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Immunogenetics of type II collagen autoimmunity and susceptibility to collagen arthritis

R. HOLMDAHL,* L. JANSSON,* M. ANDERSSON* & E. LARSSON† * *Department of Medical and Physiological Chemistry and † Department of Pathology, University of Uppsala, Uppsala, Sweden*

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SUMMARY

The MHC restriction of the antibody response and development of arthritis after immunization with autologous or heterologous type II collagens in mice have been investigated. Mice from three different H-2^q-carrying strains (DBA/1, NFR/N and B10.G) with different non-MHC genes, as well as B10-congenic strains carrying wild type H-2^q-related or H-2^r haplotypes, were susceptible for collagen arthritis. All strains tested developed an antibody response cross-reacting with autologous type II collagen after immunization with heterologous type II collagen; H-2^q predisposes for a high response against chick, rat and bovine type II collagen, H-2^r and H-2^b for a high response against bovine type II collagen. Only mouse strains with H-2^q, H-2^r, H-2^{w3} or H-2^{w17} were responders to mouse type II collagen, and only these strains developed arthritis after immunization with heterologous or autologous type II collagens. These findings indicate that the ability to mount an immune response against autologous type II collagen is a prerequisite for the susceptibility to collagen arthritis. A cross-reactive autoimmune response after immunization with various heterologous type II collagen may enhance further the development of arthritis.

INTRODUCTION

Induction of arthritis with type II collagen (CII) in mice has been described after immunization with heterologous CII (Courtenay *et al.*, 1980; Wooley *et al.*, 1981; Holmdahl *et al.*, 1985a) as well as with autologous CII (Holmdahl *et al.*, 1985, 1986b). Development of collagen arthritis after immunization with various heterologous CII is restricted to certain H-2 haplotypes (Wooley *et al.*, 1981, 1983, 1985). Thus, H-2^q haplotype mice readily develop arthritis after immunization with rat, bovine or chick CII, while H-2^r haplotype mice develop arthritis after immunization with bovine CII (Wooley *et al.*, 1983, 1985). The observed MHC restriction of arthritis susceptibility have been shown to depend on class II antigens coded from the I-A region by analysis of recombinant haplotype strains congenic on the C57BL/10Sn background (Wooley *et al.*, 1981). Furthermore, the difference in arthritis susceptibility and antibody responses against rat CII between B10.P (H-2^p) and B10.G (H-2^q) strains indicates that the critical difference is located in the IA-β chain, which is the only class II region-coded chain that differs in these strains (Holmdahl *et al.*, 1986c; Peck, Darby & Wakeland, 1983a; Peck, Smith & Jadus, 1983b).

Correspondence: Dr R. Holmdahl, Dept. of Medical and Physiological Chemistry, University of Uppsala, Box 575, S-75123 Uppsala, Sweden.

Abbreviations: CII, type II collagen; ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; MHC, major histocompatibility complex; PBS, phosphate-buffered saline.

We have investigated earlier the MHC restriction of the immune response and arthritis development after immunization with rat CII (Holmdahl *et al.*, 1986c). In this study we used three different H-2^q strains with different non-MHC genetic background (B10.G, DBA/1 and NFR/N) and a number of MHC-congenic strains on the C57BL/10Sn genetic background, including strains with H-2 haplotypes within the so called H-$2^{p/q}$ family (Klein & Figueroa, 1981; Wakeland & Klein, 1981; Wakeland & Klein 1983; Peck *et al.*, 1983a,b), e.g. the p, q, w3, w5 and w17 haplotypes. These closely related H-2 haplotypes only possess limited differences located to the Aβ chain. Firstly, we found that a high antibody response towards rat CII, cross-reactive with mouse CII, could be induced only in H-2^q strains. Secondly, we found that arthritis developed not only in H-2^q strains but also in two H-2^q-related mouse strains (B10.CAS2 and B10.SAA48).

We have now extended our investigation by analysing the response after immunization with bovine CII, chick CII and mouse CII, and have also included the B10.RIII strain with the H-2^r haplotype, which is known to be susceptible to collagen arthritis (Wooley *et al.*, 1983). It is shown that a cross-reactive autoimmune antibody response develops after immunization with heterologous CII in all strains tested. However, only H-2^q-, H-2^{w3}-, H-2^{w17}- and H-2^r-bearing strains develop an immune response after immunization with mouse CII and only these strains develop arthritis after immunization with heterologous or autologous CII.

Table 1. Some characteristics of the strains used in the study

Strain	Source	Class II genetics				
		I-A$^{\beta}$	I-A$^{\alpha}$	I-E$^{\beta}$	I-E$^{\alpha}$	Haplotype
DBA/1	Jackson	q	q	—*	—	q
NFR/N	NIH, derived from outbred Swiss mice	q	q	—	—	q
B10.G	Olac	q	q	—	—	q
B10.RIII	Jackson	r	r	r	r	r
B10.P	Tübingen†	p	p	p	p	p
B10.KEA5	Tübingen	w5	p	p	p	w5
B10.SAA48	Tübingen	w3	p	w3	w3	w3
B10.CAS2	Tübingen	w17	w17	—	—	w17
B10	Olac	b	b	b	—	b
B10.S	Olac	s	s	s	—	s
B10.D2	Olac	d	d	d	d	d

* No functional gene (Malthis *et al.*, 1983).
† Professor J. Klein, Tübingen, BRD.

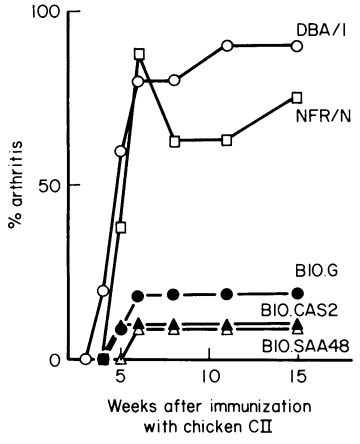

Figure 1. Incidence of arthritis in male mice from different strains after immunization with chick CII. Numbers of mice used in the experiment: DBA/1 (10), NFR/N (8), B10.G (12), B10.SAA48 (11), B10. CAS2 (11), B10.P (10), B10.KEA5 (9), B10.D2 (6), B10 (11) and B10.S (8). Only the results from strains with mice developing arthritis are indicated in the graph: DBA/1 (○—○); NFR/N (□—□); B10.G (●—●); B10.CAS2 (▲—▲); B10.SAA48 (△—△).

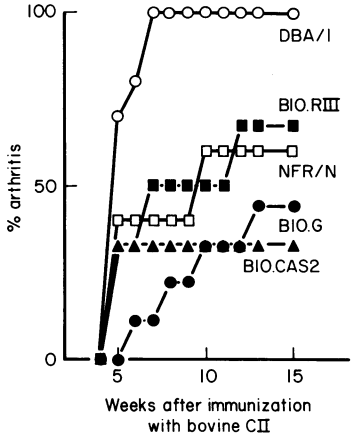

Figure 2. Incidence of arthritis in male mice from different strains after immunization with bovine CII. Numbers of mice used in the experiment: DBA/1 (10), NFR/N (5), B10.G (9), B10.RIII (6), B10.SAA48 (5), B10.CAS2 (6), B10.P (12), B10. KEA5 (4), B10.D2 (5) and B10 (3). Only the results from strains with mice developing arthritis are indicated in the graph: DBA/1 (○—○); NFR/N (□—□); B10.G (●—●); B10.RIII (■—■); B10.CAS2 (▲—▲).

MATERIALS AND METHODS

Animals

Animals were kept and bred in the animal unit at the Biomedical Center, Uppsala. The DBA/1 and B10.RIII mouse colonies were originally obtained from Jackson Laboratories Inc., Bar Habor, ME. B10.G, B10.D2, B10.S and B10 mice were originally obtained from Olac, Bicester, Oxon, U.K. Breeding pairs of B10.P, B10.CAS2, B10.SAA48 and B10.KEA5 mice were kindly donated by Dr Jan Klein, Tübingen, FRG and NFR/N mice were from National Institute of Health (NIH), Bethesda, MD (Table 1). Only male mice of each strain, at an age of 8–10 weeks, were used in the experiment.

Collagens

Rat type II collagen (CII) was prepared from a rat chondrosarcoma (Smith *et al.*, 1975), mouse CII from mouse xiphoideus, and bovine and chick from sternal cartilage by pepsin digestion and subsequent purification as described by Miller (1972). All collagens were stored lyophilized and dissolved in 0·1 M acetic acid prior to use.

Induction and quantification of arthritis

Native CII dissolved in 0·1 M acetic acid at a concentration of 1 mg/ml was emulsified in an equal volume of complete Freund's adjuvant at +4°, and 100 μl of this emulsion was injected intradermally in the skin around the root of the tail. The animals were observed weekly for the development of arthritis. Animals with erythema and swelling in at least one joint were classified as arthritic. In addition, the right hind paws from mice immunized with mouse CII were subjected to histopathologic analysis at the end the experiment. The paws were fixed in 4% formaldehyde, decalcified, sectioned and stained with haematoxylin–eosin. The sections were examined blindly and classified either for occurrence of active arthritis, with synovial hyperplasia and round cell infiltration, and/or chronic lesions, including deformed cartilage and bones as well as occurrence of fibrous pannus tissue.

Enzyme-linked immunosorbent assays (ELISAs)

Mice were bled by retro-orbital puncture. The sera were stored at −70° until use. For the quantification of anti-CII-reactive antibodies in sera, a modified standard ELISA technique was used that has been described in detail elsewhere (Homdahl *et al.*, 1986c). All tests were carried out in duplicates and the range between the duplicate values did not exceed 10% of the total absorbance value for any determination. The amounts of bound antibody were estimated after incubation with an anti-mouse kappa monoclonal antibody (187.1) (Ware, Reade & Der, 1984) coupled to alkaline phosphatase. The subsequent quantification of bound enzyme was performed with a paranitrophenol-containing substrate buffer in a Titertek multiscan spectrophotometer.

Table 2. Summary of the MHC restriction against CII based on specific antibody levels 15 weeks after immunization*

	Immunogen:	Chick		Bovine		Rat		Mouse
H2	Antigen:	Chick	Mouse	Bovine	Mouse	Rat	Mouse	Mouse
q		+++	++	+++	+++	++	++	+
r		ND	ND	+++	+++	+	+	+
w3		+	+	+	+	+	+	+
w17		++	+	+	+	+	+	+
p		++	+	−	−	+	+	−
w5		++	+	+	+	+	+	−
b		+	+	++	++	+	+	−
d		+	−	−	−	+	+	−
s		++	−	−	−	+	+	−

* Mean levels of anti CII antibodies are expressed as: <1 μg/ml (−), 1–50 μg/ml (+), 50–250 μg/ml (++) and >250 μg/ml (+++).

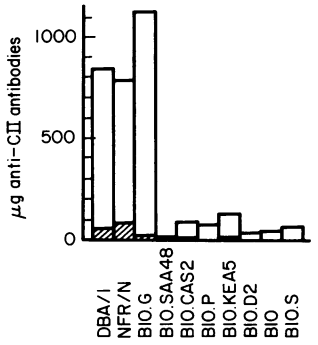

Figure 3. Amounts of antibodies reactive with mouse and chick CII in sera from mice immunized with chick CII. The filled areas in the staples represent the amounts of antibodies cross-reactive with autologous mouse CII collagen and the total areas amounts of antibodies reactive with the heterologous chick CII used for immunization.

The amounts of anti-CII antibodies in sera from immunized mice were determined from comparisons between titration curves for the serum to be tested and titration curves for affinity-purified anti-CII antibodies. These standard antibodies were obtained from sera of DBA/1 mice previously immunized with rat CII and bled 1 day after the onset of arthritis; anti-CII antibodies were isolated from these sera by affinity chromatography on Sepharose-bound rat CII (Holmdahl *et al.*, 1986c). The purified antibodies were quantified spectrophotometrically at 280 nm.

In quantifying anti-CII antibodies, serum samples and standard antibodies were titrated in parallel in ELISA. The dilution of each serum sample that gave a 50% absorbance compared with the maximum value obtained for the standard antibodies, was related to the concentration (in μg/ml) of standard antibodies required to give the same 50% absorbance value. By this method all calculations were performed using values from the steep slope of the titration curves.

RESULTS

Arthritis susceptibility of different strains after immunization with heterologous CII

The different strains were immunized with chick and bovine CII; the antibody responses and development of macroscopic arthritis were recorded, and the results are shown in Figs 1 and 2 and summarized in Table 2. In accordance with earlier experiments using rat CII (Holmdahl *et al.*, 1986c), only mice belonging to H-2^q strains (DBA/1, NFR/N, B10.G) and the q-related H-2^{w17} (B10.CAS2) and H-2^{w3} (B10.SAA48) strains developed arthritis after immunization with either bovine or chick CII. B10.RIII mice also readily developed arthritis after immunization with bovine CII, but were not included in the experiment using chick CII. However, in a separate experiment it was shown that B10.RIII mice immunized with rat CII did not develop arthritis (0 out of 10 mice followed for 15 weeks).

B10, B10.S, B10.D2, B10.P or B10.KEA5 did not show any sign of arthritis after immunization with heterologous CII. Notable is that all B10 congenic mice were relatively resistant against development of arthritis. This resistance is most likely dependent on the non-MHC genomic background since B10.G mice in all experiments developed the lowest incidence of arthritis compared with the other H-2^q haplotype-bearing mice (DBA/1 and NFR/N).

Characterization of the anti-CII antibody response after immunization with heterologous CII

The antibody responses after immunization with heterologous CII were analysed in pooled sera obtained 15 weeks after immunization; the results are shown in Figs 3 and 4. All H-2^q strains developed a relatively strong antibody response towards both bovine and chick CII. Also B10.RIII and B10 developed a high antibody response towards bovine CII, while most strains developed significant responses against chick CII. Furthermore, B10.RIII responds significantly but very weakly (compare with Holmdahl *et al.*, 1986c) to rat or mouse CII after immunization with rat CII (2 μg/ml serum 15 weeks after immunization). When the binding to mouse CII was determined a low fraction

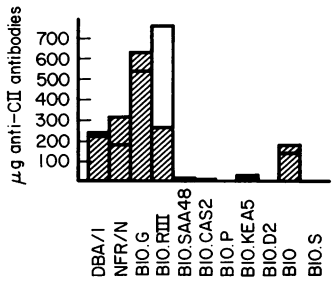

Figure 4. Amounts of antibodies reactive with mouse and bovine CII in sera from mice immunized with bovine CII. The filled areas represent the amounts of antibodies cross-reactive with autologous mouse CII and the total areas amounts of antibodies reactive with the heterologous bovine CII used for immunization.

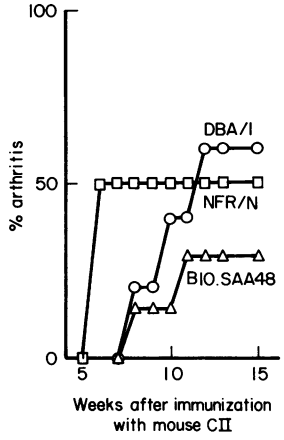

Figure 5. Incidence of arthritis in male mice from different strains after immunization with mouse CII. Numbers of mice used in the experiment; DBA/1 (10), NFR/N (4), B10.G (10), B10.RIII (6), B10.SAA48 (7), B10.CAS2 (7), B10.P (12), B10.KEA5 (3), B10.D2 (6), B10 (4) and B10.S (2). Only the results from strains with mice developing arthritis are indicated in the graph: DBA/1 (○—○); NFR/N (□—□); B10.SAA48 (△—△).

of the anti-chick CII response and a very high fraction of the anti-bovine CII response cross-reacted with mouse CII. We have shown in earlier studies using the DBA/1 mouse (Holmdahl *et al.*, 1985) that the relative degree of cross-reaction is dependent on the phylogenetic relationships between the various heterologous CII and mouse CII.

Arthritis susceptibility of different strains after immunization with mouse CII

Mouse CII emulsified in complete Freund's adjuvant was injected into male mice from different strains and the development of clinical arthritis was recorded from weekly inspections (Fig. 5). Only DBA/1 (H-2^q), NFR/N (H-2^q) and B10.SAA48 (H-2^{w3}) developed arthritis. At 15 weeks after immunization all hind paws were analysed with histopathologic examinations. Detectable inflammatory lesions are summarized in Table 3. It should be noted that the histopathologic analyses were performed only on the right hind paw and in addition only reflects a very limited part of the paws. Histopathological signs of arthritis were present in mice from all strains that exhibited clinical arthritis, but also in B10.RIII mice. As has been described earlier for the development of arthritis after immunization with mouse CII, both active and chronic lesions are detectable late after immunization. Examples of active arthritis are shown in Fig. 6a,b and chronic lesions in Fig. 6c.

Table 3. Histopathology of the right hind paw from mice immunized with mouse CII

Strain	Total no.	No. with pathology	No. with synovitis*	No. with arthritis†
DBA/1	9	8	3	5
NFR/N	4	4	1	3
B10.G	10	0	0	0
B10.RIII	6	3	2	1
B10.SAA48	7	2	2	0
B10.CAS2	7	0	0	0
B10.P	12	0	0	0
B10.KEA5	3	0	0	0
B10.D2	6	0	0	0
B10	4	0	0	0
B10.S	2	0	0	0

* Synovial hyperplasia, round cell infiltration and hyperemia.

† Active or chronic signs of inflammation of cartilage and bones usually including pannus formation and/or ankylosis.

The B10, B10.S, B10.D2, B10.P, B10.KEA5, B10.CAS2 and B10.G mice all failed to develop macroscopic or microscopic arthritis after immunization with mouse CII.

These results indicate that mice bearing certain H-2 haplotypes, such as q, r and w3, are more susceptible to mouse CII-induced arthritis than others. Similar to the results obtained after immunization with heterologous CII, it seems that the B10 non-MHC genetic background may exert a suppressive influence on the development of arthritis.

Characterization of the anti-CII antibody response after immunization with mouse CII

Sera obtained 15 weeks after immunization with mouse CII were analysed in ELISA for determination of their contents of anti-CII autoantibodies. Low (< 60 μg/ml) but definite levels of autoantibodies could be detected in some mice, summarized in Fig 7. Only mice belonging to strains bearing H-2^q (DBA/1, B10.G), H-2^{w3}, H-2^{w17} and H-2^r developed an anti-CII autoantibody response. However, many mice belonging to these strains and all of the NFR/N (H-2^q) mice did not develop detectable sera titres of antibodies reactive with mouse CII.

The CII antibody responses after immunization with mouse, rat, bovine and chick CII are summarized in Table 2.

DISCUSSION

Firstly, the MHC restriction of the immune response after immunization with heterologous CII—chick, bovine and rat—

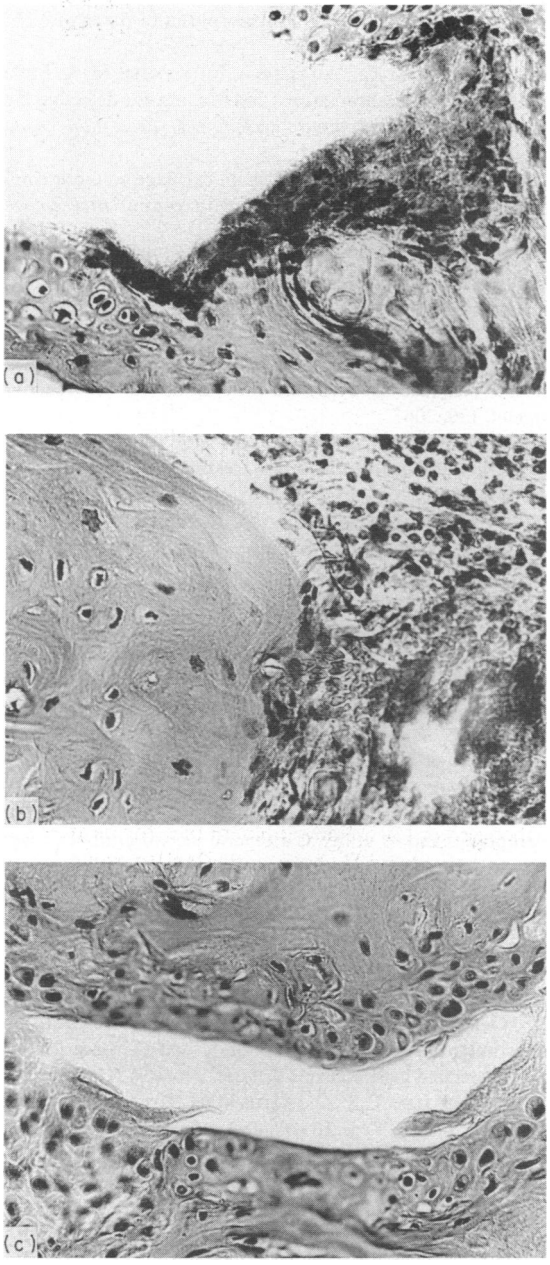

Figure 6. Examples of sections stained with haematoxyline/eosine from right front paws from mice immunized with autologous CII. Magnification × 81. In (a) is shown acute synovitis and beginning cartilage/bone erosions around the marginal zone in a joint from a B10.RIII mouse. In (b) is shown severe arthritis with dense inflammatory infiltrates and cartilage destruction in a NFR/N mouse. In (c) is shown chronic arthritis with ankylosis in a joint from a DBA/1 mouse.

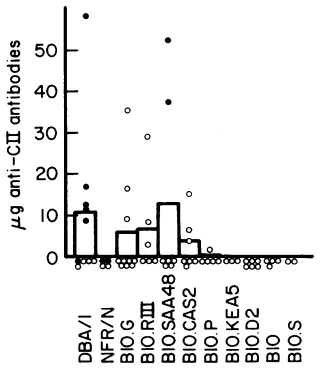

Figure 7. Amounts of antibodies reactive with mouse CII in sera from mice immunized with mouse CII. The symbols represents values of individual mice, bars mean values. Closed symbols represents occurrence of macroscopic arthritis.

was investigated. We could confirm earlier results that both H-2^q and H-2^r haplotype-bearing strains could mount a strong immune response and develop arthritis after immunization with bovine CII (Wooley *et al.*, 1983). Furthermore, as has been suggested earlier (Wooley *et al.*, 1985), strains with H-2^q and H-2^r haplotypes seem to respond to different epitopes on the CII molecule; one epitope present on bovine CII was recognized in H-2^r-haplotype mice and another epitope present on chick, rat and bovine CII was recognized in H-2^q strains. An immune response towards these epitopes after immunization with the various heterologous collagens did not only induce severe arthritis but also a strong cross-reactive antibody response towards mouse CII. However, neither the recognition of certain epitopes on heterologous CII nor development of a cross-reactive anti-mouse CII response automatically led to arthritis since, for example, the B10 (H-2^b) strains could mount a strong autoantibody response after immunization with bovine CII without developing arthritis.

Secondly, immunization with mouse CII induced both arthritis and an autoantibody response in both H-2^q- and H-2^q-related as well as in H-2^r-haplotype strains. The autoantibody response was however, very weak and did not develop at all in many H-2^q haplotype mice. We have recorded earlier a higher response in DBA/1 mice (Holmdahl *et al.*, 1985, 1986b) using another preparation of mouse CII. In these earlier works we also noted that the levels of autoantibodies did not correlate with development of arthritis. It is possible that the autoantibodies disappear from the circulation by absorbing to cartilage surfaces (Stuart & Dixon, 1983; Holmdahl *et al.*, 1986d), especially in the cases were arthritis develops. Still, an autoantibody response could only be recorded in mouse strains of H-2^q-, H-2^q-related or H-2^r haplotypes and it is notable that only mice belonging to these strains developed arthritis after immunization with various heterologous CII. Furthermore, it is possible that the more severe form of arthritis developing in the H-2^q and H-2^r strains after immunization with heterologous CII, compared to the disease developing after immunization with autologous CII, is due to the strong cross-reactive autoantibody response induced by immunization with heterologous CII.

In addition, it is also clear from the present investigation that other, non-MHC genes, influence the penetration of arthritis and immune response towards CII. This is demonstrated by the differences between the three H-2^q strains, DBA/1, NFR/N and B10.G. We have noticed previously an influence of sex chromosomes (Holmdahl, Jansson & Andersson, 1986a) and others have demonstrated influence from other non-MHC genes (Watson & Townes, 1986).

In conclusion, only haplotypes allowing an autoantibody response after immunization of the mice with mouse CII predisposes for arthritis development after immunization with autologous or heterologous CII. Since the antibody response in mice towards CII is T-cell dependent (Ranges *et al.*, 1985;

Holmdahl, Andersson & Tarkowski, 1987) this may indicate that activation of autoreactive T-helper cells is a requirement for development of collagen-induced arthritis, both after induction with autologous and with heterologous CII. Furthermore, an autoantibody response towards CII is not directly correlated to the incidence of arthritis since a strong cross-reactive anti-mouse CII antibody response develops in certain strains after immunization with bovine or chick CII without provoking an arthritic response and in other strains arthritis develops in the absence of an autoantibody response.

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