TRENTHAM, A.S. TOWNES, A.H. KANG and J.R. DAVID, *Humoral and cellular sensitivity to collagen in type II collagen-induced arthritis in rats*, J. clin. Invest. *61*, 89–96 (1978).

[11] K. MORGAN, R.B. CLAGUE, M.J. SHAW and P.J.L. HOLT, *Native type II collagen-induced arthritis in the rat. I. Incidence and humoral response to collagen*, Ann. rheum. Dis. *39*, 285–290 (1980).

[12] A.M. MACKEL, F. DELUSTRO, F.E. HARPER and E.C. LEROY, *Antibodies to collagen in scleroderma*, Arthritis Rheum. *25*, 522–531 (1982).

[13] A.M. MACKEL, F. DELUSTRO, B. DELUSTRO, H.H. FUDENBERG and E.C. LEROY, *Immune response to connective tissue components of the basement membrane*, Conn. Tiss. Res. *10*, 333–343 (1982).

[14] A.M. MACKEL, F. DELUSTRO and E.C. LEROY, *Cell-mediated immunity to homologous basement membrane (type IV) collagen in C57BL/6 mice*, Clin. Immunol. Immunopathol. *21*, 204–216 (1981).

[15] J.M. STUART, M.A. CREMER, S.N. DIXIT, A.H. KANG and A.S. TOWNES, *Collagen-induced arthritis in rats: comparison of vitreous and cartilage-derived collagens*, Arthritis Rheum. *22*, 347–352 (1979).

[16] B.H. WAKSMAN, C.M. PEARSON and J.T. SHARP, *Studies of arthritis and other lesions induced in rats by injection of mycobacterial adjuvant. II. Evidence that the disease is a disseminated immunologic response to exogenous antigen*, J. Immun. *85*, 403–417 (1960).

[17] B.H. WAKSMAN and C. WENNERSTEN, *Passive transfer of adjuvant arthritis in rats with living lymphoid cells of sensitized donors*, Int. Archs Allergy appl. Immun. *23*, 129–139 (1963).

[18] C.M. PEARSON and F.D. WOOD, *Passive transfer of adjuvant arthritis by lymph node or spleen cells*, J. exp. Med. *120*, 547–560 (1964).

[19] J.M. STUART, M.A. CREMER, A.S. TOWNES and A.H. KANG, *Type II collagen-induced arthritis in rats: passive transfer with serum and evidence that IgG anticollagen antibodies can cause arthritis*, J. exp. Med. *155*, 1–16 (1982).

[20] J. HOLOSHITZ, Y. NAPARSTEK, A. BEN-NUN and I.R. COHEN, *Lines of T-lymphocytes induce or vaccinate against autoimmune arthritis*, Science, N.Y. *219*, 56–58 (1983).

[21] R.P. CARLSON, L.J. DATKO, M. WOLF, J. CHANG and A.J. LEWIS, *Wy-41,770, [(SH-dibenzo[α,d]cyclophenpten-5-ylidene)acetic acid]: an antiinflammatory drug without major gastric irritant and prostaglandin synthetase inhibitory activity*, Fedn Proc. *41*, 1551 (1982).

[22] R.P. CARLSON, L.J. DATKO, L. O'NEILL-DAVIS, E.M. BEACH, J. CHANG, R.L. FENICHEL, M. WOLF and A.J. LEWIS, *Wy-41,770: An antiarthritic agent with immunomodulating properties*, Int. J. Immunopharmac. *4*, 314 (1982).

[23] I.M. HUNNEYBALL, Recent developments in disease-modifying anti-rheumatic drugs. In *Progress in Drug Research*, vol. 21, pp. 101–216 (Ed. ERNST JUCKER). Birkhauser Verlag, Basel 1980.

[24] C.M. PEARSON and Y.-H. CHANG, The contribution of adjuvant arthritis to the recognition of anti-rheumatic drugs. In *Recognition of Anti-Rheumatic Drugs*, pp. 229–239 (Eds D.C. DUMONDE and M.K. JASANI). MTP Press, Lancaster, England 1978.

[25] A.J. LEWIS and D.T. WALZ, *Immunopharmacology of gold*, Prog. med. Chem., in press (1982).

[26] B. GOSSLAU and H.J. BARRACH, *Enzyme-linked immunosorbent microassay for the quantification of specific antibodies to collagen types I, II, and III*, J. Immunol. Methods *29*, 71–77 (1979).

[27] S.I. RENNARD, R. BERG, G.R. MARTIN, J.M. FOIDART and P.G. ROBEY, *Enzyme-linked immunoassay (ELISA) for connective tissue components*, Analyt. Biochem. *104*, 205–214 (1980).

[28] D.E. TRENTHAM, A.S. TOWNES and A.H. KANG, *Autoimmunity to type II collagen: an experimental model of arthritis*, J. exp. Med. *146*, 857–868 (1977).

[29] M.A. CREMER, J.A. PITCOCK, J.M. STUART, A.H. KANG and A.S. TOWNES, *Auricular chondritis in rats: an experimental model of relapsing polychondritis induced with type II collagen*, J. exp. Med. *154*, 535–540 (1981).

[30] E. HAHN, R. TIMPL and E.J. MILLER, *Demonstration of a unique antigenic specificity for the collagen α(II) chain from cartilaginous tissue*, Immunology *28*, 561–568 (1975).

[31] R.B. CLAGUE, R.A. BROWN, J.B. WEISS and P.J.L. HOLT, *Solid-phase radioimmunoassay for the detection of antibodies to collagen*, J. Immunol. Methods *27*, 31–41 (1979).

[32] L. KOUROUNAKIS, R.A. NELSON JR and M.A. KUPUSTA, *The effect of cobra venom factor on complement and adjuvant-induced disease in rats*, Arthritis Rheum. *16*, 71–76 (1973).

[33] D.T. WALZ, M.J. DIMARTINO, J. FLAGIELLO, M. DECOSTE and C. WOLF, *The effect of a complement depleter and anti-arthritic agents on complement levels and inflammation in adjuvant arthritic rats*, Fedn Proc. *32*, 752 (1973).