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COLLAGEN-INDUCED ARTHRITIS IN MICE

Relationship of Collagen-Specific and Total IgE Synthesis to Disease¹

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The relationship between production of IgE and collagen-induced arthritis in mice was examined. Collagen-specific IgE was produced as a consequence of immunization of DBA/1 mice with chicken type II collagen emulsified in CFA. We observed a rise in collagen-specific IgE antibody levels at the onset of CIA clinical and histologic signs in DBA/1 mice. This rise in IgE paralleled that of IgG2a anticollagen antibodies, an isotype implicated in the pathogenesis of CIA by other laboratories. The collagen-specific IgE contained in the plasma of mice with CIA could arm basophils for Ag- (collagen) dependent degranulation. Collagen-specific IgE may thus contribute to CIA by promoting mast cell degranulation in the synovia of susceptible mice immunized with chick type II collagen; but, further work is required to establish such a role for IgE in CIA. However, genetic differences in disease susceptibility could not be accounted for by quantitative differences in collagen-specific IgE production. Further, comparable levels of IgE anticollagen antibodies were observed in animals with active CIA and after spontaneous remission, thereby confirming that the presence of such antibodies is insufficient for disease. Total IgE levels peaked just before spontaneous remission indicating active production of IL-4. IL-4 was administered to animals with CIA to determine if this lymphokine could be involved in the remission process. IL-4 facilitated remission of CIA. Enhanced total IgE production may thus be a marker for activation of Th2 cells that produce lymphokines such as IL-4 and IL-10, factors that may be involved in the spontaneous remission process.

CIA⁴ is an autoimmune arthritic disease (1) readily elicited in certain strains of rodents and subhuman primates by immunization with chick type II collagen emul-

sified in CFA. CIA exhibits many of the histologic features observed in patients with rheumatoid arthritis, including infiltration and proliferation of mononuclear cells, synovial hyperplasia, pannus formation, and often severe osteolysis with destruction of joint cartilage and architecture. CIA pathology is dependent on cell-mediated immunity (2) and production of lymphokines such as IFN- γ (3). The development of CIA is accompanied by the production of complement-fixing IgG2a antibodies reactive with type II collagen (4, 5), antibodies that can elicit arthritic signs upon passive transfer to normal murine recipients. Mice with CIA generally display arthritic signs for about 2 wk and then exhibit spontaneous remission. The mechanism of spontaneous remission has yet to be resolved.

Murine Th cells can be generally classified as Th1 or Th2 based on production of IL-2 and IFN- γ by the former, and IL-4 and IL-5 by the latter cell types (6). Lymphokines produced by Th2 cells can regulate both the production and activity of lymphokines secreted by Th1 cells, and vice versa. For example, IL-4 can inhibit IFN- γ production (7) and can counteract the effects of secreted IFN- γ (8). Also, Th2 cell-derived IL-10 inhibits lymphokine secretion by Th1 cells (6). Conversely, Th1 cell-derived IFN- γ counteracts the effects of Th2 cell-derived IL-4 (8). The extent of this reciprocal regulation remains to be fully defined. Nevertheless, it appears that the magnitude and nature of an immune response is dependent on the balance between Th1 and Th2 cell activity. This relationship between Th1 and Th2 cells has fostered the notion that Th cell activity may account for a variety of Ts cell phenomena. The finding that CIA can be suppressed by CD4⁺ T cells (9), which by virtue of CD4⁺ expression should be helper cells, may be explained by this newer view of Th cell subsets.

Inasmuch as IFN- γ stimulates IgG2a responses, and because IgG2a and IFN- γ have been directly implicated in the pathogenesis of CIA, it seems likely that Th1 cells have a role in the development of the disease. There have been no specific reports regarding a role for Th2 cells in CIA, either as a disease enhancer or inhibitor. Mounting evidence of IgE involvement in arthritis indicates that Th2 cells may contribute to this disease by promoting IgE production; this includes the finding of 1) rheumatoid factor of the IgE class in plasma of patients with active rheumatoid arthritis (10), 2) elevated numbers of IgE-sensitized mast cells in the synovial tissues of similar patients (11, 12), and 3) a transient arthritis in rats injected intraarticularly with Ag-specific IgE, and i.v. with Ag (13). A contribution of Th2 cells to the remission

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⁴ Abbreviations used in this paper: CIA, collagen-induced arthritis; BBS, borate-buffered saline; DNP-KLH, DNP-keyhole limpet hemocyanin; RBL, rat basophil leukemia cell line.

of CIA would certainly be plausible because production of IL-4 or IL-10 would inhibit production of IFN- γ and IgG2a, products known to contribute to CIA.

We initiated our studies on CIA to explore the possibility that collagen-specific IgE contributed to the disease process. During these studies, we obtained evidence that Th2 cells are activated during development of this disease. We observed that IgE antibody responses are readily elicited in mice by immunization with chick type II collagen/CFA, and that peak levels of IgE anticollagen antibodies were evident concordantly with the onset of the disease. The emergence of collagen-specific IgE paralleled that of IgG2a antibodies that have previously been implicated in CIA by other laboratories (4, 5). We also demonstrated that IgE anticollagen antibodies derived from mice with CIA could arm the Fc ϵ RI of rat basophils for collagen-induced degranulation *in vitro*. It is possible that IgE contributes to the pathogenesis of CIA by initiation of mast cell degranulation in the synovium of mice immunized with collagen/CFA.

However, two observations established that the relationship between production of IgE and development of CIA is not simple. First, genetic susceptibility and resistance to CIA versus IgE-responder phenotype cannot explain genetic differences in CIA susceptibility. Second, mice exhibiting remission of CIA displayed comparably high levels of IgE (and IgG2a) anticollagen antibody as when they were arthritic. Inasmuch as IgE has a plasma half-life of 2 to 5 days, this indicates that the IgE detected was recently formed even in the animals in remission. This result suggested that IgE production might be a marker for an element in the remission process, perhaps IL-4 production by Th2 cells. To investigate this possibility we administered IL-4 to animals with CIA. Animals that received IL-4 exhibited a hastening of CIA remission. Such results could reflect the generalized antiinflammatory properties of IL-4 (14) or the antagonism of IFN- γ function (8). IL-4 produced *in situ* by Th2 cells may be actively involved in the spontaneous remission of CIA.

MATERIALS AND METHODS

Induction of arthritis. Male DBA/1, SWR, and BALB/C mice, obtained from the Jackson Laboratories, Bar Harbor, ME were used at 8 to 12 wk of age; such mice were confirmed free of infection by common mouse pathogens such as Sendi virus and mouse hepatitis virus. The mice were immunized intradermally at the base of the tail on day 0 and again on day 28 with 20 μ g chick type II collagen, prepared exactly as described by others (2) or obtained commercially (Genzyme, Boston, MA). Collagen was dissolved in 0.1 M acetic acid/saline by incubation overnight at 4°C with gentle stirring and then emulsified in CFA (Difco, Ann Arbor, MI). The animals were examined visually twice weekly for arthritic signs of swollen and erythemic limbs. Induction of CIA-associated histopathology was assessed by evaluation of paraffin sections of normal limbs, and limbs obtained from animals with various stages of the disease essentially as described by others (15). Plasma samples were obtained by bleeding from the retroorbital sinus to monitor antibody levels.

Proteins and chemicals. The following reagents were purchased: ^{125} I and 1,2- ^{3} H(N)-serotonin binoxalate (New England Nuclear, Boston MA), BSA, chloramine T, sodium metabisulfite (Sigma Chemical Co., St. Louis, MO). DNP $_6$ -OVA, DNP $_6$ -KLH, and anti-DNP IgE mAb were prepared as previously described (16). Murine rIL-4 was expressed in a baculovirus system and prepared as described previously (17). Goat anti-mouse IgG1, IgG2a, and IgG2b were purchased from Fisher Scientific Co, Los Angeles, CA.

Quantitation of antibody levels. Collagen-specific plasma antibody levels were quantitated by RIA (18). Wells of a 96-well flexible PVC plate (Dynatech Labs, Chantilly, VA) were coated with 20 μ g/ml chick type II collagen, 5 μ g/ml DNP-KLH, or 1 μ g/ml goat anti-mouse IgE in BBS, and incubated overnight at 4°C, then washed and

blocked with 1% BSA/BBS; preliminary studies established that this is the optimal coating method for assay of IgE anticollagen antibodies. In each assay, triplicate wells were incubated with 0.1 ml/well of the individual plasma samples that had been diluted in BSA/BBS to 1/200 and 1/400 for measurement of IgE, and 1/2000 and 1/4000 for quantitation of the other Ig isotypes. A pool of arthritic plasma was constructed and assayed for anticollagen antibodies of the various isotypes by comparison with purified mAb; this pool provided a standard curve for each assay. After overnight incubation at 4°C, the wells were washed and incubated for 3 h at room temperature with radiolabeled goat antibodies (0.1 ml/well, 200 ng/ml) selectively reactive with the individual classes of mouse Ig. The plates were extensively washed and the bound radioactivity in individual wells quantitated in a gamma counter. The goat anti-mouse IgE used in these assays had no detectable cross-reactivity for the other classes of mouse Ig (18). Cross-reactivity between the various classes of mouse antibody with goat anti-mouse IgG1, IgG2a, or IgG2b, respectively, was about 1%.

Degranulation of RBL. RBL cell degranulation was assayed as previously described (18). Briefly, the granules of RBL cells were labeled with ^{3} H-serotonin by incubation with 1 μ Ci ^{3} H-serotonin/million cells in DMEM plus FCS for 2 h at 37°C. The cells were then washed, resuspended to 10^6 /ml in medium, and 0.25 ml plated into wells of a 48-well dish (Costar, Cambridge, MA). The cells were allowed to adhere by incubation at 37°C for 2 h, washed, and 0.25 ml of medium containing the indicated source of antibody added. After 1.5 h incubation, the cells were washed again, followed by addition of 0.25 ml of fresh medium alone, medium containing 25 ng/ml DNP-OVA, or 100 ng/ml chick type II collagen. The cultures were then incubated for 1 h at 37°C to allow for degranulation, which was monitored by counting of total and released ^{3} H-serotonin in a beta-counter.

RESULTS

Clinical course of CIA in DBA/1 mice immunized with chick type II collagen/CFA. DBA/1 mice were injected intradermally at the base of the tail on days 0 and 28 with 20 μ g chick type II collagen emulsified in CFA. The animals were followed for clinical signs of arthritis, i.e., joint erythema, edema, and loss of function. The data illustrated in Figure 1 were derived from a series of five independently conducted experiments. As noted by others (1), collagen-immunized DBA/1 mice exhibit disease induction starting at about 20 days postimmunization, and peaking at about 50 to 60 days into the experiment. Histologic analysis (not shown) confirmed extensive mononuclear infiltration and pannus formation as reported by others using similar techniques (15). Arthritis was usually noted in the hind limbs, but a substantial fraction of the animals exhibited disease in all four limbs. We used a four point scoring system where zero is a normal limb, 1 = marginal swelling and erythema, 2 = progressively more severe swelling, erythema, and lack of flexibility, and 3 = severe erythema, edema, and often complete fusion of the bone elements and lack of flexibility. The cumulative incidence of arthritis was on average 86% of the immunized mice, of which 92% exhibited a clinical score of 2 to 3 in at least one limb. Similar immunization of BALB/c mice did not cause CIA, confirming the requirement for an appropriate genetic background to elicit this autoimmune disease (15).

Spontaneous remission of CIA has been commonly observed in arthritic mice about 7 to 14 days after onset of the disease (1). Thus, as seen in Figure 1, the onset of remission and peak incidence follow that of arthritis by about 10 days each. Of those animals that developed CIA, an average of 84% exhibited remission. We have noted that animals in remission can redevelop arthritic signs, and again exhibit remission; CIA thus mimics RA in its cycling of exacerbation and remission of clinical signs. It should be noted that mice contaminated with pathogens

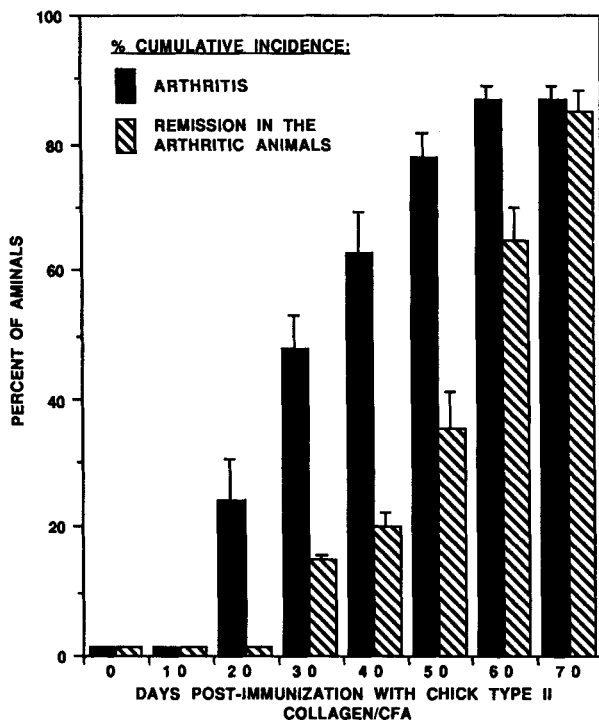


Figure 1. Clinical course of collagen-induced arthritis (CIA) in DBA/1 mice immunized with chick type II collagen/CFA. Male DBA/1 mice were immunized with 20 μ g chick type II collagen emulsified in CFA on days 0 and 28. The animals were followed for a total of 70 days for clinical signs of arthritis, joint erythema, edema, and loss of function. The data represent the means and SD of incidence of arthritis and spontaneous remission from five separate experiments using 10 to 15 mice per experiment.

such as Sendi or mouse hepatitis viruses exhibit a substantially lower incidence of remission in our work.

DBA/1 mice exhibit rise in IgG2a and IgE anticollagen antibody levels at onset of CIA. Susceptible mice exhibit a rise in IgG2a anticollagen antibody levels concurrent with development of CIA (4). Therefore, we determined the kinetics of the appearance of IgE anticollagen antibody levels as related to the onset of the disease (Fig. 2). DBA/1 mice were immunized with chick type II collagen/CFA and bled on day 35 before clinical evidence of CIA (prearthritic), and on day 45 when certain of the mice exhibited substantial arthritic signs (arthritic); all of the mice were retained for a total of 70 days to monitor the course of disease. Those mice that subsequently failed to develop clinical signs of CIA throughout the 70-day experimental period were designated as nonarthritic. On day 35, the prearthritic mice displayed low levels of IgG2a, IgG2b, and IgE anticollagen antibodies; at this time point, no differences were noted between animals that ultimately exhibited CIA (arthritic) and those that failed to display signs of clinical disease (nonarthritic). Unimmunized control animals exhibited less than 50 ng/ml of such antibodies (not shown). Importantly, at the day 45 time-point, mice that displayed arthritic signs also exhibited an approximately fivefold increase in plasma levels of both IgG2a and IgE anticollagen antibodies, with minimal increase in IgG2b anticollagen levels. Animals that remained clinically normal throughout the entire 70-day experimental period (nonarthritic) exhibited a minimal increase in such antibodies at the day-45 time-point.

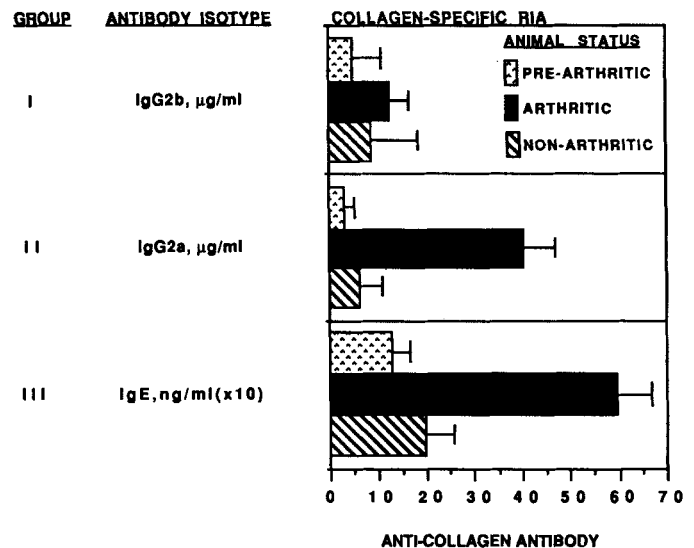


Figure 2. DBA/1 mice exhibit a rise in IgG2a and IgE anticollagen antibodies at the onset of CIA. DBA/1 mice were immunized as described in the legend to Figure 1 and bled on days 35 and 45 postprimary immunization; the animals were followed for clinical course of CIA for a total of 70 days. The plasma samples were analyzed for chick collagen-specific antibodies as described in Materials and Methods. The data are expressed as geometric means and SE from determinations from 13 prearthritic mice, of which 8 ultimately developed CIA (arthritic animals) and 5 remained clinically normal throughout the experimental period (nonarthritic).

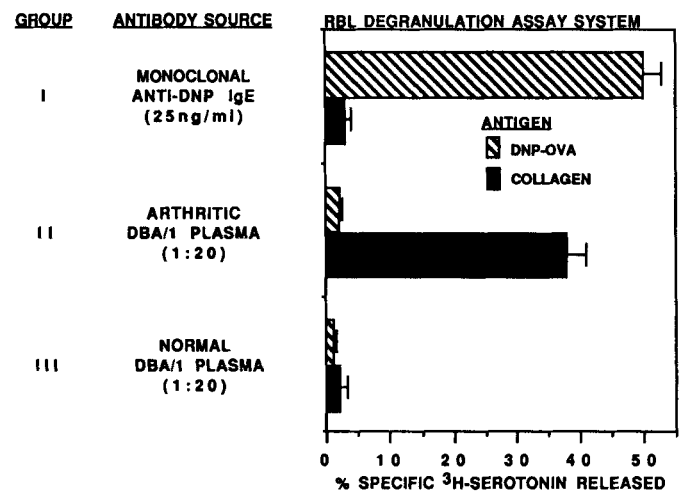


Figure 3. Plasma of mice with CIA contains collagen-specific IgE that can arm rat basophilic leukemia cells (RBL) for collagen-induced degranulation. The cytoplasmic granules of RBL cells were loaded with ^{3} H-serotonin. The cells were incubated in either medium alone, or in medium containing IgE antibodies from the indicated source. After appropriate incubation, the cells were washed and Ag added to elicit degranulation (either 25 ng/ml DNP-OVA or 100 ng/ml chick type II collagen). The data are expressed as geometric means and SE of percent specific ^{3} H-serotonin release from triplicate determinations. The percent specific release was derived by 1) subtracting spontaneous release (without Ag), which was 1208 cpm, from total cpm of ^{3} H-serotonin incorporated into the cells (22,579 cpm), and from the value obtained with addition of test Ag, 2) dividing the derived Ag-induced value by the derived total cpm-incorporated value, and 3) multiplying the quotient from step 2 by 100.

Plasma of mice with CIA contains collagen-specific IgE that can arm RBL cells for collagen-induced degranulation. To determine if anticollagen IgE from mice with CIA could bind to the high-affinity Fc ϵ RI exhibited by mast cells and arm these cells for Ag-specific degranulation, we used RBL cells in an in vitro system (Fig. 3). The cytoplasmic granules of RBL cells were labeled with ^{3} H-serotonin. The cells were then incubated in medium

alone, in medium containing arthritic or normal DBA/1 plasma, or in medium containing anti-DNP IgE as a positive control. Ag was then added (either chick type II collagen or DNP-OVA) to allow for degranulation and release of the stored ^3H -serotonin. RBL cells incubated with DNP-specific IgE at 25 ng/ml exhibited maximal release of ^3H -serotonin upon addition of DNP-OVA, which represents about 50% of the total cell-associated ^3H -serotonin. Importantly, an arthritic plasma pool was also able to readily mediate degranulation when specific Ag (collagen) was added; similar results have been obtained using plasma from individual arthritic animals. Degranulation in this system is directly related to the quantity of Ag-specific IgE used for sensitization of the RBL cells (18). The level of degranulation observed with plasma from mice with CIA was directly related to the known concentration of anticollagen IgE as determined by RIA. Normal mouse plasma showed no activity. The fact that only IgE can interact with Fc ϵ RI, and that the cells were washed between addition of antibody or Ag, makes it improbable that other antibody isotypes elicited this degranulation. These observations confirm the biologic activity and thus the pathogenic potential of anticollagen IgE produced in the course of CIA.

DBA/1 and SWR mice exhibit comparable Ag-specific IgE antibody responses. The data illustrated in Figures 2 and 3 indicate that IgE may have an important role in the pathogenesis of CIA. We wanted to determine if susceptibility and resistance to CIA could be accounted for by differences in IgE responder phenotype. Present evidence indicates that IgG2a anticollagen antibodies have a causal role in murine CIA (1, 4, 5). Thus, mice that are destined to develop CIA, such as the DBA/1 (*H-2^q*) strain, produce higher levels of anticollagen IgG2a antibodies than do mice that are resistant to disease induction. An important exception is the SWR strain of mouse (4, 19). SWR mice, which are also *H-2^q*, produce high IgG2a anticollagen antibody responses, but do not exhibit susceptibility to CIA. We compared disease-susceptible DBA/1 mice with CIA-resistant SWR mice strain for IgE responder phenotype. As shown in Figure 4, DBA/1 and SWR mice exhibited comparable IgE responses to a conventional Ag such as DNP-KLH. Both mouse strains could be considered as low IgE-responders as contrasted to the high IgE-responder BALB/c mouse strain.

Figure 5A illustrates the incidence of CIA in DBA/1 and SWR mice immunized with collagen/CFA; 80% of the DBA/1 mice exhibited arthritic signs by day 47, whereas none of the SWR animals developed the disease. The data shown in Figure 5B illustrate the anticollagen IgE responses of these same animals. Importantly, no differences in chick collagen-specific IgE could be detected between the two strains of mice at any time point. Thus, the genetic difference between DBA/1 and SWR mice that determines CIA susceptibility appears unrelated to quantitative differences in collagen-specific IgE responses.

However, as shown in Figure 5C, total IgE responses were of interest in this series of experiments. Although both mouse strains appeared comparable at the initiation and early parts of the experiment, by day 31 clear differences emerged. Thus, the DBA/1 strain exhibited higher total IgE levels at the day 31 and 47 points of analysis than did the SWR strain. It is unlikely that this difference

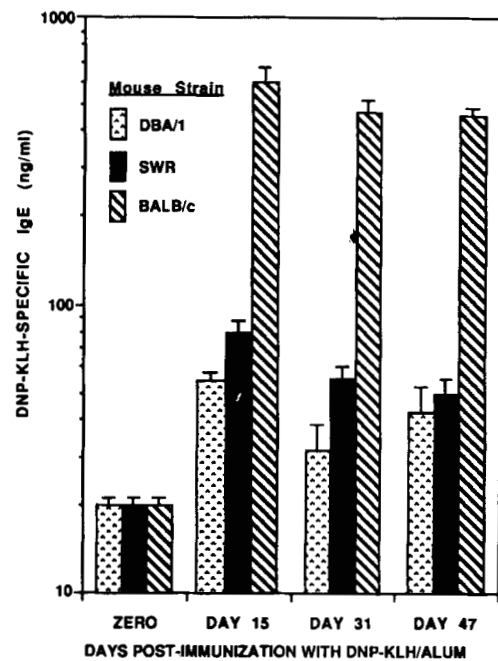


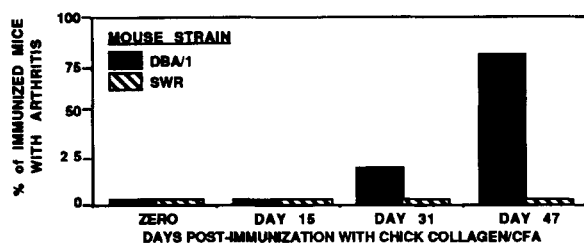
Figure 4. DBA/1 and SWR mice exhibit comparable Ag-specific IgE antibody responses. Groups of 5 DBA/1, SWR, and BALB/c mice were immunized i.p. with 10 μg DNP-KLH on 4 mg alum. The animals were bled at the indicated time points and the plasma samples analyzed for DNP-KLH-specific IgE as described in *Materials and Methods*. The data are expressed as geometric means and SE from such determinations.

in total IgE secretion reflects a difference in sensitization since comparable levels of Ag-specific IgE were noted in Figure 4B. Further, we confirmed that DBA/1 and SWR mice displayed similar IgG2a, IgG2b, and IgG1 anti-collagen antibody responses to collagen/CFA immunization (Fig. 6).

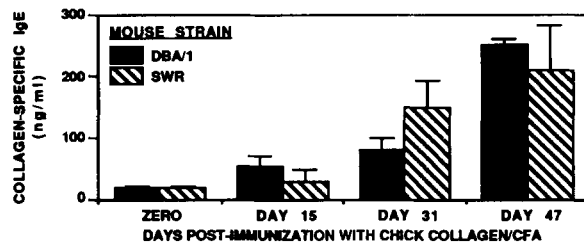
Comparable levels of collagen-specific antibody are present in plasma of mice before and after spontaneous remission of CIA. As reviewed in Figure 1, a very high percentage of DBA/1 mice with CIA will exhibit spontaneous remission. The mechanism of this remission process remains to be explored. As shown in Figure 7, however, anticollagen antibodies are probably not the limiting factor to the maintenance of the arthritic state. Arthritic animals on day 45 exhibited the same level of IgG2a, IgG2b, and IgE anticollagen antibodies as they did on day 55 after complete disappearance of arthritic signs. This points up a striking paradox about this model system, that being spontaneous remission in the face of significant anticollagen antibody levels.

Administration of IL-4 hastens remission of CIA. Inasmuch as IgE has a plasma half-life of 2–5 days, and because mice in remission exhibited high IgE levels, IL-4 must have been produced recently in situ. To test if production of IL-4 could have a determining influence on CIA course of disease, mice with CIA were injected with purified rIL-4 (Fig. 8). Comparably arthritic mice were selected at 44 days postimmunization with collagen/CFA and randomly placed into groups of five animals each. The animals were then injected on each of 4 days with saline only, or saline containing 0.1 million U of IL-4. The animals were monitored for clinical CIA. It can be seen that administration of IL-4 markedly hastened remission of CIA. Normal mice that were similarly treated with saline or IL-4 did not exhibit arthritic signs throughout the experiment. These treatments did not cause a

A. INCIDENCE OF ARTHRITIS



B. COLLAGEN-SPECIFIC IgE PRODUCTION



C. TOTAL IgE ANTIBODY PRODUCTION

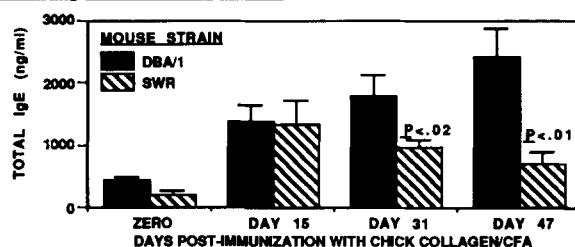


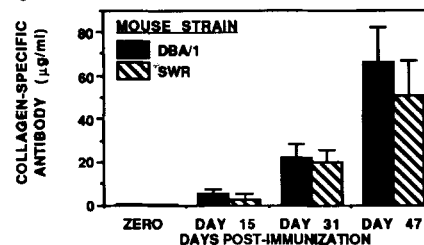
Figure 5. Incidence of CIA and plasma IgE levels of DBA/1 and SWR mice immunized with collagen/CFA. Groups of 10 DBA/1 and SWR mice were immunized with collagen/CFA as described in the legend to Figure 1, bled at the indicated time interval and followed for CIA incidence and course of disease (A). The plasma samples were analyzed for collagen-specific and total IgE levels as described in *Materials and Methods*. The data in B and C are expressed as geometric means and SE from such determinations.

change in total IgE levels in any of the experimental groups (not shown). Statistical analysis of clinical scores (*t*-test) revealed that the IL-4-treated group was significantly different from the saline-treated group on days 49 to 53 ($p < 0.05$). From day 54 until the end of the experiment, the two groups were statistically similar. An experiment of a similar design, using six animals/group, yielded nearly identical results and statistical relationships. In another experiment, IL-4 was administered on days 35 to 38 after primary immunization, just after development of clinical signs of CIA, and no effect of treatment could be noted. Another regimen of treatment was given to the same mice on days 48 to 51 and a pattern similar to that seen in Figure 8 was observed, i.e., a hastening of remission as compared to the untreated control group. This apparent lack of effect on an early stage of disease may relate to either 1) a dose of IL-4 that is unable to influence the intense inflammatory response typical of early CIA, 2) a predominant pathogenic component that is largely insensitive to potential inhibitory effects of IL-4, or 3) the possibility that the production of IL-4 is a component of the emergence of CIA signs, thus injection of IL-4 would not be expected to alter this stage of the disease.

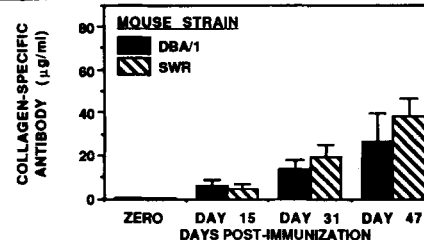
DISCUSSION

The relationship between the development and spontaneous remission of CIA and the production of IgG2a

A. IgG2a



B. IgG2b



C. IgG1

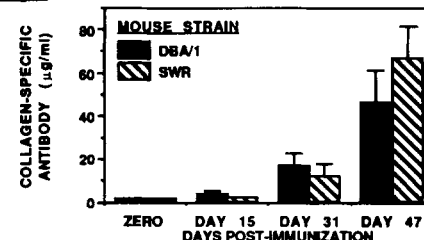


Figure 6. DBA/1 and SWR mouse strains produce comparable levels of IgG2a, IgG2b, and IgG1 when immunized with chick type II collagen/CFA. The same animals described in the legend to Figure 5 were used for these determinations. The plasma samples were analyzed for collagen-specific IgG2a, IgG2b, and IgG1 as described in *Materials and Methods*. The data are expressed as geometric means and SE from such determinations.

ANTIBODY ISOTYPE

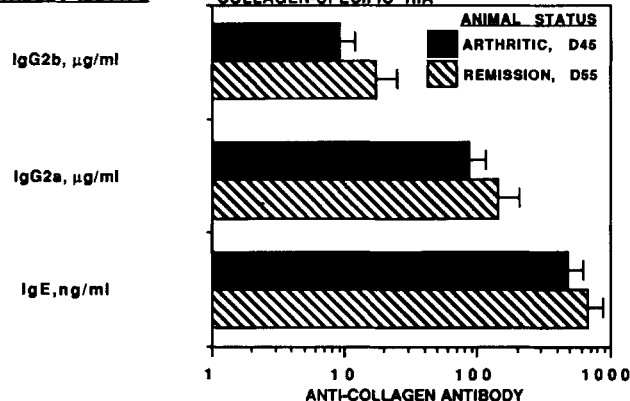


Figure 7. Comparable levels of anticollagen antibodies are present in the plasma of mice before and after spontaneous remission of CIA. DBA/1 mice, immunized as described in the legend to Figure 1, were bled on days 45 (when arthritic) and 55 (when in remission). The plasma samples were analyzed for collagen-specific IgG2a, IgG2b, and IgE as described in *Materials and Methods*. The data are expressed as geometric means and SE of determinations from six individual mice.

and IgE anticollagen antibodies is reported. We observed a clinical course and incidence of arthritis typical of that documented by others (1). The majority of the immunized DBA/1 mice (~80%) exhibited a disease characterized by clinical signs of joint erythema, edema, and loss of flexibility 20 to 40 days after primary immunization with chick type II collagen/CFA. Our histologic findings were identical to those published by many other groups (15).

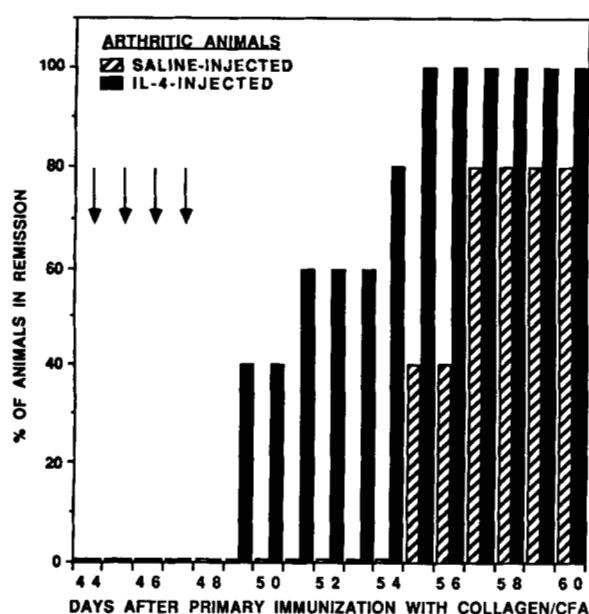


Figure 8. Administration of IL-4 hastens remission of CIA. Groups of DBA/1 mice exhibiting CIA on day 44 postprimary immunization were injected s.c. on 4 successive days with 100 μ l of saline only, or saline containing 0.1 million U of murine IL-4. The animals were followed for clinical course of CIA. The data are expressed as a percentage of animals in each group ($n = 5$) exhibiting complete clinical remission as described relative to Figure 1.

The majority of the arthritic mice (~80%) exhibited spontaneous remission, a finding also consistent with previous reports (1). CIA was not elicited in BALB/c mice, in keeping with the known genetic requirement for induction of this disease (15). Our experimental system of CIA thus conforms with that described by others.

All animals had anticollagen antibodies by 35 days after primary immunization with collagen/CFA. Mice that presented arthritic signs by day 45 also exhibited substantial increases in both IgE and IgG2a anticollagen antibody levels. Immunized animals that failed to develop clinical arthritis throughout the 70-day period failed to exhibit this rise in IgE and IgG2a anticollagen antibody levels by day 45. Production of IgE anticollagen antibodies is thus closely related in both time and quantity to the pathogenesis of CIA, as previously noted for the IgG2a class of antibody.

The potential for IgE-related pathogenicity stems in large part from its ability to bind to the high affinity Fc ϵ RI present on basophils and mast cells. The demonstration that injection of Ag-specific monoclonal IgE into the joints of rats, followed by injection of specific Ag, results in mast cell degranulation and a transient arthritic condition confirms the arthrogenic potential of IgE (13). The proposal that IgE and Fc ϵ RI-bearing cells are actively involved in CIA, although circumstantial at present, is nonetheless plausible because our results 1) establish a clear-cut association between the onset of CIA and the accumulation of plasma anti-collagen IgE, and 2) show that anticollagen IgE present in the plasma of mice with CIA is competent to mediate degranulation of RBL cells upon interaction with specific Ag. Collagen-specific IgE may accomplish a similar function by arming synovial mast cells of mice with CIA, but further work is required to establish this point. Moreover, others have reported that inhibitors of arachidonic acid metabolism suppress

CIA incidence and severity (20), as would be expected if mast cells and/or basophils were involved. It must be stated that although IgE anticollagen antibodies could act to potentiate CIA, it is also possible that such antibodies are not involved in the development of arthritis, but actually marks subsequent events, i.e., spontaneous remission.

We compared the IgE-responder status of CIA-susceptible DBA/1 mice with that of the CIA-resistant SWR mouse strain; no quantitative differences could be detected in the Ag-specific response to either DNP-KLH or chick type II collagen. We must conclude, as have others (15), that antibody responses per se are required, but insufficient, to generate CIA. However, the DBA/1 strain exhibited a substantially higher total IgE level at the time of maximal arthritic incidence, which also occurs just before the spontaneous remission process. It was observed that mice in remission exhibited comparably high levels of anticollagen antibodies as when they were arthritic. Thus, spontaneous remission does not appear to be dependent on the waning of antibody levels. Rather, spontaneous remission may well involve a dampening of the inflammatory reaction.

Inasmuch as IgE has a 2 to 5-day plasma half-life, the finding of high IgE levels in mice just after spontaneous remission is a marker for activity of IL-4-producing cells. We did not identify the IL-4-producing cells. It could be Ag-stimulated Th2 cells. It could also be mast cells, because they produce IL-4 during IgE-dependent degranulation (21). IL-4 can act at the level of mononuclear phagocytes to inhibit release of hydrogen peroxide (22), TNF, IL-1, and PGE₂ (14), and can suppress production of the chemotactic mediator IL-8 (23). IL-4 also acts at the lymphoid cell level to inhibit the action of IFN- γ (8), and can stimulate growth of IL-10-producing Th2 cells (24). Excess production of IL-4 could thus have broad activity in the spontaneous remission process. Moreover, the ability of IL-4 to inhibit the function of mononuclear phagocytes, a property yet to be demonstrated with respect to IL-10, indicates that this lymphokine might be useful in the treatment of humans with rheumatoid arthritis. Our finding that administration of IL-4 to mice with CIA hastened remission supports this notion.

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