Clin. exp. Immunol. (1992) **88,** 96–100

Vaccination and genetic experiments demonstrate that adjuvant-oil-induced arthritis and homologous type II collagen-induced arthritis in the same rat strain are different diseases

R. HOLMDAHL & C. KVICK *Department of Medical and Physiological Chemistry, Uppsala University, Uppsala, Sweden*

(Accepted for publication 5 November 1991)

SUMMARY

The DA rat is highly susceptible to induction of arthritis after immunization with homologous type II collagen (CII) emulsified in Freund's incomplete adjuvant (FIA), resulting in collagen-induced arthritis (CIA). The DA rat also develops arthritis after injection of FIA alone (oil-induced arthritis (OIA)). This finding allows a direct comparison of two different models for rheumatoid arthritis; one induced with a defined auto-immunogen and one with a pure adjuvant. Both CIA and OIA develop approximately 2 weeks after induction but OIA is a self-limited acute disease whereas CIA induced with homologous CII follows a chronic disease course. Immunization with CII leads to a strong autoantibody response to CII while injection of FIA leads to no or very limited anti-CII antibody response. The Lewis rat develops neither CIA nor OIA while F1(DA × Lewis) rats develop CIA but not OIA. Olive oil or CII emulsified in olive oil does not induce arthritis in DA rats. Pretreatment with CII in olive oil vaccinates against CIA but not OIA whereas pretreatment with FIA vaccinates against OIA but not CIA. These findings demonstrate that inclusion of CII in the adjuvant leads to a disease distinct from OIA which is characterized by a CII autoimmune response and chronicity of the disease course.

Keywords Freund's incomplete adjuvant collagen-induced arthritis autoantibodies type II collagen vaccination

INTRODUCTION

Most rat strains are highly susceptible to induction of arthritis after immunization with mycobacterial cell wall fragments suspended in Freund's incomplete adjuvant (FIA) or after immunization with heterologous type II collagen (CII) [1–3]. These induced diseases have been widely used as models for studies of rheumatoid arthritis (RA) since they share several features with the human disease such as the clinical appearance of arthritis, the histopathology, the genetic association with MHC class II genes and autoimmune responses to CII, immunoglobulin and heat shock proteins [4–6]. It is believed that the pathogenesis of these induced diseases in the rat is dependent on cross-reactions between the heterologous immunogens (CII and mycobacterial cell wall protein, respectively) and autoantigens in the rat joints [5,6]. There are however important dissimilarities to RA such as the lack of chronicity of the disease course and the need for heterologous immunogens for induction of these animal models. Recently several new models have been described which do not involve induction with heterologous immunogens. Immunization with homologous CII emulsified in adjuvant oil induces chronic arthritic diseases in both rats [7] and mice [8] (type II collagen-induced arthritis, CIA) while injection of pristane, an adjuvant oil, induces arthritis in mice [9,10]. We have found that the DA rat develops arthritis after injection of homologous type II collagen emulsified in adjuvant oil (CIA) as well as after injection with adjuvant oil alone (oil-adjuvant-induced arthritis, OIA). It is therefore important to determine whether these diseases are the same. Here we show that these models are different diseases in the sense that CIA induced with homologous CII is not the same as an adjuvant arthritic disease such as OIA.

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

MATERIALS AND METHODS

Induction and evaluation of arthritis

DA rats, originally obtained from Bantin and Kingman (UK) and Lewis rats, originally obtained from Moellegaard Labs (Roskilde, Denmark) were bred and kept at the Biomedical Centre in Uppsala. The rats were kept in a climate-controlled environment with 12 h light/dark cycles, housed in polystyrene cages containing wood shavings and fed standard rodent chow and water *ad libitum*. All experiments were performed on rats at

an age of 8–12 weeks which were age- and sex-matched before the experiments. During the experiments two or three rats were housed in each cage. The rats were screened for pathogens and found to be free from common pathogens including Sendai virus, Hantaan virus, coronavirus, reovirus, cytomegalovirus and *Mycoplasma pulmonis*. Native rat CII was prepared from a rat chondrosarcoma [11] with pepsin digestion as earlier described [12]. For induction of CIA native CII was dissolved in 0·1 M acetic acid at 4°C and emulsified 1:1 on ice with FIA (Difco, Detroit, MI) to a final concentration of 0·5 mg/ml. Rats were injected intradermally in the base of the tail with 300 μl of the emulsion. When using FIA, 150 μl was injected intradermally at the base of the tail. Arthritis development was followed by a macroscopic scoring system for the four paws ranging from 0 to 3 (1 = swelling and/or redness of one toe or finger joint, 2 = two or more joints involved and 3 = severe arthritis in the entire paw).

Quantification of antibodies in serum

Sera obtained by retroorbital puncture were collected individually and stored at −20°C until assayed. For the quantification of anti-CII reactive autoantibodies in serum, a modified standard ELISA was used [13]. Micro-ELISA plates (Dynatech, Plochingen, Germany) were coated overnight 4°C with 10 μg/ml of native rat CII in PBS. All tests were carried out in duplicates, and the standard deviations did not exceed 10%. The amount of bound antibody was estimated after incubation with a mouse serum adsorbed goat anti-rat IgG (H+L) affinity purified antibody conjugated to alkaline phosphatase (Jackson Immunoresearch Laboratories, West Groove, PA). The subsequent quantification of bound enzyme was performed with a paranitrophenol containing substrate buffer in a Titertek Multiscan® spectrophotometer. To estimate the amount of anti-CII reactive antibodies present in the serum samples, affinity purified rat anti-CII antibodies were used as standards.

Statistical analysis

Incidence of arthritis were analysed by their proportionate group frequencies (χ^2-test) and the Mann–Whitney *U*-test was used for analysis of arthritic scores. Antibody levels were analysed by Student's *t*-test.

RESULTS

OIA and CIA are differently genetically restricted

DA rats are highly susceptible for induction of CIA with homologous CII. Immunization with 150 μg native rat CII dissolved in 0·1 M acetic acid and emulsified with 150 μl FIA induces a severe and chronic arthritic disease. However, the DA rat strain is also susceptible to arthritis induced by the injection of 150 μl FIA alone or FIA emulsified with acetic acid (oil adjuvant induced arthritis, OIA) (Table 1, Fig. 1). Emulsification of FIA usually leads to milder disease compared with induction with FIA only and emulsification of FIA with heterologous immunogens such as ovalbumin does not induce any arthritis (unpublished observations). Moreover, the Lewis rat strain, which is susceptible to induction of CIA with heterologous CII, is resistant to both CIA induced with homologous CII and OIA induced with FIA alone. To investigate whether CIA, induced with homologous CII, and OIA are identical diseases we made F1 crosses between DA and Lewis. The F1(DA × Lew) developed CIA but not OIA showing that CIA can be induced in a rat resistant to OIA and that these two models are influenced by different genes (Table 1).

Table 1. Genetic restriction of OIA and CIA

Rat	*n*	Treatment	Incidence (%)	Maximal mean severity
DA	5	FIA	100	5·9
DA	10	CII/FIA	100	10·0
Lewis	5	FIA	0	
Lewis	5	CII/FIA	0	
F_1(DA × Lew)	5	FIA	0	
F_1(DA × Lew)	12	CII/FIA	83	5·6

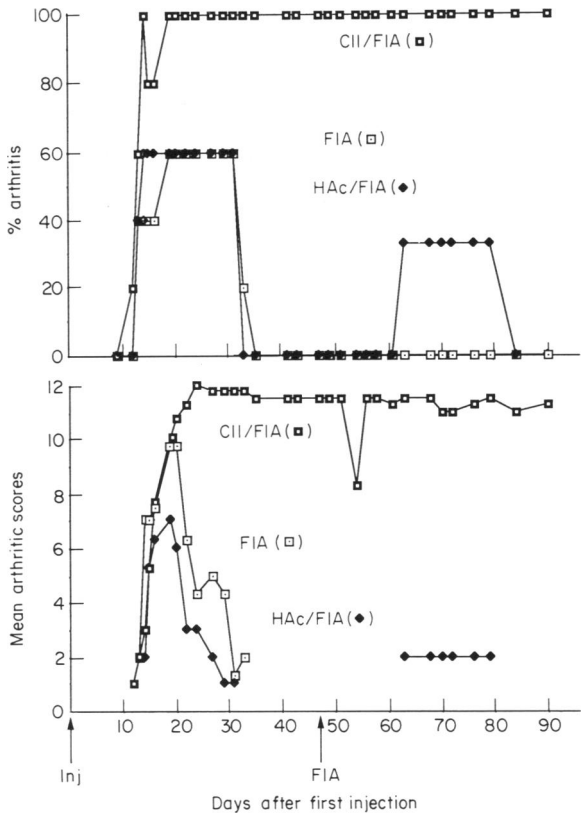

Fig. 1. Frequency and severity of arthritis after an intradermal injection of 150 μl Freund's incomplete adjuvant (FIA), 150 μg rat CII in 150 μl 0·1 M acetic acid emulsified with 150 μl FIA (CII/FIA) or 150 μl 0·1 M acetic acid emulsified in FIA (HAc/FIA) at day 0 (indicated by arrow) into DA rats (five in each group) The rats were re-injected with FIA at day 47 (indicated by arrow).

Rats recovered from OIA are resistant to a second attempt to induce OIA but not to CIA

DA rats recovered from OIA induced with FIA or FIA emulsified in 0·1 M acetic acid were challenged with a repeated injection of FIA or immunized with rat CII. These rats were resistant to OIA (Fig. 1) but not to CIA (Fig. 2). Measurements of antibody levels to rat CII in these experiments showed high levels only in sera from CII-immunized rats (Table 2). A second immunization with rat CII did not induce any detectable relapse

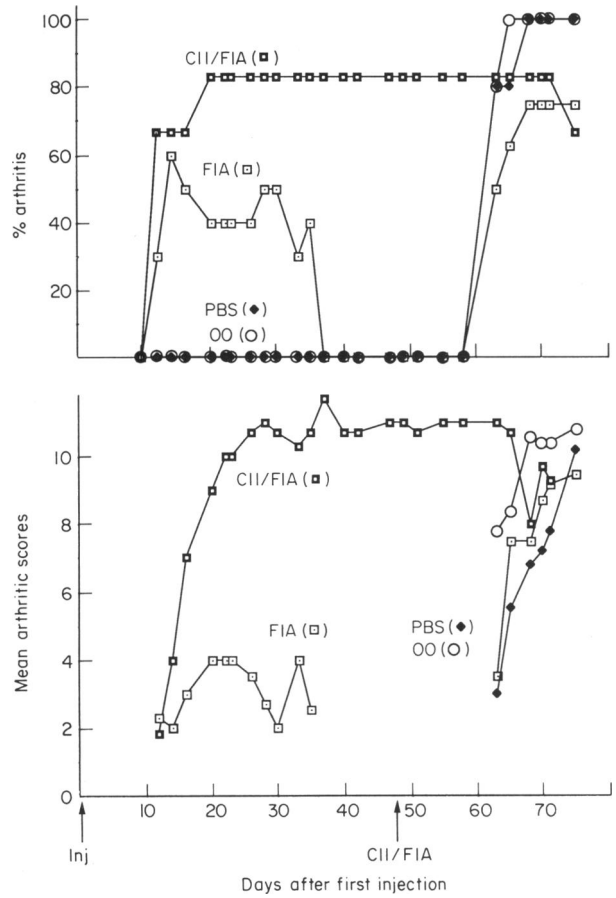

Fig. 2. Frequency and severity of arthritis after an intradermal injection of 150 μl Freund's incomplete adjuvant (FIA) (10 rats), 150 μl olive oil (OO) (five rats), 150 μg rat CII in 150 μl 0·1 M acetic acid emulsified with 150 μl FIA (CII/FIA) (10 rats) or 150 μl PBS (six rats) at day 0 (indicated by arrow) into DA rats. The rats were re-injected with CII/FIA at day 47 (indicated by arrow).

of arthritis, although this could have been difficult to detect due to the already pronounced severity, and decreased the levels of anti-CII antibody levels indicating a vaccinating effect by homologous CII.

CII in olive oil does not induce arthritis, but vaccinates against CIA but not against OIA

DA rats did not develop arthritis after injection of olive oil, which does not possess strong adjuvant activity, or after immunization with rat CII emulsified in olive oil. However, after immunization with homologous CII + FIA 35 days later, the rat-CII + olive-oil-treated rats developed less severe arthritis with a lower incidence than acetic acid + olive oil pretreated control rats (Fig. 3). Since this shows that homologous CII is able to vaccinate against an arthritis induced with an autoantigen we tried this vaccination protocol for blocking also of OIA. Pretreatment with rat CII + olive oil did not affect development of OIA as compared with a number of control treatments such as rat type I collagen + olive oil, acetic acid + olive oil and ovalbumin + olive oil (Table 3). All rats developed an acute and self-limited disease, typical of OIA. To investigate whether other joint antigens could vaccinate, the rats were also pretreated with homogenized rat chondrosarcoma tissue + olive oil. This treat-

Table 2. Anti-CII antibody levels in serum obtained from DA rats with CIA and OIA

Primary treatment (day 0)*	Anti-CII antibody levels (μg/ml ± SD) (day 20)	Secondary treatment (day 47)	Anti-CII antibody levels (μg/ml ± SD) (d75 or d86)†
FIA	0	FIA	0
HAc/FIA	0	HAc/FIA	0
CII/FIA	515 ± 552	FIA	156 ± 134
PBS	nd	CII/FIA	502 ± 172
OO	nd	CII/FIA	254 ± 68
FIA	nd	CII/FIA	770 ± 352
CII/FIA	nd	CII/FIA	123 ± 101‡

Arthritis development and the number of rats in each group are shown in Figs 1 and 2.

* FIA, Freund's incomplete adjuvant 150 μl; CII/FIA, 150 μg rat CII in 0·1 M acetic acid emulsified in 150 μl FIA, total volume 300 μl; PBS, phosphate-buffered saline 150 μl, OO, olive oil 150 μl. All injections are given intradermally at the root of the tail.

† Rats secondarily treated with FIA were bled on day 75 and rats secondarily immunized with CII/FIA were bled on day 86.

‡ Significantly lower ($P < 0.05$) compared with all other groups of rats in the same experiment (secondarily immunized with rat CII) indicating a vaccination effect by CII pretreatment.

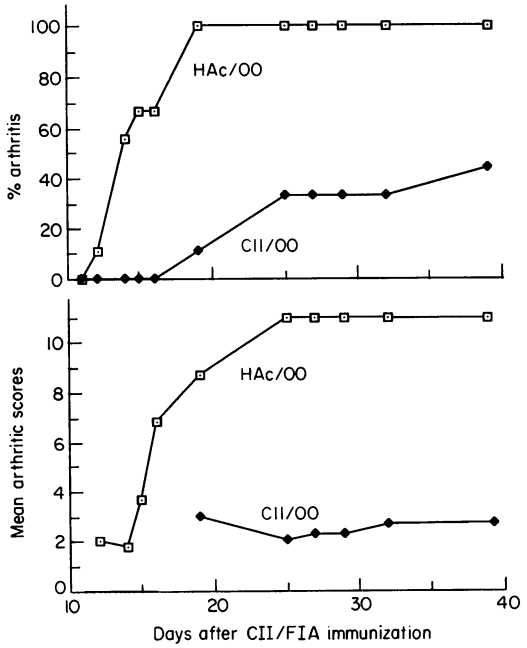

Fig. 3. Vaccination against CIA. DA rats were treated with rat CII in olive oil (CII/OO) ($n = 9$) and acetic acid in olive oil (HAc/OO) ($n = 9$). 35 days later all rats were immunized with rat CII in FIA and the development of arthritis followed by frequency in (a) and by severity in (b).

Table 3. Attempts to vaccinate against OIA with homologous collagens in olive oil

Vaccination*	*n*	Incidence (%)	Maximal mean severity
Rat CII	26	79	6·1
Rat CI	26	69	5·3
Acetic acid	8	63	5·3
Ovalbumin	9	89	7·8
RCS†	8	88	7·5

* Emulsified with olive oil and injected at the root of the tail. The collagens were dissolved in acetic acid and the ovalbumin with PBS before emulsification with olive oil. 150 μg protein and 300 μl volume was injected in each rat 35 days before induction of OIA with 150 μl FIA.

† 10% wet rat chondrosarcoma (RCS) tissue (w/v) was mixed with acetic acid. This mixture was emulsified with olive oil.

ment did not affect development of OIA suggesting that immunity to cartilage-specific antigens is not involved in the development of OIA or more likely that the pathogenetic mechanisms of OIA are partly different from those operating in CIA.

DISCUSSION

The DA strain is susceptible to induction of arthritis using FIA alone, without inclusion of mycobacterial cell wall fragments or other immunogens (OIA). The DA rat is also highly susceptible to induction of CIA after immunization with homologous CII in FIA. Thus, for the first time we are able to directly compare arthritis induced with an autoantigen and arthritis induced with a pure adjuvant without complicating effects by heterologous immunogens. Here is shown that CIA is a different disease compared with OIA, as based on several findings. Firstly, CIA develops with a chronic disease course while OIA is an acute, self-limited disease. Secondly, a strong CII autoantibody response develops in CIA but not in OIA. Thirdly, the F1(DA × Lewis) rat strain is highly susceptible for CIA but resistant to OIA. Fourthly, rats recovered from OIA are susceptible to CIA but resistant to a second attempt to induce OIA. Fifthly, using a new vaccination protocol we succeeded in vaccinating against CIA with homologous CII but using the same protocol we were not able to affect the development of OIA.

Despite these differences there are many striking similarities between CIA, induced with homologous CII, and OIA. The DA rat is susceptible to both diseases and it is likely that this strain carries genes influencing both CIA and OIA in the same way. It is also clear that activation of αβ T cells is critical in both disease models since treatment with antibodies to the αβ T cell receptor therapeutically block both diseases [14,15]. It is likely that these critically important T cells are directed at antigens specifically located in the joints since only arthritis and no signs of inflammation in other tissues are seen in both models [15]. A comparison between these models raises the question whether the observed dissimilarities are due to recognition of different joint antigens and/or if the pathogenetic mechanisms leading to arthritis are different. The present findings demonstrate that the pathogenetic mechanisms in the models are different, i.e. that the CIA, induced with homologous CII in the DA rat, is not an adjuvant arthritic disease such as OIA. As a matter of fact, these differences are interesting to consider since it might give a clue to the forces driving arthritis into a more chronic state and to the role of the very strong B cell activation to CII in the CIA. Moreover, pretreatment with homologous CII in olive oil could be shown to vaccinate against CIA induced with homologous CII. This finding might be of some interest since earlier protocols in animal models for vaccination against arthritis all involve heterologous antigens or T cells directed at heterologous antigens [16–22]. The vaccinating property of homologous CII is surprising since it could be expected that the rat is already tolerized to its own CII but apparently immunization with native CII emulsified in olive oil increases the resistance against development of arthritis after immunization with native CII in FIA. In contrast, CII vaccination does not protect against OIA. This does not necessarily imply that autoimmune reactions are not involved in OIA but may rather depend on the different pathogenesis of OIA. One possibility is that the vaccination mainly affects the strong B cell activation against CII, which is present in CIA but not in OIA, but not certain other T cell-dependent effects of importance for development of arthritis. The observed resistance against induction of OIA in rats that had been pretreated with FIA is even more obscure. This 'vaccination' effect is not induced by a previous development of arthritis *per se* since rats which failed to develop arthritis after the first injection of FIA were also 'vaccinated'. Following injection, adjuvant oil disseminates all over the reticuloendothelial system and it is possible that a refractory period of antigen-presenting cells, such as macrophages and dendritic cells, is induced. Another possibility is that adjuvant treatment leads to activation of endogenous DNA viruses, which preoccupies the immune system. It is more difficult to imagine the involvement of immunologically specific mechanisms to explain the 'vaccinating' effect since no immunogen was included in the adjuvant oil. Whatever is the mechanism it does not affect induction of CIA. 'Vaccinating' effects by pretreatment with adjuvant oil only should however be considered in the evaluation of experiments involving vaccination against various experimental autoimmune diseases. Thus, from these findings it is clear that a direct comparison between arthritis induced by immunization with a cartilage-specific autoantigen and an adjuvant arthritis type of disease demonstrates that these models for RA involve different pathogenetic mechanisms. Whether these models are critically dependent on the recognition of the same or different autoantigens could only be determined after acquiring knowledge about their different types of pathogenesis. The same is true for eventual comparisons to RA and it is likely that studies of both models will contribute to further understanding also of the human disease.

ACKNOWLEDGMENTS

This study was supported by the Swedish Medical Research Council, the National League against Rheumatism, King Gustaf V:s 80-years Foundation, Osterman foundation, Åke Wiberg foundation, Professor Nanna Svartz Research Foundation and the Craaford Research Foundation.

REFERENCES

1 Griffiths M. Immunogenetics of collagen-induced arthritis in rats. Intern Rev Immunol 1988; **4**:1–15.

2 Pearson CM. Development of arthritis, periarthritis and periostitis in rats given adjuvants. Proc Soc Exp Biol Med 1956; **91**:91–101.

3 Perlik F, Zidek Z. Breeding experiments on the frequency of adjuvant arthritis in the rat. Ann Rheum Dis 1973; **32**:72–4.

4 Trentham DE. Collagen arthritis as a relevant model for rheumatoid arthritis. Evidence pro and con. Arthritis Rheum 1982; **25**:911–16.

5 Van Eden W, Hogervorst EJM, Hensen EJ, Van Der Zee R, Van Embden JDA, Cohen IR. A cartilage-mimicking T-cell epitope on a 65K mycobacterial heat-shock protein: adjuvant arthritis as a model for human rheumatoid arthritis. Curr Top Microbiol Immunol 1989; **145**:27–43.

6 Holmdahl R, Andersson M, Goldschmidt TJ, Gustafsson K, Jansson L, Mo JA. Type II collagen autoimmunity in animals and provocations leading to arthritis. Immunol Rev 1990; **118**:193–232.

7 Larsson P, Kleinau S, Holmdahl R, Klareskog L. Homologous type II collagen-induced arthritis in rats. Characterization of the disease and demonstration of clinically distinct forms of arthritis in two strains of rats after immunization with the same collagen preparation. Arthritis Rheum 1990; **33**:693–701.

8 Holmdahl R, Jansson L, Larsson E, Rubin K, Klareskog L. Homologous type II collagen induces chronic and progressive arthritis in mice. Arthritis Rheum 1986; **29**:106–13.

9 Wooley PH, Seibold JR, Whalen JD, Chapdelaine JM. Pristane-induced arthritis. The immunologic and genetic features of an experimental murine model of autoimmune disease. Arthritis Rheum 1989; **32**:1022–30.

10 Potter M, Wax JS. Genetics of susceptibility to pristane-induced plasmacytomas in BALB/cAn: reduced susceptibility in BALB/cJ with a brief description of pristane-induced arthritis. J Immunol 1990; **127**:1591–5.

11 Smith BD, Martin GR, Dorfman A, Swarm R. Nature of the collagen synthesized by a transplanted chondrosarcoma. Arch Biochem Biophys 1975; **166**:181–6.

12 Holmdahl R, Rubin K, Klareskog L, Larsson E, Wigzell H. Characterization of the antibody response in mice with type II collagen-induced arthritis, using monoclonal anti-type II collagen antibodies. Arthritis Rheum 1986; **29**:400–10.

13 Holmdahl R, Klareskog L, Andersson M, Hansen C. High antibody response to autologous type II collagen is restricted to H-2^q. Immunogenetics 1986; **24**:84–9.

14 Goldschmidt TJ, Holmdahl R. Anti-T cell receptor antibody treatment of rats with established autologous collagen-induced arthritis. Suppression of arthritis without reduction of anti-type II collagen autoantibody levels. Eur J Immunol 1991; **21**:1327–30.

15 Holmdahl R, Goldschmidt TJ, Kleineau S, Kvick C, Jonsson R. Arthritis induced in rats with adjuvant oil is a genetically restricted, $\alpha\beta$ T-cell dependent autoimmune disease. Immunology 1992; **76:** in press.

16 Staines NA, Hardingham T, Smith M, Henderson B. Collagen-induced arthritis in the rat: modification of immune and arthritic responses by free collagen and immune anti-collagen antiserum. Immunology 1981; **44**:737–44.

17 Cremer MA, Hernandez AD, Townes AS, Stuart JM, Kang AH. Collagen-induced arthritis in rats: antigen-specific suppression of arthritis and immunity by intravenously injected native type II collagen. J Immunol 1983; **131**:2995–3000.

18 Holoshitz J, Naparstek Y, Ben-nun A, Cohen IR. Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. Science 1983; **219**:57–9.

19 Phadke K, Fouts R, Parrish JE. Collagen induced and adjuvant induced arthritis in rats. Post-immunization treatment with collagen to suppress or abrogate the arthritic response. Arthritis Rheum 1984; **27**:797–806.

20 Larsson P, Holmdahl R, Klareskog L. *In vivo* treatment with anti-CD8 and anti-CD5 monoclonal antibodies alters induced tolerance to adjuvant arthritis. J Cell Biochem 1989; **40**:1–8.

21 Billingham MEJ, Carney S, Butler R, Colston MJ. A mycobacterial 65 kD heat shock protein induces antigen-specific suppression of adjuvant arthritis but is not itself arthritogenic. J Exp Med 1990; **171**:339–44.

22 Zhang ZJ, Lee CSY, Lider O, Weiner HL. Suppression of adjuvant arthritis in Lewis rats by oral administration of type II collagen. J Immunol 1990; **145**:2489–93.