Agents and Actions, vol. 19, 5/6 (1986)

0065-4299/86/060295-11 $ 1.50+0.20/0
© 1986 Birkhäuser Verlag, Basel

Role of T lymphocytes in murine collagen induced arthritis

R. Holmdahl*, L. Klareskog*, K. Rubin*, J. Björk**, G. Smedegård**, R. Jonsson***
and M. Andersson*

* Department of Medical and Physiological Chemistry, University of Uppsala, Uppsala, Sweden; ** Division of Pharmacology, Pharmacia, Uppsala, Sweden; *** Dept. of Oral Pathology, University of Göteborg, Göteborg, Sweden

Introduction

Collagen type II induced arthritis (CIA) was first described by David Trentham and Andrew Kang in 1977 after screening different components purified from joint tissue for arthritogenic capacity in rats [1]. Intradermal injections of fractions enriched for cartilagenous collagen (mainly type II) led to the development of a severe polyarthritis about 2 weeks after immunization. CIA has subsequently been induced in a wide variety of laboratory animals, such as mice [2] and primates [3]. The mouse model for CIA has proved to be a very rewarding model for studies of autoimmune reactions that cause arthritis. Such studies have indicated that both T-cell mediated immunity and humoral immunity are involved in the pathogenesis of CIA. The model should offer possibilities both to investigate how different immune reactions may synergize in causing arthritis, and to find ways to interfere therapeutically in a multifaceted but also well defined experimental animal arthritis model. The present view mainly concentrates on studies that illuminate the role of T lymphocytes in murine CIA. With this aim we first discuss some general features of the model, secondly we describe some of our own studies on long term cultured arthritogenic collagen II reactive T helper cells, and thirdly we describe some recent studies on the influence on T-cell immunity and on CIA of two agents of therapeutic interest in arthritis.

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

1. General features of CIA in mice

Genetics

Already the first description of CIA in mice by Courtenay and coworkers indicated the influence of sex and transplantation antigens on the susceptibility to arthritis. Further studies have yielded interesting results concerning the role of both these genetic factors in CIA.

MHC restriction

The influence of transplantation antigens on anticollagen II responsiveness and arthritis development has been investigated in detail by Wooley and collaborators who showed that the susceptibility to arthritis was determined by MHC Class II molecules and that mice carrying two different haplotypes were high responders to chick and bovine collagen II and susceptible to CIA; H2-q (e.g. DBA/1, B10G) and H2-r (e.g. B10RIII) [4–6]. They also found, however, that mice belonging to strains with certain other MHC haplotypes (e.g. H2-d) responded with high antibody titers to various heterologous type II collagens but were resistent to CIA [4–6]. We have recently investigated the MHC immune response pattern to homologous type II collagen and found

in this case a better correlation between CIA susceptibility and the anti type II collagen antibody response [7]. This suggested that the development of an immune response to structures on autologous collagen II rather than to structures on the immunized heterologous collagen II was the critical feature that determined arthritis development.

Sex linkage

Courtenay *et al.* [2] found in 1980 that male mice were more susceptible to CIA than their female counterparts, but this observation was not further substantiated. When performing a larger series of experiments we found that all our collagen II preparations yielded a higher frequence of arthritis in DBA/1 males as compared to females [8]. We furthermore noted that CIA-susceptible mouse strains that carry the H2-q haplotype, but possess different non-MHC gene backgrounds, all display a male preponderance for arthritis. The high male susceptibility to CIA was found not to be dependent on arthritogenic genes located on the Y chromosome [8], something that is the case for the autoimmune disease in BXSB males [9]. Instead there is evidence that sex hormones are mainly responsible for the observed sex linkage as castrated DBA/1 females display an increased susceptibility to CIA as compared to normal females [10]. Furthermore, estrogen treatment of castrated females reinduces the low susceptibility to CIA in normal females.

Connection between CIA and anti-collagen II immune response

Both a T and a B-cell response has been reported to occur after immunization with various heterologous type II collagens and there has been a discussion on the relative contributions of T-cell and B-cell mediated immunity in the induction of CIA [8, 11–15]. We have analysed the production of anti-collagen antibodies in DBA/1 mice immunized with type II collagens from various species and found that immunization with all the available heterologous collagens induced an antibody response also to mouse type II collagen [13]. The auto anti-collagen II antibody titers were not, however, higher in arthritic than in non-arthritic mice. Mice immunized with mouse collagen II on the other hand, developed relatively low titers of auto-anti collagen II antibodies as compared to animals immunized with heterologous collagens. Analysis on individual sera from mice immunized with mouse collagen furthermore showed that there was no correlation between levels of circulating auto anti collagen antibodies and arthritis development [14]. Thus, some mice developed arthritis in spite of very low levels of circulating auto-anti-collagen II antibodies, whereas other mice with high levels of such antibodies remained healthy. Antibodies reactive with denatured collagen II were only detected in animals with severe arthritis, and these antibodies, which occurred in relatively low amounts, were only seen subsequent to arthritis development.

These findings suggested that the major part of the antibody response evoked after immunization with heterologous or homologous native type II collagen should be viewed as a conventional antibody response to an injected antigen. A minor part of the auto-anti collagen response might in addition contribute to arthritis development and/or be a consequence of the arthritis; at least from our data production of antibodies to denatured collagen appeared preferentially to be a consequence of arthritis. In view of these considerations as to the auto anti collagen II immune response in CIA, the observed differences as to levels and reactivity pattern of the anti collagen II responses in CIA and in certain cases of human RA, may not be as significant as previously assumed [11, 16].

Chronic and progressive arthritis induced with homologous type II collagen

As earlier shown by Trentham *et al.* [1] in the rat system, not only heterologous collagen II, but also homologous collagen II may be used to induce CIA, thus providing us with models where autoimmune reactions leading to arthritis can be induced without the introduction of foreign determinants on the inducing collagen. When we investigated the arthritis that could be induced in mice after injection of homologous collagen II we observed several interesting features of the induced disease that had not previously been noted after immunization with heterologous collagens [14].

The onset of clinical arthritis after immunization with homologous collagen was late and some-

times delayed for up to 5 months or longer after immunization. The first clinical sign to appear was often monoarthritis, typically in an interphalangeal joint. The disease then usually progressed slowly with an involvement of an increasing number of joints, and the course was characterized by fluctuation in intensity, with both overt and subclinical episodes of arthritis. Histopathologic findings indicated that the onset of disease in most mice was insidious and that a subclinical arthritis had often been present prior to the clinical onset.

Histopathology

We have previously described the cell pattern of arthritic joints from rats with CIA using immunohistochemical techniques [17, 18] and have now extended these studies to murine CIA (manuscript in preparation). As in the rat, the mouse synovium exhibited prominent Ia expression seen on many cells in the pannus tissue, especially close to the destroyed cartilage and bone. Most of the infiltrating lymphocytes carried the T helper phenotype but, notably, these cells were not abundant and, in later stages of disease mainly occurred at some distance from the areas where cartilage destruction hade taken place. None or very few B cells were found in the specimens investigated. These findings suggested to us that the T-cells are unlikely to be direct effectors of cartilage destruction but that they rather have critical functions in stimulating other effector cells such as macrophages to induce the cartilage and bone destruction, and that mechanisms not directly involving specific T-cell immunity may be effective in the arthritic process, at least in late stages of the disease.

Induction and perpetuation of CIA

Two types of experiments are important in the studies of the relative contributions of T- and B-cell immunity to disease development; specific impairment of functions *in vivo* and transfer of cells or antibodies to animals *in vivo*.

The impairment experiments performed so far have provided additional evidence for the participation of both T and B-cell immunity in the disease process, but have not been able to give any firm data on the relative importance of the different types of immune reactions. Evidence for the importance of T cells has been obtained from experiments showing that thymus-deficient, nude rats [17] and mice (unpublished observations) do not acquire CIA, and from the demonstration that arthritis development in DBA/1 mice can be prevented by injections of monoclonal antibodies directed against the CD4 receptor on T helper cells [19]. The corresponding experiments on the role of antibodies in CIA have demonstrated that development of CIA in rats is suppressed by anti-IgM treatment of young rats [20] and that the susceptibility to arthritis is decreased in complement-depleted rats [21].

Reconstitution experiments have involved transfer with both antibodies and lymphoid cells (see also next sections on transfer of long term cultured T cells). As to cell transfer, the first successful transfer of arthritis with lymphoid cells was reported by Trentham *et al.* [22] who showed that spleen cells from arthritic rats injected intravenously into naive recipients induced arthritis. Subsequently it has been shown that a collagen II specific T-helper cell derived lymphokine can induce arthritis after being injected into knee joints of previously healthy rats [23].

Reconstitution studies using serum fractions or purified anti-collagen antibodies have been carried out in both rats and mice and in both species synovitis can be induced by this kind of treatment [24, 25]. This antibody-mediated synovitis has been shown to be transient and to have its onset as early as 24–48 hours after the injection of antibodies. The histopathology is somewhat different from the one seen in CIA, i.e. no pannus formation with mononuclear cell infiltration or cartilage destruction is present. Instead a transient accumulation of granulocytes in the synovial tissue is seen together with immune complex deposition at the cartilage surface. Evidence that T cells are not involved in the effector phase of this antibody-mediated disease has been provided by the demonstration that collagen II antibodies induce arthritis in mouse strains that do not acquire arthritis after collagen II immunization [25].

We have in our laboratory analysed the specificity and arthritogenic properties of monoclonal antibodies reactive to mouse type II collagen [15, 26]. These studies have demonstrated that only a very mild synovitis could be induced with these monoclonals, even though they reacted strongly with

mouse type II collagen and were of a relevant, complement-fixing isotype (IgG2a). The synovitis was, however, equally mild when mixtures of monoclonal anti-collagen II antibodies with different specificities or even when affinity purified anti type II collagen antibodies from arthritic DBA/1 mice were used (unpublished observations). These studies thus suggest that anti-collagen II antibodies are not themselves sufficient to give rise to the lesions that characterize CIA.

2. Collagen II reactive T cell lines

In vitro establishment of collagen II reactive murine T helper lymphocytes

In order to investigate the role of collagen II reactive T cells in CIA we wanted to take advantage of recent techniques for culture of antigen specific T cells *in vitro*. In the late 1970s several groups succeeded in establishing T-helper cells in long term culture [27, 28]. Irun Cohen and coworkers were the first to use T cells cultured *in vitro* to investigate their effect on autoimmunity *in vivo*. Thus, Ben-Nun *et al.* [29] showed that lines of T helper cells, reactive with basic protein of nerve tissue, could transfer experimental allergic encephalomyelitis (EAE) to previously healthy rats. Subsequently, the same group also succeeded in transferring other experimental diseases such as autoimmune thyroiditis to mice [30] and adjuvant arthritis to rats [31] with the help of similarly established lines and clones of T helper cells specific for the different relevant inducing antigens. Recently, reports have also appeared from other groups which have repeated and extended some of these findings on T cell transfer of experimental autoimmune disease [32, 33].

However, whereas a few groups have succeeded in using *in vitro* established T cells for functional studies *in vivo*, many other groups have not been so successful, something that may be due to both practical and more basic problems. Thus, there are often considerable difficulties associated with the establishment *in vitro* of antigen specific T cell lines, especially against antigens towards which only weak proliferative responses are seen in primary cultures. Most cell lines that are established also tend to stop proliferating after 1–2 months in culture, and only in some of these lines can cells be rescued for further expansion. The more basic problem is associated with the possibility that the phenotype of a cell that has been cultured for a long time *in vitro* is changed so that the cell cannot fulfill its physiological functions when injected back *in vivo* (compare [34]). Thus many *in vitro* cultured cells do not migrate properly *in vivo*, but are trapped by the reticuloendothelial system to almost 100%. It is also likely that many *in vitro* cultured cells change their karyotypes and this might in particular be the case for the relatively small fraction of cells that can be propagated in long term culture. Despite the obvious need for studies on the phenotypic characteristics that permit an adequate migration *in vivo* and to find culture conditions that permit the maintenance of this phenotype *in vitro*, some functional *in vivo* experiments should be possible with our current knowledge.

We decided to establish anti collagen II reactive T cell lines for further *in vivo* work. We chose to work with the DBA/1 strain which is a high responder to collagen II and highly arthritis prone.

Our first experiments on lymph node cells from rat collagen II primed mice showed that auto anti collagen II reactive T cells are induced by this immunization procedure. In fact also priming the mice with mouse collagen II triggered collagen reactive T cells [35].

The lymph node cell cultures were then used as a source of cells for generation of T cell lines – the technique is illustrated in Figure 1. Briefly, the T cells were alternatingly selected for antigen specificity in cultures containing antigen presenting cells and antigen, and subsequently propagated in cultures containing media enriched for T cell growth factors. This protocol is known to select for T helper cells [27, 28]. A number of different collagen II reactive T cell lines were generated using this protocol and were subsequently used for specificity analysis *in vitro* and for functional studies *in vivo* [36].

T helper cells recognize homologous type II collagen in vitro

When the specific proliferative responses of lymph node cells from collagen primed mice were analysed, the responses to collagen II were remarkably weak as compared to the response to the "strong" PPD antigen [35]. This low response could be due to either a clonal abortion of T cells

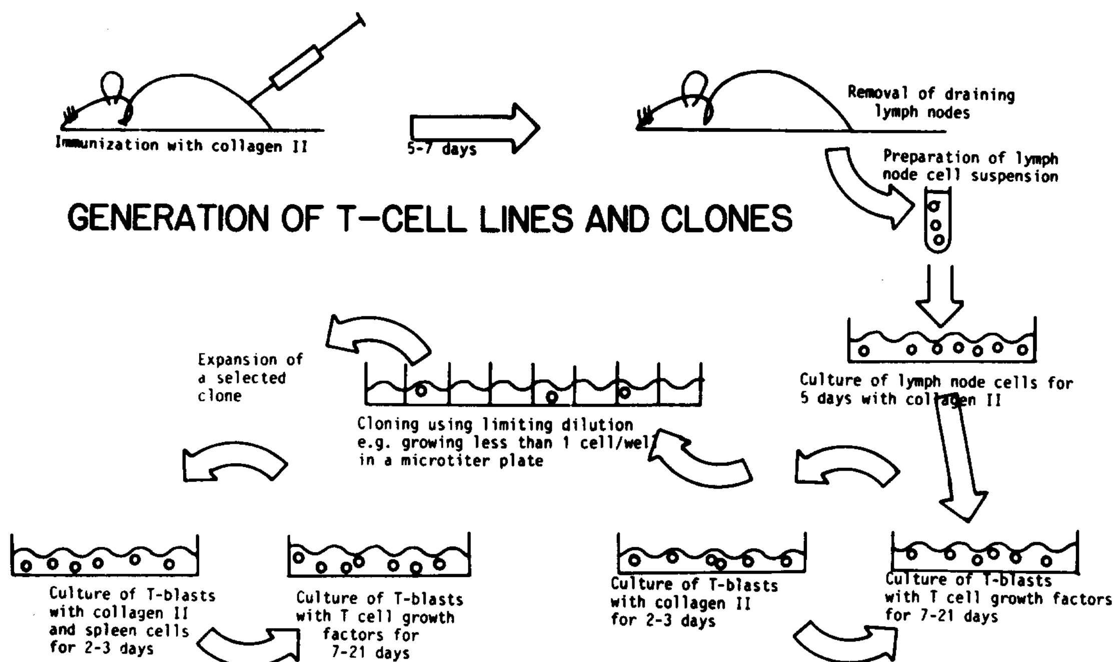

Figure 1
Summary of the procedure for generation of type II collagen reactive T cell lines and clones.

displaying a high affinity for homologous collagen or that collagen II reactive cells occur in very low numbers. We have found the latter possibility to be most likely, as the selected T cell lines, notably the DCR line, reacted with high affinity with collagen II derived from various species, including the mouse. In fact, concentrations as low as 10 ng/ml of mouse collagen II evoked a proliferative response in the DCR cells. The collagen II driven cells showed no reactivity with collagen type I, which has about 60% primary sequence homology to type II, or with synthetic collagen-like polypeptide chains, indicating that our T cells do not recognize Pro-Gly moieties. This has been suggested to be the case for anti-collagen II reactive T cells in previous studies [11, 37].

T helper cells reactive with type II collagen induce arthritis

Having established and characterized collagen II reactive T cell lines and clones, we investigated if cells from these lines and clones could induce arthritis in naive recipients. Most of this work was carried out with the DCR line or with the DCR derived clone DCR34 using the ovalbumine reactive cell line OC as a control. Before injection *in vivo* the cells were activated with antigen or mitogen (Concanavalin A). Such an activation before transfer had previously been shown to be necessary for induction of EAE in the studies by Ben Nun and coworkers [29]. Injection of high numbers of cells (up to 10×10^6) from the DCR line only occasionally induced macroscopically visible arthritis. However, when histopathological examination was performed 10 days after injection, severe lesions were detected in all animals. These lesions had an appearence similar to those seen in mild stages of conventionally induced CIA. Thus, arthritis induced with 1×10^6 DCR cells was characterized by a proliferative synovitis and by periosteitis. The most marked infiltration of mononuclear cells was seen close to the cartilage in the marginal zone. More severe lesions including pannus formation and cartilage

destruction were induced with 10×10^6 DCR cells. Interestingly, the recipients acquired T-cell transferred arthritis both after irradiation with 750 rad and without any pretreatment at all. As irradiation with 750 rad is lethal for mature lymphocytes [38], it appears that the arthritis induced by T-cells is independent of antibody producing B-lymphocytes.

An indirect indication that other cells may synergize with the injected T-cells in arthritis development is the demonstration that normal non-irradiated mice developed a more severe arthritis than the irradiated mice. This finding is in contrast to what has been reported by Holoshitz et al. [31] for adjuvant arthritis in rats, where mycobacterium reactive T helper cells induced arthritis in irradiated, but not in normal recipients. This indicates that somewhat different cellular mechanisms may be working in adjuvant arthritis as compared to CIA.

Two immediate questions arise from the results of the present T cell transfer experiments. (1) To what extent do the collagen reactive T-helper cells migrate to the joints were arthritis develops, and which mechanisms mediate the migration of T cells to the joints. (2) In which way do the T cells that have indeed entered the joint give rise to the inflammatory infiltrate and the joint destruction?

Concerning the first question as to how the injected autoreactive T helper cells home to their target in otherwise normal mice, we noticed in pilot experiments that ^{51}Cr-labelled T-cells were, to a large extent, caught by the reticuloendothelial system (RES) upon injection *in vivo*. Furthermore, we have noticed that many of our T cell lines upon prolonged culture *in vitro* change their morphologic characteristics and become highly adherent to plastic paralleling a loss of arthritogenicity in spite of the fact that they retain high reactivity against mouse collagen II *in vitro*. Nevertheless, for the arthritogenic T cell lines, a few injected T cells must obviously have migrated to the joints in order to induce the observed arthritis.

Concerning the second question as to which events in the joints are triggered by the injected collagen II reactive T cells, certain speculations can be made on the assumption that a similar pattern of cells is present in the joints after T cell transfer as is seen in conventionally induced CIA (see above); the histopathologic picture in the two states are indeed very similar. It is known that activated T helper cells secrete various lymphokines that activate inflammatory cells (reviewed in [39]). One of these lymphokines is gammainterferon (gIF) which may induce increased Ia expression on a number of different cells, including macrophages [40, 41]. This increased Ia-expression might provide a prerequisite for an enhanced presentation of antigen to infiltrating T helper cells in the joint. Antigens that may thus be presented to T cells in the synovial tissue are obviously cartilage derived molecules such as collagens that are exposed in increasing amounts during joint inflammation, for example in RA in humans [42]. A self-perpetuating inflammatory reaction, similar to the one suggested to occur in rheumatoid arthritis [43] might thus develop subsequent to the T cell transfer as depicted in Figure 2. It is also possible that B-cells producing arthritogenic antibodies can be triggered by collagen reactive T-helper cells and that these antibodies can synergize in inducing the full-blown antigen induced CIA. In fact, it has recently been shown by other investigators that type II collagen reactive T cell lines can have both B-cell stimulating and DTH stimulating properties [44, 45].

We hope that the establishment of collagen II reactive T cells will make it possible to study the molecular events involved in the activation of such potentially arthritogenic cells. The experiments carried out so far have demonstrated that the different T cell lines a) recognize both denatured and native collagens [36, 44–46], presumably due to a previous processing of the collagen molecules by accessory cells; both spleen cells and epidermal Langerhans cells have been shown to carry out such a processing [47]. b) T cell recognition is restricted to I-A^q [35, 44].

Although our T cell lines were cultured *in vitro* for at least 6 months under selective conditions before these experiments were performed they might still be heterogenous. The lines were therefore cloned using limiting dilution techniques. Two different sets of clones were derived and characterized (Table 1). One set of clones reacted with arthritogenic mouse type II collagen as well as with type II collagens from other species. This set also reacted with purified beta (II) components, but showed no reactivity with purified alpha (II) chains. This suggested that these clones (among them the previously described DCR34

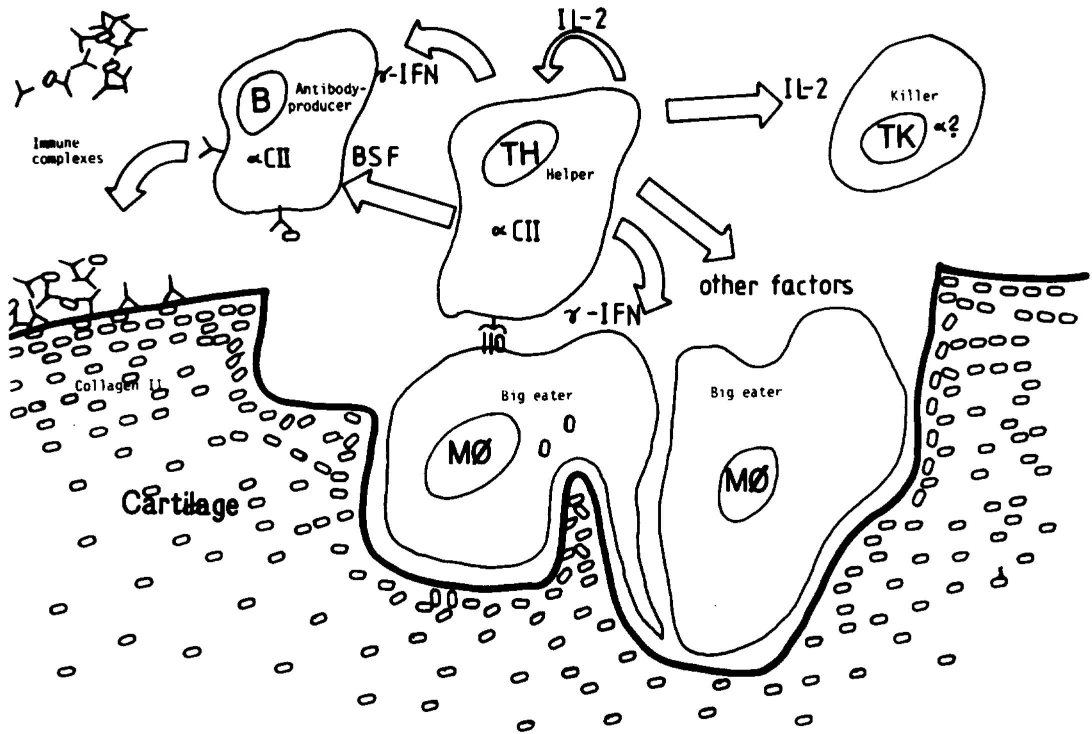

Figure 2
An hypothetical immune focus eroding the cartilage.

Table 1
Specificities of type II collagen reactive clones^{+}.

T cell clone	Stimulating antigen (10 μg/ml)					
	Native mouse C II	Native rat C II	Native bovine C II	Bovine alpha (II)	Bovine beta (II)	Native rat C I
DCR 34	116 ±2*	121±28	187 ±14	1.6± 0.2	66±12	0.8±0.1
DC4	2.2±0.7	46± 5	6.7± 2.3	nd£	n.d.	1.8±0.1
NC 18$	11 ±2	92± 2	32 ± 4	77 ±18	n.d.	3.2±0.2

$^{+}$ Measured in a (^{3}H) thymidine incorporation assay as earlier described (36).
* Mean cpm value of triplicates (mean ± SD) × 10^{3}.
£ Not determined in the present assay.
$ The antigens were used at an concentration of 20 μg/ml in the assay were NC 18 cells were tested.

clone) recognize a determinant that is either dependent on intact cross-links between collagen chains or is located in the telopeptide region of the collagen II molecule. The other set of clones reacted poorly with mouse type II collagen but more strongly with various heterologous type II collagens as well as with their purified alpha (II) chains (examples of this set of clones are the previously described DC4 and NC 18 clones). Since both DCR 34 and DC4 were derived from the arthritogenic DCR line we cannot at present conclude whether one or both of these sets of clones can induce arthritis when transferred into healthy mice *in vivo*.

It can be assumed, however, that the availability of these sets of defined collagen II reactive T helper cells clones will enable us to define in detail which structure(s) of the collagen II molecule are "arthritogenic". Such molecular studies on the requirements for activation of autoreactive, disease- inducing T cells may be particularly rewarding in murine CIA with the availability of well defined inbred mouse strains where collagen responsiveness and CIA susceptibility is dependent on minor differences in the I-A coded beta chain [7].

3. Use of murine CIA to investigate the effects of agents of interest in arthritis treatment

CIA in mice has hitherto received only a limited interest as a model for evaluation of various anti-inflammatory drugs [48], and most investigators have used the collagen induced arthritis or adjuvant arthritis in rats as models. With the growing possibilities to analyse different aspects of the arthritogenic immune reactions in mice that have been reviewed above, it is feasible, however, that CIA in mice will be a useful complement to earlier models of arthritis also in evaluation of anti-arthritic treatment regimens. We will here briefly discuss two different example of agents, estrogen and sulfasalazine, that may interact with specific parts of the immune system during arthritis development.

Suppression of CIA with estrogen

As discussed above, normal female DBA/1 mice are less susceptible to CIA than their normal male counterparts. We have recently been able to demonstrate that this suppressive effect may be mediated by estrogen by showing that administration of low doses of estradiol benzoate (0.2 μg, twice a week/mouse) to castrated females partially prevents the development of arthritis [10]. We also found that it is the T-cell mediated immunity to collagen type II that is primarily affected by the administration of estrogen. Thus, T-lymphocytes from castrated mice that had received estrogen treatment showed a diminished proliferative response when challenged with type II collagen and estrogen treatment was shown to suppress the T-cell dependent IgG anti type II collagen response but enhance the partly T-cell independent IgM anti-collagen reponse.
Interestingly there are indications that these findings may be of significance not only for our understanding of the CIA models but may also provide an additional clue to the divergent actions of estrogen on different human diseases such as systemic lupus erythematosus and rheumatoid arthritis. Thus, systemic lupus erythematosus, as well as experimental lupus in NZB/W mice, are exaggerated by estrogen treatment [49, 50] and T cell independent auto antibody production is, in these cases, stimulated by estrogen. On the other hand, it has also previously been shown that in certain situations – as in CIA – estrogens display a suppressive effect on T cell immunity [51]. It is thus tempting to speculate that a suppressive effect on T cell immunity – similar to the one seen in murine CIA – may be responsible for the findings in a number of epidemiologic studies showing that women who have received estrogen treatment are partially protected from rheumatoid arthritis [52, 53].

Modulation of CIA with sulfasalazine

Sulfasalazine (SASP) is a drug which has been claimed to have positive clinical effects in rheumatoid arthritis comparable to those of gold or penicillamine [54]. We have evaluated the effect of SASP when administered prophylactically to DBA/1 mice immunized with rat type II collagen. Treatment with SASP, both orally in a dose of 50 mg/kg and subcutaneously in a dose of 12.5 mg/kg, caused a delay in the onset of arthritis (Table 2 and Figure 3). As shown in Fig. 3, subcutaneous injections of SASP also reduced the arthritis incidence. No inhibition of the severity of

Table 2
Effects on the onset day of CIA by treatment with SASP.

Treatment	Mean onset day ± SD	Significance level (Student t-test)
Saline per orally	33.1 ± 6.8	$p < 0.05$
SASP 50 mg per orally	45.5 ± 17.3	
Saline subcutaneously	29.9 ± 7.5	$p < 0.05$
SASP 12.5 mg subcutaneously	38.7 ± 6.9	

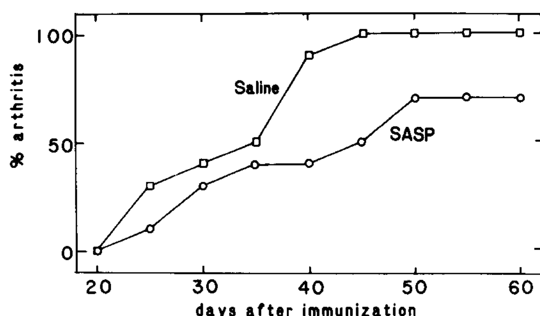

Figure 3
Development of CIA among DBA/1 mice immunized with rat type II collagen and treated orally with either 50 mg/kg SASP (○—○) or an equal volume saline (□—□). Each group consists of 10 mice.

the lesions was however observed. We have not as yet any clearcut data on the mechanisms via which SASP may affect arthritis development. Earlier data on the attenuation of T-cell proliferation *in vitro* by this drug [55] indicate, however, that SASP may affect CIA by interfering with T-lymphocytes involved in arthritis formation.

4. Summary: CIA as a model for RA?

Differences and similarities between collagen induced arthritis and rheumatoid arthritis have been widely discussed since the establishment of the collagen arthritis model and the questions on the relevance of CIA as a model for RA will necessarily unresolved remain until we acquire more knowledge on the etiology of RA. There are, however, especially from the perspective discussed in the present review, several interesting parallels between RA and CIA. Thus, in both diseases, there is strong evidence for the role of local T helper cell activation as one important link in disease development and/or propagation. This evidence comes, in the human system, both from immunomorphologic and functional studies on human rheumatoid synovial cells (see for example [43]). Irrespective of the fact that we do not know which antigen(s) are primarily responsible for the T cell activation in the human disease, the hypothesized similarities as to the role and regulation of arthritogenic T lymphocytes should make the CIA in both mice and rats interesting as models not least for the development of therapeutic agents with their primary actions on T lymphocytes. Of particular interest in this context should be the type of chronic and progressive arthritis induced in mice with homologous collagen, where not only prophylactic but also therapeutic regimens might be appropriately investigated.

Acknowledgements

This work was supported by grants from the Swedish Medical Research Council, from King Gustaf V's 80-year foundation and from the National Swedish Board for Technical Development.

References

[1] D. E. Trentham, A. S. Townes and A. H. Kang, *Autoimmunity to type II collagen: an experimental model of arthritis.* J. Exp. Med. *146*, 857–868 (1977).
[2] J. S. Courtenay, M. J. Dallman, A. D. Dayan, A. Martin and B. Mosedale, *Immunization against heterologous type II collagen induces arthritis in mice.* Nature *283*, 666–668 (1980).
[3] E. S. Cathcart, W. A. Gonnerman, K. C. Hayes, V. Agnello, A. A. Lazzari and C. Franzblau, *Experimental arthritis in a nonhuman primate. I. Induction by bovine type II collagen.* Lab. Invest. *54*, 26–31 (1986).
[4] P. H. Wooley, H. S. Luthra, J. M. Stuart and C. S. David, *Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I-region) linkage and antibody correlates.* J. Exp. Med. *154*, 688–700 (1981).
[5] P. H. Wooley, A. M. Dillon, H. S. Luthra, J. M. Stuart and C. S. David, *Genetic control of type II collagen induced arthritis in mice: factors influencing disease susceptibility and evidence for multiple MHC-associated gene control.* Transpl. Proc. Vol. *XV*, 180–185 (1983).
[6] P. H. Wooley, H. S. Luthra, M. M. Griffiths, J. M. Stuart, A. Huse and C. S. David, *Type II collagen induced arthritis in mice. IV. Variations in immunognetic regulation provide evidence for multiple arthritogenic epitopes on the collagen molecule.* J. Immunol. *135*, 2443–2451 (1985).
[7] R. Holmdahl, L. Klareskog, M. Andersson and C. Hansen, *High antibody response to autologous type II collagen is restricted to $H\text{-}2^q$.* Immunogenetics *24*, 84–89 (1986).
[8] R. Holmdahl, L. Jansson and M. Andersson, *Female sex hormones suppress development of collagen induced arthritis.* Arthritis Rheum. (in press) (1986).
[9] E. D. Murphy and J. B. Roths, *A Y-chromosome associated factor in strain BXSB producing accelerated autoimmunity and lymphoproliferation.* Arthritis Rheum. *22*, 1188–1194 (1979).
[10] R. Holmdahl, L. Jansson, B. Meyerson and L. Klareskog, *Collagen arthritis as a model for estrogen induced protection against T cell dependent inflammatory joint disease.* (submitted for publication).
[11] J. M. Stuart, A. S. Townes and A. H. Kang, *Nature and specificity of the immune response to collagen in type II collagen induced arthritis in mice.* J. Clin. Invest. *69*, 673–683 (1982).
[12] W. C. Watson and A. S. Townes, *Genetic susceptibility to murine collagen II autoimmune arthritis. Proposed relation-*

ship to the IgG2 autoantibody subclass response, complement C5, major histocompatibility complex (MHC) and non MHC-loci. J. Exp. Med. *162*, 1878–1891 (1985).

[13] R. Holmdahl, L. Jansson, D. Gullberg, K. Rubin, P.-O. Forsberg and L. Klareskog, *Incidence of arthritis and autoreactivity of anti-collagen antibodies after immunization of DBA/1 mice with heterologous and autologous collagen II.* Clin. exp. Immunol. *62*, 639–646 (1985).

[14] R. Holmdahl, L. Jansson, E. Larsson, K. Rubin and L. Klareskog, *Homologous type II collagen induces chronic and progressive arthritis in mice.* Arthritis Rheum. *29*, 106–113 (1986).

[15] R. Holmdahl, K. Rubin, L. Klareskog, E. Larsson and H. Wigzell, *Characterization of the antibody response in mice with type II collagen induced arthritis, using monoclonal anti-type II collagen antibodies.* Arthritis Rheum. *29*, 400–410 (1986).

[16] J. M. Stuart, A. S. Townes and A. H. Kang, *Collagen autoimmune arthritis.* Ann. Rev. Immunol. *2*, 199–218 (1984).

[17] L. Klareskog, R. Holmdahl, E. Larsson and H. Wigzell, *Role of T-lymphocytes in collagen II induced arthritis in rats.* Clin. exp. Immunol. *51*, 117–125 (1983).

[18] R. Holmdahl, K. Rubin, L. Klareskog, L. Dencker, G. Gustafsson and E. Larsson, *Appearence of different lymphoid cells in synovial tissue and in peripheral blood during the course of collagen II induced arthritis in rats.* Scand. J. Immunol. *21*, 197–204 (1985).

[19] G. E. Ranges, S. Sriram and S. M. Cooper, *Prevention of type II collagen-induced arthritis by in vivo treatment with anti-L3T4.* J. Exp. Med. *162*, 1105–1110 (1985).

[20] S. E. Helfgott, H. Bazin, A. Dessein and D. E. Trentham, *Suppressive effects of anti-μ serum on the development of collagen arthritis in rats.* Clin. Immunol. Immunopathol. *31*, 403–411 (1984).

[21] K. Morgan, R. B. Clague, M. J. Shaw, S. A. Firth, T. M. Twose and P. J. L. Holt, *Native type II collagen-induced arthritis in the rat: the effect of complement depletion by cobra venom factor.* Arthritis Rheum. *24*, 1356–1362 (1981).

[22] D. E. Trentham, R. A. Dynesius and J. R. David, *Passive transfer by cells of type II collagen-induced arthritis in rats.* J. Clin. Invest. *62*, 359–366 (1978).

[23] S. M. Helfgott, R. Dynesius-Trentham, E. Brahn and D. E. Trentham, *An arthritogenic lymphokine in the rat.* J. Exp. Med. *162*, 1531–1545 (1985).

[24] J. M. Stuart, M. A. Cremer, A. S. Townes and A. H. Kang, *Type II collagen induced arthritis in rats. Passive transfer with serum and evidence that IgG anticollagen antibodies can cause arthritis.* J. Exp. Med. *155*, 1–16 (1982).

[25] J. M. Stuart and F. J. Dixon, *Serum transfer of collagen-induced arthritis in mice.* J. Exp. Med. *158*, 378–393 (1983).

[26] R. Holmdahl, T. Moran and M. Andersson, *A rapid and efficient immunization protocol for production of monoclonal antibodies reactive with autoantigens.* J. Immunol. Methods *83*, 379–384 (1985).

[27] M. H. Schreier, N. N. Iscove, R. Tees, L. Aarden and H. von Boehmer, *Clones of killer and helper T cells: growth requirements, specificity and retention of function in long term culture.* Immunol. Rev. *51*, 315–336 (1980).

[28] A. Ben Nun and I. R. Cohen, *Experimental autoimmune encephalomyelitis (EAE) mediated by T cell lines: Process of selection of lines and characterizations of the cells.* J. Immunol. *129*, 303–310 (1982).

[29] A. Ben Nun, H. Wekerle and I. R. Cohen, *The rapid isolation of clonable antigen specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis.* Eur. J. Immunol. *11*, 195–199 (1981).

[30] R. Maron, R. Zerubavel, A. Friedman and I. R. Cohen, *T Lymphocyte line specific for thyroglobulin produces or vaccinates against autoimmune thyroiditis in mice.* J. Immunol. *131*, 2316–2322 (1983).

[31] J. Holoshitz, Y. Naparstek, A. Ben-Nun and I. R. Cohen, *Lines of T lymphocytes induce or vaccinate against autoimmune arthritis.* Science *219*, 56–60 (1983).

[32] C. Linington, S. Izumo, M. Suzuki, K. Uyemura, R. Meyerman and H. Wekerle, *A permanent rat T cell line that mediates experimental allergic neuritis in the Lewis rat in vivo.* J. Immunol. *133*, 1946–1950 (1984).

[33] J. Trotter, S. Sriram, L. Rassenti, C.-H. Chou, R. B. Fritz and L. Steinman, *Characterization of T cell lines and clones from SJL/J and (Balb/cxSJL/J)F_1 mice specific for myelin basic protein.* J. Immunol. *134*, 2322–2327 (1985).

[34] J. Hall, *The study of circulating lymphocytes in vivo, a personal view of artifice and artifact.* Immunol. Today *6*, 149–152 (1985).

[35] R. Holmdahl, *Collagen induced arthritis; pathogenetic mechanisms and relevance for rheumatoid arthritis.* Comprehensive summaries of Uppsala dissertations from the faculty of Medicine 2 (1985).

[36] R. Holmdahl, L. Klareskog, K. Rubin, E. Larsson and H. Wigzell, *T lymphocytes in collagen II induced arthritis in mice. Characterization of arthritogenic collagen II specific T cell lines and clones.* Scand. J. Immunol. *22*, 295–306 (1985).

[37] L. F. Rosenwasser, R. S. Bhatnagar and J. D. Stobo, *Genetic control of the murine T lymphocyte proliferative response to collagen: analysis of the molecular and cellular contributions to immunogenicity.* J. Immunol. *124*, 2854–2862 (1980).

[38] G. Doria, G. Agarossi and L. Adorini, *Selective effects of ionizing radiations on immunoregulatory cells.* Immunol. Rev. *65*, 23–53 (1983).

[39] P. H. Krammer, B. Echtenacher, D. Gemsa, U. Hamann, L. Hultner, B. Kaltmann, U. Kees, C. Kubelka and F. Macucci, *Immune-interferon (IFN-gamma), macrophageactivating factors (MAFs) and colony stimulating factors (CSFs) secreted by T cell clones in limiting dilution microcultures, long term cultures, and by T cell hybridomas.* Immunol. Rev. *76*, 5–28 (1983).

[40] M. G. Scher, D. I. Beller and E. R. Unanue, *Demonstration of a soluble mediator that induces exudates rich in Ia-positive macrophages.* J. Exp. Med. *152*, 1684–1698 (1980).

[41] M. Nakamura, T. Manser, G. D. N. Pearson, M. J. Daley and M. L. Gefter, *Effect of IFN-gamma on the immune response in vivo and on gene expression in vitro.* Nature *307*, 381–382 (1984).

[42] L. Klareskog, O. Johnell, A. Hulth, R. Holmdahl and K. Rubin, *Reactivity of monoclonal anti-collagen II antibodies with cartilage and synovial tissue in rheumatoid arthritis and osteoarthritis.* Arthritis Rheum. *29*, 730–738 (1986).

[43] L. Klareskog, U. Forsum, A. Scheynius, D. Kabelitz and H. Wigzell, *Evidence in support of a self-perpetuating HLA-DR dependent delayed type cell reaction in rheumatoid arthritis.* Proc. Natl. Acad. Sci. USA, *79*, 3632–3636 (1982).

[44] J. T. Hom, J. M. Stuart and J. M. Chiller, *Murine T cells reactive to type II collagen. I. Isolation of lines and clones and*

characterization of their antigen-induced proliferative responses. J. Immunol. *136,* 769–775 (1986).

[45] J. T. Hom, J. M. Stuart, J. Tovey and J. M. Chiller, *Murine T cells reactive to type II collagen. II. Functional characterization.* J. Immunol. *136,* 776–782 (1986).

[46] M. Dallman and G. Fathman, *Type II collagen-reactive T-cell clones from mice with collagen-induced arthritis.* J. Immunol. *135,* 1113–1118 (1986).

[47] A. Scheynius, R. Holmdahl and L. Klareskog, *Diminished activation of T helper cells by antigen presentation with DTH-activated epidermal cells.* Immunology (in press) (1986).

[48] J. M. Stuart, *Collagen-induced arthritis: A new animal model for the evaluation of antiarthritic drugs.* Adv. Inflam. Res. *7,* 263–277 (1984).

[49] P. Jungers, M. Dougados, C. Pelissier, F. Kuttenn, F. Tron, P. Lesavre and J.-F. Bach, *Influence of oral contraceptive therapy on the activity of systemic lupus erythematosus.* Arthritis Rheum. *25,* 618–623 (1982).

[50] A. D. Steinberg, K. A. Melez, E. S. Raveche, J. P. Reeves, W. A. Boegel, P. A. Smathers, J. D. Taurog, L. Weinlein and M. Duvic, *Approach to the study of the role of sex hormones in autoimmunity.* Arthritis Rheum. *22,* 1170–1176 (1979).

[51] C. J. Grossman, *Interactions between the gonadal steroids and the immune system.* Science *227,* 257–260 (1985).

[52] J. P. Vandenbroucke, H. A. Valkenburg, J. W. Boersma, A. Cats, J. J. M. Festen, O. Huber-Bruning and J. J. Rasker, *Oral contraceptives and rheumatoid arthritis: Further evidence for a preventive effect.* Lancet *ii,* 839–842 (1982).

[53] S. J. Wingrave and C. R. Kay, *Reduction in incidence of rheumatoid arthritis associated with oral contraceptives.* Lancet *i,* 569–571 (1978).

[54] V. C. Neuman, K. A. Grindulis, S. Hubball, B. McConkey and V. Wright, *Comparison between penicillamine and sulphasalazine in rheumatoid arthritis: Leeds-Birmingham trial.* Br. Med. J. *287,* 1099–1102 (1983).

[55] A. T. M. M. Ali, G. S. Basran and J. Morley, *Mode of action of sulphasalazine: an alternative view.* Lancet *i,* 506–507 (1982).