

## SYNERGY BETWEEN ADJUVANT ARTHRITIS AND COLLAGEN-INDUCED ARTHRITIS IN RATS

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Immunization of certain strains of rats or mice with native type II collagen in incomplete Freund's adjuvant induces an inflammatory polyarthritis, collagen-induced arthritis (CIA)<sup>1</sup> (1, 2). Although the pathogenesis of this experimental disease is still incompletely understood, antibodies directed against epitopes on the collagen molecules in articular cartilage appear to play an important role (3–6). A similar but clinically and histologically distinct joint disease, adjuvant arthritis (AA), is produced in rats by intradermal injection of complete Freund's adjuvant (CFA) or other materials with immunoadjuvant activities similar to that of CFA (7–9). We (10, 11) and others (12, 13) have recently provided evidence from adoptive transfer studies that AA is primarily mediated by cellular immune mechanisms.

In this paper we report that the pathogenetic mechanisms of AA and CIA synergize to produce an extraordinarily severe arthritic process. This synergy provides evidence that AA and CIA are induced by fundamentally different mechanisms; furthermore, it provides a model for studying the interaction of humoral and cellular immune effector mechanisms in the pathogenesis of joint inflammation.

### Materials and Methods

*Animals.* Outbred Wistar rats from Royalhart Lab Animals, New Hampton, NY, were used for the production of antibody. In all other experiments, specific pathogen-free inbred LEW/SsN rats, obtained through the Frederick Cancer Research Center, Frederick, MD, from Charles River Breeding Laboratories, Inc., Wilmington, MA, were used.

*Collagen and Antibody Preparations.* Native bovine type collagen (CII) was purified from bovine nasal septum (1, 4). Affinity-purified anticollagen IgG (anti-CII) was prepared

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<sup>1</sup>*Abbreviations used in this paper:* AA, adjuvant arthritis (or arthritic); anti-CII, affinity-purified antibody to native bovine type II collagen; CFA, complete Freund's adjuvant; CIA, collagen-induced arthritis (or arthritic); CII, native bovine type II collagen; Con A, concanavalin A; LN, lymph node; NREGG, normal rat gamma globulin; OVA, ovalbumin; PHA, phytohemagglutinin.

as previously described in detail (4). Affinity-purified anti-ovalbumin (anti-OVA) antibody was prepared in a similar manner from the pooled serum of rats immunized with OVA. Antibody concentrations were measured by enzyme-linked immunoassay, as previously described (4), and adjusted to 6 mg/ml. Normal rat gamma globulin (NRGG) was prepared from normal rat serum by the method of Stuart et al. (3).

**Induction of Arthritis.** AA was induced with 100% incidence in 5–10-wk-old LEW rats of either sex by a single intradermal injection into the tail, of a suspension of 0.6 mg *Mycobacterium butyricum* (Difco Laboratories, Inc., Detroit, MI) in oil (CFA), prepared as previously described (11). CIA was induced with >90% incidence by a modification of the method of Stuart et al. (3); in brief, rats were given two intradermal injections, 1 wk apart, of 0.2 mg CII in incomplete Freund's adjuvant into the tail.

**Cell Cultures.** Lymph node (LN) and spleen cell suspensions, prepared as previously described (11), were cultured 1–3 d (usually 2 d) in Dulbecco's modified Eagle's medium (M. A. Bioproducts, Walkersville, MD) containing 4.5 g/liter of glucose, 10 mM Hepes, 50 µg/ml penicillin, 50 µg/ml amikacin (Bristol Laboratories Div., Bristol-Myers Co., Syracuse, NY), 5% horse serum (K.C. Biological, Inc., Lenexa, KA), and 2 µg/ml concanavalin A (Con A) (Miles-Yeda, Rehovot, Israel), at  $2 \times 10^6$  cells/ml in 150-cm<sup>2</sup> flasks, at 37°C in a humidified 95% air/5% CO<sub>2</sub> atmosphere. In some experiments, phytohemagglutinin (PHA-M; Difco Laboratories, Inc.), 1% vol/vol, was substituted for Con A in cultures of AA lymphoid cells.

**Injections.** 6–10-wk-old LEW/SsN rats of either sex were given intravenous injections of anti-CII or control antibodies in phosphate-buffered saline and/or lymphoid cells in serum-free culture medium. Cells irradiated just before transfer were exposed to either 1,000 or 1,500 rad from a <sup>137</sup>Cs source (J. L. Shepherd & Associates, Glendale, CA).

**Evaluation of Arthritis.** After injection of cells and/or antibody, rats were clinically evaluated three to six times per week until the end of the experiment. In initial experiments, it became clear that diffuse, symmetric involvement of every joint of the four paws was a major effect of the synergistic arthritis. To quantitate this arthritis most accurately, a scoring system was devised in which each joint was separately evaluated for swelling and erythema; this system is outlined in Table I. The knee was excluded because neither swelling nor erythema of the knee could be reliably quantitated by physical examination.

TABLE I  
Scoring System for Clinical Evaluation of Arthritis

Part	Joint score range	
	Swelling*	Erythema <sup>‡</sup>
Forepaw		
Wrist	0–4	0–2
Hand/MCP joints	0–8	0–4
Digits	0–8	0–4
Hindpaw		
Ankle	0–8	0–4
Mid-tarsals	0–8	0–4
MTP joints	0–10	0–2
Digits	0–10	0–2

Maximum scores: forepaw, 30; hindpaw, 48; rat, 156. MCP, metacarpophalangeal; MTP, metatarsophalangeal.

\* The wrists, ankles, and mid-tarsals were each considered as separate right and left compartments, each compartment with half the total maximum possible score. Each MCP and MTP joint and each digit were given a score of 0–2. The first forepaw digits were not scored separately, but were considered part of the wrist.

<sup>‡</sup> Erythema was scored in each region as follows: 4, deep violaceous; 3, purple; 2, red; 1, pink; 0, normal.

Examinations were carried out under constant fluorescent lighting by either of two observers (JDT or SLL), with interobserver variation of the joint scores of <15%.

**Radiography.** Radiographs were prepared as previously described (10), and scored blindly by one observer (MLM); abnormalities were graded as follows: periosteal reaction, 0–3 (none, slight, moderate, marked); erosions, 0–3 (none, few, many small, many large); joint space narrowing, 0–3 (none, minimal, moderate, marked); joint space destruction, 0–3 (none, minimal, extensive, ankylosis).

**Histopathology.** Tissues were fixed in 10% neutral-buffered formalin. Larynges and ears were embedded in paraffin. As previously described (4), paws were decalcified in EDTA, then embedded in methacrylate and sectioned at 3–4  $\mu\text{m}$ ; sections were stained with a modified Giemsa (American Histolabs, Rockville, MD).

**Immunofluorescence.** Frozen sections were prepared from hindpaws and studied for immunoglobulin and C3 deposition, as previously described in detail (4). Formal evaluation of immunofluorescence and histopathology was carried out blindly by one observer (RAM).

**Statistics.** Paired means were compared by Student's *t* test. Three or more group means were compared by one-way analysis of variance, with an assumed significance level of  $P < 0.05$  (14).

## Results

**Synergy Between Passively Administered Cells and Antibody.** (Tables II–IV, and Figs. 1 and 2). Synergy between AA and CIA was observed when normal LEW rats were intravenously given varying amounts of anti-CII, followed within 1 d by varying numbers of adjuvant-sensitized, Con A-stimulated (AA/Con A) syngeneic LN or spleen cells. The arthritis induced by anti-CII and AA/Con A cells appeared 3–6 d after cell transfer. In most instances, every joint of the four paws showed severe swelling and erythema (Fig. 1). The arthritis peaked in severity within 2 wk of cell administration; after 3 wk, the erythema generally began to subside; residual deformity, including widespread joint ankylosis, was the rule after 6 wk or more. In rats not given anti-CII, AA/Con A LN or spleen cells induced a moderately severe, asymmetric arthritis that tended to affect primarily the ankles, midtarsal regions, and, to a lesser extent, the wrists; the small joints of the hind- and forepaws were much less often involved (Fig. 1; Table II, group E, and Table III, group L), as previously described (10, 11).

In rats given both AA/Con A spleen cells and anti-CII, increasingly severe arthritis resulted with coadministration of increasing doses of AA/Con A lymphoid cells and increasing doses of anti-CII (Fig. 2; Table II, groups C and D; and data not shown). In contrast, in rats given AA/Con A LN cells (Table III, group J) no significant differences in the severity of the arthritis resulted from doses of anti-CII of 6, 12, or 24 mg (separate peak joint scores,  $130 \pm 15$ ,  $124 \pm 21$ , and  $150 \pm 8$ , respectively;  $P > 0.05$ ). This may have been due to the generally larger doses of LN cells given. Some synergy was also observed when large numbers of uncultured LN or spleen cells were transferred directly from AA donors to recipients simultaneously given anti-CII (Table II, groups H and I; Table III, groups M and N), although the arthritis was significantly less severe than that of rats receiving anti-CII and AA/Con A cells.

Given without cells, 24 mg of anti-CII reproducibly induced within 24 h a mild, symmetrical hindfoot arthritis that rarely lasted >1 wk (Table II, group A). A dose of 12 mg of anti-CII induced a similar arthritis in some recipients and no detectable arthritis in others. As previously observed (4), doses of 3 or 6

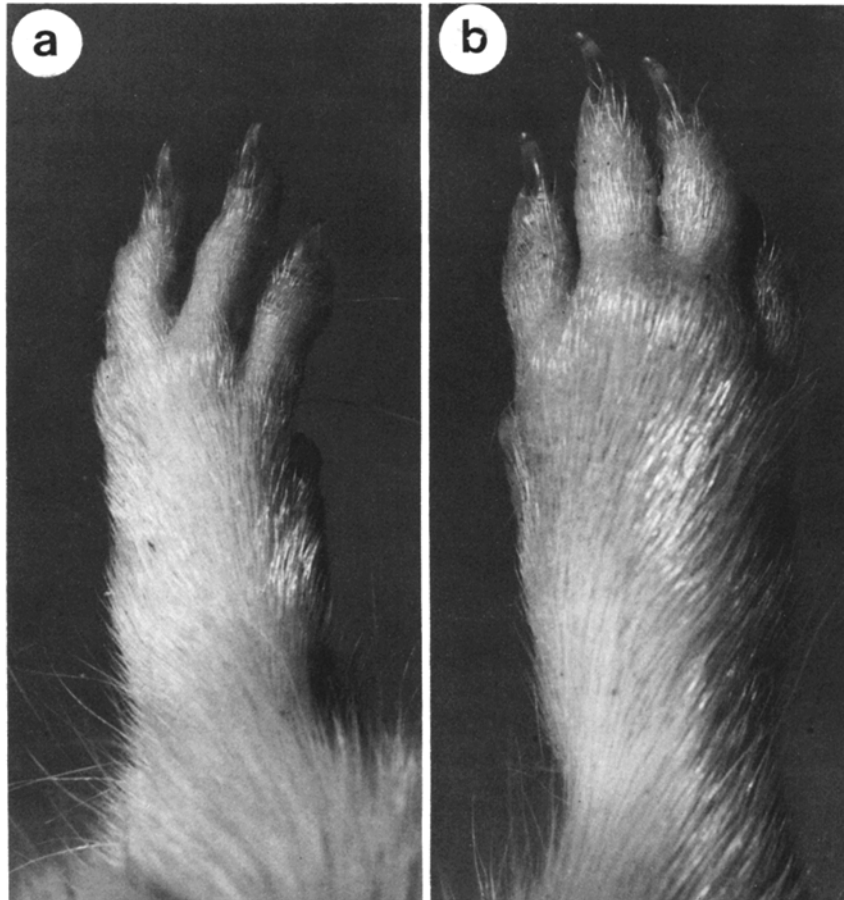


FIGURE 1. Forepaws of representative rats receiving  $1 \times 10^8$  AA/Con A LN cells (*a*), or the same dose of cells together with 12 mg anti-CII (*b*), photographed 13 d after passive transfer. In *a*, there was mild swelling and erythema of the wrist and ulnar three MCP joints. In *b*, there was diffuse soft tissue swelling and intense erythema of the entire paw, with prominent synovitis of all the metacarpophalangeal and interphalangeal joints. This degree of diffuse swelling and erythema, in both hind and forepaws, is typical of the synergy between AA and CIA.

mg anti-CII caused no detectable arthritis (Table II, group B). The ability of administered anti-CII to synergize with AA/Con A cells persisted for at least 4 wk, the longest time interval examined (Table III, group K).

The synergy between anti-CII and AA lymphoid cells in the induction of arthritis showed specificity. No synergy was seen when either NREG in doses up to 100 mg or 24 mg of affinity-purified anti-OVA was given with AA/Con A cells (Table IV, groups O–Q). Con A–stimulated lymphoid cells from LEW rats with CIA showed neither arthritogenicity when given alone nor synergy when given with anti-CII, nor was there evidence of synergy between anti-CII and Con A–stimulated cells from normal LEW rats (Table IV, groups T–W).

By far the most severe arthritis and most striking synergy were seen in recipients of anti-CII and AA LN or spleen cells cultured with Con A; AA cells

TABLE II  
*Synergy Between Anti-CII and AA Spleen Cells in the Induction of Passively Transferred Arthritis*

Group	AA spleen cells given	Cell dose ( $\times 10^{-7}$ )	Anti-CII dose (mg)	Arthritis incidence	Peak joint score	
					Mean $\pm$ SD*	Range
A	None	—	12–24	10/13	7 $\pm$ 4	2–16
B	None	—	3–6	0/5		
C	Con A–stimulated	1.5–5.4	12–24	22/22	140 $\pm$ 16	99–156
D	Con A–stimulated	1.5–5.4	6	10/10	107 $\pm$ 22	70–144
E	Con A–stimulated	2.4–6.6	0	24/26	23 $\pm$ 12	7–44
F	Con A–stimulated, irradiated <sup>‡</sup>	4.0	12	2/3	8 $\pm$ 3	5–10
G	Con A–stimulated, irradiated	4.0	0	0/2		
H	Not cultured	20–34	6	6/7	19 $\pm$ 5	13–25
I	Not cultured	20–34	0	0/6		

Tables II–IV summarize passive transfer experiments using 19 separate groups of cell donors and four separate preparations of anti-CII. AA cells were harvested 10–12 d after inoculation of donors with CFA. Cells were given 1 min to 1 d after antibody; viable cells were counted just before transfer.

\* Rats that failed to develop arthritis (i.e., scores of 0) were excluded from this calculation. Significant differences ( $P < 0.05$  by analysis of variance) are represented by the symbol  $>$ , insignificant differences by the symbol  $=$ , as follows: group C  $>$  D  $>$  E = H  $>$  F = A  $>$  B = G = I.

<sup>‡</sup> 1,500 rad irradiation.

TABLE III  
*Synergy Between Anti-CII and AA LN Cells in the Induction of Passively Transferred Arthritis*

Group	AA LN cells given	Cell dose ( $\times 10^{-7}$ )	Anti-CII dose (mg)	Arthritis incidence	Peak joint score	
					Mean $\pm$ SD*	Range
J	Con A–stimulated	4.5–13.0	6–24	20/20	132 $\pm$ 19	90–156
K <sup>‡</sup>		12.6	12	3/3	85 $\pm$ 26	74–107
L		6.2–12.2	0	13/15	22 $\pm$ 14	3–42
M	Not cultured	27–30	6	2/3	60 $\pm$ 0	60
N		27–30	0	2/2	20 $\pm$ 12	9–32

Procedures were as described in the legend to Table II, except for group K, as noted below.

\* Group J  $>$  K  $>$  M  $>$  L = N.

<sup>‡</sup> AA/Con A LN cells were given 4 wk after anti-CII.

cultured with PHA-M were much less effective in synergizing with anti-CII than AA cells cultured with Con A (Table IV, groups R and S, and data not shown). Similar levels of [<sup>3</sup>H]thymidine incorporation were stimulated by Con A and PHA-M in AA LN and spleen cells (data not shown). Irradiation of AA/Con A cells abrogated their ability to synergize with anti-CII (Table II, groups F and G; see also Fig. 4).

*Synergy Between Active and Passive Disease.* To determine if the synergy between anticollagen antibody and AA cells could also be observed in actively

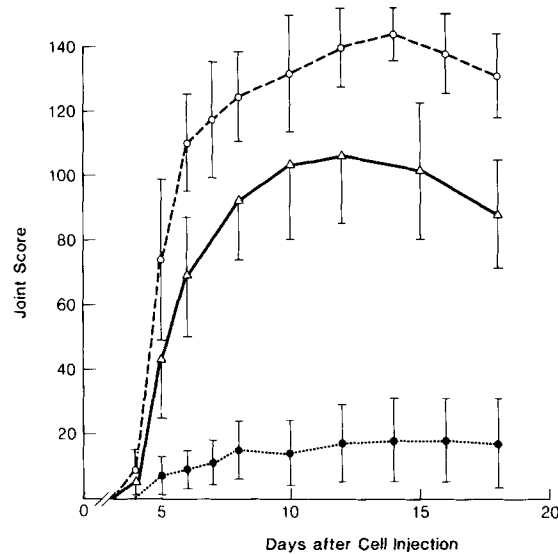


FIGURE 2. Early course of arthritis passively transferred by AA/Con A spleen cells in the presence or absence of coadministered anti-CII. Data are taken from groups C, D, and E listed in Table II; all rats for which complete time course data were obtained are included. The three groups were significantly different at all time points past day 5 ( $P < 0.05$ ). A similar time course was observed in the respective recipients of AA/Con A LN cells with and without anti-CII, except that there were no significant differences between the two LN cell recipient groups receiving 6 and 12 mg of anti-CII (see text). Bars represent  $\pm 1$  SD in this and subsequent figures. (O) Anti-CII, 12 mg,  $2.4-5.4 \times 10^7$  cells ( $n = 14$ ); ( $\Delta$ ) anti-CII, 6 mg,  $2.4-5.4 \times 10^7$  cells ( $n = 10$ ); ( $\bullet$ ) no antibody,  $2.4-6.6 \times 10^7$  cells ( $n = 20$ ).

TABLE IV  
Specificity of Both AA/Con A Cells and Anticollagen Antibody in the Synergistic Induction of Passively Transferred Arthritis

Group	Cells given*	Cell dose ( $\times 10^{-7}$ )	Antibody	Antibody dose	Arthritis incidence	Peak joint score	
						Mean $\pm$ SD <sup>†</sup>	Range
O	AA spleen/Con A	5.0	Anti-OVA	24	2/2	5 $\pm$ 3	2-8
P	AA LN/Con A	11.0-12.0	Anti-OVA	24	3/4	5	5-6
Q	AA LN/Con A	10.0-13.0	NRGG	12-100	3/6	7 $\pm$ 1	6-8
R	AA LN/PHA	7.8-9.0	Anti-CII	6	3/4	27 $\pm$ 19	5-39
S	AA LN/PHA	7.8-9.0	None	—	2/3	5 $\pm$ 2	3-7
T	CIA spleen/Con A	4.6-5.5	Anti-CII	12	4/5	9 $\pm$ 8	3-16
U	CIA spleen/Con A	4.6	None	—	0/3		
V	CIA LN/Con A	7.5	Anti-CII	12	0/5		
W	Normal LN/Con A	8.6-12	Anti-CII	12	1/2	8	8

Procedures were as described in the legend to Table II, except as described below.

\* CIA cells were harvested 13 d after primary immunization of donors with CII, by which time all donors had developed arthritis.

<sup>†</sup> Groups R, U, and V are significantly different from the other six groups.

induced arthritis, rats with actively induced AA or CIA were reciprocally given either anti-CII or AA/Con A spleen cells. Within 2 d of administration of 12 mg of anti-CII to rats with established AA, a marked, sustained increase in arthritic severity occurred (Fig. 3). Normal rat gamma globulin had no significant effect. Similarly, administration of  $5 \times 10^7$  AA/Con A spleen cells to rats with established CIA produced a marked, sustained increase in arthritic severity (Fig. 4), while irradiated AA/Con A spleen cells from the same cultures had no effect. Administration of  $5 \times 10^7$  Con A-stimulated AA spleen cells to rats with established, actively induced AA had no effect on the severity or course of the disease (data not shown).

*Synergy Between the Two Active Diseases.* To determine if synergy could occur between actively induced AA and CIA, conventional doses of CFA and CII were separately administered to groups of rats, either simultaneously or 2 wk apart. As shown in Fig. 5, the arthritic severity of rats simultaneously given CFA and CII exceeded the sum of the arthritic scores of rats given each agent alone. Rats that received the two agents 2 wk apart showed biphasic arthritic responses, with arthritic scores that were essentially additive when compared with the scores of rats receiving each agent alone. Rats receiving both CFA and CII consistently showed diffuse, symmetric involvement of the small joints of the forepaws that was not seen in either of the two control groups.

*Radiographic Evaluation of Passively Transferred Arthritis.* Rats given arthritogenic doses of anti-CII alone showed no radiographic changes (results not

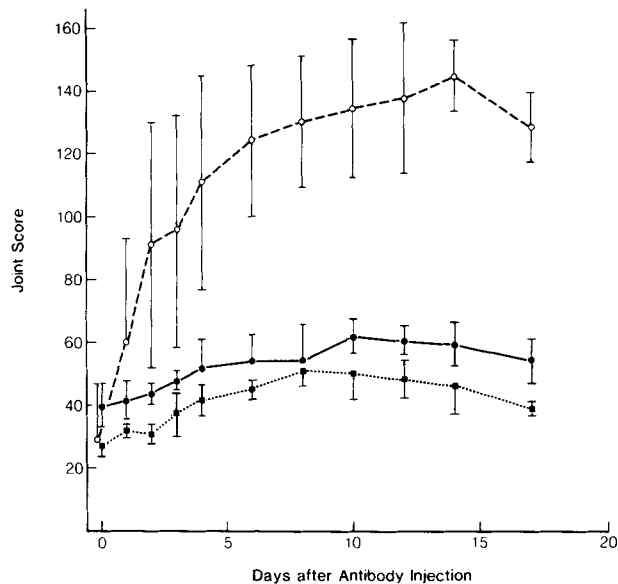


FIGURE 3. Synergistic effect of anticollagen antibody on actively induced AA. 10 rats were injected with CFA on day -13; on day 0, all rats had arthritis and were randomly selected to receive intravenously either 12 mg of anti-CII (four rats), 12 mg of NRGG (four rats), or nothing (two rats). One rat in the group receiving anti-CII died after anesthesia for roentgenography on day 13. The anti-CII-treated group was significantly different from the other two groups at each point past day 2 ( $P < 0.05$ ). Similar results were obtained in a second experiment. (○) Anti-CII, 12 mg; (●) NRGG, 12 mg; (■) control.

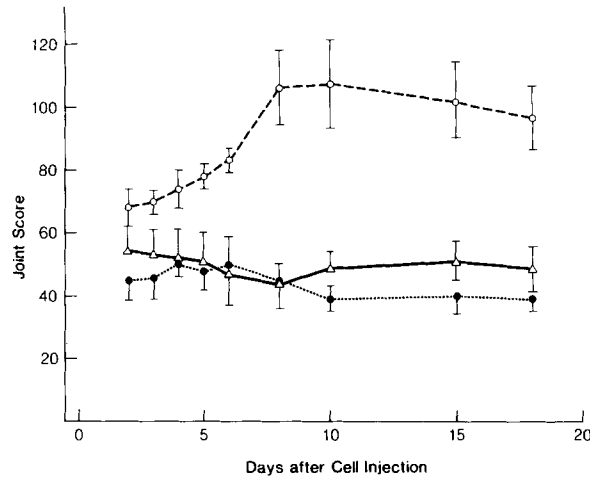


FIGURE 4. Synergistic effect of AA/Con A spleen cells on actively induced CIA. 10 rats were given 0.2 mg CII in incomplete Freund's adjuvant on day -34 and day -27; all rats developed arthritis. On day 0, rats were randomly selected to receive intravenously  $5 \times 10^7$  AA/Con A spleen cells nonirradiated (three rats) or irradiated at 1,000 rad just before transfer (three rats), or nothing (four rats). The AA/Con A spleen cell-treated group was significantly different from the other two groups at each point past day 2. Similar results were obtained in a second experiment, in which AA/Con A spleen cells were transferred on day 0 to rats immunized with CII on day -13 and day -6. (○) AA/Con A spleen cells,  $5 \times 10^7$ ; (△) AA/Con A spleen cells,  $5 \times 10^7$ , 1,000 rad; (●) control.

shown). In contrast, radiographic abnormalities developed in animals receiving AA/Con A cells alone or in conjunction with anti-CII. These results are summarized in Table V and illustrated in Figs. 6 and 7.

In rats given AA/Con A cells alone, abnormalities consisted of asymmetric soft tissue swelling and small erosions, periosteal thickening, and minimal joint space narrowing, predominantly localized to the proximal areas of the paws (Figs. 6a, 7a). At 3 mo, these rats had developed definite joint space narrowing of the intertarsal joints (Fig. 6b); forepaw changes were not apparent (Fig. 7b).

In contrast to the mild changes induced by AA/Con A cells alone, marked changes were present in the recipients of both AA/Con A cells and anti-CII. At 19 d, changes in these rats consisted of diffuse soft tissue swelling that included the digits, diffuse demineralization of bone, marked periosteal thickening, and cystic enlargement of bone. Extensive erosions produced narrowing or pseudo-widening of all joint spaces (Figs. 6c, 7c). By 7 wk, soft tissue swelling had diminished, bones were denser, and bony enlargement was more pronounced. Joint space destruction, pseudo-widening, ankylosis, and subluxation were widespread. These changes persisted upon examination at 3 mo (Figs. 6d, 7d). In rats given both CFA and CII (Fig. 5), radiographic changes were qualitatively similar to those shown in Figs. 6c, 6d, 7c, and 7d (data not shown).

**Immunofluorescence.** Rats injected with 12 mg anti-CII, with or without AA/Con A cells, and sacrificed on the second or fourth day after injection, showed bright linear fluorescence for IgG along the articular surfaces of their intertarsal joints; slight but definite fluorescence for C3 was also present in several of these rats. These findings were very similar to those previously reported (4). Rats



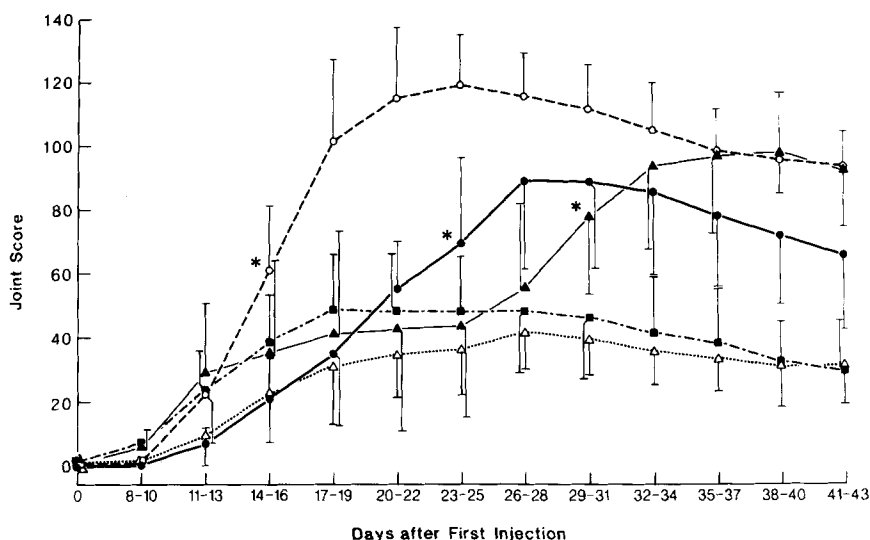


FIGURE 5. Synergy between actively induced AA and actively induced CIA. Data have been pooled from a total of three experiments. In each experiment, rats were divided into five groups and given: CFA alone, CII in IFA alone, CFA and CII together, CFA followed 2 wk later by CII, and CII followed 2 wk later by CFA. Rats given CII were boosted 1 wk after the first injection, as described in Materials and Methods. Each point represents the composite mean  $\pm$  SD arthritis score for all arthritic animals in the indicated treatment group during the time period indicated. 2 of 14 rats given CII alone failed to develop arthritis and have been excluded from the computations; all rats in all other groups developed arthritis. (\*) Significantly different from both control groups at this and all subsequent time points. (■) CFA alone ( $n = 13$ ); ( $\Delta$ ) CII alone ( $n = 12$ ); (○) CFA + CII on day 0 ( $n = 14$ ); ( $\blacktriangle$ ) CFA on day 0, CII on day 14 ( $n = 14$ ); ( $\bullet$ ) CII on day 0, CFA on day 14 ( $n = 14$ ).

TABLE V  
Radiographic Evidence for Synergy between AA Lymphoid Cells and Anti-CII Antibody

Group	Radiographs	Days after cell transfer		
		19-21	50	97
A (AA cells alone)	Forepaws	2.0 $\pm$ 2.7	1.4 $\pm$ 2.3	0 $\pm$ 0
	Hindpaws	3.3 $\pm$ 2.5	8.4 $\pm$ 8.3	9.4 $\pm$ 10.4
	Total	5.3 $\pm$ 4 (12)	9.9 $\pm$ 8.3 (7)	9.4 $\pm$ 10.4 (7)
B (AA cells + anti-CII)	Forepaws	18.0 $\pm$ 6.9	17.9 $\pm$ 6.9	16.6 $\pm$ 8.4
	Hindpaws	21.5 $\pm$ 3.1	21.5 $\pm$ 3.4	21.6 $\pm$ 3.2
	Total	39.5 $\pm$ 8.5 (16)	39.4 $\pm$ 8.6 (10)	38.2 $\pm$ 10.6 (9)

All rats were given either  $3-4 \times 10^7$  AA/Con A spleen cells or  $1 \times 10^8$  AA/Con A LN cells; rats in group B also received 6-24 mg anti-CII. Radiographs taken at the time points indicated were scored blindly as described in Materials and Methods. Data represent mean  $\pm$  SD of the scores for all rats in each group. Numbers in parentheses represent the number of rats radiographed in each group at each time point. All paired comparisons between groups A and B at each time point are significant at  $P < 0.001$ .

receiving AA/Con A cells alone had no detectable IgG or C3 binding to joint structures.

**Histopathology.** Intertarsal joints from rats given anti-CII alone, AA/Con A cells alone, or both agents together, were examined serially (Fig. 8). By day 2 after the administration of AA/Con A cells plus anti-CII, pathologic changes



**FIGURE 6.** Serial radiographic appearance of the hindpaws of two representative rats given  $3 \times 10^7$  AA/Con A spleen cells, alone (*a* and *b*), or  $1 \times 10^6$  AA/Con A LN cells together with 24 mg anti-CII (*c* and *d*). Panels *a* and *c* were taken 19 d, and *b* and *d* 97 d, after cell transfer. (*a*) There was diffuse soft tissue swelling about the ankles and plantar fascia. Periosteal thickening was present in the distal tibia, over the tarsal bones, and on the calcaneus. The tibiotalar and intertarsal joints were indistinct. (*b*) There was no soft tissue swelling. There was increased density of the enlarged distal tibia and calcaneus, with ankylosis of the intertarsal joints. The digits showed normal

posture. (*c*) There was diffuse soft tissue swelling, as well as striking cystic enlargement of the distal tibiae, calcanei, tarsal bones, metatarsals, and proximal phalanges. There were extensive erosions of the tibiotarsal and intertarsal joints, and marked destructive changes in all the metatarsophalangeal and interphalangeal joints, with marked pseudowidening. (*d*) Soft tissue swelling was no longer evident. There was complete destruction of the ankle and intertarsal joints, with extensive periosteal proliferation of the metatarsals and proximal phalanges and with widespread bony ankylosis.

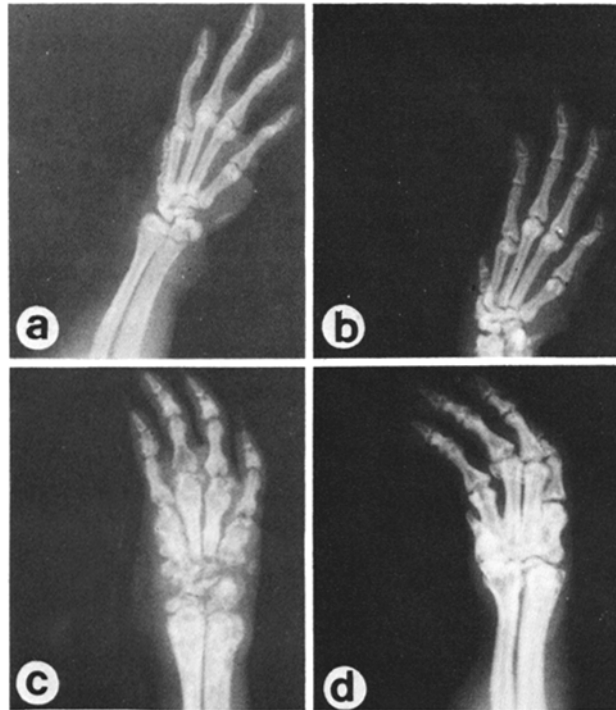
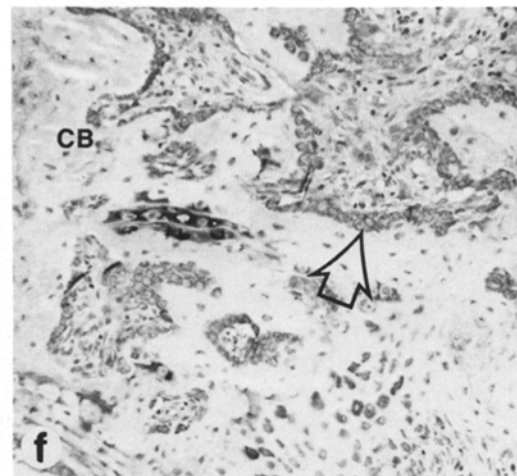
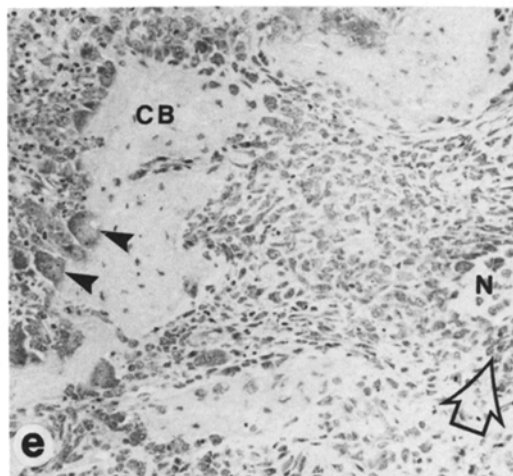
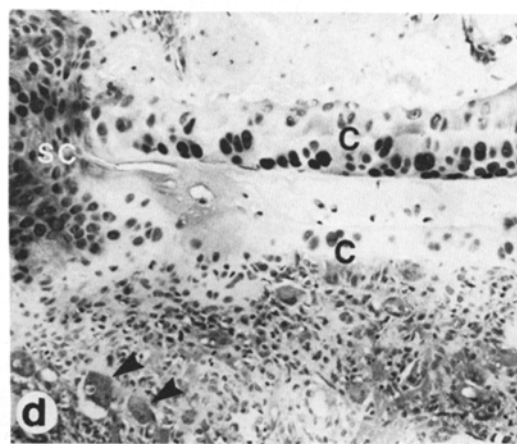
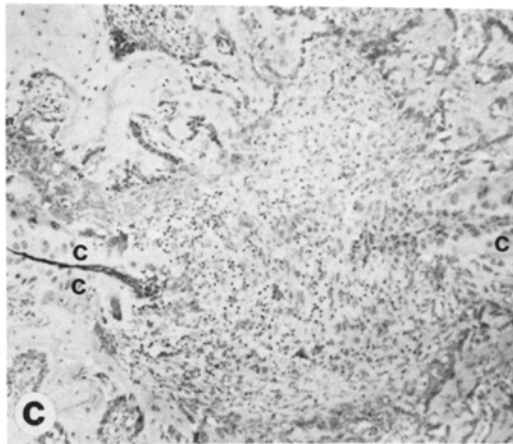
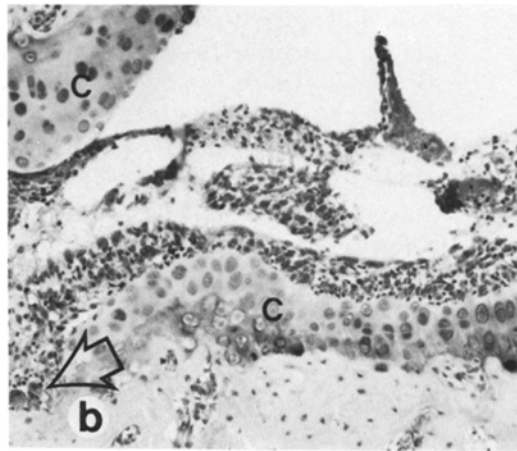
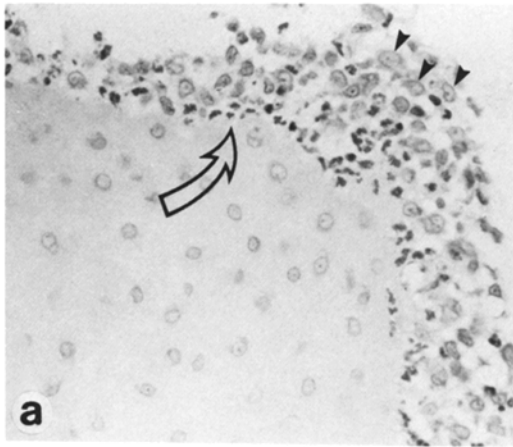


FIGURE 7. Serial radiographic appearance of the forepaws of the two representative rats shown in Fig. 6. See Fig. 6 legend for identification of panels (a–d). (a) The only abnormality was soft tissue swelling of the left wrist. (b) There was slight widening of the distal radius due to periosteal proliferation; the distal third and fourth metacarpals were slightly enlarged; the distal carpal row on the right had fused. (c) The soft tissues were diffusely swollen. The shafts of the ulna, radius, distal metacarpals and proximal phalanges showed extensive bony enlargement. Large erosions of the carpal and carpometacarpal joints result in shortening of the carpal space. There was extensive destruction of the metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints. (d) The proximal metacarpals had undergone bony ankylosis. The proximal phalanges remained enlarged and some erosions persisted in the MCP and PIP joints. The carpal bones had almost completely dissolved, and bony lysis had also caused shortening of the distal ulna and radius.

FIGURE 8. Effects of  $7 \times 10^7$  AA/Con A LN cells plus 12 mg anti-CII antibody, days 4–10 after antibody and cell administrations (a–c); effects of  $4 \times 10^7$  AA/Con A spleen cells with or without 6 mg anti-CII, 21 d after antibody and cell administration (d–f). (a) Day 4, cells plus antibody. Neutrophils were closely applied to the articular surface of the cartilage, and lay in superficial erosions (arrow). A mantle of macrophages (arrowheads) covered the layer of neutrophils. Giemsa,  $\times 25$ . (b) Day 6, cells plus antibody. There was extensive erosion of articular cartilage (C), with layering of neutrophils on the articular surface and exudate within the joint space. Osteoclasts (arrowheads) are associated with resorption of subchondral bone at the margin of the articular cartilage. Giemsa,  $\times 10$ . (c) Day 10, cells plus antibody. There was extensive destruction of articular cartilage (C), and of subchondral bone. Giemsa,  $\times 6.3$ . (d) Day 21, cells plus antibody. There was extensive destruction of articular cartilage (C) and subchondral bone, with the formation of partial synchondrosis (SC). Little inflammation was present, associated with areas of active bone resorption (arrowheads indicate osteoclasts). All intertarsal joints in this group were similarly involved. Giemsa,  $\times 10$ . (e) Day 21, cells plus antibody. Osteoneogenesis and bone resorption were marked and extensive. CB, original bone cortex being eroded by osteoclasts (closed arrowheads). N, new bone; osteoblasts (open arrowhead) surround the new matrix. Giemsa,  $\times 10$ . (f) Day 21, cells alone. Osteoneogenesis was present but less marked than in e. CB, original bone cortex; arrowhead, osteoblasts. Giemsa,  $\times 10$ .



consisted of infiltration of a few neutrophils into mildly edematous synovium. By day 4, destructive lesions were evident in articular cartilage (Fig. 8*a*). By day 6, there was frank exudation into the joint space, more extensive erosion of articular cartilage by neutrophils, and marginal erosion of subchondral bone (Fig. 8*b*); these destructive changes were more extensive on day 10 (Fig. 8*c*). By day 21, the acute, exudative changes had subsided; residual destruction of cartilage and cortical bone, and florid osteoneogenesis, were characteristic findings (Fig. 8, *d* and *e*).

Rats receiving AA/Con A cells alone had minimal inflammatory changes by day 4; these had progressed only slightly by day 10. By day 21, mild osteoneogenesis was apparent (Fig. 8*f*). Rats receiving anti-CII alone showed minimal inflammatory changes on day 4 only. No pathologic changes were found in the larynges or ears of any rat, whether treated with anti-CII, AA/Con A cells, or both.

### Discussion

Although adjuvant arthritis has been extensively studied since it was first observed three decades ago (7), no specific inciting or target antigen has been convincingly implicated in the pathogenesis of the disease. Nonetheless, the evidence that a cell-mediated immune response is primarily responsible for AA is quite strong, and several investigators have demonstrated that lymphoid cells derived from adjuvant-treated rats can adoptively transfer inflammatory arthritis (10–13, 15–17). In most of these systems, some manipulation of either the transferred cells or the recipients or both has been necessary to obtain reproducible transfer of arthritis. Such manipulation has included coadministration of transferred cells with a subarthritogenic dose of adjuvant (17), treatment of the transferred cells with Con A *in vitro* (10, 11), transfer of mycobacterial antigen-specific T cell lines (12, 13), and immunosuppression of the recipients (10, 12, 13). In all of these systems, the passively transferred arthritis generally is less severe and involves fewer joints than in conventional, actively induced AA.

One interpretation of these results posits that the manipulations are necessary to overcome the resistance of the normal host synovium to the induction of inflammation by the transferred cells, in contrast to actively induced AA, in which the pro-inflammatory adjuvant has been shown to disseminate to joint tissues (18). We reasoned that, if local synovial alterations promote arthritis induced by AA lymphoid cells, then the administration of antibody to type II collagen, which has been shown to bind rapidly *in vivo* wherever there is articular cartilage (19) and to produce transient inflammation in large joints (3–5), should augment the severity of the arthritis passively transferred with AA cells.

This prediction was borne out in the experiments described in this paper; in normal rats given anti-CII and AA lymphoid cells, we observed a striking, synergistic arthritis as a specific and dose-dependent effect of the two components. Compared with the arthritis induced by transfer of either AA cells alone or anti-CII alone, this synergy was manifested clinically by earlier and more abrupt onset of arthritis, and by more prominent, diffuse, and symmetric swelling and erythema; histological manifestations were an earlier, more diffuse, and far more phlogistic and erosive appearance; and radiographic manifestations were

more diffuse and intensive joint destruction and reactive osteoneogenesis. Similar results were seen when rats with active AA or CIA were given the reciprocal passive component, and when both active diseases were induced in the same hosts.

From these data we may draw the following conclusions: (a) antibody to type II collagen, bound to articular cartilage, can serve as a latent arthritogen in virtually any synovial joint by interacting with specifically stimulated cellular components; (b) lymphoid cells from AA rats have a substantially greater capacity than do lymphoid cells from CIA rats to induce severe, destructive arthritis; (c) AA and CIA are pathogenetically distinct diseases.

Several possible mechanisms can be envisioned for the observed synergy. The antibody on the articular surface probably serves to focus within the joint a variety of cells bearing Fc receptors. Complement fixation by the bound IgG could be expected to increase synovial vascular permeability and neutrophil migration into the joint; this in turn would be expected to render the joint more susceptible to the entry and pro-inflammatory effects of cytokine-secreting lymphoid cells.

The passive transfer experiments presented here did not address the relative effector roles of the administered cells and of recruited host cells; although the vast majority of the administered cells were lymphocytes or lymphoblasts, the predominant cells in the early lesions of the synergistic passive arthritis were neutrophils and macrophages. Indeed, we have only indirect evidence that the administered AA cell population contained effector cells that themselves traffic to joints. We have observed that AA/Con A LN or spleen cells induce passive arthritis equally well in intact, splenectomized, or sham-splenectomized recipients; however, our efforts by a variety of methods to directly demonstrate specific trafficking of AA/Con A lymphoid cells to joints have all failed (unpublished results). At present, we speculate that relatively few AA effector cells are necessary for induction of arthritis in a particular joint, especially in the case of synergy with anti-CII.

Con A-stimulated cells from rats with CIA were found not to synergize with anti-CII, nor were they arthritogenic when given alone (Table IV). In other experiments, we obtained the same negative results with large numbers ( $>5 \times 10^8$ ) of CIA LN and spleen cells transferred directly, and with CIA cells cultured in vitro with type II collagen (unpublished results). These results conflict with those of Trentham et al. (20), who observed that large numbers of CIA LN and spleen cells could transfer arthritis in LEW rats. While the explanation for this difference is not apparent, there is substantial evidence for the participation of cell-mediated immune mechanisms in the pathogenesis of collagen-induced arthritis (21–23), including the direct demonstration of T lymphocytes in the synovium of rats with CIA (24). However, the data from our passive transfer experiments suggest that, in AA, the destructive capacity of the cellular immune responses is substantially greater than in CIA.

The conclusion that AA and CIA have different pathogenetic mechanisms is consistent with the findings of several investigators, who have reported that significant levels of anti-CII do not develop in AA rats (25–27), that arthritogenic cloned antimycobacterial T cells do not react with type II collagen (13), and that

AA and CIA are not susceptible to cross-tolerization (21, 25, 26). While there have also been reports of suppression of AA by administration of CII (27, 28), these reports are not necessarily in conflict with our findings or conclusions, since the mechanism of this suppression may not be antigen-specific.

We have found only one other report of a positive interaction between humoral and cellular mechanisms in the passive transfer of experimental autoimmune disease (29). However, in complex human diseases, it is likely that several independent events contribute to disease expression. Synergistic mechanisms such as we have demonstrated may operate in human rheumatoid arthritis, in which accumulations of both intrasynovial immune complexes and inflammatory mononuclear cells are readily observed (30). It is conceivable that the effect of anti-CII in our synergy model is not specific; rather, the critical factor may be a high density of intrasynovial immunoglobulin of the proper isotype, which, independently of its antigenic specificity, provides a matrix of in situ immune complexes that promotes cell-mediated inflammation and destruction, analogous to the situation in rheumatoid arthritis (30, 31). Further experimentation with this model should produce better understanding of its mechanisms and its application to human disease.

#### Summary

Adjuvant arthritis (AA) in rats is susceptible to cell-mediated passive transfer. Collagen-induced arthritis (CIA) in rats is susceptible to passive transfer with antibody to type II collagen. We report here the development of strikingly severe arthritis in Lewis rats as the result of synergy between passively transferred antibody to type II collagen from rats with CIA and concanavalin A (Con A)-stimulated lymph node or spleen cells from syngeneic rats with AA. Similar synergy was seen in rats with AA given anticollagen antibody, in rats with CIA given Con A-stimulated adjuvant spleen cells, and in rats actively immunized with CII and complete Freund's adjuvant. The synergistic process caused a very severe polyarthritis, characterized by marked swelling and erythema in all the joints of the distal extremities, with histologic and radiographic evidence of early, extensive erosion of articular cartilage. Synergy was apparent if the lymphoid cells from AA rats were given up to 1 mo after a single injection of anticollagen antibody. No synergy was seen when normal rat immunoglobulin or antiovalbumin antibody was substituted for anticollagen antibody, when Con A-stimulated lymphoid cells from normal rats or donors with CIA were used, or when Con A-stimulated AA lymphoid cells were irradiated before transfer. Synergy between separate immune effector mechanisms may represent a general phenomenon in the pathogenesis of inflammatory joint disease.

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