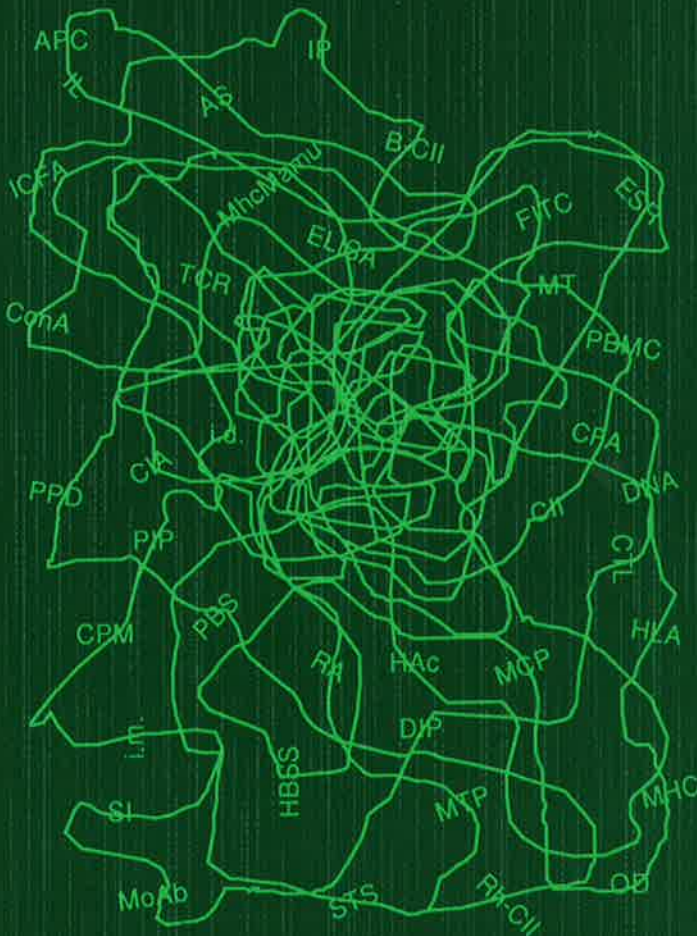


I 1860
XVI
BAKK
1993

Collagen-Induced Arthritis in the Rhesus Monkey

B16

Relations between aspects of autoimmunity
and disease development



N.P.M. Bakker

**COLLAGEN-INDUCED ARTHRITIS
IN THE RHESUS MONKEY**

**Relations between aspects of autoimmunity
and disease development**

**COLLAGEN-INDUCED ARTHRITIS
IN THE RHESUS MONKEY**

**Relations between aspects of autoimmunity
and disease development**

een wetenschappelijke proeve op het gebied van
de Medische Wetenschappen,
in het bijzonder de geneeskunde

Proefschrift

ter verkrijging van de graad van doctor
aan de Katholieke Universiteit Nijmegen,
volgens besluit van het College van Decanen
in het openbaar te verdedigen
op dinsdag 27 oktober 1992
des namiddags te 1.30 uur

door

Nicolaas Petrus Maria Bakker

geboren 30 juli 1958 te Bovenkarspel

1992

Druk: Pasmans, s'Gravenhage

Promotor : Prof. Dr. L.B.A. van de Putte

Co-promotor : Dr. M. Jonker (ITRI-TNO)

The work described in this thesis was performed at the TNO Institute of Applied Radiobiology and Immunology (ITRI), Rijswijk, The Netherlands. This thesis is considered to be a publication of this Institute. The costs of this publication were partly defrayed by ITRI-TNO.

Further financial support for printing of this thesis was provided by: 'Het Nationaal Reumafonds', the 'Dr. Saal van Zwanenberg-stichting', Becton and Dickinson and Celltech Limited.

Aan mijn ouders

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Bakker, Nicolaas Petrus Maria

**Collagen-induced arthritis in the rhesus monkey :
relations between aspects of autoimmunity and disease
development / Nicolaas Petrus Maria Bakker. - [S.l. :
s.n.] ('s-Gravenhage : Pasmans). - Ill.
Proefschrift Nijmegen. - Met lit. opg. - Met samenvatting
in het Nederlands.
ISBN 90-9005389-1
Trefw.: arthritis.**

CONTENTS

	pg.
List of abbreviations	9
CHAPTER 1 General introduction	11
1.1 The relevance of an arthritis model in the rhesus monkey	11
1.2 Spontaneous arthritic manifestations in nonhuman primates	14
1.3 Experimentally induced arthritis in nonhuman primate species	14
1.4 Type II collagen	16
1.5 Type II collagen immunity in humans	16
1.6 Type II collagen induced arthritis in rodents	18
1.7 Aim of the study	20
CHAPTER 2 Experimental immune mediated arthritis in rhesus monkeys. A model for human rheumatoid arthritis?	31
CHAPTER 3 Collagen induced arthritis in an outbred group of rhesus monkeys comprising responder and non-responder animals. Relation between the course of arthritis and collagen-specific immunity.	51
CHAPTER 4 Induction of type II collagen-specific antibody production in blood lymphocyte cultures of rhesus monkeys (<i>Macaca mulatta</i>) with collagen-induced arthritis using the immobilized native antigen.	69
CHAPTER 5 Acquired resistance to type II collagen induced arthritis in rhesus monkeys is reflected by a T cell low-responsiveness to the antigen.	81

CHAPTER 6	Resistance to collagen-induced arthritis in a nonhuman primate species maps to the MHC class I region.	95
CHAPTER 7	Concluding remarks	105
-	Samenvatting	108
-	Curriculum vitae	111
-	List of Publications	112
-	Nawoord	115

List of abbreviations

APC	antigen presenting cell
AS	ankylosing spondylitis
B-CII	bovine type II collagen
BSA	bovine serum albumin
CFA	complete freunds adjuvant
CIA	collagen induced arthritis
CII	type II collagen
CPM	counts per minute
CRP	C-reactive protein
ConA	conconavalin A
CTL	cytotoxic T cell
DIP	distal interphalangeal (joint)
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
ESR	erythrocyte sedimentation rate
FITC	fluorescein isothiocyanate
HAc	acetic acid
HBSS	Hanks' Balanced Salt Solution
HLA	human leucocyte antigen
i.d.	intradermal
ICFA	incomplete freunds adjuvant
IL	interleukin
i.m.	intramusculair
IP	interphalangeal (joint)
MCP	metacarpophalangeal (joint)
MHC	major histocompatibility complex
MhcMamu	MHC of the rhesus monkey
MT	Mycobacterium tuberculosis
MoAb	monoclonal antibody
MTP	metatarsophalangeal (joint)
OD	optimal density
PPD	purified protein derivate
TCR	T cell receptor
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered salt solution
PIP	proximal interphalangeal (joint)
RA	rheumatoid arthritis
Rh-CII	rhesus monkey type II collagen
SI	stimulation index
STS	soft tissue swelling

CHAPTER 1

GENERAL INTRODUCTION

1.1 The relevance of an arthritis model in the rhesus monkey

The autoimmune rheumatic diseases, from which RA is probably the best known, comprise a group of chronic disorders forming one of the most challenging problems of modern medical research. In spite of considerable research, the etiology of RA and other human arthritic diseases remains obscure creating a great obstacle for developing specific therapies.

During the last years several observations provided more insight in the pathogenic mechanisms involved in human chronic arthritis. A fundamental discovery was the association existing between the susceptibility to develop particular rheumatic diseases and certain genes localized within the MHC (1-3). The human MHC, designed HLA, encodes several classes of polymorphic gene products. Among them are the class I (HLA-A, -B and -C) and class II (HLA-DR, -DQ and -DP) region products. An allele encoded by the HLA-DR region, named HLA-DR4, seems to be associated with a progressive form of severe RA (4). It is not clear yet where such type of associations are based on, but it is known that MHC class I and II molecules are membrane bound glycoproteins which can bind and present (self) peptides to (autoreactive) T lymphocytes. The polymorphism of class I and class II molecules is not randomly located but amino acid sequence variability is concentrated at positions involved in the interaction with either epitopes of the antigen and/or the TCR (5, 6). Therefore, the MHC plays a pivotal role in controlling the immune response.

The involvement of T cells in RA is a second important feature of the disease, although their exact influence is still under discussion (7). The potential role of autoreactive T helper lymphocytes is substantiated by immunohistological studies of RA synovium, where perivascular aggregates of CD4⁺ T cells, closely situated next to APC, can be demonstrated (8, 9). In addition, the role of T cells in RA is supported by the finding that partial elimination or inhibition of the function of these cells, by a variety of techniques, leads in some patients to remission of the disease (10). Furthermore, evidence for involvement of specified subsets of T cell are suggested by observations of T cell oligoclonality in affected joints of RA

patients (11, 12), although these data are debated (13).

A last crucial RA related finding is the fact that RA can be classified among the so called immune complex diseases because of the presence of autoantibodies and immunocomplexes in synovial fluid, tissue and sera (14).

So, the conclusion can be drawn that in the pathogenesis of RA the allelic variation of the MHC genes, together with a certain autoreactive T and B cell repertoire, constitutes important factors which may influence susceptibility (or resistance) to the disease.

Autoimmune diseases can be regarded as the clinical result of a disregulated immune system that has lost its immunologic tolerance to an autoantigen. Immunologic tolerance to an autoantigen may be achieved by deletion or inactivation of autoreactive T cells during their differentiation in the thymus (15, 16). To understand autoimmunity it is important to know how autoreactive T cells escape thymic depletion and which factors control how these autoreactive T cells (and also autoreactive B cells) become or remain activated.

Animal models are indispensable in studying factors governing maintenance or breaking of self tolerance and the components which subsequently regulate the development to a clinical manifested autoimmune disease. Rodent models have proven to be valuable, since they have given information about the autoantigens involved, associations with the MHC and regulatory mechanisms behind autoimmunity (17). However, most information is based on observations performed in a limited number of inbred strains.

Profound differences between the immune systems of rodents and man have been described. One of the consequences is that extrapolation of experimental data from rodent studies to man has to be done with caution. In this respect nonhuman primates may provide a more reliable system and can play an important role. Extrapolation of data obtained in nonhuman primates to man might be more relevant because of their close phylogenetic relationship and because they share many biologic and immunologic similarities.

The knowledge of the MHC systems in different nonhuman primate species has rapidly expanded (18, 19). On basis of these data it has been shown that some alleles of different, but related, species are more similar to each other than the MHC alleles within a species (20). This high degree of shared MHC similarity, observed between different primate species, has also functional significance as reflected by successful antigen presentation across a species barrier (21). The current knowledge of the MHC of the rhesus monkey (*Macaca mulatta*), named *MhcMamu* (22) and located on chromosome 2, is for a great deal based on serologic information (23,

24). In this way 27 different class I molecules (13 A loci, 14 B loci) could be identified as well as 10 different Mamu-DR molecules. Recently, restriction fragment length polymorphism analysis has shown that most of the serologically defined DR specificities can be divided into subtypes (25).

The close phylogenetic relationship between rhesus monkeys and man is also reflected by the high structural conservation of many surface structures which can be found on several types of PBMC (26-28). Many of these surface molecules have important functions in the immune response (29). In addition, Ahmed-Ansari et al. (30) has shown that certain rhesus monkey T cell subtypes, phenotyped with specified anti-human MoAb, have similar immunologic functions as their human counterparts.

Similarities between the immune systems of man and rhesus monkey make a rhesus monkey arthritis model suitable to test the efficacy and safety of a new generation of immunomodulating / anti-rheumatic agents. Especially biological response modifiers with an exclusive biological activity in primates might be suitable candidates. Most biological response modifiers have multiple biological effects, so results obtained *in vitro* cannot be directly extrapolated to the therapeutic efficacy in humans. This point again stresses that monkey studies can be an essential prerequisite for the successful application of drugs in man. Examples of biological response modifiers, which already have been applied in monkeys, are human interferon preparations (31) and human-specific MoAb directed to several surface molecules (32-34).

The disadvantages of the monkey model are ethical and technical. Also the relatively high costs of experiments with rhesus monkeys prevent the use of large numbers of animals. Nevertheless, rhesus monkeys have been used, and in some areas with increasing frequency, as animal models for human infectious diseases (35), nowadays especially in the case of acquired immunodeficiency syndrome (AIDS) (36), bone marrow- (37), heart- and kidney transplantation (38) and autoimmunity (39).

In all these studies the most important reason for using rhesus monkeys was that the collected experimental data can be extrapolated to the clinical situation with more confidence than data obtained in rodent models.

1.2 Spontaneous arthritic manifestations in nonhuman primates

Nonhuman primates are our closest living relatives. As in the human population, spontaneous manifestations of arthritis, although described by only a few papers, do occur in these animals.

One report describes spontaneous AS in two old rhesus monkeys (> 18 year old) and the manifestation of hyperosteoic AS in one rhesus monkey (40). Benditt & Eriksen (41) have described three rhesus monkeys with inflammatory activity of the joints resembling RA involving knee joints and the proximal IP joints. All monkeys had amyloidosis, the extracellular deposition of amyloid fibrils in many organs. Also other studies reported the occurrence of amyloidosis in association with chronic arthritis in respectively the rhesus monkey (42) and baboon (43).

Concerning the etiology of arthritis in nonhuman primates a role of certain pathogens has been suggested. In 5 rhesus monkeys with chronic arthritis three showed an ulcerative enterocolitis associated with *Shigella flexnerii* (44). Moreover, four had rheumatoid factor present and four also amyloidosis. The description given of the knee joints, both gross and microscopic, resembled known manifestations of RA. Another case was provided by a young male gorilla that developed spontaneously systemic and articular manifestations similar to RA (45). This arthritic activity however, was likely associated with a mycobacterial infection and was successfully treated with the antibiotic tetracycline.

A more elaborate study in 152 rhesus monkeys showed in the joints of only 1 animal histological changes which resembled closely those found in human RA (46).

In conclusion, signs of spontaneous (especially RA like) arthritis can be found in monkeys, but more study will be necessary to obtain detailed insight in the incidence and forms of arthritis in these animals.

1.3 Experimentally induced arthritis in nonhuman primate species

Only a few publications have described the attempts to elicit experimentally arthritis in monkeys. To demonstrate whether cells, present in synovial fluid or synovial membrane of RA patients, are involved in the etiology of RA, twenty-five baboons were injected intravenously and intra-articularly with these cells. No signs of arthritis were recognized during 3 years of observation (47). A study with marmosets (*Callithrix jacchus*) showed that intra-articular injection of a mixture of

methylated-BSA plus *Bordetella pertussis* results in chronic synovitis (48).

The first case of experimental induced arthritis in monkeys, which was induced by extra-articular immunization, appeared in the literature in 1986. It was shown that six female squirrel monkeys (*Saimiri sciureus*) immunized with native B-CII in CFA developed arthritis 3 to 6 weeks later (49). This CIA was symmetrical and involved mainly the IP and MCP joints. Although three monkeys suffered from an extremely bad condition and died, the other three showed a spontaneously remission of their arthritis. All monkeys developed antibody titers to native B-CII, as was measured by the passive hemagglutination technique. In the same study three male cebus monkeys (*Cebus albifrons*), also sensitized with B-CII in CFA, did not develop arthritis and these animals had a significantly weaker antibody response to B-CII as compared to the (CIA susceptible) squirrel monkeys (49). The relation between the capability to produce a high titer of anti-CII antibodies and the development of CIA seemed also present in cynomolgus monkeys (*Macaca fascicularis*) (50). In cynomolgus monkeys the capability to produce anti-CII antibodies was clearly sex-linked; only females developed arthritis together with a high titer of antibodies to autologous CII (50).

The induction of CIA in rhesus monkeys, by immunization with native B-CII in CFA, has also been reported (51-53). In all studies rhesus monkeys were immunized systemically, but the way of immunization differed. Yoo and coworkers (51) induced CIA in all immunized female rhesus monkeys by injecting the animals i.m. in the posterior thigh with 2.0 mg native B-CII in CFA followed by 2 booster immunizations of 1.0 mg B-CII in ICFA. In contrast to cynomolgus monkeys, susceptibility to CIA in rhesus monkeys seems not sex-linked because Rubin et al. (53) showed that all 7 male rhesus monkeys, upon several i.d. immunizations with 1.0 mg B-CII, developed arthritis. CIA could also be induced in rhesus monkeys by implantation of nitrocellulose filters coated with B-CII in the peritoneal cavity (54). Thus, the use of CFA as adjuvant is not crucial for the induction of CIA in rhesus monkeys. This may be important because it has been reported that CFA itself has arthritogenic capacities in rats (55). As expected, CFA elicits a more severe arthritis in rhesus monkeys while immunization with the CII coated nitrocellulose filters results only in a sub-clinical arthritis (54). This might be explained by the fact that nitrocellulose functions only as a slow release device of the antigen, but not as adjuvant.

Till now, CII is the only described arthritogenic antigen which after extra-articular immunization induces arthritis in nonhuman primates. The resistance of the cebus monkey and the clear sex-linked susceptibility in the cynomolgus monkey

raises questions whether susceptibility versus resistance to CIA in nonhuman primates is genetically controlled.

1.4 Type II collagen

In general, collagen molecules are characterized by their organization in triple-helical fibrils containing peptides with repeating glycine-X-Y triplets and the presence of hydroxyproline and hydroxylysine (56). At present, at least 13 types of collagen differing in amino acid composition have been identified. Most of these collagens have a structural function in the extracellular matrix of connective tissues.

CII is the main protein of cartilage and can also be found in a few other tissues such as the vitreous body of the eye. CII can be isolated from cartilage by limited pepsin-digestion followed by a series of precipitations from solutions of varying pH and ionic strengths (57). The most striking feature of CII is its high content of hydroxylysine and glycosidically bound carbohydrate. CII is synthesized by chondrocytes as a procollagen molecule with non-collagenous amino- and carboxy peptides. These peptides are removed by extracellular processing and subsequently the molecules are organized into fibrils; fine fibrils around the chondrocytes and thicker ones distributed elsewhere in the cartilage (58). The CII fibrils form a network which contains proteoglycans, the molecules which together with enclosed tissue fluid give articular cartilage its specific elasticity and resistance to compression (59).

CII is a molecule which, upon i.d. immunization in different experimental animals, elicits an autoimmune mediated arthritis (see Chapters 1.3 and 1.6). From an immunologic point of view it is important that certain immunogenic epitopes of CII are exposed at the surface of intact cartilage (60) and because no basal membrane or other barrier in the synovium prevents proteins from entering the synovial fluid (61), cartilage may be a target for binding of anti-CII antibodies.

1.5 Type II collagen immunity in humans

Two prominent questions arising in the study of human arthritis are 1) whether autoimmune reactions to cartilage components occur and 2) to which extent such reactions contribute to the initiation or perpetuation of the inflammatory process in the joint. In this respect many studies are performed to investigate autoimmunity to

CII in humans.

Little is known about CII-reactive T cells in humans. The most direct evidence for the presence of CII-reactive T cells in healthy individuals was recently provided by Lacour et al. (62). In human arthritis, there are indications that T cells, proliferating upon *in vitro* exposure to CII, accumulate in joints of RA patients (63,64).

Concerning antibodies directed to CII, elevated serum levels were measured in patients with bilateral progressive sensorineural hearing loss (65) and in a variety of rheumatic diseases like RA (66, 67) relapsing polychondritis (68) and AS (69). Deposition of CII reactive antibodies have been observed in synovial tissue (70), synovial fluid (71) or cartilage (72) of some patients with RA. The assumption that CII reactive antibodies may play a role during early stages of RA is based on longitudinal studies indicating that these antibodies could be measured preferentially in patients with early symptoms of RA (73) and were absent in pre-illness sera (74). The specificity of the anti-CII antibody response in RA has been investigated in further detail because it is assumed that antibodies directed to conformational epitopes, which can be found on intact, native CII are involved in the initiation of the disease (60). On the other hand, the presence of antibodies directed to non-conformational epitopes, present in the primary structures of CII, suggest that denatured CII (i.e. CII-fragments eroded from the cartilage surface, for instance due to the inflammatory process) may function as autoantigen involved in perpetuation of joint inflammation. But this differentiation in the humoral immune response to CII is difficult to establish, because most studies reported the presence of antibodies directed to both conformational- and linear structures of CII in different stages of RA (75, 76).

Studies involved in mapping of the B-cell epitopes on CII suggest that there are several immunogenic epitopes. The humoral immune response is directed to different parts on the molecule, both containing common (species non-specific) epitopes as well as species-specific epitopes (72, 76, 77). It is reported by some investigators that the production of anti-CII antibodies in RA patients may be under the genetic control of the HLA-DR1 and -DR4 immune response gene products (78,79), but these findings are contradicted by data from others (80, 81).

It is far from clear yet whether anti-CII antibodies are involved in the pathogenesis of RA, but there is strong evidence for their arthritogenic capacities because purified anti-CII serum antibodies from a RA patient were found to induce arthritis in naive mice (82).

1.6 Type II collagen induced arthritis in rodents

While animals like guinea pigs are insensitive to CIA (83), polyarthritis can be induced in certain strains of rats and mice by i.d. injection of native heterologous or homologous CII (84-87). CIA induced by heterologous CII (mainly from bovine or chicken origin) is a monophasic disease, whereas immunization with homologous CII usually results in a perpetuating arthritis (88).

The arthritic activity is usually most severe in the ankle joints, but also the knee joints can be affected (86,89). The very early histopathology of the affected joint in CIA shows an infiltration of CD4+ T cells and a proliferation of synovial lining cells, with an increased expression of MHC class II antigens (90-93). Also MHC class II- and Fc-receptor expressing macrophages and relatively few B cells and plasma cells can be found in the hyperplastic synovial lining layer (93). Subsequent development of arthritis can be divided into two stages. First a massive infiltration of PBMC and severe oedema of synovium and extra-articular tissue. During the second stage, a pannus tissue is formed with activated macrophages and T cells and a few dendritic cells and fibroblasts (94). It appeared that macrophages are important effector cells in the joint destruction and that activated T cells as well as immune complexes are involved in the induction of the macrophage proliferation and activation (91).

Generally it is considered that the histopathology of the established inflamed joint in CIA resembles that of the chronic proliferative synovitis characteristic of human RA (84,88,95-97). Both in CIA and RA prostaglandin E2 and proteases, such as collagenase contribute to the destruction of cartilage (98,99), as well as the activity of neutrophils and reactive oxygen species (100-102). Considerable evidence indicates that CIA is initiated by an immune response to CII that is regulated both in rats and mice by the MHC (96,103,104). More precisely, in rats the responsiveness to B-CII and the development of arthritis is linked to the RT1u MHC locus (105). In mice, the linkage has been mapped to the class II region (103). Holmdahl et al. (106) have shown that only strains expressing I-Aq or I-Ar (or closely linked) molecules developed arthritis, accompanied by a humoral response to mouse-CII, upon immunization with heterologous- or mouse-CII. Comparison of the I-A-beta first domain exons of the susceptible H-2q mice with the H-2p mice (CIA-resistant) resulted in the identification of a site on the I-A-beta chain that is involved in susceptibility for CIA (107). Analysis of the nucleotide sequences coding for the first domains (this region may be important in antigen presentation) of the I-Aq and I-Ar molecules revealed high similarities (108,109).

The association between MHC and CIA, together with the necessity to immunize with, for antibodies important, conformationally intact ("native") collagen to induce CIA, suggest that both B and T cells mediate anti-CII immunity and are both crucial for development of CIA (110). The importance of antibodies in the induction of CIA has been demonstrated by the observation that anti-CII enriched immunoglobulin fractions, obtained from arthritic rats and mice, as well as anti-CII MoAb can induce arthritis when transferred to healthy animals (111-113) or nude rats (114). In contrast to the arthritis induced by immunization with CII, the arthritis induced in these transfer experiments is not restricted to certain MHC haplotypes and has a transient character without destructive lesions in the joint (115). The involvement of anti-CII antibodies in CIA is also supported by the observation that complement plays a crucial role in the initiation of CIA (116-118). Moreover, the disease can be inhibited by pretreatment with anti-IgM antibodies (119) or applying anti-idiotypic MoAb's directed to an idiotope on anti-CII antibodies (120).

It seemed that B cells producing anti-CII antibodies are easily activated after the primary immunization with CII (121). The produced antibodies are mainly of the IgG2a isotype (122), suggesting the activation of memory B cells. There are indications that anti-CII antibodies, which can have rheumatoid factor specificity (122, 123) or binding capacities to the activated complement factor C1q (124), recognize different epitopes on the CII molecule (125, 126). Until now 2 major immunogenic and arthritogenic B cell epitopes have been localized in the region of the CII molecule designated as CB11 (= peptide of 272 amino acids obtained by cleavage of CII by cyanogen bromide) (127, 128).

Evidence supporting the role of T cells was obtained from the following observations: a) development of disease can be prevented by treating the animals with anti-thymocyte serum or with antibodies directed to CD4, the TCR, MHC class II molecules or the IL2 receptor (129-133) b) it is impossible to induce CIA in nude rats (91) and mice (134) c) arthritis can be induced in normal mice via adoptive transfer of CII-specific T cell lines (135-138) d) cyclosporin treatment inhibits CIA and the anti-CII antibody response (139, 140). T cells expressing certain V-beta allotypes are thought to play a central role in CIA (141, 142). Whether susceptibility to CIA is associated with the usage of particular V-beta elements is yet not certain. *In vivo* experiments showed that depletion of T cells expressing V-beta-8 (thought to be relevant for the induction of CIA) did not alter CIA (143). Other arguments against the central role of certain TCR V-beta allotypes came from studies that showed that CIA could be induced in the susceptible DBA/1 mouse but not in the SWR mice. Both mice strains are MHC identical and showed a CII-dependent

proliferative T cell response after immunization with CII (144). This shows that both mice strains do not lack anti-CII T cells. Even bone marrow transplantation from DBA/1 to SWR had no influence on the resistance for CIA in the SWR strain (145). The genetic factor(s) responsible for the resistance to CIA in SWR mice are unknown.

Concerning immunoregulation to the autoantigen CII, it is shown in naive healthy mice that anti-CII T cells exist in the periphery (146), suggesting that a regulatory system maintains these autoreactive T cells in an anergic stage. The existence of such a regulatory system is also indicated by studies in which antigen-specific hyporesponsiveness to CII and resistance to CIA could be induced by pretreating the animals intravenously, orally or intraperitoneally with soluble CII or collagen-coupled spleen cells (147-150). Probably, when CII is not administered into the skin in its native constitution but when it is administered along another route or applied in its denatured form, an (antigen) specific mechanism is installed or activated which facilitates resistance to CIA. The nature of such a hypothesized mechanism is not known, but there is some evidence that lymphocytes may be involved. By *in vivo* administration studies with MoAb's directed to CD4⁺ and CD8⁺ T cells Williams et al. (151) showed that both T cell subsets govern in resistance to CIA; CD4⁺ cells in an early- and CD8⁺ cells in a later stage. Other studies have shown that lymphocytes are directly involved in suppressing CIA by suppressing CII-reactive antibody production (152) and that suppression to CIA can be transferred to naive animals with both spleen cells and CD4⁺ cells isolated from CII-immunized animals (137, 153-156).

Concerning CII derived epitopes operating in the immunoregulation of CIA, it is likely that next to epitopes involved in the triggering of CIA (127, 128) other epitopes are involved in resistance cq. suppression of CIA. Myers et al. (157) identified a CII-derived peptide of 25 amino acids which could induce tolerance and suppression of disease. The suggestion that T cells mediate suppression to CIA is also supported by the finding that both cyclophosphamide and cyclosporin treatment can abrogate suppression to CIA (158, 159).

1.7 Aim of the study

The aim of this study was to establish an experimental arthritis model in the rhesus monkey (*Macaca mulatta*), to compare the clinical and pathologic parameters with human arthritis and to investigate the association of relevant immunologic para-

meters with the induced arthritis.

The study has been performed with a panel of outbred rhesus monkeys of the colony of the Institute of Applied Radiobiology and Immunology-TNO (ITRI-TNO), Rijswijk, The Netherlands.

One part of this study comprising an identification of humoral and cellular compartments of the immune system which influence the onset and course of the experimentally induced arthritis. To this purpose, the antibody repertoire, antigen specific T cell proliferation and some cytokine profiles were monitored in responder and non-responder rhesus monkeys. The Institute contains a primate colony in which all animals are characterized for their *MhcMamu*-A, -B and -DR locus alleles. For this reason a second part of this study was focussed on identifying MHC related susceptibility or resistance genes associated with experimental induced arthritis. This was done by comparing the MHC repertoire of responder and non-responder animals. Identification of MHC immune response genes associated with CIA will allow the possibility to study their biological role in autoimmunity.

REFERENCES

1. Wordsworth B.P., Lanchbury J.S.S., Sakkas L.I., Welsh K.I., Panayi G.S., Bell J.I. HLA-DR4 subtype frequencies in rheumatoid arthritis indicate that DR-beta1 is the major susceptibility locus within the HLA class II region. *Proc. Natl. Acad. Sci. USA* **86**:10049 (1989)
2. Harley J.B., Reichlin M., Arnett F.C., Alexander E.L., Bias W.B., Provost T.T. Gene interaction at HLA-DQ enhances autoantibody production in primary Sjogren's syndrome. *Science* **232**:1145 (1986)
3. Benjamin R., Parham P. Guilt by association: HLA-B27 and Ankylosing Spondylitis. *Immunol. Today* **11**:137 (1990)
4. Van Zeben D., Hazes J.M.W., Zwinderman A.H., Cats A., Schreuder G.M.T., D'Amato J., Breedveld F.C. Association of HLA-DR4 with a more progressive disease course in patients with rheumatoid arthritis. Results of a followup study. *Arthritis Reum.* **34**:822 (1991)
5. Buus S., Sette A., Colon S.M., Miles C., Grey H.M. The relation between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides. *Science* **235**:1353 (1987)
6. Bjorkman P.J., Saper M.A., Samraoui B., Bennett W.S., Strominger J.L., Wiley D.C. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature* **329**:512 (1987)
7. Firestein G.S., Zvaifler N.J. How important are T cells in chronic rheumatoid synovitis? *Arthritis Reum.* **33**:768 (1990)
8. Janossy G., Panayi G., Duke O., Bofill M., Poulter L.W., Goldstein G. Rheumatoid arthritis: a disease of T-lymphocytes/macrophage immunoregulation. *Lancet* **II**: 839 (1981)
9. Warren C.J., Howell W.M., Bhambhani M., Cawley M.I.D., Smith J.L. An investigation of T-cell subset phenotype and function in the rheumatoid synovium using in situ hybridization of IL-2 mRNA. *Immunology* **72**:250 (1991)

10. Kingsley G., Panayi G., Lanchbury J. Immunotherapy of rheumatic diseases-practice and prospects. *Immunol. Today* **12**:177 (1991)
11. Stamenkovic I., Stegnagno M., Wright K.A., Krane K.A., Amento S.M., Colvin R.B., Dudquesnoy R.J., Kurnick J.T. Clonal dominance among T-lymphocyte infiltrates in arthritis. *Proc. Natl. Acad. Sci. USA* **85**:1179 (1988)
12. Paliard X., West S.G., Lafferty J.A., Clements J.R., Kappler J.W., Marrack P., Kotzin B.L. Evidence for the effects of a superantigen in rheumatoid arthritis. *Science* **253**:325 (1991)
13. Van Laar J.M., Miltenburg A.M.M., Verdonk A.M.M., Daha M.R., de Vries R.R.P., van der Elsen P.J., Breedveld F.C. Lack of T cell oligoclonality in enzyme-digested synovial tissue and in synovial fluid in most patients with rheumatoid arthritis. *Clin. Exp. Immunol.* **83**:352 (1991)
14. Natvig J.B., Randen I., Thompson K., Forre O., Munthe E. The B cell system in the rheumatoid inflammation. New insights into the pathogenesis of rheumatoid arthritis using synovial B cell hybridoma clones. *Springer Semin. Immunopathol.* **11**:301 (1989)
15. Kappler J.W., Roehm N., Marrack P. T cell tolerance by clonal elimination in the thymus. *Cell* **49**: 373 (1987)
16. Kisielow P., Bluthman H., Staerz U.D., Steinmetz M., von Boehmer H. Tolerance in T cell receptor transgenic mice involves deletion of nonmature CD4⁺8⁺ thymocytes. *Nature* **332**:35 (1988)
17. Holmdahl R., Andersson M., Goldschmidt T.J., Gustafsson K., Jansson L., Mo J.A. Type II collagen autoimmunity in animals and provocations leading to arthritis. *Immunol. Rev.* **118**:193 (1990)
18. Lawlor D.A., Ward F.E., Ennis P.D., Jackson A.P., Parham P. HLA-A and B polymorphisms predate the divergence of humans and chimpanzees. *Nature* **335**:268 (1988)
19. Bontrop R.E., Otting N., Broos L.A.M., Noort M.C., Kenter M., Jonker M. RFLP analysis of the HLA-, ChLA, and RhLA-DQ alpha chain gene regions: conservation of restriction sites during evolution. *Immunogenetics* **30**:432 (1989)
20. Mayer W.E., Jonker M., Klein D., Ivanyi P., Van Seventer G., Klein J. Nucleotide sequences of chimpanzee MHC class I alleles: evidence for trans-species mode of evolution. *EMBO Journal* **7**:2765 (1988)
21. Bontrop R.E., Elferink D.G., Otting N., Jonker M., De Vries R.P. Major histocompatibility complex class-II restricted antigen presentation across a species barrier: conservation of restriction determinants in evolution. *J. Exp. Med.* **172**:53 (1990)
22. Klein J., Bontrop R.E. et al. Nomenclature for the major histocompatibility complexes of different species: a proposal. *Immunogenetics* **31**:217 (1990)
23. Van Vreeswijk W., Roger J.H., D'Amario J., Balner H. The major histocompatibility complex of rhesus monkeys, RhL-A. VII. Identification of five new serologically defined antigens. *Tissue Antigens* **9**:17 (1977)
24. Roger J.H., Van Vreeswijk W., Balner H. The major histocompatibility complex of the rhesus monkey. XIII. Current knowledge of DR and other B-cell specific antigens. *J. Immunogenetics* **7**:333 (1980)
25. Slierendregt B.L., Otting N., Jonker M., Bontrop R.E. RFLP analysis of the rhesus monkey MHC class II DR subregion. *Human Immunol.* **30**:11 (1991)
26. Haynes B.F., Dowell B.L., Hensley L.L., Gore I., Metzgar R.S. Human T cell antigens expression by primate cells. *Science* **215**:298 (1982)
27. Letvin N.L., King N.W., Reinherz E.L., Hunt R.D., Lane H., Schlossman S.F. T lymphocyte surface antigens in primates. *Eur. J. Immunol.* **13**:345 (1983)
28. Jonker M., Slingerland W. Reactivity of monoclonal antibodies specific for human CD markers with rhesus monkey leucocytes. In *Leucocyte Typing IV*, pp. 1058-1063. Oxford University Press, Oxford (1989)
29. Springer T.A. Adhesion receptors of the immune system. *Nature* **346**:425 (1990)
30. Ahmed-Ansari A., Brodie A.R., Fultz P.N., Anderson D.C., Sell K.W., McClure H.M. Flow microfluorometric analysis of peripheral blood mononuclear cells from nonhuman primates: correlation of phenotype with immune function. *Am. J. Primatol.* **17**:107

(1989)

31. Schellekens H., Van der Meide P.H., Nooter K. Interferon studies in non-human primates. M. Revel (ed.) Clinical aspects of interferons. Kluwer Academic Publishers, Boston. (1988)
32. Jonker M. The importance of non-human primates for preclinical testing of immunosuppressive monoclonal antibodies. *Immunology* 2:427 (1990)
33. Jonker M., van Lambalgen R., Mitchell D.J., Durham S.K., Steinman L. Successful treatment of EAE in rhesus monkeys with MHC class II specific monoclonal antibodies. *J. Autoimmunity* 1:399 (1988)
34. Waldman T.A. Monoclonal antibodies in diagnosis and therapy. *Science* 252:1657 (1991)
35. McClure H.M. Non-human primate models for human disease. *Adv. Vet. Sci. Comp. Med.* 28:267 (1984)
36. Letvin N.L., Daniel M.D., Sehgal P.K., Desrosiers R.C., Hunt R.D., Waldron L.M., Mackey J.J., Schimdt D.K., Chalifoux L.V., King N.W. Induction of AIDS-like disease in Macaque monkeys with T-cell tropic retrovirus STLV-III. *Science* 230:71 (1985)
37. Bekkum, D.W. van. The rhesus monkey as a preclinical model for bone marrow transplantation. *Trans. Proc.* 10:105 (1978)
38. Marquet R.L., Heystek G.A., van Bekkum D.W. Heterotopic heart and kidney transplantation in rhesus monkeys. In Goldsmith E.L., Moor-Jankowski J. (eds). *Medical Primatology*. Basel, Karger, p.125 (1972)
39. Lambalgen van R., Jonker M. Experimental allergic encephalomyelitis in rhesus monkeys. I. Immunological parameters in EAE resistant and susceptible rhesus monkeys. *Clin. Exp. Immunol.* 68:100 (1987)
40. Sokoloff L., Snell K.C., Stewart H.L. Spinal ankylosis in old rhesus monkeys. *Clin. Orthop.* 61:285 (1968)
41. Benditt E.P., Eriksen N. Chemical characteristics of the substance of typical amyloidosis in monkeys. *Acta. Pathol. Microbiol. Scand.(A)*. 80:103 (1972)
42. Casey H.W., Kirk J.H., Splitter G.A. General amyloidosis in a rhesus monkey. *Lab. Anim. Sci.* 22:587 (1972)
43. Gillman J., Gilbert C. Primary amyloidosis in the baboon (*Papio ursinus*). *Acta. Med. Scand.* 152:155 (1955)
44. Chapman, W.L., Crowell W.A. Amyloidosis in rhesus monkeys with rheumatoid arthritis and enterocolitis. *J. Am. Vet. Ass.* 171:855 (1977)
45. Brown T.M., Clark H.W., Bailey J.S., Gray C.W. A mechanistic approach to treatment of rheumatoid type arthritis naturally occurring in a gorilla. *Trans. Amer. Clinical & Climatological Assn.* 82:227 (1971)
46. Bywaters, E.G.L. Observations on chronic polyarthritis in monkeys. *J. R. Soc. Med.* 74:794 (1981)
47. Mackay J.M.K., Sim A.K., McCornick J.N., Marmion, B.P., McCraw A.P., Duthie J.J.R., Gardner D.L. Aetiology of rheumatoid arthritis: an attempt to transmit an infective agent from patients with rheumatoid arthritis to baboons. *Ann. Rheum. Dis.* 42:443 (1983)
48. Hunneyball I.M. Investigations into the induction of chronic experimental arthritis in the common Marmoset (*Callithrix jacchus*). *Rheumatol. Int.* 3:69 (1983)
49. Cathcart E.S., Hayes K.C., Gonnerman W.A., Lazzari A.A., Franzblau C. Experimental arthritis in a non-human primate. I. Induction by bovine type II collagen. *Lab. Invest.* 54:26 (1986)
50. Terato K., Arai H., Shimozuru Y., Fukuda T., Tanaka H., Watanabe H., Nagai Y., Fujimoto K., Okubo F., Cho F., Honjo S., Cremer M.A. Sex-linked differences in susceptibility of cynomolgus monkeys to type II collagen-induced arthritis. *Arthritis Rheum.* 32:748 (1989)
51. Yoo T.J., Stuart J.M., Takeda T., Sudo, N., Floyd R.A., Ishibe T., Olson G., Orchik D., Shea, J.J., Kang A.H. Induction of type II collagen autoimmune arthritis and ear disease in monkey. *Ann. New York Acad. Sciences* 475:341 (1986)
52. Yoo T.J., Kim S.Y., Stuart J.M., Floyd R.A., Olson G.A., Cremer M.A., Kang A.H. Induction of arthritis in monkeys by immunization with type II collagen. *J. Exp. Med.* 168:777 (1988)

53. Rubin A.S., Healy C.T., Martin L.N., Baskin G.B., Roberts E.D. Experimental arthropathy induced in rhesus monkeys (*Macaca mulatta*) by intradermal immunization with native bovine type II collagen. *Lab. Invest.* **57**:524 (1987)
54. Healy C.T., Martin L.N., Roberts E.D., Rubin A.S. Methods in laboratory investigation. Experimental arthropathy induced in rhesus monkeys and DBA/1 mice by a novel method: intraperitoneal implantation of type II collagen adsorbed onto nitrocellulose filters. *Lab. Invest.* **60**:462 (1990)
55. Pearson C.M. Development of arthritis, peri-arthritis and periostitis in rats given adjuvants. *Proc. Soc. Exp. Biol. Med.* **91**:95 (1956)
56. Martin G.R., Timpl R., Muller P.K., Kuhn K. The genetically distinct collagens. *TIBS* p. 285-286, Elsevier (1985)
57. Trelstad R.L., Kang A.H., Igarashi S., Gross J. Isolation of two distinct collagens from chick cartilage. *Biochemistry* **9**:4993 (1970)
58. Kelly W.N., Harris E.D., Ruddy S., Sledge C.B. *Textbook of Rheumatology*, 2nd edn. Sanders, Philadelphia, PA (1985)
59. Poole C.A., Flint M.H., Beaumont B.W. Morphological and functional interrelationships of articular cartilage matrices. *J. Anat.* **138**:113 (1984)
60. Mayne R. Cartilage collagens. What is their function, and are they involved in articular disease? *Arth. Rheum.* **32**:241 (1989)
61. Levick J.R. Permeability of rheumatoid and normal human synovium to specific plasma proteins. *Arthritis Rheum.* **24**:1550 (1981)
62. Lacour M., Rudolphi U., Schesier M., Peter H.H. Type II collagen-specific human T cell lines established from healthy donors. *Eur. J. Immunol.* **20**:931 (1990)
63. Klareskog L., Forsum U., Scheynius A., Kabelitz D., Wigzell H. Evidence in support of a self-perpetuating HLA-DR- dependent delayed-type cell reaction in rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA* **79**:3632
64. Londei M., Savill C.M., Verhoef A., Brennan F., Leech Z.A., Duance V., Maini R.N., Feldmann M. Persistence of collagen type II-specific T-cell clones in the synovial membrane of a patient with rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA* **86**:636 (1989)
65. Helfgott S.M., Moscicki R.A., Martin J.S., Lorenzo C., Kieval R., McKenna M., Nadol J., Trentham D.E. Correlation between antibodies to type II collagen and treatment outcome in bilateral progressive sensorineural hearing loss. *Lancet* **337**:387 (1991)
66. Andriopoulos N.A., Mestecky J., Miller E.J., Bradley E.L. Antibodies to native and denatured collagens in sera of patients with rheumatoid arthritis. *Arthritis Rheum.* **19**:613 (1976)
67. Charriere G., Hartmann D.J., Vignon E., Ronziere M.C., Herbage D., Ville G. Antibodies to types I, II, IX and XI collagen in the serum of patients with rheumatic diseases. *Arthritis Rheum.* **31**:325 (1988)
68. Ebringer R., Rook G., Swana G.T., Bottazzo G.F., Doniach D. Autoantibodies to cartilage and type II collagen in relapsing polychondritis and other rheumatic diseases. *Ann. Rheum. Dis.* **40**:473 (1981)
69. Claque R.B., Shae M.J., Holt P.J.J. Incidence of serum antibodies to native type I and type II collagens in patients with inflammatory arthritis. *Annals Rheum. Dis.* **39**:201 (1980)
70. Tarkowski A., Klareskog L., Carlsten H., Herberts P., Koopman W. Secretion of antibodies to type I and II collagen by synovial tissue cells in patients with rheumatoid arthritis. *Arthritis Rheum.* **32**:1087 (1989)
71. Klareskog L., Olsson T. Autoimmunity to collagen II and myelin basic protein: comparative studies in humans and rodents. *Immunol. Rev.* **118**:285 (1990)
72. Terato K., Shimozuru Y., Katayama K., Takemitsu Y., Yamashita I., Miyatsu M., Fujii K., Sagara M., Kobayashi S., Goto M., Nishioka K., Miyasaka N., Nagai Y. Specificity of antibodies to type II collagen in rheumatoid arthritis. *Arthritis Rheum.* **33**:1493 (1990)
73. Pereira R.S., Black C.M., Duance V.C., Jones V.E., Jacoby R.K., Welsh K.I. Disappearing collagen antibodies in rheumatoid arthritis. *Lancet* **ii**:501 (1985)
74. Mottonen T., Hannonen P., Oka M., Rautiainen J., Jokinen I., Arvilommi H., Palosuo T., Aho K. Antibodies against native type II collagen do not precede the clinical onset of

- rheumatoid arthritis. *Arthritis Rheum.* **31**:776 (1988)
75. Morgan K., Clague R.B., Collins I., Ayad S., Phinn S.D., Lennox Holt P.J. A longitudinal study of anticollagen antibodies in patients with rheumatoid arthritis. *Arthritis Rheum.* **32**:139 (1989)
 76. Boissier M.C., Chiocchia G., Texier B., Fournier C. Pattern of humoral reactivity to type II collagen in rheumatoid arthritis. *Clin. Exp. Immunol.* **78**:177 (1989)
 77. Buckee C., Morgan K., Ayad S., Collins I., Clague R.B., Holt P.J.L. Diversity of antibodies to type II collagen in patients with rheumatoid arthritis: detection by binding to alpha-chains and to cyanogen bromide peptides. *Brit. J. Rheumatol.* **29**:254 (1990)
 78. Solinger A.M., Bhatnagar R., Stobo J.D. Cellular, molecular, and genetic characteristics of T cell reactivity in man. *Proc. Natl. Acad. Sci. USA* **78**:3877 (1981)
 79. Rowley M., Tait B., Mackay I.R., Cunningham T., Philips B. Collagen antibodies in rheumatoid arthritis. Significance of antibodies to denatured collagen and their association with HLA-DR4. *Arthritis Rheum.* **29**:174 (1986)
 80. Dyer P.A., Clague R.B., Klouda P.T., Firth S., Harris R., Holt P.J.L. HLA antigens in patients with rheumatoid arthritis and antibodies to native type II collagen. *Tissue Antigens* **20**:394 (1982)
 81. Wooley P.H., Luthra H.S., O'Duffy J.D., Bunch T.W., Moore S.B., Stuart J.M. Anti-type II collagen antibodies in rheumatoid arthritis. *Tissue Antigens* **23**:263 (1984)
 82. Wooley P.H., Luthra H.S., Singh S.K., Huse A.R., Stuart J.M., David C.S. Passive transfer of arthritis to mice by injection of human anti-type II collagen antibody. *Mayo. Clin. Proc.* **59**:737 (1984)
 83. Hernandez A.D., Cremer M.A., Townes A.S., Stuart J.M., Kang A.H. The immune response of guinea pigs to type II collagen: poor cross-reactivity with homologous type II collagen accounts for resistance to collagen-induced arthritis. *Immunology* **63**:619 (1988)
 84. Trentham D.E., Townes A.S., Kang A.H. Autoimmunity to type II collagen: an experimental model of arthritis. *J. Exp. Med.* **146**:857 (1977)
 85. Stuart J.M., Cremer M.A., Dixit S.N., Kang A.H., Townes A.S. Collagen-induced arthritis in rats. Comparison of vitreous and cartilage-derived collagens. *Arthritis Rheum.* **22**:347 (1979)
 86. Courtenay J.S., Dallman M.J., Dayan A.D. Martin A., Mosedale B. Immunization against heterologous type II collagen induces arthritis in mice. *Nature* **283** (1980)
 87. Boissier M.C., Carlioz A., Fournier C. Experimental autoimmune arthritis in mice. II. Early events in the elicitation of the autoimmune phenomenon induced by homologous type II collagen. *Clin. Immunol. Immunopathol.* **48**:225 (1988)
 88. Holmdahl R., Jansson L., Larsson E., Rubin K., Klareskog L. Homologous type II collagen induces chronic and progressive arthritis in mice. *Arthritis Rheum.* **29**:106 (1986)
 89. 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.* **33**:693 (1990)
 90. Caulfield J.P., Hein A., Dynesius-Trentham R., Trentham, D.E. Morphologic demonstration of two stages in the development of type II collagen-induced arthritis. *Lab. Invest.* **46**:321 (1982)
 91. Klareskog L., Holmdahl R., Larsson E., Wigzell H. Role of T lymphocytes in collagen II induced arthritis in rats. *Clin. Exp. Immunol.* **51**:117 (1983)
 92. Breedveld F.C., Trentham R.D., De Sousa M., Trentham D.E. Collagen arthritis in the rat is initiated by CD4⁺ T cells and can be amplified by iron. *Cell. Immunol.* **121**:1 (1989)
 93. Holmdahl R., Jonsson R., Larsson P., Klareskog L. Early appearance of activated CD4 positive T lymphocytes and Ia- expressing cells in joints of DBA/1 mice immunized with type II collagen. *Lab. Invest.* **58**:53 (1988)
 94. Holmdahl R., Tarkowski A., Jonsson R. Involvement of macrophages and dendritic cells in synovial inflammation of collagen induced arthritis in DBA/1 mice and spontaneous arthritis in MRL/Lpr mice. *Autoimmunity* **8**:271 (1991)
 95. Morgan K., Clague R.B., Shaw M.J., Holt P.J.L. Native type I collagen-induced arthritis in the

- rat. I. Incidence and humoral response to collagen. *Ann. Rheum. Dis.* **39**:285 (1980)
96. Griffiths M.M., Eichwald E.J., Martin J.H., Smith C.B., Dewitt C.W. Immunogenetic control of experimental type II collagen-induced arthritis. I. Susceptibility and resistance among inbred strains of rats. *Arthritis Rheum.* **24**:781 (1981)
 97. Trentham D.E. Collagen arthritis as a relevant model for rheumatoid arthritis. Evidence pro and con. *Arthritis Rheum.* **8**:911-916 (1982)
 98. Prickett J.D., Trentham D.E., Robinson D.R. Dietary fish oil augments the induction of arthritis in rats immunized with type II collagen. *J. Immunol.* **132**:725 (1984)
 99. Westmacott D., Hawkes J.E., Wadsworth J., Cashin C.H., Cline A., Bradshaw D., Bloxham D.P. Prostaglandin E2 and collagenase release by cultured talus from type II collagen arthritis rats. *Agents Actions* **19**:320 (1986)
 100. Schrier D., Gilbertsen R.B., Lesch M., Fantone J. The role of neutrophils in type II collagen arthritis in rats. *Am. J. Pathol.* **117**:26 (1984)
 101. Beauchamp C., Gilbertsen R.B., Fantone J.C., Menapace D.P. Disease modifying activity of superoxide dismutase in collagen-induced arthritis in the rat. *Fred. Proc.* **42**:1377 (1983)
 102. T Hart B.A., Simons J.M., Knaan-Shanzer S., Bakker N.P.M., Labadie R.P. Antiarthritic activity of the newly developed neutrophil oxidative burst antagonist apocynin. *Free Biol. Med.* **9**:127 (1990)
 103. Wooley P.H., Luthra H.S., Stuart J.M., David C.S. Type II collagen induced arthritis in mice. I. Major histocompatibility complex (I-region) linkage and antibody correlates. *J. Exp. Med.* **154**:688 (1981)
 104. Holmdahl R., Klareskog L., Andersson M., Hansen C. High antibody response to autologous type II collagen is restricted to H-2q. *Immunogenetics* **24**:84 (1986)
 105. Griffiths M.M., DeWitt C.W. Immunogenetic control of experimental collagen-induced arthritis in rats. II. ECIA susceptibility and immune response to type II collagen (calf) are linked to RTI. *J. Immunogenet.* **8**:463 (1981)
 106. Holmdahl R., Jansson L., Andersson M., Larsson E. Immunogenetics of type II collagen autoimmunity and susceptibility to collagen arthritis. *Immunology* **65**:305 (1988)
 107. Holmdahl R., Karlsson M., Andersson M.E., Rask L., Andersson L. Localization of a critical restriction site on the I-A-beta chain that determines susceptibility to collagen -induced arthritis. *Proc. Natl. Acad. Sci. USA* **86**:9475 (1989)
 108. Banerjee S., Hillman K., McCormick D.J., Luthra H.S., David C.S. Proliferative inhibition of an I-Aq restricted type II collagen T cell clone by I-A(alpha)q 71-85 synthetic peptide. *Arthritis Rheum.* **31**:532 (1988)
 109. Holmdahl R., Andersson M., Goldschmidt T.J., Gustafsson K., Jansson L., Mo J.A. Type II collagen autoimmunity in animals and provocations leading to arthritis. *Immunol. Rev.* **118**:193 (1990)
 110. Seki N., Sudo Y., Yoshikoka T., Sugihara S., Fujitsu T., Sakuma S., Ogawa T., Hamaoka T., Senoh H., Fujiwara H. Type II collagen-induced murine arthritis. I. Induction and perpetuation of arthritis require synergy between humoral and cell-mediated immunity. *J. Immunol.* **140**:1477 (1988)
 111. Stuart J.M., Cremer M.A., Yownes A., Kang A. 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 (1982)
 112. Kerwar S., Englert M.E., McReynolds R., Landes M., Lloyd J., Oronsky A., Wilson F. Type II collagen-induced arthritis. Studies with purified anticollagen immunoglobulin. *Arthritis Rheum.* **26**:1120 (1983)
 113. Holmdahl R., Rubin K., Klareskog L., Larsson E., Wigzell H. Characterization of the antibody response during collagen II induced arthritis in mice using anti-collagen II antibodies. *Arthritis Rheum.* **29**:400 (1986)
 114. Takagishi K., Kaibara N., Hotokebuchi T., Arita C., Morinaga M., Arai K. Serum transfer of collagen arthritis in congenitally athymic nude rats. *J. Immunol.* **6**:3864 (1985)
 115. Stuart J.M., E.J. Dixon. Serum transfer of collagen induced arthritis in mice. *J. Exp. Med.* **158**:378 ((1983)
 116. Morgan K., Claque R.B., Shaw M.J., Firth S.A., Trevor M.T., Holt P.J.L. Native type II

- collagen-induced arthritis in the rat. The effect of complement depletion by cobra venom factor. *Arthritis Rheum.* **24**:1356 (1981)
117. Kerwar S.S., Bauman N., Oronsky A.L., Sloboda A.E. Studies on type II collagen induced polyarthritis in rats. Effect of complement depletion. *J. Immunopharmacology* **3**:323 (1982)
 118. Watson W.C., Townes A.S. Genetic susceptibility to murine collagen II autoimmune arthritis. Proposed relationship to the IgG2 autoantibody subclass response, complement C5, major histocompatibility complex (MHC) and non-MHC loci. *J. Exp. Med.* **162**:1878 (1985)
 119. Helfgott S.M., Bazin H., Dessein A., Trentham D.E. Suppressive effects of anti-u serum on the development of collagen arthritis in rats. *Clin. Immunol. Immunopathol.* **31**:403 (1984)
 120. Nordling C., Holmdahl R., Klareskog L. Down-regulation of collagen arthritis after in vivo treatment with a syngeneic monoclonal anti-idiotypic antibody to a cross-reactive idiotope on collagen II auto-antibodies. *Immunology* **72**:486 (1991)
 121. Holmdahl R., Andersson M., Tarkowski A. Origin of the autoreactive anti-type II collagen response I. Frequency of specific and multispecific B cells in primed murine lymph nodes. *Immunology* **61**:369 (1987)
 122. Holmdahl R., Nordling C., Rubin K., Tarkowski A., Klareskog L. Generation of monoclonal rheumatoid factors after immunization with collagen II-anti-collagen II immune complexes. An anti-idiotypic antibody to anti-collagen II is also a rheumatoid factor. *Scand. J. Immunol.* **24**:197 (1986)
 123. Punjabi C.J., Wood D.D., Wooley P.H. A monoclonal anti-type II collagen antibody with cross-reactive anti-IgG specific for the F(ab)2 fragment. *J. Immunol.* **141**:3819 (1988)
 124. Heinz H.P., Rubin K., Laurell A.B., Loos M. Common epitopes in C1q and collagen type II. *Mol. Immunol.* **26**:163 (1990)
 125. Holmdahl R., Bailey C., Enander I., Mayer R., Klareskog L., Moran T., Bona C. Origin of the reactive anti-type II collagen response. II. Specificities, antibodies isotypes and usage of V gene families of anti-type II collagen B cells. *J. Immunol.* **142**:1881 (1989)
 126. Wooley P.H., Luthra H.S., Griffiths M.M., Stuart J.M., Huse A., David C.S. Type II collagen-induced arthritis in mice IV. Variations in immunogenetic regulation provide evidence for multiple arthritogenic epitopes on the collagen molecule. *J. Immunol.* **4**:2443 (1985)
 127. Terato K., Hasty K.A., Cremer M.A., Stuart J.M., Townes A.S., Kang A.H. Collagen-induced arthritis in mice. Localization of an arthritogenic determinant to a fragment of the type II collagen molecule. *J. Exp. Med.* **162**:637 (1985)
 128. Burkhardt H., Holmdahl R., Deutzmann R., Wiedemann H., Von der Mark H., Goodman S., Von der Mark K. Identification of a major antigenic epitope on CNBr-fragment 11 of type II collagen recognized by murine autoreactive B cells. *Eur. J. Immunol.* **21**:49 (1991)
 129. Brahn E., Trentham D.E. Effect of antithymocyte serum on collagen arthritis in rats: evidence that T cells are involved in its pathogenesis. *Cell. Immunol.* **86**:421 (1984)
 130. Ranges G., Sriram S., Cooper S.M. Prevention of type II collagen-induced arthritis by in vivo treatment with anti-L3T4. *J. Exp. Med.* **162**:1105 (1985)
 131. Goldschmidt T.J., 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.* **21**:1327 (1991)
 132. Wooley P.H., Luthra H.S., Lafuse W.P., Huse A., Stuart J.M., David C.S. Type II collagen-induced arthritis in mice. III. Suppression of arthritis by using monoclonal and polyclonal anti-Ia antisera. *J. Immunol.* **134**:2366 (1985)
 133. Banerjee S., Wei B., Hillman K., Luthra H.S., David C.S. Immunosuppression of collagen-induced arthritis in mice with an anti-IL-2 receptor antibody. *J. Immunol.* **141**:1150 (1988)
 134. Holmdahl R. Autoreactive B cells in collagen-induced arthritis (ed. T.F. Kresina) Marcel Dekker Inc. (1990)

135. Holmdahl R., Klareskog L., Rubin K., Larsson E., Wigzell H. 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 (1985)
136. Holmdahl R., Klareskog L., Rubin K., Bjork J., Smedegard G., Jonsson R., Andersson M. Role of T lymphocytes in murine collagen induced arthritis. *Agents and Actions* 19:295 (1986)
137. Kakimoto K., Katsuki M., Hirofuji T., Iwata H., Koga T. Isolation of T cell line capable of protecting mice against collagen-induced arthritis. *J. Immunol.* 140:78 (1988)
138. Brahn E., Trentham D.E. Experimental synovitis induced by collagen-specific T cell lines. *Cell. Immunol.* 118:491 (1989)
139. Kaibara N., Hotokebuchi T., Takagishi K., Katsuki I. Paradoxical effects of cyclosporin A on collagen arthritis in rats. *J. Exp. Med.* 158:2007 (1983)
140. Henderson B., Staines N.A., Burrai I., Cox J.H. The anti-arthritic and immunosuppressive effects of cyclosporin on arthritis induced in the rat by type II collagen. *Clin. Exp. Immunol.* 57:51 (1984)
141. Banerjee S., Haqqi T.M., Luthra H.S., Stuart J.M., David C.S. Possible role of VB T cell receptor genes in susceptibility to collagen-induced arthritis in mice. *J. Exp. Med.* 167:832 (1988)
142. Haqqi T.M., David C.S. T-cell receptor VB genes repertoire in mice possible role in resistance and susceptibility to type II collagen-induced arthritis. *J. Autoimmunity* 3:113 (1990)
143. Goldschmidt T.J., Jansson L., Holmdahl R. In vivo elimination of T cells expressing specific T-cell receptor VB chains in mice susceptible to collagen-induced arthritis. *Immunology* 69:508 (1990)
144. Andersson M., Goldschmidt T.J., Michaelsson E., Larsson A., Holmdahl R. T-cell receptor V-beta and complement component C5 play no significant role for the resistance to collagen-induced arthritis in the SWR mouse. *Immunology* 73:191 (1991)
145. Fujita M., Mishima M., Iwabuchi K., Katsume C., Gotohda T., Ogasawara K., Mizuno Y., Good R.A., Onoe K. A study on type II collagen-induced arthritis in allogenic bone marrow chimaeras. *Immunology* 66:422 (1989)
146. Andersson M., Holmdahl R. Analysis of type II collagen-reactive T cells in the mouse. I. Different regulation of autoreactive vs. non-autoreactive anti-type II collagen T cells in the DBA/1 mouse. *Eur. J. Immunol.* 20:1061 (1990)
147. Cremer M.A., Hernandez A.D., Townes A.S., Stuart J.M., Kang A.H. Collagen-induced arthritis in rats: antigen-specific suppression of arthritis and immunity by intravenously injected native type II collagen. *J. Immunol.* 131:2995 (1983)
148. Englert M.E., Landes M.J., Oronsky A.L., Kerwar S.S. Suppression of type II collagen-induced arthritis by intravenous administration of type II collagen or its constituent peptide alpha1(II)CB10. *Cell. Immunol.* 87:357 (1984)
149. Nagler-Anderson C., Bober L.A., Robinson M.E., Siskind G.W., Thorbecke G.J. Suppression of type II collagen-induced arthritis by intragastric administration of soluble type II collagen. *Proc. Natl. Acad. Sci. USA* 83:7443 (1986)
150. Butler L., Simmons B., Zimmerman J., Deriso P., Phadke K., Hom J. Regulation of cellular and humoral immune responses to collagen type I or collagen type II. *Immunology* 63:611 (1988)
151. Williams R.O., Whyte A., Waldman H. Resistance to collagen-induced arthritis in DBA/1 mice by intraperitoneal administration of soluble type II collagen involves both CD4⁺ and CD8⁺ T lymphocytes. *Autoimmunity* 4:237 (1989)
152. Rahman J., Staines N.A. Contribution of the spleen, lymph nodes and bone marrow to the antibody response in collagen-induced arthritis in the rat. *Clin. Exp. Immunol.* 85:48 (1991)
153. Brahn E., Trentham D.E. Antigen-specific suppression of collagen arthritis by adoptive transfer of spleen cells. *Clin. Immunol. Immunopathol.* 31:124 (1984)
154. Burrai I., Henderson B., Knight S.C., Staines N.A. Suppression of collagen type II-induced arthritis by transfer of lymphoid cells from rats immunized with collagen. *Clin. Exp.*

Immunol. 61:368 (1985)

155. Kresina T.F., Moskowitz R.W. Adoptive transfer of suppression of arthritis in the mouse model of collagen-induced arthritis. *J. Clin. Invest.* 75:1990 (1985)
156. Myers L.K., Stuart J.M., Kang A.H. A CD4 cell is capable of transferring suppression of collagen-induced arthritis. *J. Immunol.* 143:3976 (1989)
157. Myers L.K., Stuart J.M., Seyer J.M., Kang A.H. Identification of an immunosuppressive epitope of type II collagen that confers protection against collagen-induced arthritis. *J. Exp. Med.* 170:1999 (1989)
158. Kaibara N., Morinaga M., Arita C., Hotokebuchi T., Takagishi K. Serum transfer of collagen arthritis to cyclosporin-treated, type II collagen-tolerant rats. *Clin. Immunol. Immunopathol.* 35:252 (1985)
159. Arai K.L., Kaibara N., Takagishi K., Hatakebuchi T., Arita C. Reversal of antigen-induced resistance to collagen arthritis by cyclophosphamide. *Clin. Immunol. Immunopathol.* 43:325 (1987)

CHAPTER 2

EXPERIMENTAL IMMUNE MEDIATED ARTHRITIS IN RHESUS MONKEYS A model for human rheumatoid arthritis ?

N.P.M. Bakker, M.G.M Van Erck, C. Zurcher¹, P. Faaber²,
A. Lemmens³, M. Hazenberg⁴, R.E. Bontrop and M. Jonker

- 1 C. Zurcher MD, TNO-IVVO Institute, Leiden, The Netherlands.
- 2 P. Faaber PhD, Department of Rheumatology, Sint Radboud Hospital, University of Nijmegen, The Netherlands.
- 3 A. Lemmens MD, Department of Radiologic diagnosis, Sint Radboud Hospital, University of Nijmegen, the Netherlands.
- 4 M. Hazenberg, PhD, Department of Immunology, Erasmus University, Rotterdam, The Netherlands.

SUMMARY

The induction of experimental arthritis in rhesus monkeys was studied by i.d. immunization of B-CII and antigens derived from MT, *Streptococcus pyogenes* and *Eubacterium aerofaciens*. The tested bacterial antigens proved to be not arthritogenic. In this study B-CII induced clinical arthritis in 50% of the rhesus monkeys. CIA in rhesus monkeys proved to be a potential model to study clinical, serologic, histological, genetic and immunologic features associated with human RA.

INTRODUCTION

In recent years, strong evidence has accumulated that human RA is an autoimmune-mediated arthropathy (1). To understand more about the factors contributing to arthritis in susceptible individuals, animal models have been used to investigate the pathogenesis of the disease. A widely used model in rats is adjuvant arthritis, inducible by immunization with MT (2). There is evidence for a role of *Mycobacterium* in patients with RA. In these patients T cell reactivity to MT has been reported (3) and patients with advanced cancer, treated with BCG immunotherapy, may develop arthritic symptoms (4). Arthritis in rats has been induced by administration of cell walls derived from group A streptococci (5) or from *Eubacterium* species (6). In human patients Streptococcal infections occasionally give rise to acute rheumatic fever (7). The induction of arthritis by *Eubacterium* cell wall fragments, a major component of the human anaerobic faecal flora, suggests a possible etiological role of intestinal bacteria in RA (8). A non-bacterial rodent model is represented by CIA. Antibodies to CII are found in sera derived from patients with relapsing polychondritis (9) and in some instances for RA (10, 11). Thus, the immune response to CII may be an event of general importance in the pathogenesis of human RA. All the above mentioned arthritis models in rodents share common clinical and histological features with human RA. Nevertheless, the relevance of these experimental rodent models for human arthritis is debated (12). The study of arthritis in rhesus monkeys is, because of its close phylogenetic relationship with man (13) interesting, and may reflect many similar immunologic features of the human disease. The natural occurrence of arthritis in non-human primates has been described in the gorilla (14) and in rhesus monkeys (15, 16, 17). It is possible to induce arthritis in rhesus monkeys (18, 19) and squirrel monkeys (20) by i.d. injection of B-CII. In this study arthritogenic properties of a number of

bacterial antigens and B-CII in the rhesus monkey were investigated. Next to clinical observations, rontgenological and histological examinations were performed. Moreover, a number of hematological, immunologic and serologic parameters was measured. The aim of this study was to compare the clinical pathologic and immunologic parameters with those observed in human RA in order to examine the relevance of experimentally induced arthritis in the rhesus monkey as a model for immune mediated arthritis in man.

MATERIALS AND METHODS

Animals

All rhesus monkeys (*Maccaca mulatta*) are unrelated and were born and raised in the Rijswijk TNO Primate Center. The female monkeys were 3.0 to 21.0 years of age weighing 3.9-5.8 kg. The male monkeys were 4.0 to 21.0 years of age weighing 4.4-9.3 kg. The animals were examined, weighed and bled under ketamin anesthesia. If an animal was experiencing severe pain it was given twice a day 0.06 mg Buprenorfine (Temgesic, Warrick B.V., The Netherlands) by i.m. injection.

Collagen preparation

B-CII was prepared from either bovine joint cartilage (kindly donated by Dr. W. van der Berg, University of Nijmegen, The Netherlands) or nasal septum cartilage. Purification of collagen from both sources was performed in the same manner. The cartilage was dissected free of surrounding connective tissue and bone and extracted in 4M guanidine chloride for 24h at 4°C. The precipitate, obtained by centrifugation, was subjected to a limited pepsin digestion (1 mg/ml) for 48 h in 0.1 M HAc at 20°C in the presence of gentamicine (1 µl/ml). The supernatant was collected by centrifugation and adjusted to pH 7.4. Purification of CII was performed by salt precipitation with 20% NaCl during 48h at 4°C. After centrifugation the precipitate was dissolved into 0.5 M HAc and dialyzed against the same solvent during 5h. This solution was centrifugated, the supernatant was collected and a second precipitation step was performed with 5% NaCl at 4°C overnight. The precipitated collagen was collected by centrifugation and washed with distilled water and subsequently stored lyophilized at -20 °C until use.

Bacterial antigens

Heat-killed MT H37Ra was purchased from Difco Laboratories (Detroit, USA). Cell

wall fragments of *Streptococcus pyogenes* and *Eubacterium aerofaciens* were prepared as described previously (5).

Immunization procedures

All antigens were injected i.d. on the lower back.

Bacterial antigens (Table 1) Five rhesus monkeys were injected with 10 mg MT H37Ra preparation, emulsified in ICFA (Difco, Detroit, USA).

Four monkeys were injected with an emulsion of *Streptococcus pyogenes* cell walls in ICFA. Two of these monkeys received in total 2.5 mg and two received 5.0 mg. All of these four monkeys were challenged at day 105 by immunization with 4.0 mg *Streptococcus pyogenes* cell wall fragments emulsified in ICFA.

Four rhesus monkeys were injected with an emulsion of *Eubacterium aerofaciens* in CFA. Two of these monkeys received 5.0 mg and two received 10.0 mg.

Collagen (Table 1) For the primary immunization CII, isolated from bovine joint, was dissolved in 0.1 M HAc and emulsified in CFA (Difco, Detroit, USA). Six rhesus monkeys received 3.0 mg of CII, whereas two others received 1.0 mg CII. Four of these were challenged on day 87 with 3.0 mg CII, isolated from bovine joint, dissolved in 0.1 M HAc without adjuvant. The other monkeys were challenged at day 94 with 3.0 mg CII isolated out of the nasal septum. This CII was dissolved in 0.1 M HAc and emulsified in ICFA.

Clinical assessment of arthritis

Monkeys were observed daily for the presence of arthritis and a clinical score was recorded weekly for the IP joints. Swelling of the MCP and MTP joints, tarsal and carpal joints, elbows, knees, shoulders, hips and spinal joints were documented but not scored on a scale.

Radiographic examinations

Radiographic projections were made (with a Kodak X-Omatic cassette, single fine screen) of hands, wrists, feet, ankles, knees and elbows. The first projections were made as soon as the first clinical signs of arthritis were observed. The follow-up projections were made at about 3 weeks intervals.

Blood parameters

Every 14 days the following parameters were measured:

- hematological parameters: ESR, total leucocytes, differential white blood cell counts, erythrocyte count, platelet count, hemoglobin and hematocrit.
- biochemical parameters: albumin, total protein, urea, alkaline phosphatase, uric acid, glucose and transferrin.
- acute phase reactants: CRP, alpha-1-anti-trypsin, haptoglobin and complement component C3.
- immunologic parameters: levels of total IgG, IgA and IgM.

Autoimmune parameters

To detect IgG, IgA and IgM isotypes of rheumatoid factor, specific conjugated anti-human IgG, IgA and IgM antibodies were used in an ELISA assay. Before use, the anti-human conjugates were first tested for cross-reactivity with rhesus monkey immunoglobulins. The IgM rheumatoid factor titer was also determined by latex agglutination. Human IgG-coated latex particles were purchased from Behringwerke AG, Marburg, Germany. Circulating immune complexes were measured with the C1q-binding assay (21).

Humoral antibody response to collagen

Titration of serum antibody to B-CII (sample used for primary immunization), was performed by ELISA. Flexible 96 well assay plates (Falcon 3911, Oxnard, CA) were coated overnight at 4 °C with antigen (25 µg/ml) 25 µl/well. Plates were postcoated with 200 µl 3% BSA in PBS for 60 min. Serum samples (1:200) were added, 20 µl/well, and incubated for 2 h at 37 °C. Subsequently, a peroxidase-conjugated rabbit anti-monkey IgG-H+L chains fraction (Nordic, Tilburg, The Netherlands) was added, 20 µl/well, at a 1:10,000 dilution and the plates were incubated for 2 h at 37°C. Substrate (O-phenyl-enediamine, Kodak, Rochester, NY) was added and the reaction was stopped by adding 2N H₂SO₄. The plates were read on a Titertek Multiskan Plus Mark II.

Necropsy and histology

Rhesus monkey 1JT was euthanized on day 122 during a period of active clinical arthritis, because of the severe state of the disease. The knee and elbow joints were opened and the gross lesions were documented. After fixation, the IP-joints of hand and feet were separately embedded in paraffin and sections of 3 µm were stained with hematoxylin-phloxine-saffron (HPS).

RESULTS

Clinical observations

Arthritis induction Rhesus monkeys immunized with MT, *S. pyogenes* or *E. aerofaciens*, did not demonstrate clinical signs of arthritis (Table 1).

Four of the eight rhesus monkeys (1 female and 3 males), immunized with CII did not reveal any clinical signs of arthritis. Four developed a symmetrical polyarthritis mainly located in IP-joints of hand and feet (Fig.1).

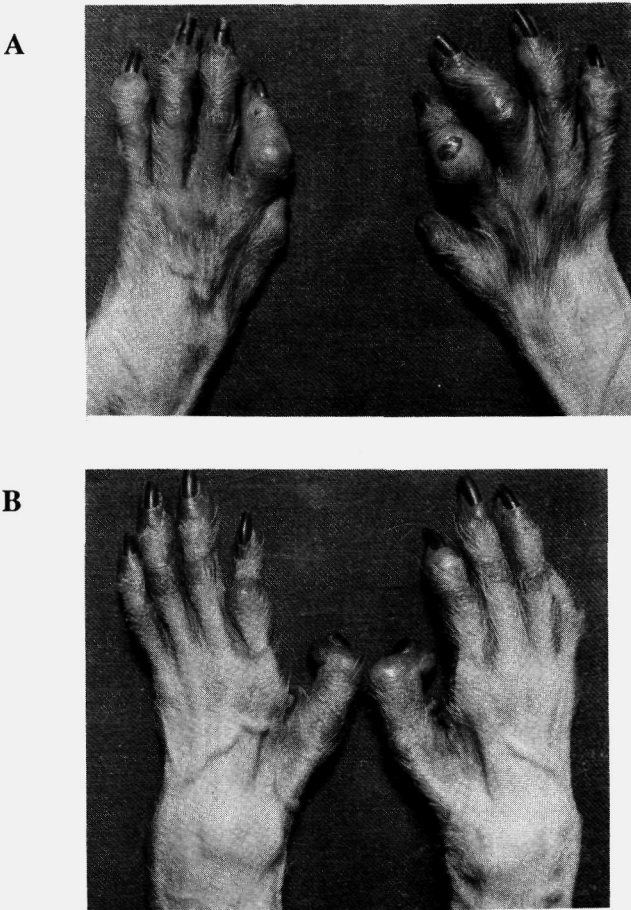


Figure 1. Soft tissue swelling and erythema in hands (A) and feet (B) of rhesus monkey M15, 30 days after onset of disease.

Table 1

Incidence of clinical arthritis in rhesus monkeys after intradermal injection of antigens

monkey	sex	Mamu-DR	antigen	primary immunization		second immunization			
				dose (mg/ml)	adjuvant	arthritis	dose (mg/ml)	adjuvant	arthritis
KD	M	5/5	MT	10.0	ICFA	-			
Z	M	3/3	MT	10.0	ICFA	-			
M6	M	8/2	MT	10.0	ICFA	-			
Y	F	8/3	MT	10.0	ICFA	-			
2813	F	3/3	MT	10.0	ICFA	-			
1RW	M	-/4	SP	2.5	ICFA	-	4.0	ICFA	-
1TD	M	5/8	SP	2.5	ICFA	-	4.0	ICFA	-
1TM	M	5/3	SP	5.0	ICFA	-	4.0	ICFA	-
1WY	F	1/2	SP	5.0	ICFA	-	4.0	ICFA	-
1UC	M	2/1	EA	5.0	CFA	-			
1UI	M	2/8	EA	5.0	CFA	-			
1XE	M	5/2	EA	10.0	CFA	-			
1WJ	F	5/5	EA	10.0	CFA	-			
1NR	M	5/101	CII	1.0 ^a	CFA	-	3.0 ^a	-	-
1RO	F	3/3	CII	1.0 ^a	CFA	-	3.0 ^a	-	-
1UY	M	1/2	CII	3.0 ^a	CFA	-	3.0 ^a	-	-
M14	M	2/8	CII	3.0 ^a	CFA	-	3.0 ^b	ICFA	-
1OX	F	-/8	CII	3.0 ^a	CFA	+	3.0 ^a	-	-
M15	M	3/8	CII	3.0 ^a	CFA	+	3.0 ^b	ICFA	-
1JT	F	3/2	CII	3.0 ^a	CFA	-	3.0 ^b	ICFA	+
1KM	F	3/1	CII	3.0 ^a	CFA	+	3.0 ^b	ICFA	(+)

37

M = male; F = female; Mamu-DR = serologically defined equivalent of the HLA-DR type of the rhesus monkey; - = DR blank; MT = *Mycobacterium tuberculosis*; SP = *Streptococcus pyogenes*; EA = *Eubacterium aerofaciens*; CII = bovine type II collagen; ICFA = incomplete Freund's adjuvant; CFA = complete Freund's adjuvant. ^aCII isolated from the bovine joint; ^bCII isolated from the nasal spectrum.

In this limited number of CII immunized animals, no correlation was found between the Mamu-DR type and the incidence or severity of the arthritis (Table 1). The very first signs of disease in all animals were: decreased mobility, severe apathy and poor appetite associated with loss of weight. The most severe inflammatory activity was observed during the first 14 days after onset of arthritis (Table 2).

Table 2

Clinical arthritis and radiographic scores of the distal interphalangeal (DIP) and proximal interphalangeal (PIP) joints of hands and feet

monkey	day (a.i/a.o.)	FINGERS		FEET	
		DIP (CS/RS)	PIP (CS/RS)	DIP (CS/RS)	PIP (CS/RS)
1OX	28/0	1/A	1/A	2	3
	42/14	2/A-B	3/A	2/A	3
	50/22	2-4	2	2-4	2-3
	64/36	4	1	4	2
	72/44	4	1	4	2-4
	86/58	4/C	1/A	4/A	4/A-B
	92/64	4	1	4-5	4
	106/78	4/C	1/A	4/A	4/A
M15	21/0	2/A	2/A-B	1/A	1/A
	28/7	2-3	2-3	1-2	1-2
	34/13	2-3	3	2	2
	41/20	2-4	2-3	2	1-2
	55/34	4	2-4	2-4	1
	62/41	5/D	4/B-D	4-5/C-D	1/B-C
1JT	104/0	1/A	1/A-B	2/A	2/B
	111/7	3	2-3	3	3
	118/14	3	2-3	3	3
	122/18	3/B	2/B	3/B	3/B-C
1KM	41/0	1	1	1/A	1-2/A
	48/7	1-2/A	2/A-B	2/A	2/A
	55/14	2	1-2	2	2
	69/28	1-2	1	2-4	1
	77/36	1/A	1/A-B	4/C	1/C
	83/42	1	1	4	1
	118/77	1	1	4	1
	125/84	1	1	2-4	1
	140/99	1	1	2-4	1
	153/112	1	1	2-4	1
	160/119	1	1	1	1

a.i = after immunization; a.o. = after onset of disease; CS = clinical score; RS = radiographic score. Clinical score: 1 = normal, 2 = light STS with redness, 3 = severe STS with redness, 4 = loss of flexion and extension, 5 = contracture. Radiographic score: A = normal, B = joint space narrowing, C = erosion, D = destruction, E = bony ankylosis

Clinical arthritis was first palpable as STS of the IP-joints in hands and feet. Only monkey 1OX showed a clinical arthritis which started with STS of the carpal joints 6 days before it was palpable in the IP joints. STS of carpal and tarsal joints was only observed 20-30 days after onset of arthritis. Especially STS of the carpal joints was correlated with severity of STS of the IP-joints. No clinical signs of arthritis were observed in the MCP- and MTP-joints, nor in shoulders or hips in any of the monkeys. 1JT showed, 10 days before its death, also STS of both elbows and both knees. M15 and 1JT developed a strong STS of the metatarsal-tarsus region of both feet (Fig.1).

Onset / course arthritis The time of onset of the first signs of arthritis after primary immunization, as well as the duration of clinical signs varied among animals (Fig.2).

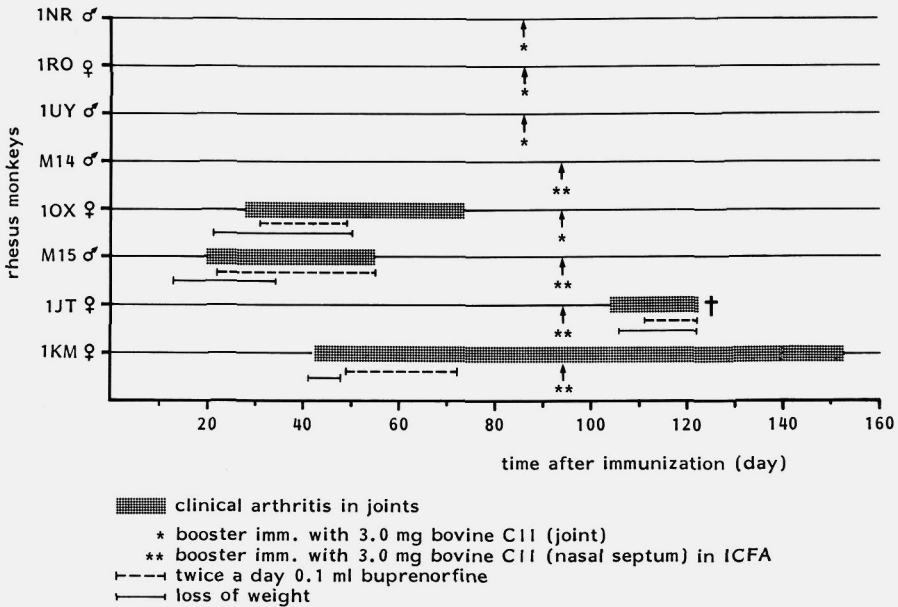


Figure 2. Onset and duration of clinical signs of arthritis in the joints.

Three monkeys (M15, 1OX and 1KM) developed arthritis within 42 days after primary immunization. Only one monkey (1JT) showed the first clinical signs 10 days after the second immunization. Two animals, M15 and 1OX showed an acute, self-limiting arthritis of respectively 35 and 46 days. The severe STS, especially of the DIP of hands and feet, resulted in contracture of these joints after a period of about three weeks (Table 2). The course of arthritis was explosive in 1JT. The state of disease and loss of weight (25% in the last week), after 18 days of arthritis, was so severe that it was necessary to euthanize the animal. The course of disease in monkey 1KM which developed arthritis with a sub-acute character for more than 100 days was exceptional. Slight arthritic activity was most prominent in the DIP-joints of the feet after a flare-up of inflammation on day 84. This moderate arthritis in 1KM did not result in loss of function, except in the DIP joints of the feet due to the continual slight inflammation (Table 2).

Radiographic findings

Radiographic manifestations reflecting the inflammatory process were found in all arthritic monkeys. Early radiographic manifestations of the inflammatory process were STS and narrowing of the interosseus space, most marked in the IP-joints of hands and feet (Table 2). For example in rhesus monkey M15 (Table 2, Fig.3), after a period of about 40 days, the inflammatory activity resulted in marginal erosions of the cartilage and ultimately in total destruction of cartilage and of the underlying bone structure. Severe STS in M15 and 1JT of the metatarsal-tarsus region at both feet resulted in focal erosions in the tarsus and carpus.

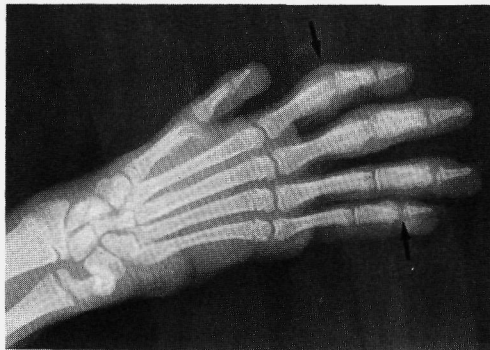


Figure 3. Radiographic changes in the right hand of rhesus monkey M15, 41 days after onset of disease.

Erosion of bone of the processus styloideus of the radius was observed in 1JT and 1KM. MCP and MTP joints showed no radiographical abnormalities. Only monkey M15 showed some erosive lesions on day 41 after the onset of arthritis in MCP1 of the left hand. No abnormalities were found in the radiographs of other joints such as knees, elbows, shoulders and hips.

Macroscopic observations

Necropsy was only performed on monkey 1JT 18 days after active arthritis. All large diarthrodial joints were examined. Both knees and elbow joints showed synovial hyperplasia accompanied by a yellowish discoloration of the synovial fluid. The synovial hyperplasia was accompanied by strong edema and hyperaemia. The articular cartilage in both knees showed small lesions.

Histology

Compared to a normal IP-joint of a non-affected rhesus monkey with smooth cartilage surface and normal synovial tissue, several abnormalities were seen in arthritic IP-joints (Fig.4).



Figure 4. IP joint of rhesus monkey 1JT, 18 days after onset of clinical arthritis in the joint (x40).

The cartilage layer was thinner than normal with an irregular surface. The synovium was hyperplastic with neovascularization and infiltration of mononuclear cells and an increased number of fibroblasts. There was clear pannus formation with erosion of cartilage and subchondral bone. The joint space was widened and contained fibrin, necrotic debris and granulocytes.

Laboratory findings

ESR and CRP The presence and degree of inflammation was mainly reflected by the ESR and the CRP levels (Fig.5). The highest levels were measured in the period just after the onset of arthritis. In 1KM the ESR and CRP were persistently slightly elevated during the relatively long period of arthritis.

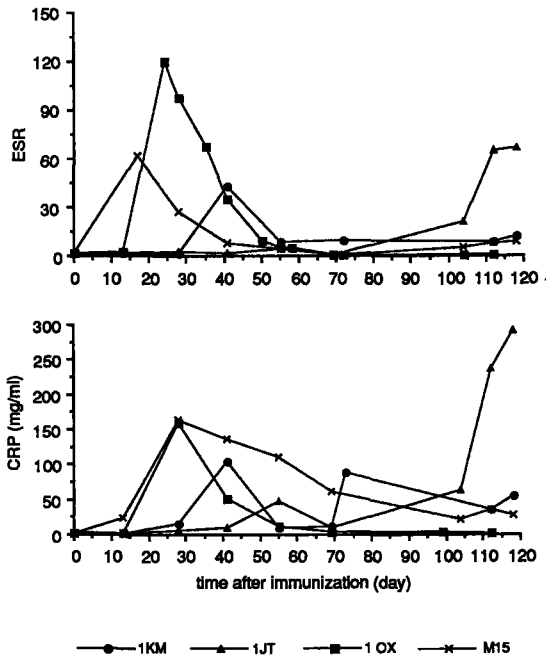


Figure 5. ESR and CRP levels in the arthritic monkeys.

Only the parameters listed below showed values outside the normal range in the arthritic monkeys (Table 3). All other parameters (see Materials and Methods) remained within the normal ranges (results not shown).

Hemoglobin/erythrocytes/hematocrit Anemia was present in three monkeys with arthritis, except in 1JT. This anemia was presented by decreased values of hemoglobin associated with reduced counts of erythrocytes and hematocrit. Lowered values were detected just before or during the period the first clinical symptoms of arthritis were observed in the monkeys.

Table 3**Blood parameters measured in the arthritic rhesus monkeys which were associated with arthritic activity**

blood parameter (normal range) ^{a)}	arthritic monkey	days after onset arthritis ^{b)}	measured values
hemoglobin (6.0-9.1 mmol/l)	1OX	-6 - 22	4.7 - 5.6
	M15	-4 - 48	4.4 - 5.2
	1KM	0 - 28	4.9 - 5.8
hematocrit (0.30-0.44 l/l)	1OX	-4 - 30	0.23 - 0.28
	M15	-4 - 48	0.23 - 0.27
	1KM	0 - 14	0.24 - 0.24
erythrocytes (4.0-6.4X10 ¹² /l)	1OX	-4 - 14	3.6 - 3.9
	M15	-4 - 34	3.4 - 3.8
	1KM	0 - 14	3.7 - 3.9
thrombocytes (192-605 10 ⁹ /l)	1OX	0 - 14	625 - 737
	1JT	14	622
albumin (34-46 g/l)	1OX	0 - 22	25 - 28
	M15	7 - 20	19 - 19
	1KM	0	29
	1JT	-49 8 - 14	27 28 - 32
alkaline phosphatase (64-414 U/l)	1OX	0 - 44	610 - 1361
	M15	20 - 48	750 - 791
	1KM	0 - 77	433 - 678
	1JT	-49 -35	441 - 619
C3 (0.8-1.7 g/l)	1OX	0 - 14	1.8 - 2.0
	M15	7 - 48	1.8 - 2.0
	1KM	0 - 77	1.8 - 2.5
	1JT	-49 7 - 14	2.3 2.0 - 2.1
leucocytes (3.4-14.3X10 ⁹ /l)	1OX	-6 - 7	18.7 - 27.2

a) as determined for the rhesus monkeys in TNO Primate Center, Rijswijk, The Netherlands

b) day 0 = first day rhesus monkey showed symptoms of clinical arthritis

Leucocytes Leucocytosis was noted in 1OX. There was no evidence of septic arthritis, as this animal had no fever and the bacteriological cultures of blood were negative.

Thrombocytes Thrombocytosis was observed in 1OX and 1JT during the period of severe active arthritis.

Albumin Hypoalbuminemia was noted in all monkeys which developed arthritis. The lowest values could be detected during the period of clinical arthritis, and was generally not correlated with the severity of arthritis.

Serum alkaline phosphatase Increased serum alkaline phosphatase levels were associated with active arthritis, except in 1JT.

C3

Elevated levels were found in 1OX and 1KM during the period of clinical arthritis. In M15 levels remained elevated after the period of active arthritis.

Immunoglobulins In the monkeys which developed arthritis, the total IgG and IgA levels were raised after the onset of arthritis (Fig.6).

Autoimmune parameters Immune complexes were not found and none of the assays resulted in detection of rheumatoid factors in the sera of non-arthritic or arthritic rhesus monkeys.

Sub-clinical inflammatory activity

In the period before the second immunization, i.e. day 49-35 before onset of clinical arthritis in 1JT, some of the blood parameters showed values which normally were associated with clinical arthritis in the other 3 monkeys. In this period 1JT showed a slightly elevated ESR and CRP level (Fig.5), decreased albumin and increased C3 and alkaline phosphatase levels (Table 3). In addition, an increase of total IgG was observed (Fig.6). Because these parameters were normally associated with clinical arthritis these findings were indicative for sub-clinical symptoms of inflammation.

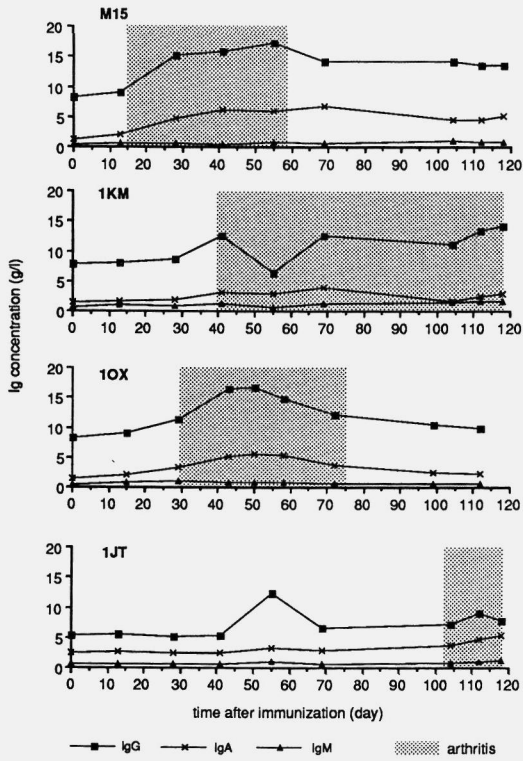


Figure 6. Levels of total IgA, IgM and IgG in the arthritic rhesus monkeys.

Humoral antibody response to type II collagen

All eight monkeys immunized with B-CII developed IgG antibody titers specific for this antigen (Fig. 7). No significant differences between arthritic and non-arthritic monkeys were observed.

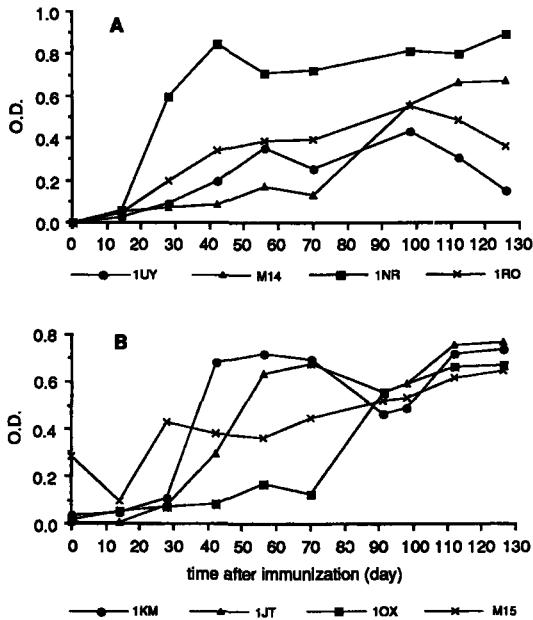


Figure 7. The anti-B-CII IgG antibody levels in non-arthritic (A) and arthritic (B) rhesus monkeys.

DISCUSSION

In contrast to rodent models in which arthritis can be induced in genetically susceptible strains with MT (2) and cell wall fragments of *Streptococcus* (5) or *Eubacterium* species (6), these antigens did not result in clinical arthritis in rhesus monkeys. This failure to induce arthritis may be due to preexisting immunity to these bacterial antigens or to the relatively small number of monkeys used in this experiment, which therefore may lack the required MHC-immune response gene(s).

As reported, B-CII proved to be arthritogenic in 50% of the rhesus monkeys. CIA in rhesus monkeys in this study is comparable to other studies (18,19) with respect to the affected joints. In all studies CIA is most prominent in the IP-joints of hands and feet and observed to a lesser extent in the carpal and tarsal joints. Rubin et al. (18) reported that only one out of seven male monkeys developed clinical signs of arthritis, but that after necropsy all monkeys revealed evidence of articular inflammation. Yoo et al. (19) reported clinical signs in all female monkeys. In contrast to these two studies, in which several booster immunizations were applied

to induce arthritis, in the present study only one immunization induced clinical or subclinical arthritis in 50% of the animals (3 females, 1 male). So, CIA in rhesus monkeys exhibits a clear variation in susceptibility and severity of the disease. Susceptibility or resistance to CIA in monkeys may possibly be influenced by sex or could be dependent on the immunization protocol. We checked the CII preparation by sodium dodecyl sulphate-gel electrophoresis for contamination, but we can not exclude contamination of the CII preparation with other cartilage derived collagens like type IX and type XI, from which it is shown that they have arthritogenic capacities (22). Genetic factors may also play an important role in the susceptibility to CIA. Genetic factors seem to control susceptibility to CIA in rats (23) and mice (24, 25). In human RA genetic factors, in particular MHC class II genes, have been reported to be associated with the disease (26, 27). This association is not absolute, indicating that other factors may play a role in the etiology and/or pathogenesis of human RA.

Genetic factors, other than sex, may also play a role in the susceptibility or resistance to CIA in rhesus monkeys. The number of animals in this study was too small to draw any firm conclusions.

Although CIA in rhesus monkeys is not self-perpetuating, clear clinical and radiographical similarities with human rheumatoid arthritis exist. For example, the peripheral diarthrodial joints are often inflamed symmetrically. The major radiographically abnormalities due to inflammation, like STS, narrowing of the joint space and marginal bony erosions are also observed in human RA (28). The histopathology showed synovial cell hyperplasia, neovascularization, mononuclear cell infiltration and pannus formation. These synovitis-related features were also reported in rodents (12) and in other CIA-nonhuman primates studies (18, 19, 20), and resembles human RA (1).

Several blood parameters were found to be associated with the arthritic activity in rhesus monkeys. ESR and CRP proved to be the most relevant ones for inflammatory activity. Increased ESR and CRP values have also been found in patients with RA (29, 30). Rheumatoid factors and immune complexes were not detected in the serum of arthritic monkeys in this study or other rodent models (12). Possibly the formation of immune complexes is restricted to the synovial tissue and can not be detected in serum. Next to an elevated level of total IgG and total IgA, the arthritic monkeys also developed hypoalbuminemia. A polyclonal increase of gamma globulin serum levels is frequently observed in seropositive RA patients, along with a reduction in the concentration of albumin (31). Other observed abnormalities include anemia, leucocytosis, thrombocytosis, and elevated alkaline

phosphatase levels. Anemia, usually moderate in degree, is seen in RA (1), whereas leucocytosis is common in juvenile arthritis and in RA patients with an acute and severe disease onset or acute exacerbations (32). Correlations between thrombocytosis and disease activity, anemia and leucocytosis have been noted in RA (33). In arthritic monkeys, highest levels of C3 were associated with periods of severe clinical arthritis. It is not clear whether C3 must be considered as an acute phase protein (a result of inflammatory activity) or that it plays a role in the pathogenesis of CIA. Binding of antibodies to the Rh-CII in the articular cartilage may form intra-articular immune complexes, and subsequently activate the complement cascade (34). C3 levels are usually normal in RA patients, but they are sometimes elevated as much as four times the normal mean values (35).

In accordance with other studies (18, 19), rhesus monkeys developed a IgG antibody response against heterologous CII which was not associated with clinical arthritis. A possible reason for the non-correspondance between anti-CII IgG antibodies and disease-activity can be that other isotypes are involved or because of the existence of multiple non-arthritisogenic epitopes on the B-CII molecule and a small number of arthritisogenic ones. Some studies have shown that anti-CII antibodies do not precede the clinical onset of human RA (36). However, significant levels were found at the first signs of disease, but had disappeared by the time a diagnosis of RA was made (37). Both in human RA and CIA in rhesus monkeys anti-CII antibodies may be important as a component in the destruction of articular cartilage. Next to antibodies, it is known that T cells play an important role in the pathogenesis of CIA in rodents (38,39) and that CII-specific T cell clones persist in the synovial membrane of RA patients (40). Preliminary results from this laboratory also indicate that T cells are involved in the immune response to CII.

In conclusion the monkey model resembles many features associated with active inflammatory stages of human RA. Since in this study only 50% of the rhesus monkeys developed arthritis, there may be a genetic factor for susceptibility, as is known in the case of human RA. Because the close phylogenetic relationship between man and monkey and the similarities of their immune systems, this rhesus monkey arthritis model may elucidate relevant information on mechanisms contributing to human arthritic diseases. Moreover, immunomodulating therapies, for instance pre-testing of MoAb's to cell surface antigens shared by humans and rhesus monkeys, can be performed in rhesus monkeys with a larger predictive value for clinical results than those tested in rodents.

REFERENCES

1. Kelley W.N., Harris E.D., Ruddy S., Sledge C.B. Textbook of Rheumatology. Second Edition. W.B. Sanders Compagny, Philadelphia (1985)
2. Pearson C.M. Experimental models in rheumatoid disease. *Arthritis Rheum.* 7:80 (1964)
3. Holoshitz J., Drucker I., Yaretsky A., Eden W. van, Klajman A., Lapidot Z., Frenkel A., Cohen I.R. T lymphocytes of rheumatoid arthritis patients show augmented reactivity to a fraction of mycobacteria cross-reactive with cartilage. *Lancet* II:305 (1986)
4. Torisu M., Miyahara T., Shinohara N., Ohsato K., Sonozaki H. A new side effect of BCG immunotherapy - BCG-induced arthritis in man. *Cancer Immunol. Immunother* 5:77 (1978)
5. Cromartie W.J., Craddock J.G., Schwab J.H., Anderle S.K., Yang C.H. Arthritis in rats after systemic injection of streptococcal cells or cell walls. *J. Exp. Med.* 146:1585 (1977)
6. Severijnen A.J., Kleef van R., Hazenberg M.P., Merwe van de J.P.: Cell wall fragments from major residents of the human intestinal flora induce chronic arthritis in rats. *J. Rheumatol.* 16:106 (1989).
7. Cantazarro F.J., Stenson C.A., Morris A.J., Chamowitz R., Rammelkamp C.H., Stolzer B.L., Perry W.D. Role of the streptococcus in the pathogenesis of rheumatic fever. *Am. J. Med.* 17:749 (1954)
8. Greenstein A.J., Janowitz H.D., Sachar D.B. The extra-intestinal complications of Crohn's disease and ulcerative colitis. A study of 700 patients. *Medicine* 55: 401 (1976)
9. Foidart J.M., Shigeto A., Martin G.R., Zizic T.M., Barnett E.V., Lawley T.J., Katz S.I. Antibodies to type II collagen in relapsing polycondritis. *N. Engl. J. Med.* 299:1203 (1978)
10. Charriere G., Hartmann D.J., Vignon E., Ronziere M.C., Herbage D., Ville G. Antibodies to types I, II, IX and XI collagen in the serum of patients with rheumatic diseases. *Arthritis and Rheum.* 31:325 (1988)
11. Pereira R.S., Black C.M., Duance V.C., Jones V.E., Jacoby R.K., Welsh K.I. Disappearing collagen antibodies in rheumatoid arthritis. *Lancet* II:501 (1985)
12. Trentham D.E. Collagen arthritis as a relevant model for rheumatoid arthritis. *Arthritis and Rheum.* 25: 911 (1982)
13. Balner H. The major histocompatibility system of subhuman primate species, The major histocompatibility system in man and animals. Edited by D Gotze. Springer Verlag, Berlin (1977)
14. Brown T.Mc., Clark H.W., Bailey J.S., Gray C.W. A mechanistic approach to treatment of rheumatoid type arthritis naturally occurring in a gorilla. *Transactions of the American Clinical and Climatological Association* 82:227 (1971)
15. Benditt E.P. and Eriksen N. Chemical characteristics of the substance of typical amyloidosis in monkeys. *Acta path. microbiol. scand.* Section a 80, suppl 233:103 (1972)
16. Chapman W.L., Crowell W.A. Amyloidosis in rhesus monkeys with rheumatoid arthritis and enterocolitis. *JAVMA* 171:855 (1977)
17. Bywaters E.G.L. Observations on chronic polyarthritis in monkeys. *J. Royal Soc. Med.* 74:794 (1981)
18. Rubin A.S., Healy C.T., Martin L.N., Baskin G.B., Roberts E.D. Experimental arthropathy induced in rhesus monkeys (*Macaca mulatta*) by intradermal immunization with native bovine type II collagen. *Lab. Invest.* 57:524 (1987)
19. Yoo T.J., Kim S.Y., Stuart J.M., Floyd R.A., Olson G.A., Cremer M.A., Kang A.H. Induction of arthritis in monkeys by immunization with type II collagen. *J. Exp. Med.* 168:777 (1988)
20. Cathcart E.S., Hayes K.C., Gonnerman W.A., Lazzari A.A., Franzblau C. Experimental arthritis in a nonhuman primate. *Lab. Invest.* 54:26 (1986)
21. Zubler R.H., Lange G., Lambert P.H. et al. Detection of immune complexes in untreated sera by a modified 125I-C1q binding test. *J. Immunol.* 116:232 (1976)
22. Morgan K., Evans H.B., Firth S.A., Smith M.N., Ayad S., Weiss J.B., Holt P.J.L. 1-alpha-2-alpha-3-alpha collagen is arthritogenic. *Ann. Rheum. Dis.* 42:680 (1983)

23. Griffiths M.M., Eichwald E.J., Martin J.H. et al. Immunogenetic control of experimental type II collagen-induced arthritis. I. Susceptibility and resistance among inbred strains of rats. *Arthritis Rheum.* **24**:781 (1981)
24. Watson W.C., Townes A.S. Genetic susceptibility to murine collagen II autoimmune arthritis. *J. Exp. Med.* **162**:1878 (1978)
25. Holmdahl R., Jansson L., Andersson M., Larsson E. Immunogenetics of type II collagen autoimmunity and susceptibility to collagen arthritis. *Immunology* **65**:305 (1988)
26. Strom H., Moller E.: HLA and rheumatoid arthritis. Arthritis study of five families. *Tissue Antigens* **18**:92 (1981)
27. Khan M.A., Kusher I., Braun W.E. et al. Clinical and HLA studies in multiple case studies with rheumatoid arthritis. *Tissue Antigens* **18**:136 (1981)
28. Renick D., Niwayama G. *Diagnosis of bone and joint disorders. Second Edition.* W.B. Saunders Company, Philadelphia (1988)
29. Short C.L., Bauer W., Reynolds W.E. Red-cell, white-cell, and differential counts, Rheumatoid Arthritis. Edited by CL Short, W Bauer, WE Reynolds. Harvard University Press, Cambridge MA, pp 349 (1957)
30. Pepys M.B. C-reactive protein (CRP), serum amyloid P-component (SAP) and serum amyloid A protein (SAA) in autoimmune disease, *Clinics in Immunology and Allergy. Volume 1(1)* Edited by EJ Holborrow. W.B. Saunders, Eastbourne (1981)
31. Rodnan G.P., Schumacher H.R. *Primer on the rheumatic diseases.* Arthritis Foundation, Atlanta (1983)
32. Toumbis A. et al. Clinical and serological observations in patients with juvenile rheumatoid arthritis and their relatives. *J. Pediatr.* **62**:463 (1963)
33. Selroos O. Thrombocytosis in rheumatoid arthritis. *Scand. J. Rheumatol.* **1**:136 (1972)
34. Jasin H.E. Autoimmunity specificities of immune complexes requested in articular cartilage of patients with rheumatoid arthritis and osteoarthritis. *Arthritis Rheum.* **28**:241 (1985)
35. Morrow J., Isenberg D. *Autoimmune Rheumatic Disease.* Blackwell Scientific Publications, Oxford (1987)
36. Mottonen T., Hannonen P., Oka M., Rautiainen J., Jokinen I., Arvilommi H., Palosuo T., Aho K. Antibodies against native type II collagen do not precede the clinical onset of rheumatoid arthritis. *Arthritis Rheum.* **31**:776 (1988)
37. Pereira R., Black C.M., Duance V.C., Jones V.E., Jacoby R.K., Welsh K.I. Disappearing collagen antibodies in rheumatoid arthritis. *Lancet* **II**:501 (1985)
38. Holmdahl R., Klareskog L., Rubin K., Larsson E., Wigzell H. T lymphocytes in collagen II-induced arthritis in mice. *Scand. J. Immunol.* **22**:295 (1985)
39. Brahn E., Trentham D.E. Effect of antithymocyte serum on collagen arthritis in rats: evidence that T cells are involved in its pathogenesis. *Cell. Immunol.* **86**:421 (1984)
40. Londei M., Savill C.M., Verhoef A., Brennan F., Leech Z.A., Duance V., Maini R.N., Feldmann M. Persistence of collagen type II-specific T-cell clones in the synovial membrane of a patient with rheumatoid arthritis. *Proc.Natl.Acad.Sci.USA* **86**:636 (1989)

CHAPTER 3

COLLAGEN INDUCED ARTHRITIS IN AN OUTBRED GROUP OF RHESUS MONKEYS COMPRISING RESPONDER AND NON-RESPONDER ANIMALS **Relation between the course of arthritis and collagen-specific immunity**

N.P.M. Bakker, M.G.M. van Erck, C.A.D. Botman,
M. Jonker and L.A. 't Hart

Arthritis Rheum. 34:616 (1991)

SUMMARY

It is speculated that the autoimmune response to CII is a driving force in the pathogenesis of human RA. In this report we describe the relationship between induction of collagen arthritis and the CII-specific humoral as well as cellular immune response in rhesus monkeys. Ten of fourteen monkeys immunized with B-CII developed polyarthritis. Susceptible animals showed a T cell response to B-CII; resistant animals did not. After the primary immunization, the humoral response to B-CII, as well as to Rh-CII, was dominated by antibodies of the IgM isotype in the susceptible animals and by antibodies of the IgG isotype in the resistant animals. Because of the close phylogenetic relationship between rhesus monkey and humans these data contribute valuable information about the role of CII-specific immunity in the pathogenesis of human RA.

INTRODUCTION

The pathogenesis of chronic inflammatory arthritis is unknown. One hypothesis is that CII, a major constituent of the joint-cartilage, is involved as auto-antigen in the pathogenesis of arthritis. In support of this hypothesis is the finding of CII-specific T cells and anti-CII antibody producing B cells in the synovial membrane of patients with RA (1,2). CII-specific antibodies have also been found in sera of RA patients (3). Arthritis that shares a number of clinical, hematologic, serologic, and radiographic features with RA in humans (6) can be induced in rhesus monkeys by i.d. immunization with B-CII (4-6). This experimental model can provide valuable information about human arthritis because of the close phylogenetic relationship between rhesus monkey and humans (7). Most data concerning CIA have been obtained with inbred strains of rodents (8). In these models it has been shown that both cellular and humoral immunity play a role in the pathogenesis of this experimental disease (9,10). The antibody response to CII is under the control of the MHC (11), and CIA cannot be induced in animals with either inherited or experimentally impaired T cell function (12-14). Direct evidence for the role of T cells and antibodies has been gained from studies demonstrating that the disease can be induced in naive animals by passive transfer of T cells (15) or IgG antibodies (16) derived from collagen immunized animals. In the present study the mechanism(s) leading to CIA were investigated in a group of fourteen randomly selected rhesus monkeys, seven males and seven females, that had been immunized

with B-CII. The course of the arthritis and the development of humoral and cellular immunity to collagen were assessed. Arthritic activity was assessed by several laboratory parameters of inflammation, including serum levels of IL-6. The proliferative response of PBMC to B-CII was absent in the 4 monkeys that did not develop arthritis, low in monkeys that developed arthritis only after the primary immunization, and high in monkeys that also developed an arthritis flare upon booster immunization. Moreover, a correlation was found between the severity of arthritis and the serum concentration of collagen-specific IgM. The relevance of these findings for understanding the pathogenesis of CIA are discussed below.

MATERIALS AND METHODS

Animals

The rhesus monkeys (*Macaca mulatta*) were born and raised in the ITRI-TNO Primate Center, except for monkeys BB25 and BB37 which were born in the Zoo Beekse Bergen, Arnhem, The Netherlands. All monkeys were typed for Mamu-DR antigens according to standard serologic techniques (17). The female monkeys were 3 to 21 years of age, weighed 2.8-5.8 kg. The male monkeys were 4 to 21 years of age weighed 3.9-9.3 kg. The animals were examined, weighed and bled under ketamin anesthesia. Animals experiencing pain were given 0.06 mg Buprenorfine twice daily by i.m. injection (Temgesic, Warrick, Amstelveen, The Netherlands). The experiments were approved by the TNO ethical committee for animal experiments. Throughout the experiments the animals were under veterinary care.

Collagen

Native B-CII, isolated from bovine articular cartilage, was a kind gift from dr. J. van Kampen, dr. Jan van Breemen Institute, Amsterdam, The Netherlands. Native B-CII from the bovine nasal septum and native type II collagen from rhesus monkey nasal septum and articular cartilage were isolated as described elsewhere (6).

Arthritis induction

The animals were immunized by i.d. (skin of their back) injection of 1 ml of an 1:1 emulsion of purified B-CII dissolved in 0.1M HAc and CFA (Difco Lab., Detroit, MI). For booster immunizations the B-CII solution was emulsified with ICFA or PBS solution (See Figure 2 for doses).

The severity of arthritis was assessed by the presence of: a) decreased mobi-

lity and apathy with slight STS, b) moderate STS with redness, or c) severe STS with redness. Serologic and macroscopic parameters associated with arthritis were previously investigated in 8 of the 14 monkeys of the present study, the findings of which have been published elsewhere (6).

Laboratory parameters of inflammation

The ESR and the serum levels of CRP were determined by standard procedures (SSDZ Hospital, Delft, The Netherlands). Serum levels of IL-6 were assessed according to the proliferation of the IL-6 dependent murine hybridoma cell line B9 (19). The recombinant IL-6 used as a standard and the B9 cell line were a kind gift from dr. L.A. Aarden (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands).

Mononuclear cell proliferation assay.

PBMC were isolated from heparinized blood by density gradient centrifugation using lymphocyte separation medium (Organon Teknika, Durham, NC). The cells from the interphase of the gradient were washed twice with HBSS (Gibco, Paisley, UK) and resuspended in culture medium, consisting of HEPES-buffered RPMI-1640 (Gibco) supplemented with 200 mM L-glutamine, 0.15 % NaHCO₃, 200 U/ml penicillin, 200 U/ml streptomycin and 20% heat-inactivated pooled nonimmune rhesus monkey serum. PBMC were cultured for 6 days in 96 well round-bottom plates (Greiner, Labortechnik, Hamburg, Germany) in a humidified atmosphere containing 5% CO₂ at 37 °C at a concentration of 5 x 10⁴ cells per well. Antigen and mitogens were added in the optimal concentrations: 34 µg/ml ConA (Pharmacia, Uppsala, Sweden) and 50 µg/ml B-CII. For dissolution, B-CII in RPMI-1640 was heated 30 min at 90 °C. Cell proliferation was measured by the incorporation of [³H]thymidine (0.5 µCi per well) during the final 8 hours of the culture. The results represent the mean cpm of triplicate cultures and are expressed as SI = cpm in mitogen or antigen stimulated culture divided by cpm in cultures containing medium alone.

ELISA for collagen-specific antibodies

High-activated 96 well polyvinyl chloride plates (Titertek; Flow, Ayrshire, UK) were coated overnight at 4 °C with 10 µg/ml B-CII or Rh-CII in PBS (50 µl per well). Wells were postcoated by incubation for 60 min at 37 °C with 1% BSA in PBS (100 µl/well). Subsequently, different dilutions of (absorbed) rhesus monkey sera were added (100 µl/well). After thorough washing the binding of IgM and IgG

antibodies was detected with alkaline-phosphatase conjugated antibodies directed toward human IgM or IgG (1 $\mu\text{g}/\text{ml}$ PBS-1% BSA) (Tago, Burlingame, CA). The plates were developed using a substrate solution with 0.5 mg p-nitrophenyl phosphate (Sigma) per 0.1 ml of glycine-NaOH buffer containing 1 mM MgCl_2 and 1 mM ZnCl_2 . Light absorbance was read at 405 nm using a Titertek multiscan ELISA reader. Control experiments showed that the binding of the respective conjugates to human IgM and IgG could be reduced to background levels by preincubation with 1% rhesus monkey serum in PBS (data not shown). These conjugates are therefore considered to be highly cross-reactive with rhesus monkey immunoglobulin isotypes.

Absorption of anti-CII antibodies

For absorption studies native B-CII and Rh-CII were immobilized on Affigel-15 beads (BioRad, Richmond, CA). Briefly, 500 μl of thoroughly washed beads were mixed with 1 ml of a 10 or 5 mg/ml solution of collagen in 0.1 M HAc, containing 0.3 M NaCl to reduce non-specific binding. The mixture was incubated for 60 min at room temperature with gentle stirring. After washing once with 0.1 M HAc and twice with PBS, the beads were postcoated for 60 min at room temperature with 1% BSA in PBS. Control beads were coated with BSA. To determine whether immobilized collagen could still be recognized by serum antibodies the following control experiment was done. Affigel beads (250 μl) coated with B-CII at 5 mg/ml were mixed with 1 ml of different dilutions in PBS/1% BSA of pooled sera from B-CII immunized rats. After 60 min of incubation at room temperature with gentle stirring, the vials were centrifugated. The residual B-CII-specific IgG in the supernatant was determined by ELISA using alkaline phosphatase-conjugated goat-anti-rat IgG (1.25 $\mu\text{g}/\text{ml}$; Sigma). The titer of B-CII-specific IgG in serum from a B-CII immunized rat was reduced from 1:12,800 to 1:1,600 by this absorption procedure (Fig.1). Thus, immobilized collagen still can be recognized by serum antibodies.

The beads were used to estimate the amount of Rh-CII specific and B-CII specific antibodies in the sera of B-CII immunized monkeys. To this end, 150 μl of rhesus monkey sera diluted 1:1,500 in PBS was preincubated with 100 μl Rh-CII or B-CII coated beads. After 2 hours of incubation at 37 $^\circ\text{C}$, the supernatants were centrifugated, and the residual Rh-CII specific IgG and IgM antibodies were determined by ELISA.

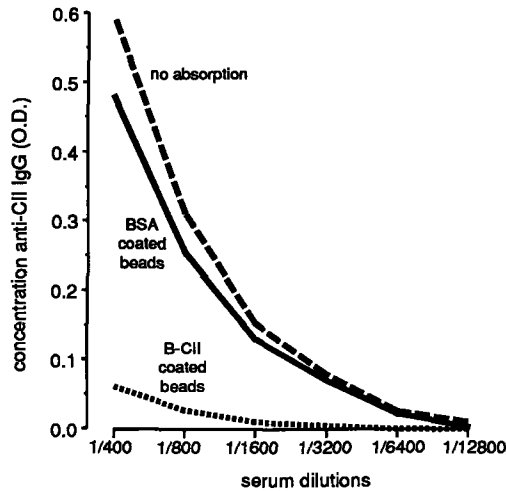


Figure 1. Recognition of immobilized CII by IgG from immune rat serum (see Material and Methods for details).

RESULTS

Induction and course of clinical arthritis

Nine of the 14 immunized monkeys (5 of 7 females and 4 of 7 males) developed symmetric polyarthritis after the first immunization with B-CII. Monkey 1JT developed clinical symptoms only after a booster immunization (Fig. 2). In all monkeys with arthritis the clinical activity was mainly located in the IP joints of the hands and feet (6), and the most severe inflammation occurred during the first 21 days after onset of arthritis. Complete remission occurred in all surviving animals. The time of onset, the severity and duration of the clinical arthritis varied significantly among the animals. Based on the severity of the clinical arthritis, three groups were distinguished (Fig. 2). Group I animals (BB25, 2BN, BB37, 1WF and 1JT) had severe disease activity, with arthritic scores of 3, within 14 days of the onset of clinical signs, and it was necessary to euthanize the animals.

Group II animals (1KM, D3, M15, 1OX and L65) developed a milder form of arthritis. Since a flare of clinical arthritis might be induced by a booster immuniza-

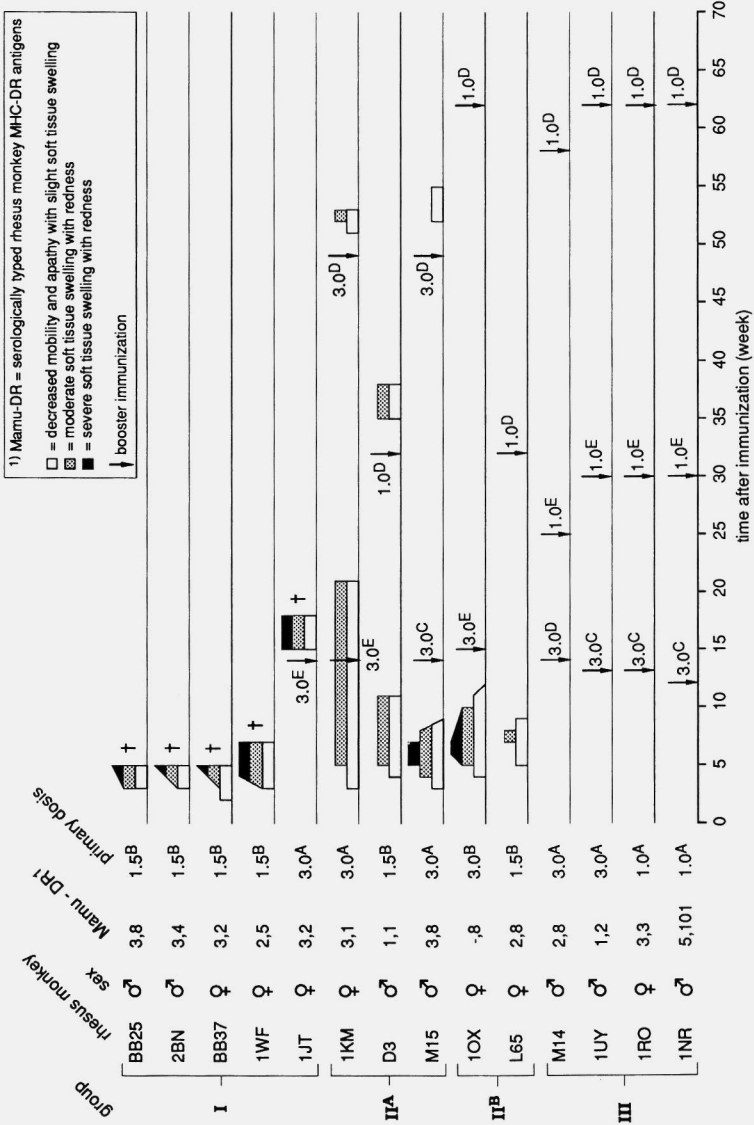


Figure 2. Incidence, time of onset and clinical course of CIA in rhesus monkeys, grouped according to the severity of clinical arthritis. A = B-CII (mg) from joint cartilage in CFA, B = B-CII (mg) from nasal cartilage in CFA, C = B-CII (mg) from joint cartilage in PBS, D = B-CII (mg) from joint cartilage in ICFA, E = B-CII (mg) from nasal cartilage in ICFA.

Table 1

IL-6, ESR, and CRP levels in rhesus monkeys immunized with bovine type II collagen

group	rhesus monkey	weeks after immunization	clinical Arthritis	IL-6 (U/ml)	ESR (mm/hr)	CRP (mg/ml)
I	BB25	1	-	122	1	1
		3	+	320	43	380
	2BN	1	-	85	2	1
		3	+	233	20	186
	BB37	1	-	44	1	1
		3	+	492	53	230
	1WF	1	-	56	1	1
		3	+	197	34	302
	1JT	1	-	57	1	1
		17	+	213	9	35
IIA	1KM	1	-	28	1	1
		7	+	150	43	105
	D3	1	-	<1	2	1
		5	+	545	58	334
	M15	1	-	42	1	1
		5	+	168	27	163
IIB	1OX	1	-	N.D.	2	1
		4	+	246	120	159
	L65	1	-	13	1	1
		5	-/+	91	5	91
III	M14	1	-	42	2	1
		6	-	123	2	1
	1UY	-2	-	25	1	1
		5	-	<1	3	1
	1RO	-2	-	28	3	1
		5	-	33	2	1
	1NR	-2	-	<1	2	1
		5	-	<1	2	1

N.D. = not done
 See Figure 2 for description of groups.

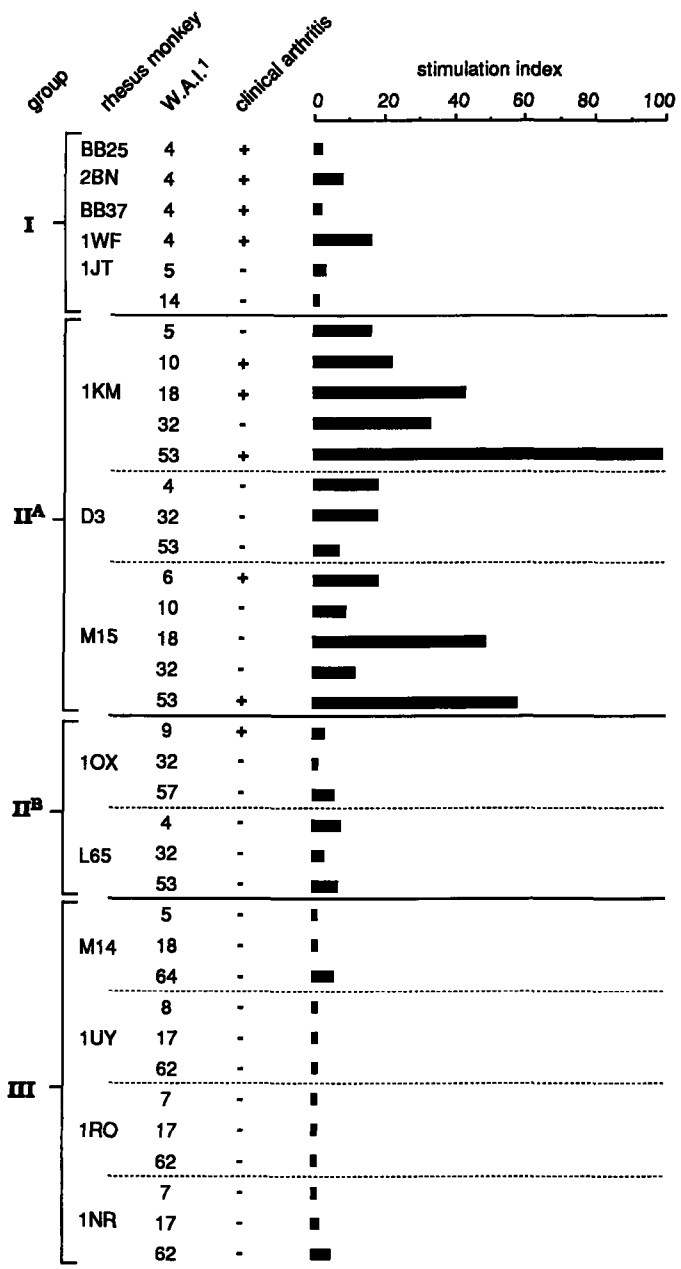


Figure 3. Proliferation of PBMC from group I, II and III rhesus monkeys in cultures with B-CII.
 1) W.A.I. = weeks after immunization. See Figure 2 for description of groups.

immunization, group II was divided into subgroups IIA (1KM, D3 and M15) and IIB (L65 and 1OX) for further studies. Arthritis could not be induced in group III animals (M14, 1UY, 1RO and 1NR). No correlation was found between the serologically defined Mamu-DR antigens and the development of arthritis (Fig. 2). The inflammation in the rhesus monkeys with CIA was monitored by changes in ESR, CRP and IL-6 levels. As shown in Table 1, all these parameters were enhanced in group I and II animals. In group III, only monkey M14 had an increase in the IL-6 level, but the ESR and CRP levels remained at the background level. These negative laboratory parameters exclude the presence a subclinical arthritis in group III animals, as has been described elsewhere (6) in monkey 1JT before the booster injection.

Cellular immune response

The proliferative response of PBMC to B-CII was assessed at various intervals after immunization (Fig.3). Because of the poor condition of the group I animals, sufficient blood for isolation of PBMC could be taken only once during the arthritis. Two animals in this group (2BN and 1WF) showed a significant proliferative response during week 4, but in the other three animals, the SI was low. Group III animals lacked a proliferative response to B-CII, even after specific challenge. Animals M14 and 1NR showed only a weak response at 64 weeks, even after repeated immunizations. However, all animals in this group showed a normal proliferative response to ConA and to a panel of antigens (data not shown). Cells obtained from the three group IIA animals (1KM, D3 and M15) showed a high proliferative response to B-CII after the first immunization. This response remained high or was further increased after the booster immunizations, which induced a flare of the arthritis.

In contrast, the proliferative response remained low in the two animals of group IIB (1OX and L65); these animals did not develop a flare of clinical arthritis after the booster immunizations.

Isotype and specificity of collagen-specific antibodies

Sera from the animals in each group were tested for the presence of antibodies to B-CII (no sera were available from monkey 2BN). After the primary immunization, B-CII-specific antibodies in Group I animals were mainly of the IgM isotype, whereas those in group III animals were almost exclusively IgG (Table 2).

Table 2

Isotype of antibodies to bovine type II collagen (B-CII) in the sera of rhesus monkeys immunized with B-CII*

group/ monkey	weeks after immunization													
	0 - 2		3 - 5		6 - 8		9 - 11		12 - 14		17 - 19		35	
	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
I														
BB25	0.00	0.03	0.15	1.90	0.78	2.17								
BB37	0.00	0.02			0.78	1.56								
1WF	0.00	0.03	0.40	1.91	0.89	1.38								
1JT			0.11	0.22			0.68	0.10	2.77**	0.45**				
IIA														
1KM	0.00	0.00	0.17	0.31	0.42	2.46	2.19	0.20			2.84**	0.70**		
D3	0.01	0.00	0.03	0.40	1.30	1.66	1.74	1.64					2.50**	1.60**
M15	0.00	0.00	1.60	2.02	1.24	1.39	1.79	0.59			2.55**	0.85**		
IIB														
1OX	0.00	0.00	0.09	0.36	1.16	0.76	1.49	0.56	1.26	0.50	1.05**	0.27**	2.50**	0.93**
L65	0.00	0.04			0.85	0.97	2.21	0.98						
III														
M14			0.03	0.09	0.11	0.08	0.12	0.08			2.33**	0.20**		
1UY			0.00	0.00	0.30	0.19	0.27	0.10	1.03	0.65	1.14**	0.17**		
1RO			0.00	0.00	1.07	0.08	0.96	0.07	1.66**	0.26**	1.22**	0.11**		
1NR			0.00	0.00	2.96	0.43	2.97	0.24			2.99**	0.16**		

*Values are optical density units, as determined by enzyme-linked immunosorbent assay. Sera were not available from monkey 2BN (group I). See Figure 2 for description of groups.
 ** = after booster immunization

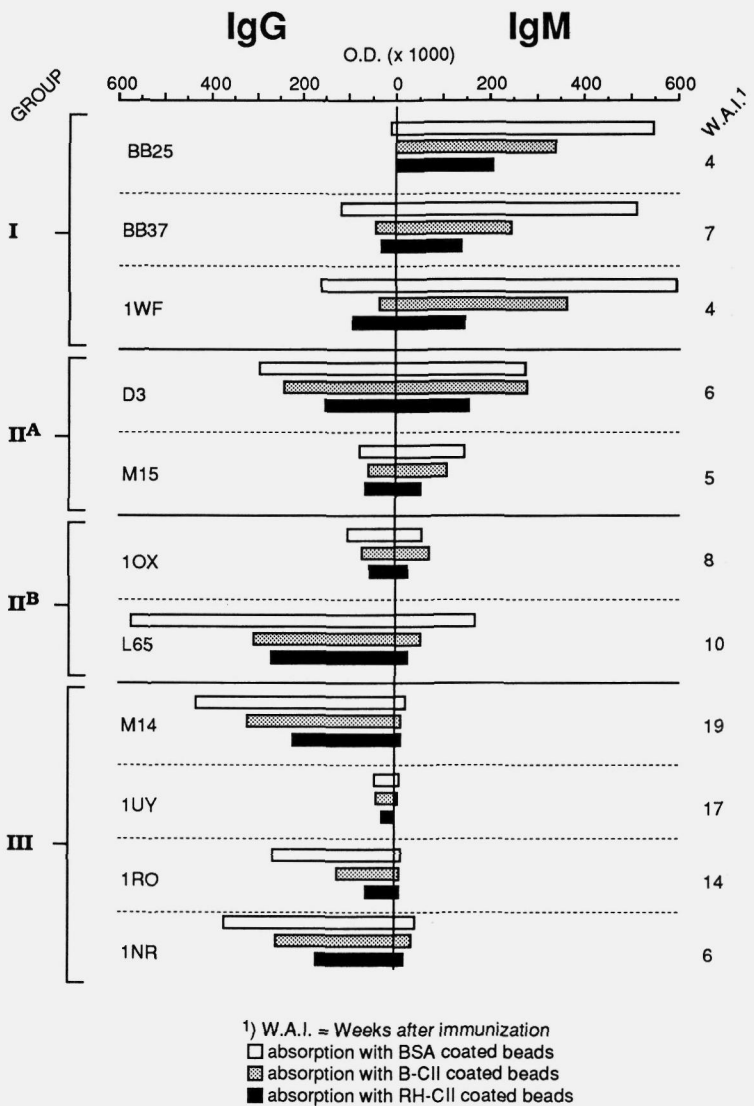


Figure 4. Isotype of Rh-CII-specific antibodies in absorbed sera from group I, II and III rhesus monkeys. See Figure 2 for description of groups.

B-CII specific antibodies in group II animals were of the IgG, as well as the IgM isotype, but IgG antibodies dominated after week 9. High levels of anti-B-CII antibodies of the IgM isotype were found at weeks 3-5 after the primary immuni-

zation in group I monkeys (except 1JT) and in monkey M15 of group II, which had developed severe clinical arthritis by that time (Fig.2).

To differentiate Rh-CII-specific antibodies from species non-specific antibodies, absorbing studies were performed on sera obtained at the onset of arthritis (except for D3). As shown in Fig. 4, absorption with Rh-CII-coated beads removed a considerably higher percentage of IgM antibodies in group I and II sera than with B-CII-coated beads.

The difference was less distinct for the IgG antibodies. In group III animals there was a more efficient absorption of IgG antibodies with Rh-CII coated beads than with B-CII coated beads. The results from this procedure suggest that the majority of the Rh-CII-specific antibodies in group I animals were of the IgM isotype, while Rh-CII specific antibodies in group III animals were predominantly of the IgG isotype. Rh-CII-specific antibodies in group II animals were both of the IgM and the IgG isotype.

DISCUSSION

It has been suggested by several investigators that auto-immunity to CII may be involved in the initiation and/or perpetuation of the inflammatory process in human RA (1-3). In the present study, the possible relation between CIA and the humoral and cellular immunity to CII in rhesus monkeys was investigated.

Based on the course of clinical arthritis after a single immunization with B-CII, three groups could be discerned: a group that developed severe arthritis (group I), a group with moderate arthritic symptoms (group II), and a non-arthritic group (group III). Three of the four monkeys without arthritis were males. The lower incidence of arthritis in male monkeys has also been reported by Terato et al. (20), who studied cynomolgous monkeys (*Macaca fascicularis*), but the group we studied is too small to conclude that there is a statistically significant sex-linked sensitivity for CIA in rhesus monkeys.

With respect to the role of cellular immunity in the initiation of CIA, our results show a significant difference in T cell reactivity to B-CII between the arthritic group I and group II animals and the non-arthritic group III animals after the first immunization. In group III animals, the proliferative response of T cells to B-CII was completely negative. B-CII induced T cell proliferation was observed in cells from arthritic monkeys, but we found no apparent correlation between the level of T cell reactivity and the severity of arthritis. The T cell reactivities to B-CII in

group IIB animals, which failed to develop an arthritis flare after booster immunization, were low compared with those in group IIA animals. It should be noted that only the animals of group IIA had showed an arthritis flare upon booster immunization. These data suggest that CII-reactive T cells are involved in the initiation of CIA and that the flare of disease activity is correlated with a relatively strong T cell reactivity to CII. A slight proliferative T cell response to B-CII was observed in two non-arthritic monkeys (1NR and M14) after 60 weeks. This may be an indication of a breakdown of the cellular non-responsiveness to B-CII after several booster immunizations with the antigen. This also indicates that in these animals, CII-reactive T cells are part of the T cell repertoire. Findings from studies of mice support the existence of anti-CII T cells in the normal T cell repertoire (21).

Clear differences in the isotype distribution of CII-recognizing antibodies were found in each group. The severity of arthritis after the triggering immunization corresponded with the serum level of B-CII recognizing antibodies of the IgM isotype. Holmdahl et al. (22) provided evidence that the isotype of the anti-CII antibodies in mice immunized with native CII is governed by class II MHC antigens (H-2q). This could also be the case in our outbred population of rhesus monkeys. However, no correlation was found between arthritis and the serologically typed Mamu-DR antigens. A definitive answer for a possible correlation between MHC antigens and CIA in rhesus monkeys requires a more detailed study of the *MhcMamu* system. The Rh-CII-specific antibodies in the group of monkeys with severe arthritis (group I) were mainly of the IgM isotype. In contrast, antibodies to Rh-CII in the non-arthritic animals of group III were predominantly IgG, while sera of group II animals, with a relatively mild arthritis, contained Rh-CII-specific antibodies of the IgM and IgG isotype. These data suggest that Rh-CII-specific IgG antibodies are less important for the initiation of clinical arthritis, but that antibodies of the IgM isotype may be responsible for the initiation of CIA. Our results on the necessity of IgM antibodies for the development of CIA contrast with data from rodent studies. Stuart et al. (16) established that a mild form of CIA can be induced in naive rats by passive transfer of purified IgG from collagen-immunized rats. Holmdahl et al. (21) demonstrated that the MHC-restricted anti-CII response in mice is dominated by IgG antibodies. However, the initiation of CIA resembles in a number of aspects an immune-complex disease, with the involvement of complement and neutrophils (23-26). It has been well established that IgM binding of one antibody molecule to an epitope on the cartilage can activate the classical pathway of the complement cascade and thus trigger inflammation. In contrast, because at least a pair of monomeric IgG molecules is necessary for activation of the classical

complement pathway, a high epitope density is required, and such high density might not be present on intact cartilage.

Based on all these data, we propose that the initiation of CIA in rhesus monkeys depends on CII-specific T cells, possible in concert with CII-specific antibodies of the IgM isotype. The different distribution of CII-specific antibody isotypes between the monkeys might be governed by different cytokine profiles. Because activated anti-CII T cells are only observed in the arthritic monkeys, particularly during a disease flare, T cell derived lymphokines are of interest. In this respect, IL-4, IL-6 and interferon-gamma may be important lymphokines. It is known that IL-4 plays a role in the immunoglobulin isotype switching (27), and that it can inhibit IgM secretion *in vitro* (28). IL-6, which was found at high levels only in the arthritic monkeys, is a cytokine that, *in vivo*, can induce polyclonal B cell activation and autoantibody production (29). Finally, treatment with interferon-gamma greatly exacerbates CIA in rodents (30) and enhances antigen-specific IgM production by activated B cells (28). A second hypothesis for the diversity of immune response to CII among the several groups is based on the observation that only in animals with a relative high proliferative T cell response to B-CII an arthritis flare could be induced by booster immunization with B-CII (group IIA). Usually, the disease has an acute and limited course, despite the presence of high quantities of CII. These results indicate that suppression of the immune response at the level of CII-reactive T cells may play a role in the induction and/or pathogenesis of CIA in rhesus monkeys. This suppressive mechanism might be responsible for the low production of arthritogenic IgM antibodies to Rh-CII in the non-arthritic group III monkeys. In this context, interesting results have been obtained in mice studies showing that T cells are involved in resistance to CIA (31) or that protection against CIA can be accomplished with a CII-specific T cell line (32).

The findings of our present studies might have implications for understanding the pathogenesis of human RA. The elucidation of the initiating event in RA is still seriously hampered by the fact that the responsible antigen(s) is not known. CII-specific T cells and B cells secreting predominantly CII-specific IgG antibodies have been found in the synovium (1,2) and antibodies of the IgM and the IgG isotype have been found in plasma (33), of RA patients. It is tempting to speculate, based on our data, that IgM antibodies bind to CII in the joint cartilage and, thus, form the focus of an inflammatory reaction. As a consequence of the cartilage damage, arthritogenic "self" epitopes might become available (34), which may be recognized by infiltrating autoreactive T cells. These are, then, the cells that are primarily responsible for the chronic nature of RA. This attractive animal model

unifies the contribution of humoral and cellular autoimmunity to collagen in the pathogenesis of RA.

REFERENCES

1. Londei M., Savill C.M., Verhoef A., Brennan F., Leech Z.A., Duance V., Maini R.N., Feldmann M. Persistence of collagen type II-specific T cell clones in the synovial membrane of a patient with rheumatoid arthritis. *Proc. Natl. Acad. Sci.* **86**:636 (1989)
2. Tarkowski A., Klareskog L., Carlsten H., Herberts P., Koopman W.J. Secretion of antibodies to type I and II collagen by synovial tissue cells in patients with rheumatoid arthritis. *Arthritis Rheum.* **32**:1087 (1989)
3. Morgan K., Claque R.B., Collins I., Ayad S., Phinn S.D., Lennox Holt P.J. A longitudinal study of anticollagen antibodies in patients with rheumatoid arthritis. *Arthritis Rheum.* **32**:139 (1989)
4. Rubin A.S., Healy C.T., Martin L.N., Baskin G.B., Roberts E.D. Experimental arthropathy induced in rhesus monkeys (*Macaca mulatta*) by intradermal immunization with native bovine type II collagen. *Lab. Invest.* **57**:524 (1987)
5. Yoo T.J., Kim S.Y., Stuart J.M., Floyd R.A., Olson G.A., Cremer M.S.A., Kang A.H. Induction of arthritis in monkeys by immunization with type II collagen. *J.Exp.Med.* **168**:777 (1988)
6. Bakker N.P.M., van Erck M.G.M., Zurcher C., Faaber P., Lemmens A., Hazenberg M., Bontrop R.E., Jonker M. Experimental immune-mediated arthritis in rhesus monkeys. A model for human rheumatoid arthritis? *Rheumatol.Int.* **10**:21 (1990)
7. Balner H. The major histocompatibility system of subhuman primate species, The major histocompatibility system in man and animals. Edited by D Gotze. Berlin. Springer Verlag (1977)
8. Holmdahl R., Andersson M.E., Goldschmidt T.J., Jansson L., Karlsson M., Malmstrom V., Mo J. Collagen induced arthritis as an experimental model for rheumatoid arthritis. Immunogenetics, pathogenesis and autoimmunity. *APMIS* **97**: 575 (1989)
9. Trentham D.E., Townes A.S., Kang A.H., David J.R. Humoral and cellular sensitivity to collagen in type II collagen induced arthritis in rats. *J.Clin.Invest.* **61**: 89 (1978)
10. Seki N., Sudo Y., Yoshioka T., Sugihara S., Fujitsu T., Sakuma S., Ogawa T., Hamaoka T., Senoh H., Fujiwara H. Type II collagen-induced murine arthritis 1. Induction and perpetuation of arthritis require synergy between humoral and cell-mediated immunity. *J.Immunol.* **140**:1477 (1988)
11. Holmdahl R., Klareskog L., Andersson M., Hansen C. High antibody response to autologous type II collagen is restricted to H-2q. *Immunogenetics* **24**:84 (1986)
12. Banerjee S., Haoqi T.M., Luthra H.S., Stuart J.M., David C.S. Possible role of V β cell receptor genes in susceptibility to collagen-induced arthritis in mice. *J.Exp.Med.* **167**:832 (1988)
13. Brahn E., Trentham D.E. Effect of antithymocyte serum on collagen arthritis in rats. Evidence that T cells are involved in its pathogenesis. *Cell.Immunol.* **86**:421 (1984)
14. Ranges G.E., Sciram S., Cooper S.M. Prevention of type II collagen-induced arthritis by in vivo treatment with anti-L3T4. *J.Exp.Med.* **162**:1105 (1985)
15. Holmdahl R., Klareskog L., Rubin K., Larsson E., Wigzell H. 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 (1985)
16. Stuart J.M., Cremer M.A., Townes A.S., Kang A.H. 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 (1982)
17. Roger J.H., van Vreeswijk W., Balner H. The major histocompatibility complex of rhesus monkeys. XII. Current knowledge of DR and other B cell specific antigens. *Immunogenetics* **7**:333 (1980)

18. Takai Y., Seki N., Senoh H., Yokota T., Lee F., Hamaoka T., Fujiwara H. Enhanced production of interleukine-6 in mice with type II collagen-induced arthritis. *Arthritis Rheum.* **32**:594 (1989)
19. Helle M., Brakenhoff J.P.J., De Groot E.R., Aarden L.A. Interleukine 6 involved in interleukine 1-induced activities. *Eur.J.Immunol.* **18**:957 (1988)
20. Terato K., Arai H., Shimozuru Y., Fukuda T., Tanaka H., Watanabe H., Nagai Y., Fujimoto K., Okubo F., Cho F., Honjo S., Cremer M.A. Sex-linked differences in susceptibility of cynomolgous monkeys to type II collagen arthritis. Evidence that epitope-specific immune suppression is involved in the regulation of type II collagen autoantibody formation. *Arthritis Rheum.* **32**:748 (1989)
21. Holmdahl R., Bailey C., Enander I., Mayer R., Klareskog L., Moran T., Bona C. Origin of the autoreactive anti-type II collagen response. II. Specificities, antibody isotypes and usage of V-gene families of anti-type II collagen B cells. *J.Immunol.* **142**:1881 (1989)
22. Holmdahl R., Jansson L., Andersson M., Larsson E. Immunogenetics of type II collagen autoimmunity and susceptibility to collagen arthritis. *Immunology* **65**:305 (1988)
23. Clague R.B., Morgan K., Shaw M.J., Firth S.A., Twose T., Holt P.J.L. Native type-II collagen-induced arthritis: an antibody antigen complex mediated disease. *Ann.Rheum.Dis.* **39**:599 (1980)
24. Morgan K., Clague R.B., Shaw M.J., Firth S.A., Twose T.M., Lennox Holt P.J. Native type II collagen-induced arthritis in the rat. The effect of complement depletion by cobra venom factor. *Arthritis Rheum.* **24**:1356 (1981)
25. Schrier D., Gilbertsen R.B., Lesch M., Fantone J. The role of neutrophils in type II collagen arthritis in rats. *Am. J.Pathol.* **117**:26 (1984)
26. Hart B.A., Simons J.M., Knaan-Shanzer S., Bakker N.P.M., Labadie R.P. Anti-arthritic activity of the newly developed neutrophil oxidative burst antagonist apocynin. *Free Rad. Biol. Med.* **9**:127 (1990)
27. Boom W.H., Liano D., Abbas A.K. Heterogeneity of helper/inducer T lymphocytes II. Effects of interleukine 4- and interleukine 2-producing T cell clones on resting B lymphocytes. *J.Exp.Med.* **167**:1350 (1988)
28. Snapper M.S., Paul W.E. Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. *Science* **236**:944 (1987)
29. Kishimoto T. The biology of interleukine-6. *Blood* **74**:1 (1989)
30. Mauritz N.J., Holmdahl R., Johnsson R., van der Meide P., Schynius A., Klareskog L. Treatment with interferon gamma triggers onset of collagen arthritis in mice. *Arthritis Rheum.* **31**:1297 (1988)
31. Williams R.O., Whyte A., Waldmann H. Resistance to collagen-induced arthritis in DBA/1 mice by intraperitoneal administration of soluble Type II collagen involves both CD4+ and CD8+ T lymphocytes. *Autoimmunity* **4**:237 (1989)
32. Kakimoto K., Katsuki M., Hirofujii T., Iwata H., Koga T. Isolation of T cell line capable of protecting mice against collagen-induced arthritis. *J.Immunol.* **140**:78 (1988)
33. Clague R.B., Shaw M.J., Holt P.J.L. Incidence and correlation between serum IgG and IgM antibodies to native type II collagen in patients with chronic inflammatory arthritis. *Ann.Rheum.Dis.* **40**:6 (1981)
34. Klareskog L., Johnell O., Hulth A., Holmdahl R., Rubin K. Reactivity of monoclonal anti-type II collagen antibodies with cartilage and synovial tissue in rheumatoid arthritis and osteoarthritis. *Arthritis Rheum.* **29**:730 (1986)

CHAPTER 4

INDUCTION OF TYPE II COLLAGEN-SPECIFIC ANTIBODY PRODUCTION IN BLOOD LYMPHOCYTE CULTURES OF RHESUS MONKEYS (*Macaca mulatta*) WITH COLLAGEN-INDUCED ARTHRITIS USING THE IMMOBILIZED NATIVE ANTIGEN

L.A. 't Hart, C.A.D. Botman and N.P.M. Bakker

Clin. exp. Immunol. **83**: 375 (1991)

SUMMARY

PBMC from rhesus monkeys, previously immunized with B-CII to induce arthritis were cultured with the same antigen. Because the native protein is poorly soluble in culture medium a heating step is often used. The antigen in this form induced PBMC proliferation, but epitopes for the induction of antibody production and arthritis were lost. To keep the native protein intact it was coated on affigel beads. With the immobilized antigen specific antibody production could be induced.

INTRODUCTION

RA is often associated with cellular and humoral autoimmunity to CII (1-4). The possible role of the autoimmunity to a major constituent of the joint cartilage, however, is only poorly understood. In recent years CIA has been established as an animal model to investigate the mechanism(s) by which experimentally induced (auto)immunity to CII can lead to arthritis (5).

We have investigated in rhesus monkeys the different contribution of humoral and cellular immunity to CII to the susceptibility to CIA (6). In that study we determined the capacity of PBMC from animals immunized with B-CII to proliferate and produce antibodies in culture with the same antigen. A complicating factor in such studies is that B-CII in its native form is poorly soluble in culture medium and therefore gives unreliable results. Many investigators dissolve the protein in culture medium by heating briefly (7). However, immunization of rats with a heated B-CII solution emulsified in CFA induced cellular but not humoral immunity to the native protein (unpublished data, see also 8). Neither did it evoke arthritis, indicating that heating destroys B-CII epitopes which are indispensable for the induction of arthritis as well as humoral immunity. Similarly, we could demonstrate the induction of proliferation, but not antibody production in cultures of rhesus monkey PBMC with heated B-CII.

In the present study we have developed a procedure for the induction of specific antibody production in cultures of rhesus monkey PBMC by immobilization of the antigen in its native form.

MATERIALS AND METHODS

Animals

All rhesus monkeys were bred and raised at the TNO Primate Center in Rijswijk. The animals used for this study had been immunized several times with B-C11 (6). The isolation of B-CII from bovine nose septum has been described in the same report. Blood for serum production or for the isolation of PBMC was obtained from immunized monkeys which developed arthritis (susceptible animals: 1KM, M15, D3, L65) or which failed to develop any clinical or serologic sign of inflammation (resistant animals: 1RO, M14).

Isolation and culture of PBMC

The animals were anesthetized with 0.1 ml/kg of a mixture of 9 volumes ketamine (10 mg/ml; Chassot & Cie AG, Bern, Switzerland) and 1 volume vetranquil (10 mg/ml acepromazine and 5 mg/ml chlorbutanol; Compagnie Rousselot, Paris, France). Blood was taken in heparinized (thromboliquine) vacutainers (Becton Dickinson, Meylan Cedex, France) and diluted with an equal volume of HBSS (Gibco Ltd., Paisley, Scotland). PBMC were isolated by density gradient centrifugation on lymphocyte separation medium (Organon Technika Corporation, Durham, NC). The cells from the interphase of the gradients (PBMC) were washed twice with HBSS. PBMC were cultured in a humidified atmosphere containing 5% CO₂ in 96-well round-bottomed plates (Greiner Labortechnik, Nürtingen, Germany) at a concentration of 40,000 cells/well. The culture medium consisted of carbonate-buffered RPMI 1640 containing 200 mM L-glutamine, 200 U/ml penicillin, 200 IU/ml streptomycin and 10% (vol.) heat-inactivated fetal calf serum (all from Gibco). For stimulation with soluble B-CII the protein was routinely dissolved in RPMI 1640 by heating for 30 min at 90°C. Proliferation was assessed by counting the number of viable cells in each culture. Results were expressed by the S.I.

Coating of affigel beads

Affigel-15 beads (Bio-Rad Laboratories, Richmond, CA) contain N-hydroxy-succinimide groups which couple to free amino residues in a protein. Usually this will not disturb the conformation of the molecule and hence conformational epitopes will remain intact. For this study the affigel beads were washed thoroughly with 0.05 M HAc. Routinely one volume of the washed beads was mixed with one volume of 5 mg B-CII per ml 0.05 M HAc for coating. The mixture also contained

0.3 M NaCl to reduce non-specific binding (manufacturer's instruction). When B-CII concentrations <5 mg/ml were used for coating the beads, the stimulation of antibody production was reduced, while beads coated with higher B-CII concentrations had only a slightly higher stimulatory capacity (not shown). After 2 h incubation at room temperature under gentle stirring the beads were centrifuged. After removal of the supernatant the beads were washed at least five times with PBS, pH 7.4. For post-coating the whole procedure was repeated with 1% BSA in PBS. Beads coated only with BSA functioned as controls in all assays.

ELISA for CII-specific antibodies in monkey immune sera

The assay was performed exactly as described by 't Hart et al. (9).

Detection of antibody production induced by the beads

Beads recovered from cell cultures were washed once with PBS and then incubated for 1 min with 0.1% Triton X-100 in demineralized water to lyse adherent cells. After the beads had been washed thoroughly with PBS-0.1% Tween 20 (at least five times) binding of immunoglobulin was determined by incubation for 1 h at 37°C with alkaline-phosphatase-conjugated affinity-purified goat antibodies directed to heavy chains of human IgM and IgG (Tago, Burlingham, CA). After thorough washing with PBS-0.1% Tween 20, the beads were incubated for about 30 min at 37°C with substrate solution. This solution consisted of 0.5 mg p-nitro-phenyl-phosphate (Sigma Chemical Company, St. Louis, MO) per ml 0.1 M glycine-NaOH buffer (pH 10.4), containing 1 mM MgCl₂ and 1 mM ZnCl₂. Colour development was read at 405 nm.

RESULTS

Recognition of native and heated B-CII by monkey immune sera

Immune-sera from four susceptible B-CII-immunized monkeys at 200-fold dilution were pre-incubated with different concentrations of native or heated B-CII. The amount of free IgG antibodies to native B-CII after pre-incubation was determined with ELISA. As shown in Fig. 1, heating considerably reduced the capacity of B-CII to deplete specific IgG from monkey serum. The same experiment was then done with immobilized native B-CII. The results in Fig. 2 show that the B-CII-coated beads effectively removed specific IgG from rhesus monkey immune sera. Control

beads coated with BSA did not remove B-CII-specific IgG. These data show that heating of B-CII destroyed epitopes recognized by specific IgG antibodies, but these remain intact in the (immobilized) native protein.

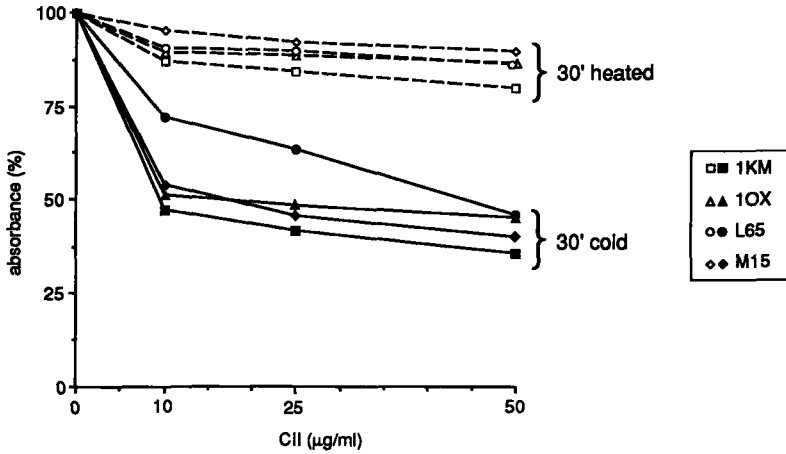


Figure 1. Destruction of anti-CII IgG antibody epitopes by heating of B-CII. Immune sera were incubated with heat-denatured (open symbols) or native (closed symbols) B-CII. After 60 min incubation the amount of free IgG antibody to native B-CII was determined with ELISA. Results are given as the percentage absorbance (in this ELISA at 405 nm). Results were normalized by defining the measured IgG in sera without B-CII at 100%.

Stimulation of PBMC with soluble B-CII

In a first experiment the capacity of different concentrations of heated B-CII to stimulate proliferation and antibody production in cultures of PBMC was determined. As shown in Table 1(a) a low but significant proliferation was observed in cultures of PBMC from the B-CII-immunized animals 1KM and M15. This proliferation was absent in PBMC from the non-immunized animal Z, or from animal TD which had been immunized with an unrelated antigen. No B-CII-specific antibodies were detectable with ELISA in any of the cultures.

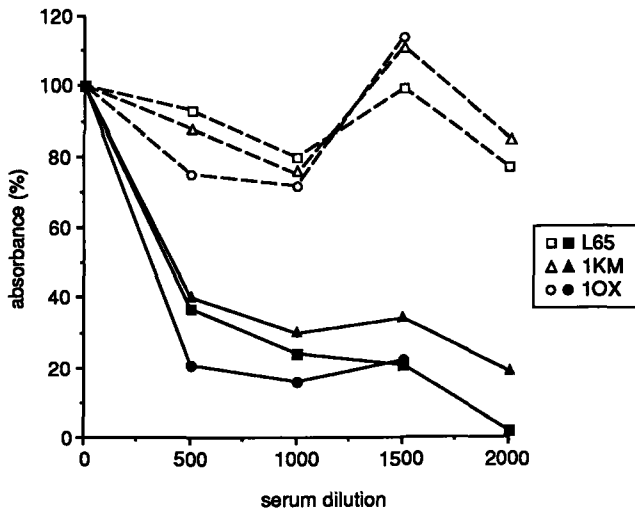


Figure 2. Absorption of antibodies from rhesus monkey serum with B-CII-coated beads. One volume of B-CII- (closed symbols) or BSA-coated (open symbols) beads (settled bed volume) was incubated at 37°C with 3 volumes of differently diluted immune sera from three monkeys. After 60 min incubation the amount of free IgG antibodies to B-CII was determined with ELISA. Results are given as the percentage absorbance (in this ELISA at 405 nm). The results from the different animals were normalized by defining the measured IgG in sera without B-CII at 100%.

In a second experiment, PBMC from monkeys 1KM and M15 were again stimulated with different antigen concentrations. In this experiment recombinant IL2 was added as well, aimed at enhancing the production of B cell activation and maturation factors by B-CII-activated T cells. Despite a strongly increased proliferation (Table 1b) in the supernatants of these cultures, again no B-CII-specific antibodies were detectable with ELISA.

Stimulation of PBMC with immobilized B-CII

PBMC from four B-CII-immunized animals were cultured with B-CII-coated beads. PBMC were obtained from two susceptible (D3 and 1KM) and two resistant animals (1RO and M14). The sera of both groups of animals contain a high amount of B-CII-specific IgG. In this experiment the beads of three identical culture wells were pooled. The results in Fig. 3 show that B-CII-coated beads induced only PBMC from susceptible animals to antibody production, while no antibody production was

detectable in cultures with BSA-coated beads. Only antibody molecules binding to the beads were found. Antibodies in the culture medium were not detectable.

This finding was reproduced in three further experiments. Triplicate PBMC cultures were stimulated with 25 μ l settled bed volume of B-CII-coated beads in a total culture volume of 150 μ l. After culture, the beads from each well were developed separately. Again the supernatants of the cultures did not contain B-CII-specific antibodies detectable with ELISA, but development of the beads revealed binding of B-CII-specific antibodies (Table 2).

Table 1.

Stimulation of PBMC with soluble C-II

(a) Experiment I		stimulation index			
C-II (μ g/ml)	Z	TD	M15	1KM	
0	1.0	1.0	1.0	1.0	
2.5	1.1	1.1	1.4	2.3	
5.0	0.8	0.5	1.4	2.8	
10.0	1.1	0.5	1.8	2.5	
20.0	0.8	0.6	2.6	4.3	
40.0	0.7	0.3	3.3	6.8	

(b) Experiment II		stimulation index							
C-II (μ g/ml)	-	monkey 1KM rIL2 (U/ml)			-	monkey M15 rIL2 (U/ml)			
		2	5	10		2	5	10	
0	1.0	17.5	35.5	38.3	1.0	9.6	12.6	12.8	
10	1.5	26.5	42.3	40.5	1.6	15.4	18.4	18.8	
20	2.1	32.8	44.8	45.0	1.8	14.8	19.2	19.6	
40	1.8	30.3	28.0	41.8	1.8	14.8	17.2	17.0	

PBMC were cultured in triplicate with different doses of B-CII in the absence or presence (only in experiment II) of recombinant IL-2 that was added at day 0. After 6 days of culture the number of cells per well was counted, and the stimulation index calculated. Donor Z was a non-immune control monkey; donor TD had been immunized with streptococcal cell walls; donors M15 and 1KM were monkeys immunized with B-CII which had developed CIA (susceptible animals).

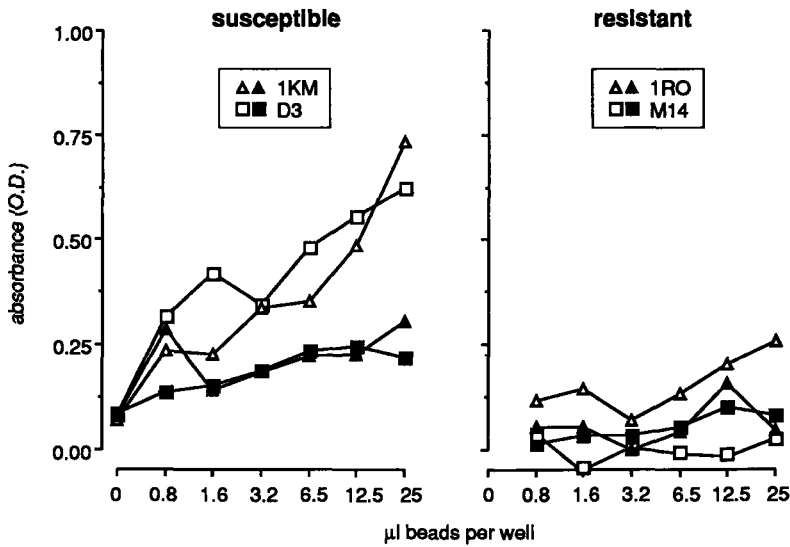


Figure 3. Induction of antibody production with B-CII-coated beads. PBMC of four monkeys were cultured with different amounts of B-CII- (open symbols) or BSA-coated (closed symbols) beads. Monkeys 1KM and D3 were susceptible to CIA induction (a), while 1RO and M14 were resistant (b). The beads recovered from triplicate cultures were pooled and developed analogous to ELISA plates, as described in Materials and Methods. The results show the light absorbance in arbitrary units (OD).

Table 2

Stimulation of PBMC with immobilized CII

coating	OD (405 nm)		
	monkey L65	monkey 1KM	monkey M15
BSA-coated beads	98 ± 15	75 ± 12	83 ± 1.9
B-CII-coated beads	397 ± 52	215 ± 10	304 ± 2.3

PBMC from three CII-immunized susceptible rhesus monkeys were cultured in triplicate for 8 days with 25 μ l affigel-15 beads (settled bed) per 150 μ l culture medium. After culture the binding of IgM and IgG was determined. Results are expressed in arbitrary units (OD x 10³; mean ± S.D.).

DISCUSSION

From a group of 14 rhesus monkeys immunized with B-CII 10 developed CIA. These were regarded as susceptible animals. In four animals no CIA could be induced, although these have a high titre of B-CII-specific serum antibodies (10). The latter animals are therefore regarded as resistant to CIA. Using these two groups of animals the role of the immunity raised against CII in the course of CIA has been investigated. The results of this study are published separately (6). The present report deals with a methodological problem we encountered in that study.

As also shown in Table 1, PBMC from susceptible animals were induced to proliferate in culture with B-CII, although the stimulation remained low. We were unable, however, to detect specific antibody production in these cultures even in the presence of added IL2. The fact that B-CII is only a weak antigen might, particularly, be explained by the fact that B-CII shows a high degree of structural homology with autologous CII. Hence, the immunologic response to B-CII might be suppressed under the influence of tolerance to self-CII. Addition of IL2 gives a strong enhancement of PBMC proliferation.

Our present results indicate that the heating step, routinely used by many investigators to dissolve B-CII in culture medium, destroys epitopes which are necessary for recognition by the B cell antigen receptor. Analysis with SDS-PAGE showed that the linear alpha-chains of CII remains largely intact (not shown). The destroyed determinants are therefore likely conformational epitopes. Because T cells do not recognize conformational epitopes this will not affect the proliferation of this cell type. This explanation is supported by a recent observation in rats that immunization with heat-denatured B-CII induced cellular immunity to the native B-CII, as was tested by delayed type hypersensitivity, but no humoral immunity, as measured by serum antibodies. Moreover, animals immunized with the heated antigen failed to develop arthritis, indicating also that arthritogenic determinants are destroyed.

It has been demonstrated by Logtenberg et al. (11) that PBMC from patients with autoimmune thyroiditis can be induced to produce anti-thyroglobulin antibodies in culture with the autoantigen, when it is coated on the surface of affigel beads. In the present study we have immobilized B-CII in its native form on affigel beads. Our results show that the immobilized native antigen effectively depleted antibodies from monkey immune sera, indicating that epitopes recognized by antibody molecules remained intact. PBMC from susceptible animals were cultured with the B-CII-coated beads or with BSA-coated beads as control antigen. No

specific antibodies were found in the culture supernatant, but development of the B-CII-coated beads revealed attached IgG antibodies. In cultures with BSA-coated control beads IgG binding was absent. We conclude therefore that immobilized native B-CII induced specific antibody production in PBMC cultures from susceptible monkeys. We were unable, however, to induce significant antibody production by PBMC obtained from resistant animals. At present, we do not have an explanation for this phenomenon. As was shown previously (6) B-CII-induced PBMC proliferation was also absent in the animals. An explanation might thus be that B-CII-reactive T and B cells can be subjected to suppression. The absence of antibody production in this resistant group, however, allowed us to conclude that the antibodies binding to the B-CII-coated beads are not plasma antibodies that are passively transferred with the cells, but are produced in the culture.

In our view the method described in this paper enables the study of B cell immunity to different forms of native CII in rheumatoid arthritic patients at the cellular level.

REFERENCES

1. Clague, R.B., Shaw, M.J. and Holt, P.J.L. (1981). Incidence and correlation between serum IgG and IgM antibodies to native type II collagen in patients with chronic inflammatory arthritis. *Ann. rheum. Dis.* 40, 6.
2. Klareskog, L., Johnell, O., Hulth, A., Holmdahl, R. and Rubin, K. (1986). Reactivity of monoclonal anti-type II collagen antibodies with cartilage and synovial tissue in rheumatoid arthritis and osteoarthritis. *Arthritis Rheum.* 29, 730.
3. Morgan, K., Clague, R.B., Collins, I., Ayad, S., Phinn, S.D. and Holt, P.J.L. (1989). A longitudinal study of anti-collagen antibodies in patients with rheumatoid arthritis. *Arthritis Rheum.* 32, 139.
4. Tarkowski, A., Klareskog, L., Carlsten, H., Herberts, P. and Koopman, W.J. (1989). Secretion of antibodies to types I and II collagen by synovial tissue cells in patients with rheumatoid arthritis. *Arthritis Rheum.* 32, 1087.
5. Trentham, D.E. (1982). Collagen arthritis as a relevant model for rheumatoid arthritis. Evidence pro and con. *Arthritis Rheum.* 25: 911.
6. Bakker, N.P.M., Van Erck, M.G.M., Botman, C.A.D., Jonker, M. and 't Hart, B.A. (1991). Collagen-induced arthritis in an outbred group of rhesus monkeys comprising responder and non-responder animals. Relation between the course of arthritis and collagen-induced immunity. *Arthritis Rheum.* 34: 1991.
7. Holmdahl, R., Klareskog, L., Rubin, K., Larsson, E. and Wigzell, H. (1985). 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.
8. Stuart, J.M., Townes, A.S. and Kang, A.H. (1981). Nature and specificity of the immune response to collagen in type II collagen-induced arthritis. *J. clin. Invest.* 69, 673.
9. 't Hart, L.A., Simons, J.M., Knaan-Shanzer, S., Bakker, N.P.M. and Labadie, R.P. (1990). Anti-arthritic activity of the newly developed neutrophil oxidative burst antagonist apocynin. *Free Radical Biol. Med.* 9: 127.
10. Bakker, N.P.M., Van Erck, M.G., Zurcher, C., Faaber, P., Lemmens, A., Hazenberg, M.,

- Bontrop, R.E. and Jonker, M. (1990a). Experimental immune-mediated arthritis in rhesus monkeys. A model for human rheumatoid arthritis? *Rheumatol. Int.* 10, 21.
11. Logtenberg, T., Kroon, A., Gmelig-Meyling, F.H.J. and Ballieux, R.E. (1986). Production of anti-thyroglobulin antibody by blood lymphocytes from patients with autoimmune thyroiditis, induced by the insolubilized autoantigen. *J. Immunol.* 136, 1236.

CHAPTER 5

ACQUIRED RESISTANCE TO TYPE II COLLAGEN INDUCED ARTHRITIS IN RHESUS MONKEYS IS REFLECTED BY A T CELL LOW-RESPONSIVENESS TO THE ANTIGEN

N.P.M. Bakker, M.G.M. Van Erck, L.A. 't Hart and M. Jonker

Clin. exp. Immunol. 86: 219 (1991)

SUMMARY

Ten out of fourteen rhesus monkeys developed arthritis after a single immunization with B-CII. In contrast to primary resistant monkeys, arthritic animals showed a B-CII specific T cell proliferation during the induction phase of the disease. All surviving animals showed a full remission of the disease. Two monkeys acquired resistance to CIA after one period of disease, but in three animals a booster immunization with B-CII induced a slight flare-up. It is demonstrated that B-CII immunized rhesus monkeys have the capacity to restore resistance to CIA. The development of resistance to CIA is reflected by a decreased T cell responsiveness to B-CII. It is shown that the lack of IL2 plays a role in B-CII-induced T cell low-responsiveness. A potential role of CD8⁺ T cells in the down-regulation of the T cell response to B-CII is discussed.

INTRODUCTION

The autoantigen CII might be involved in the pathogenesis of human RA, because anti-CII antibodies and CII-reactive T cells are found in serum and joint fluids of patients (1-3). A possible causal relation between autoimmunity to CII and arthritis is supported by the fact that rodents and monkeys immunized with heterologous or autologous CII may develop polyarthritis (4-7).

Because of the close phylogenetic relationship between rhesus monkeys and man (8), the non-human primate arthritis model can provide valuable information on the regulation of autoimmunity to CII. As reported earlier, 70% of randomly selected rhesus monkeys develop CIA after a single i.d. immunization with B-CII, dividing the animals into susceptible and primary resistant animals (9). All susceptible animals showed 1) a predominance of serum anti-CII antibodies of the IgM isotype and 2) a B-CII specific induced proliferation of T cells *in vitro*. On the basis of whether a flare-up of arthritis occurred following a booster immunization with B-CII, the susceptible monkeys were divided into animals with acquired resistance and 'flare-up' animals (9). Since autoimmunity to CII causes arthritis in the animal model, a preexisting or induced suppression of the immune response to CII could prevent or abrogate arthritis. The 'flare-up' animals showed a slight disease activity during the flare-up, indicating that animals may have developed a decreased responsiveness to CII.

This study has investigated how resistance to CIA is regulated in B-CII-

immunized rhesus monkeys. Susceptible monkeys seem to acquire resistance to CIA, reflected by a decreased *in vitro* proliferative response of T cells to B-CII. Both in primary resistant and in susceptible animals the low-proliferative response of T cells to B-CII could be reversed by exogenous IL2.

MATERIALS AND METHODS

Animals

Rhesus monkeys (*Macaca mulatta*) were born and raised in the ITRI-TNO Primate Center, except for animals BB25 and BB37 which were born in the Zoo Beekse Bergen, Arnhem, The Netherlands. The female monkeys were 3 to 21 years of age, weighing 2.8 to 5.8 kg. The male monkeys were 4 to 21 years of age weighing 3.9 to 9.3 kg. Monkey KD and M16 were immunized with MT H37Ra (7). The other monkeys received B-CII. All animals were examined, weighed and bled under ketamine anesthesia.

Collagens

Native B-CII, isolated from bovine articular cartilage, was a kind gift from Dr. J. van Kampen, Dr. Jan van Breemen Institute, Amsterdam, The Netherlands. Native B-CII from the bovine nasal septum as well as the native CII from rhesus monkey nasal septa and articular cartilage (Rh-CII) were isolated as previously described (7).

Arthritis induction

Animals were immunized with 1.0 ml of an emulsion (1:1) of purified B-CII dissolved in 0.1M HAc and CFA (Difco Lab., Detroit, USA) by i.d. injections in the back. For the booster immunizations the B-CII solution was emulsified with ICFA or PBS solution.

B-CII doses in the primary and booster immunizations are given in Fig.1. Disease severity was quantified by scoring:

- a) decreased mobility and apathy with slight STS, b) moderate STS with redness, c) severe STS with redness.

T cell proliferation assay

PBMC were isolated, cultured (5×10^4 cells/well) for 6 days and assayed for antigen specific T cell proliferation as described previously (9). Mitogens and

antigens were applied in the following concentrations; ConA (Pharmacia, Uppsala, Sweden) at 33.3 µg/ml and purified protein derivative (PPD) from MT (Statens Seruminstitut, Copenhagen, Denmark) at 10 µg/ml. Both B-CII and Rh-CII were applied at 50 µg/ml. When indicated, recombinant-human-IL2 (r-IL2)(CIBA-CEIGY, Switzerland) was added after day 3 of culture in a final concentration of 5 U/ml, which gave the highest enhancing effect on the CII-induced T cell proliferation.

Two-color flow cytometry

For two-color flow cytometry cultured cells were incubated with fluorochrome-labelled MoAb: Leu3a/PE (anti-CD4), Leu2a/PE (anti-CD8) and anti-IL2-receptor/FITC (all Becton Dickinson) or with unconjugated GM11 (ITRI-TNO) an anti-Mamu-DR MoAb in combination with a fluoresceinated goat anti-mouse IgG (CLB, Amsterdam, the Netherlands). The cells were incubated for 30 minutes on ice. After washing, the cells were analysed with a fluorescence-activated cell sorter (FACScan; Becton Dickinson). Lymphocytes were gated on forward and right-angle scatter for lymphocyte characteristics to minimize inclusion of other leukocytes in the cell count. From each sample, 5,000 cells were analysed. Cells were scored as positive or negative staining according to their fluorescence with reference to unlabelled control samples. The frequencies of positive cells were expressed as the percentage of all PBMC counted.

RESULTS

Arthritis induction

10 out of 14 monkeys developed clinical arthritis after a single immunization with B-CII in CFA (9). Five monkeys were euthanized due to the severity of the disease. The surviving animals were boosted with B-CII and analysed for exacerbation of the disease. As shown in Fig. 1, the monkeys in group A (1KM, D3 and M15) developed a flare-up of the clinical arthritis twice after a booster immunization. Compared to the disease activity observed during the first flare-up, the activity during the second flare-up was lower for animals 1KM and D3. Thus these animals seem to acquire resistance to CIA. Animals in group B (1OX and L65) developed an arthritis after primary immunization, but thereafter showed complete resistance to CIA. The group C animals (M14, 1UY, 1RO and 1NR) never developed any signs of clinical arthritis and therefore were regarded as primary resistant.

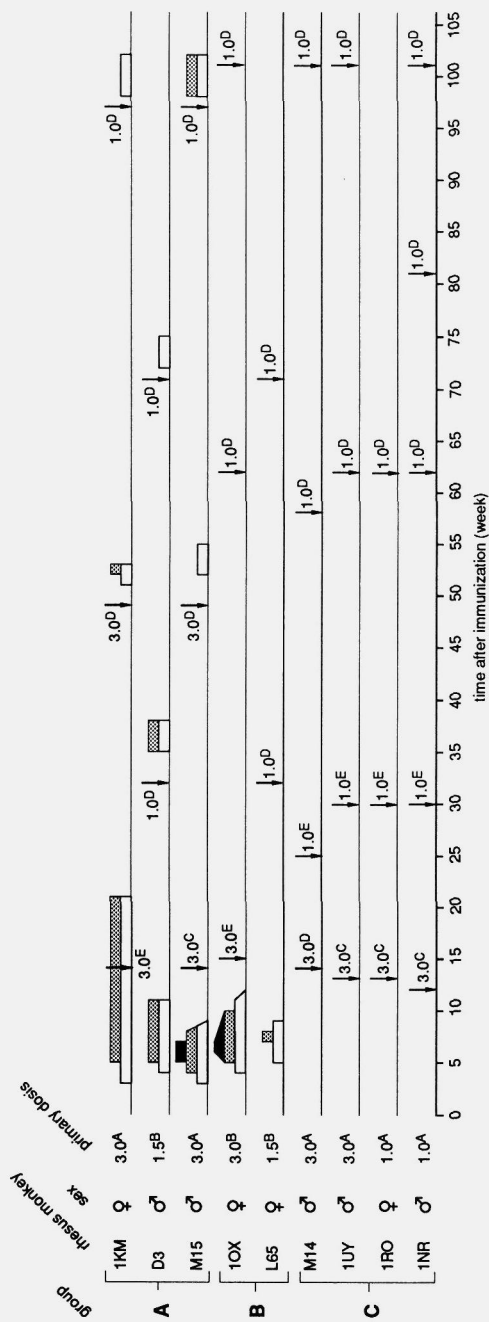


Figure 1. Incidence, onset and course of CIA in group A, B and C rhesus monkeys. □ = decreased mobility and apathy with slight STS; ■ = moderate STS with redness; ■ = severe STS with redness. Booster immunization is given with an arrow (↓): A = B-CII (mg) from joint cartilage in CFA, B = B-CII (mg) from nasal cartilage in CFA, C = B-CII (mg) from joint cartilage in PBS, D = B-CII (mg) from joint cartilage in ICFA, E = B-CII (mg) from nasal cartilage in ICFA.

Table 1

Highest measured B-CII induced T cell proliferation measured in rhesus monkeys (groups A, B and C) at various periods after the primary immunization.

group	monkey	weeks after primary immunization											
		0-25		25-50		50-75		75-100		100-125			
		M1	CII ²	M	CII	M	CII	M	CII	M	CII	M	CII
A	IKM	143	6195*(43)	92	2944 (32)	27	22723*(100)	255	3470*(14)	274	3017 (11)		
	D3	1017	20415 (20)	487	9643 (20)	196	3590* (18)	409	3296 (8)	1161	3181 (3)		
	M15	64	3723* (58)	387	3899 (10)	79	4734** (60)	427	3239* (8)	271	1738 (6)		
B	IOX	501	1726 (5)	447	1622 (4)	401	6191* (15)	504	1539 (3)	140	370* (3)		
	L65	355	3425 (10)	657	3392 (5)	699	5382* (8)	1864	1838 (1)	nd ³	nd		
C	M14	115	138 (1)	138	265 (2)	162	1317 (8)	157	280 (2)	56	99* (2)		
	IUY	169	338 (2)	214	96 (1)	934	1328 (1)	59	702 (12)	301	320* (1)		
	IRO	159	333 (2)	155	207 (1)	907	999 (1)	437	302 (1)	nd	nd		
	INR	198	285 (1)	113	265 (2)	599	3186 (5)	78	936 (12)	568	644* (1)		

Numbers represent cpm. Numbers in parentheses represent the SI, which = cpm from cells cultured with CII / cpm from cells cultured only in culture medium.

1) M = culture medium, no antigen

2) CII = bovine type II collagen, conc.: 50ng/ml

3) nd = not done

*) Measured within 4 weeks after the booster immunization with B-CII in the given period (Fig.1).

#) Measured during arthritic activity

Antigen specific T cell proliferation

As demonstrated before (9), only the arthritic monkeys out of group A and B showed after the primary immunization a proliferative T cell response to B-CII (Table 1). The T cell response (reflected in the highest measured SI-value during a certain period) is relatively high between week 50 - 75 after the primary immunization in two of the three group A monkeys (1KM and M15). During this period the animals showed their first flare-up of CIA. After week 75, during the second (compared with the first flare-up relatively slight) flare-up activity, these animals showed a decreasing T cell responsiveness to B-CII. The first flare-up of CIA in group A monkey D3 was in the period of week 25 - 50 after the primary immunization, after which this animal also showed a decreasing T cell response to B-CII. This phenomenon was also observed in the monkeys with a complete acquired resistance to CIA (group B). The low-responsiveness to B-CII in these animals was, compared with the 'flare-up' animals, reached at an earlier stage. This indicates a relation between the existence of anti-CII T cell activity and the possibility to induce a flare-up. After 50 weeks the primary resistant animals out of group C, except 1RO, occasionally showed a T cell proliferation to B-CII. There was no correlation between arthritis activity and the spontaneous (no antigen/mitogen) proliferation of PBMC.

Proliferation and expression of activation markers on B-CII stimulated T subsets

To investigate in the 'flare-up' animals during the period in which they restore resistance to CIA which T cell subset proliferated to B-CII and to study the level of activation of T cell subsets between the different groups of monkeys, PBMC were isolated in week 70 - 75 after the primary immunization and cultured for 6 days in the presence or absence of B-CII. The CD4⁺/CD8⁺ ratio's and the expression of the activation markers Mamu-DR⁺ and IL2R on CD4⁺ and CD8⁺ T cells are shown in Table 2. In the 'flare-up' animals (group A) and in monkey L65 (group B) B-CII induced a relative increase of CD8⁺ T cells (lower CD4⁺/CD8⁺ ratio) and an increased expression of both Mamu-DR antigens and IL2R. The same, but to a lesser extent, was also observed in the primary resistant monkey 1NR. Comparing both T cell subsets, B-CII induced higher percentages of CD8⁺ T cells which expressed both activation markers than of CD4⁺ T cells.

Table 2

Percentage of Mamu-DR and IL2R+ cells in PBMC of rhesus monkeys (groups A, B and C) after *in vitro* stimulation with B-CII

group	rhesus monkey	CD4/CD8		CD4+ Mamu-DR+ (%)		CD8+ Mamu-DR+ (%)		CD4+ IL2R+ (%)		CD8+ IL2R+ (%)	
		M ¹	CII ²	M	CII	M	CII	M	CII	M	CII
A	1KM	1.9	0.9	9	19	13	27	8	16	1	17
	D3	1.1	0.8	8	18	12	20	8	11	6	18
	M15	1.3	0.9	12	16	13	25	8	29	1	23
B	1OX	1.8	1.8	9	10	9	8	4	7	2	3
	L65	1.1	0.3	17	38	22	71	7	4	1	42
C	M14	1.8	1.7	9	8	10	9	7	8	2	2
	1UY	1.1	1.3	14	15	15	15	6	5	9	1
	1RO	1.3	1.2	6	7	8	7	7	8	3	6
	1NR	2.3	1.3	7	9	4	12	7	9	2	9

1) M = culture medium, no antigen

2) CII - bovine type II collagen, conc.: 50 µg/ml

The role of IL2 in the proliferative response of T cells to B-CII and Rh-CII

Addition of human r-IL2 after day 3 of culture enhanced the B-CII and the Rh-CII induced proliferation of T cells in all monkeys, although low in 1OX and M14 (Figure 2).

To investigate if the effect of r-IL2 was specific for CII, similar experiments were done in cultures with PPD. PBMC were used from the B-CII (in CFA) immunized monkeys 1KM and M15 and from monkeys KD and M6, which had been immunized with MT 2.5 years prior to the experiment. The MT immunized animals, which were used for control purposes, responded to PPD but not, as expected, to B-CII (Table 3). Also no effect of IL2 on the culture with B-CII was seen. In contrast,

the PPD induced proliferation could be enhanced by exogenous IL2 in the MT animals and also in monkey M15.

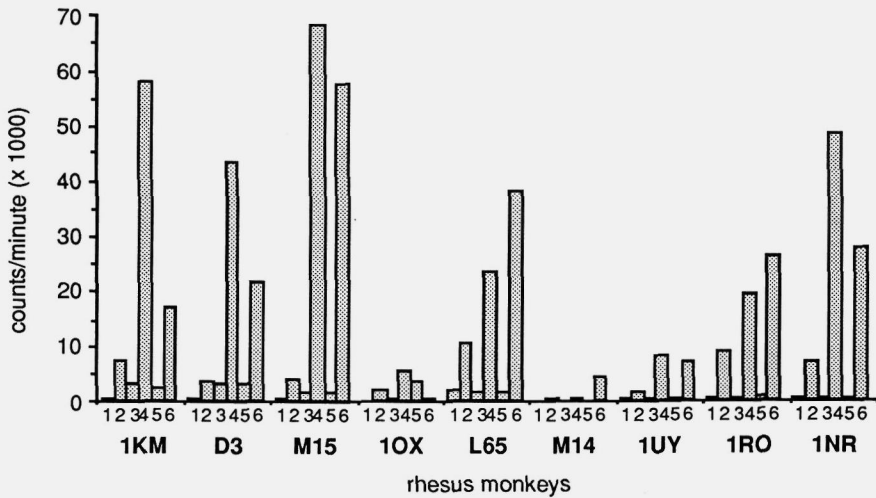


Figure 2. B-CII and Rh-CII induced proliferation of PBMC of rhesus monkeys after the last booster immunization with B-CII and the effect of exogenous recombinant IL2. 1, medium; 2, medium + IL2; 3, B-CII (50 µg/ml); 4, B-CII + IL2; 5, Rh-CII (50 µg/ml); 6, Rh-CII + IL2. Human recombinant-IL2 was added to the culture on day 4, final concentration 5 U/ml.

DISCUSSION

The present study describes the development of resistance to CIA in rhesus monkeys. The results demonstrate that 1) rhesus monkeys acquire resistance to CIA after one period of arthritis, which was incomplete in the so called 'flare-up' animals, 2) acquired resistance is reflected by a decreased in vitro T cell response to B-CII, 3) B-CII induces a slight T cell proliferation and a preferential activation and proliferation of CD8⁺ T cells in the 'flare-up' animals, 4) low-responsiveness of T cells to B-CII as well as to the autoantigen Rh-CII was reversed by addition of r-IL2 in all monkeys.

These findings provide a basis for understanding the role of cellular immunity to CII in the course of CIA. For the triggering of the disease after the primary immunization are, next to anti-CII IgM antibodies, CII-reactive T cells involved (9).

The T cell low-responsiveness to B-CII was reached at an earlier stage in the animals with complete resistance (group B) compared with the 'flare-up' animals.

Table 3

Enhancing effect of r-IL2 on the proliferation of PBMC of B-CII and MT immunized rhesus monkeys.

	IL-2 ¹ -/+	B-CII monkeys		MT monkeys	
		1KM	M15	KD	M6
medium	-	617 ²	2203	932	674
	+	3325	4044	2714	1332
ConA ³	-	23238	56284	36538	61939
	+	45214	67691	58395	73589
B-CII ⁴	-	4065	10309	953	1015
	+	24921	31396	2271	1178
PPD ⁵	-	677	3319	5587	8740
	+	2801	18196	25883	30457

1) Human recombinant IL2 was added on day 4, final concentration 5 U/ml.

2) Numbers represent counts per minute.

3) ConA, concentration: 33.3 µg/ml

4) B-CII, concentration: 50 µg/ml

5) Tuberculin purified protein derivative, concentration: 10 µg/ml

Because at the time of boosting/flare-up activity the B-CII induced T cell proliferation in the 'flare-up' animals was relative high and the serum levels of anti-CII IgM antibodies were low in all monkeys (data not shown), it is likely that CII reactive T cells play an important role in the induction of the flare-up activity.

B-CII induced an activation and proliferation of both CD4⁺ and CD8⁺ T cells in the 'flare-up' animals and in L65 which acquired complete resistance to CIA. There is direct evidence from adoptive transfer experiments in rodents that both CD4⁺ and CD8⁺ T lymphocytes can induce resistance to CIA (10-12). A role for CD8⁺ T cells in the resistance to CIA in rodents has been suggested in studies in which resistance was induced by administration of CII intravenously (13),

intraperitoneally (14) or orally (15). It has been described that antigen-specific suppressor activity can be mediated *in vitro* by CD8⁺ T cells (16,17). This may also be the case in monkey L65 where a low percentage of CD4⁺ T cells and a high percentage of activated CD8⁺ T cells were found after stimulation with B-CII *in vitro* (Table 2).

Antigen-induced T cell proliferation involves both the expression of receptors and the secretion of IL2 (18). The fact that addition of (a low-mitogenic concentration) IL2 after day 3 of culture enhanced T cell proliferation illustrates that the low-responsiveness was not due to a reduced capacity to express IL2-R on the CII-reactive T cells, but that there was a primary lack of IL2 in the culture medium. Accordingly, we could not measure IL2 in supernatants of CII stimulated PBMC (results not shown).

Low-proliferating (activated) CD8⁺ T cells in the cell cultures (Table 2) may be responsible for the lack of IL2 in the supernatant because they strongly absorb this lymphokine (19). These observations are in line with data from studies in humans demonstrating that antigen-specific unresponsiveness in autoimmune and other diseases can be reversed by the addition of exogenous IL2 (20-22). As in tuberculosis patients (23), PPD-induced T cell proliferation in rhesus monkeys could also be enhanced by adding IL2, proving that insufficiency of IL2 *in vitro* plays also a role in T cell low-responsiveness to bacterial antigens. It has been described in human RA that a defective regulation of the IL2 production may play a role in the impaired immunoregulation (24). Our present results show that in normal healthy monkeys an experimentally induced autoimmunity to CII is quickly restored to normal possibly by the activation of potentially immunosuppressive CD8⁺ T cells. Defects found in the suppressor T cell compartment of RA patients (25,26) might impair this regulatory circuit and therefore facilitate exacerbations.

REFERENCES

1. Tarkowski, A., Klareskog, L., Carlsten, H., Herberts, P., Koopman, W.J. Secretion of antibodies to type I and II collagen by synovial tissue cells in patients with rheumatoid arthritis. *Arthritis Rheum.* **18**:1087 (1989)
2. Morgan, K., Claque, R.B., Collins, I., Ayad, S., Phinn, S.D. Lennox Holt, P.J. A longitudinal study of anticollagen antibodies in patients with rheumatoid arthritis. *Arthritis Rheum.* **32**:139 (1989)
3. Londei, M., Savill, C.M., Verhoef, A., Brennan, F., Leech, Z.A., Duance, V., Maini, R.N., Feldmann, M. Persistence of collagen type II-specific T cell clones in the synovial membrane of a patient with rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA* **86**:636 (1989)
4. Trentham, D.E., Townes, A.S., Kang, A.H. Autoimmunity to type II collagen: an experimental

- model of arthritis. *J. Exp. Med.* **146**:857 (1977)
5. Courtenay, J.S., Dallman, M.J., Dayan, A.D., Martin, A., Mosedal, B. Immunization against heterologous type II collagen induces arthritis in mice. *Nature* **283**:666 (1980)
 6. Yoo, T.J., Kim, S.Y., Stuart, J.M. Induction of arthritis in monkeys by immunization with Type II collagen. *J. Exp. Med.* **168**: 777 (1988)
 7. Bakker, N.P.M., van Erck, M.G., Zurcher, C., Faaber, P., Lemmens, A., Hazenberg, M., Bontrop, R.E., Jonker, M. Experimental immune mediated arthritis in rhesus monkeys. A model for human rheumatoid arthritis. *Rheumatol. Int.* **10**:21 (1990)
 8. Balner, H. The major histocompatibility system of subhuman primate species, The major histocompatibility system in man and animals (edited by D.Gotze). Springer Verlag, Berlin (1977)
 9. Bakker, N.P.M., van Erck, M.G.M., Botman, A.D., Jonker, M., 't Hart, L.A. Collagen induced arthritis in an outbred group of rhesus monkeys comprising responder and non-responder animals. Relation between the course of arthritis and collagen-specific immunity. *Arthritis Rheum.* **34**:616 (1991)
 10. Kresina, T.F., Moskowitz, R.W. Adoptive transfer of suppression of arthritis in the mouse model of collagen-induced arthritis. Evidence for a type II collagen-specific suppressor T cell. *J.Clin. Invest.* **75**:1990 (1985)
 11. Kakimoto, K., Katsuki, M., Hirofuji, T., Iwata, H., Koga, T. Isolation of a T cell line capable of protecting mice against collagen-induced arthritis. *J. Immunol.* **140**:78 (1988)
 12. Myers, L.K., Stuart, J.M., Kang, A.H. A CD4 cell is capable of transferring suppression of collagen-induced arthritis. *J. Immunol.* **143**:3976 (1989)
 13. Staines, N.A., 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 anti-serum. *Immunology* **44**:737 (1981)
 14. Williams, R.O., Whyte, A., Waldmann, H. Resistance to collagen-induced arthritis in DBA/1 mice by intraperitoneal administration of soluble type II collagen involves both CD4+ and CD8+ T lymphocytes. *Autoimmunity* **4**:237 (1989)
 15. Nagler-Anderson, C., Bober, L.A., Robinson, M.E., Siskind, G.W., Thorbeake, G.J. Suppression of type II collagen-induced arthritis by intragastric administration of soluble type II collagen. *Proc. Natl. Acad. Sci. USA* **83**:7443 (1986)
 16. Nishimura, Y., Sasazuki, T. Suppressor T cells control the HLA-linked low responsiveness to streptococcal antigen in man. *Nature* **302**:67 (1983)
 17. Bloom, B.R., Mehra, V. Immunological unresponsiveness in leprosy. *Immunol. Rev.* **80**:6 (1984)
 18. Cantrell, D.A., Smith, K.A. The interleukin 2 T cell system: a new cell growth model. *Science* **224**:1321 (1984)
 19. Palacios, R. Mechanism of T cell activation: role and functional relationship of HLA-DR antigens and interleukins. *Immunol. Rev.* **63**: 73 (1982)
 20. Emery, P., Panayi, G.S., Nouri, M.E. Interleukin-2 reverses deficient cell-mediated immune responses in rheumatoid arthritis. *Clin. Exp. Immunol.* **57**:123 (1984)
 21. Essery, G., Feldmann, M., Lamb, J.R. Interleukin-2 can prevent and reverse antigen-induced unresponsiveness in cloned human T lymphocytes. *Immunology* **64**:413 (1988)
 22. Chizzolini, C., Geinoz, A., Schrijvers, D. Interleukin-2 reverses T cell unresponsiveness to *Plasmodium falciparum* antigen in malaria immune subjects. *Cell. Immunol.* **128**:1 (1990)
 23. Shiratsuchi, H., Okuda, Y., Tsuyuguchi, I. Recombinant human interleukin-2 reverses in vitro-deficient cell-mediated immune response to tuberculin purified protein derivative by lymphocytes of tuberculous patients. *Infect. Immun.* **55**:2126 (1987)
 24. Miyasaka, N., Nakamura, T., Russell, I.J., Talal, N. Interleukin 2 deficiencies in rheumatoid arthritis and systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **31**:109 (1984)
 25. Emery, P., Gentry, C.K., Mackay, I.R., Muirden, K.D., Rowley, M. Deficiency of the suppressor inducer subset of T lymphocytes in rheumatoid arthritis. *Athritis Rheum.* **30**:849 (1987)
 26. Chattopadhyay, C., Chattopadhyay, H., Narvig, J.B., Michaelsen, T.E., Mellbye, O.J. Lack of

suppressor cell activity in rheumatoid synovial lymphocytes. *Scand. J. Immunol.* **10**:309
(1979)

CHAPTER 6

RESISTANCE TO COLLAGEN-INDUCED ARTHRITIS IN A NONHUMAN PRIMATE SPECIES MAPS TO THE MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I REGION

By Nicolaas P.M. Bakker, Monique G.M. van Erck, Nel Otting,
Neubury M. Lardy,¹ Riet C. Noort, Bert A. 't Hart, Margreet Jonker,
and Ronald E. Bontrop

¹Central Laboratory of the Red Cross Bloodtransfusion Service, Amsterdam, The Netherlands

J. Exp. Med. 175: 933 (1992).

SUMMARY

CIA is an experimentally inducible autoimmune disorder which is, just like several forms of human arthritis, influenced by a genetic background. Immunization of young rhesus monkeys (*Macaca mulatta*) with CII-induced CIA in about 70% of the animals. One MHC class I allele was present only in young animals resistant to CIA and absent in arthritic animals. This strong association suggests that the MHC class I allele itself, or a closely linked gene, determines resistance to CIA. The mechanism controlling the resistance to CIA becomes less efficient in aged animals since older rhesus monkeys, which were positive for the resistance marker, developed a mild form of arthritis. At the cellular level it is demonstrated that resistance to CIA is reflected by a low-responsiveness of T cells to CII. This association between a specified MHC class I allele and resistance to an autoimmune disease points at the importance of the MHC class I region in the regulation of the immune response to an autoantigen.

INTRODUCTION

The highly polymorphic MHC region, which is known in man as the HLA-system, plays a pivotal role in controlling the immune response to foreign pathogens. The MHC class I and II glycoproteins bind peptides derived from intracellular enzymatic degradation of proteins and subsequently present them on the cell surface to CD8⁺ cytotoxic T lymphocytes and CD4⁺ (primarily helper) T cells respectively (1,2). MHC class I and II region encoded susceptibility genes for several autoimmune diseases have been documented in man and experimental animal models (3-7). MHC genes associated with resistance to autoimmune disorders are relative rare and have until now only been mapped to the class II region (8,9).

CIA is an experimental animal model for studying immunoregulatory mechanisms underlying an autoimmune disease. Upon i.d. immunization with CII, the major cartilage protein, rhesus monkeys as well as rodents develop CIA which shares several pathologic features with human RA (10,11). Human RA is associated with the HLA-DR1 and -DR4 serotypes (12,13), while in mice susceptibility to CIA has been mapped to a critical site of an I-A allele encoded by the MHC class II region (14).

In this study the genetic influence of MHC genes on the development of CIA in rhesus monkeys was investigated. The current knowledge of the MHC of the

rhesus monkey, recently named *MhcMamu* (15), located on chromosome 2, is primarily based on serological data. Allo-antisera allow the detection of 13 Mamu-A and 14 Mamu-B locus products while more than 10 allelic specificities are encoded by the Mamu-DR locus (16,17). Recently the knowledge of the MHC systems in different non-human primate species has expanded rapidly. These studies demonstrate that MHC polymorphisms are inherited in a trans-species mode of evolution (18,19) which means that the evolution of MHC alleles does not start at the inception of a species, but is rather a process in which a major group of alleles is passed on in the phylogeny from one species to another. The major implication of this finding is that some alleles of different species are more related to each other than the MHC alleles within a species. This type of relationship may have functional implications as is reflected by antigen presentation studies across a species barrier (20). Therefore, the identification of MHC genes or alleles as markers for susceptibility or resistance to autoimmune disorders in non-human primate models may be relevant for understanding the pathogenesis and immunoregulation of human autoimmune diseases.

MATERIAL AND METHODS

Animals

Most rhesus monkeys (*Macaca mulatta*) used in this study were born and raised at the ITRI-TNO Primate Center. Monkeys which were born outside the Primate Center are BB25 and BB37, born in the Zoo Beekse Bergen, Arnhem, The Netherlands; 3974 and 3982, born in Bethesda Primate Center, USA and monkeys 2822, 2774 and 3215 were imported from India at a young age. Monkeys 4062, 4070, 4116 and 4106 were imported from Birma at a young age. All monkeys were typed for *MhcMamu* antigens using standard serological techniques (16). The animals used in the experiments were randomly selected from the colony. The sex and age of the animals are given in Table 1. The animals were examined, weighed and bled under ketamin anesthesia. When an animal suffered from pain 0.06 mg Buprenorfine was given twice daily by i.m. injection (Temgesic, Warrick BV, The Netherlands). The experiments have been approved by the TNO ethical committee for animal experiments. Throughout the experiments the animals were under veterinary control.

Table 1

Resistance to collagen-induced arthritis in rhesus monkeys is associated with Mamu-A26

rhesus monkey	sex	age [§] (year)	<i>MhcMamu</i> @			immuni- zation dose* (mg)	anti-CII** T-cell response	arthritis***
			A	B	DR			
<u>young animals</u>								
BB25	M	6.0	-,-	10,-	3,8	1.5	+	+++
2BN	M	3.5	11,18	9,19	3,4	1.5	+	+++
BB37	F	3.0	-,31	-,-	3,2	1.5	+	+++
1WF	F	4.0	11,32	9,28	2,5	1.5	+	+++
1JT	F	6.5	11,24	19,33	3,2	1.5	+	+++
1KM	F	6.0	11,14	1,19	3,1	3.0	+	++
D3	M	4.0	17,18	3,28	1,1	1.5	+	++
M15	M	4.0	11,2	6,21	3,8	3.0	+	++
1OX	F	5.0	11,13	9,9	-,8	3.0	+	++
L65	F	5.0	-,2	-,-	2,8	1.5	+	++
4062	M	6.0	11,20	10,-	2,3	1.0	+	++
4116	M	7.0	18,29	33,-	2,4	1.0	+	++
4070	M	8.0	2,31	6,-	2,-	1.0	+	+
8827	M	1.5	2,11	10,27	1,2	1.0	+	+(s)
8765	F	3.0	17,32	10,27	101,8	1.0(r)	+	+(s)
8684	F	4.0	2,17	10,22	8,1	1.0(r)	-	+(s)
8675	M	3.0	2,11	6,10	1,3	1.0	-	-
M14	M	4.0	11,26	6,19	2,8	3.0	-	-
1UY	M	3.5	11,26	19,27	1,2	3.0	-	-
1RO	F	4.5	24,26	6,-	3,3	1.0	-	-
1NR	M	5.5	11,26	21,23	5,101	1.0	-	-
8769	M	2.0	11,26	27,19	1,2	1.0	+	-
8781	F	3.0	2,26	19,23	3,3	1.0(r)	-	-
4106	F	6.0	-,26	6,-	8,5	1.0(r)	-	-
<u>old animals</u>								
2774	M	>23	11,32	10,19	3,101	1.0	+	++
3974	F	>23	2,13	6,10	4,1	1.0	+	+++
3982	F	>22	14,32	6,23	4,5	1.0	+	+++
3215	F	>23	13,26	9,6	5,3	1.0	+	+(s)
2822	F	>22	31,26	33,6	1,3	1.0	+	+

Collagens

Native B-CII, isolated from bovine articular cartilage, was a kind gift from dr. J. van Kampen, dr. Jan van Breemen Institute, Amsterdam, The Netherlands. The isolation of B-CII and Rh-CII, respectively from the bovine and rhesus monkey nasal septa has been described elsewhere (10).

Arthritis induction

The animals were immunized by i.d. injection on the back with 1 ml of an emulsion (1:1) of purified B-CII or Rh-CII dissolved in 0.1M HAc and CFA (Difco Lab., Detroit, USA). CII doses in the primary immunizations are given in Table 1. The disease severity was quantified in an arthritic score as explained in the legend of Table 1.

Proliferation of mononuclear cells

PBMC were isolated from heparinized blood by density gradient centrifugation (LSM, Organon, Durham, USA). The cells from the interphase of the gradient were washed twice with HBSS (Gibco Ltd., Paisley, Scotland) and resuspended in culture medium, consisting of HEPES-buffered RPMI-1640 (Gibco) supplemented with L-glutamine (200 mM; Gibco), 0.15 % NaHCO₃, 200 U/ml penicillin, 200 U/ml streptomycin (both Gibco) and 20% heat-inactivated pooled nonimmune rhesus monkey serum. PBMC were cultured for 6 days in 96 wells round-bottom plates

legend Table 1:

Sex: M = male; F = female.

\$Age at time of immunization. (In captivity rhesus monkeys can reach the maximum of 30-35 years of age).

@MhcMamu-A, -B and -DR locus alleles have been identified serologically (16). The A26 allele has been printed bold.

*All rhesus monkeys are immunized with B-CII, only the (r) marked animals were immunized with Rh-CII.

**The CII-dependent T cell proliferation is scored as negative (-) if the stimulation indices (cpm from cells cultured with CII/cpm from cells cultured only in culture medium) was <2 during the first 25 weeks after the primary immunization with CII. A positive score (+) is given when the stimulation indices are >2.

The anti-CII T cell response in the responding animals ranged from 1,500 to 23,000 cpm, whereas the non-responder animal reached levels of 300 cpm, which is approximately equal to the controls, done with medium with no antigen.

***The arthritic score is as follows: +++ severe STS of all small joints of hands and feet and of the wrists and ankle joints, ++ moderate STS of most small joints of hands and feet and wrists and ankle joints, + moderate STS only of wrists and ankle joints, +(s) sub-clinical arthritis only manifest by elevated ESR and CRP levels and the detection of anti-CII T cell response and IgM antibodies (30).

(Greiner, Labortechnik, Germany) in a humidified atmosphere containing 5% CO₂ at 37°C at a concentration of 5 x 10⁴ cells per well. Denatured CII was added in the optimal concentration of 50 µg/ml. Cell proliferation was measured by the incorporation of [³H]thymidine (0.5 µCi per well) during the final 8 hours of the culture.

RESULTS AND DISCUSSION

After immunization of 24 MHC typed young animals (<6.5 years of age) with heterologous or homologous CII, 16 young animals developed CIA (Table 1). No apparent association was observed between susceptibility, sex and immunization dose of CII or between Mamu-DR and CIA. However, 7 out of 8 resistant animals shared the same serologically defined MHC class I allele (Mamu-A26), which was absent in all 18 young animals that developed CIA (P<0.00002). Only one of the resistant animals (8675) lacks the Mamu-A26 allele (Table 1). To our knowledge this is the first demonstration that resistance to an autoimmune disease, that can be evoked by immunization with a soluble antigen, is associated with the MHC class I region. So far, resistance markers for autoimmune diseases have been exclusively linked to the MHC class II region (8,9). The Mamu-A26 associated resistance seems unique for CIA since this phenomenon was not observed in rhesus monkeys immunized with myelin basic protein to evoke experimental autoimmune encephalomyelitis (data not shown). Because in both models immunization was performed with CFA, it is unlikely that the Mamu-A26 associated resistance is due to the adjuvant.

It is generally accepted that during ageing the capacity of the immune system to maintain tolerance to autoantigens decreases. To study whether the association between Mamu-A26 and resistance to CIA holds true in old animals, 1 male and 4 female monkeys of more than 22 years of age were immunized with CII. The Mamu-A26 positive old animals (3215 and 2822) developed arthritis, but the severity of the disease was clearly less than in the Mamu-A26 negative animals (Table 1).

Using biochemical methods the MHC class I gene products were characterized in more detail. One dimensional isoelectrofocussing (1D-IEF) of class I products, immunoprecipitated by using the anti-MHC class I monoclonal antibody W6/32, demonstrated that the Mamu-A and -B serotypes correlate with isoelectric point differences (manuscript in preparation). In addition, such experiments also

showed that all Mamu-A26 positive animals share one product with similar isoelectric point which was absent in the Mamu-A26 negative animals.

There appears to exist a strong correlation between the Mamu-A26 allele and resistance to develop CIA. The question arises, whether Mamu-A26 itself confers resistance, or whether it functions as a marker for a closely linked regulator gene. At present it is not known whether the Mamu-A alleles are equivalent to the HLA-A or -B alleles. Therefore we can not exclude that other genes, like some of the HLA-B associated transcripts (BAT), exhibit polymorphism and may be linked to Mamu-A26 (21-23). In this respect it is of interest that some BAT genes encode for collagen-like structures (24). A potential problem with any association study is population stratification. In this case, the A26 allele may mark a subset of rhesus monkeys resistant to CIA because of other genes in that subset. In this respect we are studying Vb usage in TCR diversity in order to elucidate whether other polymorphic gene systems may play a role in resistance to CIA. The alternative option is that the Mamu-A26 allele determines resistance by itself. MHC class I molecules present peptides from intracellular origin to cytotoxic T lymphocytes (1), whereas soluble antigens derived from extracellular pathogens are usually presented by MHC class II molecules (2). When the Mamu-A26 allele itself is involved in resistance to CIA the question has to be answered if Mamu-A26 molecules are able to bind self-peptides from CII, which is a soluble antigen. This is not unlikely because it has been documented recently that peptides derived from soluble proteins can complex with class I molecules (25). As shown in Table 1 resistance to CIA in rhesus monkeys is accompanied by *in vitro* CII-specific T cell low-responsiveness. The binding of peptides from CII by Mamu-A26 molecules and subsequent presentation to regulator CD8 positive T cells, which can eliminate antigen presenting cells such as CII reactive B cells and cells of the macrophage lineage (26), may be an effective mechanism in the prevention of CIA. After repeated immunizations with CII, all arthritic monkeys acquire resistance to the disease, which is also reflected by a T cell low-responsiveness to CII. Therefore it is possible that other class I alleles, although less efficiently than A26, mediate resistance to CIA in a similar manner.

Findings in the human population on associations between certain HLA class I alleles and protection to disease are mainly observed for infectious diseases (27,28). On the other hand, there exists a strong counterpart of the MHC class I linked resistance to CIA in nonhuman primates, namely the association between HLA-B27 and AS (3,4), which is an inflammatory disease predominantly affecting the joints of the spine and the pelvis. Although MHC class I determines in AS

susceptibility and in CIA resistance to disease, both associations are remarkably strong. At present it is disputed whether AS is an autoimmune or an infectious disease (29). Apart from that the antigen which causes AS has not been identified. However, in the case of CIA disease is evoked by a known autoantigen, namely CII. Therefore, this arthritis model is most suitable to investigate the role of MHC class I genes in the immunoregulation of autoimmune arthritis in primates.

REFERENCES

1. Townsend, A.R.M., J. Rothbard, F.M. Goth, G. Bahadur, D. Wraith, and A.J. McMichael. The epitopes of influenzanucleoprotein recognized by cytotoxic T lymphocytes can be defined with short synthetic peptides. *Cell* 44:959 (1986).
2. Buus, S., A. Sette, S.M. Colon, C. Miles, and H.M. Grey. The relation between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides. *Science* 235:1353 (1987).
3. Schlosstein, L., P.I. Terasaki, R. Bluestone, and C.M. Pearson. High association of a HL-A antigen, w27, with ankylosing spondylitis. *N. Engl. J. Med.* 288:704 (1973).
4. Caffrey, M.F.P., and D.C.O. James. Human lymphocyte antigen association in ankylosing spondylitis. *Nature* 242:121 (1973).
5. Harley, J.B., M. Reichlin, F.C. Arnett, E.L. Alexander, W.B. Bias, and T.T. Provost. Gene interaction at HLA-DQ enhances autoantibody production in primary Sjogren syndrome. *Science* 232:1145 (1986).
6. Sollid, L.M., G. Markussen, J. Ek, H. Gjerde, F. Vartdal, and E. Thorsby. Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer. *J. Exp. Med.* 169:345 (1989).
7. Hammer, R.E., S.D. Maika, J.A. Richardson, J.P. Tang, and J.D. Taurog. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human beta2m: an animal model of HLA-B27-associated human disorders. *Cell* 64:1099 (1990).
8. Schreuder, G.M.T., M.G.J. Tilanus, R.E. Bontrop, G.J. Bruining, M.J. Giphart, J.J. van Rood, and R.R.P. de Vries. HLA-DQ polymorphism associated with resistance to type I diabetes detected with monoclonal antibodies, isoelectric point differences, and restriction fragment length polymorphism. *J. Exp. Med.* 164:938 (1986).
9. Todd, J.A., J.I. Bell, and H.O. McDevitt. HLA-DQ-beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* 329:599 (1987).
10. Bakker, N.P.M., M.G. van Erck, C. Zurcher, P. Faaber, A. Lemmens, M. Hazenberg, R.E. Bontrop, and M. Jonker. Experimental immune mediated arthritis in rhesus monkeys. A model for human rheumatoid arthritis? *Rheumatol. Int.* 10:21 (1990).
11. Holmdahl, R., M. Andersson, T.J. Goldschmidt, K. Gustafsson, L. Jansson, and J.A. Mo. Type II collagen autoimmunity in animals and provocations leading to arthritis. *Immunol. Rev.* 118:193 (1990).
12. Gregersen, P.K., J Silver, and R.J. Winchester. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum.* 30:1205 (1987).
13. Nepom, G.T., P. Byers, C. Seyfried, L.A. Healey, K.R. Wilske, D. Stage, and B.S. Nepom. HLA genes associated with rheumatoid arthritis. Identification of susceptibility alleles using specific oligonucleotide probes. *Arthritis Rheum.* 32:15 (1989).
14. Holmdahl, R., M. Karlsson, M.E. Andersson, L. Rask, and L. Andersson. Localization of a critical restriction site on the I-A-beta chain that determines susceptibility to collagen-induced arthritis in mice. *Proc. Natl. Acad. USA* 86:9475 (1989).
15. Klein, J., R.E. Bontrop, R.L. Dawkins, H.A. Erlich, U.B. Gyllensten, E.R. Heise, P.J. Jones, P.

- Parham, E.K. Wakelande, and D.I. Watkins. Nomenclature for the major histocompatibility complexes of different species: a proposal. *Immunogenetics* 31:217 (1990).
16. Van Vreeswijk, W., J.H. Roger, J. D'Amaro, and H. Balner. The major histocompatibility complexes of rhesus monkeys, RhL-A. VII. Identification of five new serologically defined antigens. *Tissue Antigens* 9:17 (1977).
 17. Sliereendregt, B.L., N. Otting, M. Jonker, and R.E. Bontrop. RFLP analysis of the rhesus monkey MHC class II DR subregion. *Human Immunol.* 30:11 (1991).
 18. Mayer, W.E., M. Jonker, D. Klein, P. Ivanyi, G. van Seventer, and J. Klein. Nucleotide sequences of chimpanzee MHC class I alleles: evidence for trans-species mode of evolution. *EMBO J.* 7:2765 (1988).
 19. Lawlor, D.A., E. Warren, F.E. Ward, and P. Parham. Comparison of class I MHC alleles in humans and apes. *Immunol. Rev.* 113:147 (1990).
 20. Bontrop, R.E., D.G. Elferink, N. Otting, M. Jonker, and R.R.P. de Vries. Major histocompatibility complex class II-restricted antigen presentation across a species barrier: conservation of restriction determinants in evolution. *J. Exp. Med.* 172:53 (1990).
 21. Spies, T., M. Bresnahan, and J.L. Strominger. Human major histocompatibility complex contains a minimum of 19 genes between the complement cluster and HLA-B. *Proc. Natl. Acad. Sci. USA* 86:8955 (1989).
 22. Sargent, C.A., I. Dunham, J. Trowsdale, and R.D. Campbell. Human major histocompatibility complex contains gene for the major heat shock protein HSP70. *Proc. Natl. Acad. Sci. USA* 86:1968 (1989).
 23. Ciccone, E., M. Colonna, O. Viale, D. Pende, C.D. Donato, D. Reinharz, A. Amoroso, M. Jeannet, J. Guardiola, A. Moretta, T. Spies, J. Strominger, and L. Moretta. Susceptibility or resistance to lysis by alloreactive natural killer cells is governed by a gene in the human major histocompatibility complex between BF and HLA-B. *Proc. Natl. Acad. Sci. USA* 87:9794 (1990).
 24. Banerji, J., J. Sands, J.L. Strominger, and T. Spies. A gene pair from the human major histocompatibility complex encodes large proline-rich proteins with multiple repeated motifs and a single ubiquitin-like domain. *Proc. Natl. Acad. Sci. USA* 87:2374 (1990).
 25. Moore, M.W., F.R. Carbone, and M.J. Bevan. Introduction of soluble protein into the class I pathway of antigen processing and presentation. *Cell* 54:777 (1988).
 26. Lanzavecchia, A. Antigen-specific interaction between T and B cells. *Nature* 314:537 (1985).
 27. De Vries, R.R., P.M. Khan, L.F. Bernini, E. van Loghem, and J.J. van Rood. Genetic control of survival in epidemics. *J. Immunogenet.* 6:271 (1979).
 28. Hill, A.V.S., C.E.M. Allsopp, D. Kwiatkowski, N.M. Anstey, P. Twumasi, P.A. Rowe, S. Bennett, D. Brewster, A.J. McMichael, and B. M. Greenwood. Common West African HLA antigens are associated with protection from severe malaria. *Nature* 352:595 (1991).
 29. Gaston, J.S.H. How does HLA-B27 confer susceptibility to inflammatory arthritis? *Clin. Exp. Immunol.* 82: 1 (1990).
 30. Bakker, N.P.M., M.G.M. van Erck, C.A.D. Botman, M. Jonker, and B.A. 't Hart. Collagen-induced arthritis in an outbred group of rhesus monkeys comprising responder and nonresponder animals. Relationship between the course of arthritis and collagen-specific immunity. *Arthritis Rheum.* 34:616 (1991).

CHAPTER 7

CONCLUDING REMARKS

After David Trentham in 1977 showed for the first time that rats develop a polyarthritis upon immunization with CII, an increasing number of investigators have studied CII and its relationship to CIA and human arthritis.

In this thesis various aspects of CIA in the rhesus monkey are discussed. By comparing responder (susceptible) and nonresponder (resistant) animals it was possible to associate several clinical and immunologic parameters with the disease.

B-CII as well as homologous CII proved to be arthritogenic in the rhesus monkey (Chapters 2 and 6). It has been shown that, after primary immunization with B-CII the activity of CII-reactive T cells (monitored by *in vitro* proliferation to the antigen), together with the production of anti-CII IgM antibodies correlates with susceptibility to CIA (Chapter 3). Despite high levels of circulating anti-CII IgG antibodies resistant monkeys showed no disease symptoms. On the basis of these findings it is hypothesized that there is a direct relationship between the activity of (certain subtypes of) CII-reactive T cells, the subsequent production of B cell activating- and differentiating lymphokines, and thus the preferential production of anti-CII IgM or IgG antibodies. Anti-CII antibodies, particularly of the IgM isotype, are able to trigger the onset of disease by 1) binding to epitopes of self-CII located at the surface of intact cartilage in the joint, and 2) subsequently activating the complement cascade, resulting in chemotaxis of leucocytes into the joint.

The observation that autoimmunity to CII can lead to clinical polyarthritis in the rhesus monkey, and that anti-CII antibodies are present in several forms of human arthritis, suggests that CII is one of the autoantigens involved in triggering and / or perpetuating the autoimmune response in human (chronic) arthritis. This study shows that serum levels of anti-CII IgM antibodies in patients should be investigated as an early marker of joint inflammation.

In rhesus monkeys no significant association was found between susceptibility to CIA and certain MHC class II genes. In mice only animals that express the MHC class II I-A_q or I-A_r allelic varieties develop CIA. This suggests that in inbred mice strains the interaction between a restricted number of CII peptides and MHC

molecules is relevant for triggering arthritis. The restricted immune response to CII may be due to the inbred character (i.e. homozygotic and less allelic variation) of the mice strains used in these studies. Possibly outbred rhesus monkeys develop a much more heterogenous immune response to CII, so that an association between certain MHC class II genes and susceptibility to CIA is, in comparison to inbred rodents, difficult to find. The MHC class II region of the rhesus monkey is currently being characterized at the DNA- and at the protein level. This analysis indicates a) that there is a high degree of similarity between certain MHC-DR alleles of the rhesus monkey and man and b) that class II allelic variation is more diverse than assumed earlier on the basis of serological typing techniques. Further analysis of the Mamu-class II genes may reveal an association between susceptibility to CIA and certain class II genes or MHC haplotypes.

As well as susceptibility to CIA, this study has focussed on aspects of resistance to the disease. Resistant animals produced anti-CII antibodies that were predominantly of the IgG isotype after primary immunization with B-CII. This may represent a secondary B cell response in the resistant animals (Chapter 2). Given that CII is a T cell dependent antigen, these data show that resistance to CIA is not due to the absence of CII-reactive T and B cells. It is more likely that in the resistant animals the anti-CII B cells have undergone a triggering prior to immunization, potentially mediated by activated CII-reactive T cells. The anergic state of the anti-CII T cells (defined by the low *in vitro* CII-dependent T cell proliferation) in the resistant animals could be the result of such an activation because it has been shown that in susceptible animals, which later acquired resistance to CIA, anti-CII T cells became anergic after prolonged exposure to the antigen (Chapter 5). An attractive explanation for the induction of anergy *in vivo*, may be that antigen is recognized in the absence of costimulatory signals, such as those induced by lymphokines (Nossal G., 1989, Science 245:147). Self antigens can be presented by antigen-presenting cells which lack the costimulatory activity, such as resting B cells, and the interacting T cell will become anergic. One aspect of anergy that is of importance when discussing pathological autoimmunity is the eventual capability of the anergic autoreactive T cell to overcome this non-reactive state. This study showed that addition of exogenous IL-2 allowed the restoration of the proliferative response of anti-CII T cells (Chapter 5). Thus, anergic CII-reactive T cells are not inert but may exert potential effector functions if properly activated. A consequence of the reversibility of the anergic state of the autoreactive T cells in the presence of IL2 may be the risk of developing pathological autoimmunity during, for example, infection.

A mechanism for the active maintenance of anergy would appear to be necessary, given that the tolerant state of CII-reactive T cells appears to be relatively easily reversible. Antigen-specific suppression of the activation of these autoreactive T cells could be mediated by direct cell-cell contact or by soluble factors. By defining the factors involved in suppressing CIA, we may find tools that are valuable in the therapy of human arthritis. This study shows that the cellular immune response to B-CII is down-regulated and that monkeys can regain their tolerance for CIA. The mechanism is unknown, although CD8⁺ cells may be involved in the selective downregulation of anti-CII T cell activity (Chapter 5).

The finding described in Chapter 6, that a certain MHC class I allele is associated with resistance to CIA, is unique. Why is it that MHC class I associated resistance to an (experimental) autoimmune disease has never been found before? There may be several reasons. The lack of such an association in humans may be because MHC class I associated resistance only becomes manifest when analysing pathological autoimmunity induced by active immunization with CII or other autoantigens in CFA, as done in the rhesus monkeys. A second possibility is that investigators have always been more focussed on susceptibility markers. For example, in RA a lot of work has been performed on the HLA-DR4 variants and -DR1 associations which support the concept of a shared epitope as the functional unit that forms the molecular basis for disease susceptibility. Further analysis of the Mamu-class I locus, i.e. by DNA-sequencing of the different alleles, will possibly reveal the existence of a human equivalent of the Mamu-A26 allele. When a human homologue of the Mamu-A26 allele is found, epidemiological studies in the human population may reveal an (positive or negative) association with arthritis.

In conclusion, CIA in rhesus monkeys is an unique model in which the influence of the MHC on CIA can be studied. Future studies of the role of Mamu-A26 in resistance to CIA include analysis of the role of antigen-specific T cells in CIA. In particular, determination of the factors that suppress autoreactive T cell activity may allow the development of immunization protocols that prevent or suppress autoimmune arthritis in humans.

SAMENVATTING

Deze studie met rhesusapen laat zien dat na een eenmalige immunisatie met type II collageen (CII) van rund of rhesusaap (beide geëmulgeerd in compleet Freund's adjuvant) in de huid een deel van de dieren een polyarthritis ontwikkelden (Hfst. 2 en 6). Door voor collageen-geïnduceerde artritis (CIA) gevoelige en resistente dieren met elkaar te vergelijken was het mogelijk de inductie en verloop van de ziekte te associëren met verschillende klinische en immunologische parameters. Zo bleek dat alleen bij de gevoelige dieren *in vitro* een door CII geïnduceerde proliferatie van CII-reactieve T lymfocyten kon worden gemeten (Hfst. 3). In serum van gevoelige dieren konden, naast de aanwezigheid van anti-CII IgG antistoffen, anti-CII antistoffen van het IgM type worden gemeten na de eerste immunisatie met CII (Hfst. 3). De observatie dat autoimmuniteit tegen CII in de rhesusaap kan leiden tot een polyarthritis en dat anti-CII antistoffen aanwezig zijn in verschillende vormen van humane artritis, suggereert dat CII een van de autoantigenen is betrokken bij het ontstaan of onderhouden van de (chronische) gewrichtsontsteking bij de mens.

Resistente dieren bleken, naast de afwezigheid van een CII afhankelijke T cel proliferatie, alleen CII-bindende antistoffen van het IgG type te produceren. CII is een T cel afhankelijk antigeen en dus is het onwaarschijnlijk dat resistentie voor CIA in de rhesusaap het resultaat is van de afwezigheid van CII-reactieve T cellen. De afwezigheid van anti-CII IgM antistoffen, maar de aanwezigheid van anti-CII IgG antistoffen, heeft eerder het karakter van een secundaire B cel activiteit (Hfst. 3). Door de apen meerdere malen te immuniseren met CII bleek dat de voor CIA gevoelige dieren een (gedeeltelijke) resistentie tegen de ziekte hadden opgebouwd, gepaard gaande met een verlaagd proliferatief vermogen van de CII-reactieve T cellen (Hfst. 5).

Verder bleek dat, naast CD4⁺ T cellen, ook CD8⁺ T cellen sterk worden geactiveerd door CII. Dit zou een indicatie kunnen zijn dat CD8⁺ T cellen betrokken zijn bij de selectieve down-regulatie van de anti-CII T cel activiteit (Hfst. 5). De aan- resp. afwezigheid van IL2 speelt in deze geïnduceerde verlaagde T cel proliferatie ook een rol. Door aanwezigheid van CII en door toevoeging van een relatieve lage concentratie IL2 kon *in vitro* het laag-prolifererende vermogen van de CII-reactieve T cellen worden opgeheven (Hfst. 5).

Een opvallend aspect van verschillende humane autoimmuunziekten, zoals bij reumatoïde artritis (RA) en multiple sclerose, is dat de gevoeligheid voor deze

ziekten geassocieerd blijkt te zijn met de aanwezigheid van bepaalde klasse II allelen van het polymorfe zgn. major histocompatibility complex (MHC). Aangezien bekend is dat MHC moleculen een functie hebben bij de presentatie van peptiden aan T cellen, suggereert deze vinding dat (voor RA en multiple sclerose tot nu toe onbekende) antigenen een rol spelen bij de pathogenese van de ziekte.

Omdat in het rhesusaap arthritis model het ziekte inducerend antigeen (CII) bekend is en alle dieren getypeerd zijn voor hun (op de mens gelijkende) MHC, bestond speciale belangstelling voor associaties tussen gevoeligheid voor CIA en de aanwezigheid van bepaalde MHC klasse II allelen. Maar in het gebruikte panel apen werd geen significante associatie gevonden tussen gevoeligheid voor CIA en de aanwezigheid van bepaalde MHC klasse II allelen. Echter, er werd een MHC klasse I allel (A26) gevonden, dat geassocieerd bleek te zijn met resistentie voor CIA. Verdere studie van het MHC klasse I locus van de rhesusaap, o.a. door bepaling van de nucleotide volgorde van het DNA dat codeert voor het A26 eiwit, zal duidelijk maken of er een ortholoog allel aanwezig is bij de mens. Indien dit het geval is zullen epidemiologische studies moeten uitwijzen of er in de menselijke populatie een (positief of negatief) associatie bestaat tussen de aanwezigheid van dit allel en bepaalde vormen van arthritis.

Concluderend, CIA in de rhesusaap blijkt een relevant model te zijn om de invloed van het MHC op de ziekte te bestuderen. In de nabije toekomst zal de rol van het MHC klasse I A26 allel in de resistentie voor CIA nader worden bestudeerd. De hypothese is dat er een effectief antigeen-specifiek suppressor mechanisme werkzaam is in de A26 positieve dieren dat de CII-reactieve T cellen inactiveert en daardoor de aap beschermt voor ziekte. In dit verband zal eerst worden gekeken of MHC klasse I moleculen in staat zijn peptiden van CII te binden en te presenteren aan T cellen. Dit soort onderzoek zal inzicht geven in de factoren die leiden tot suppressie cq. regulatie van autoreactieve T cellen. Inzichten in het mechanisme verantwoordelijk voor resistentie voor CIA in de rhesusaap kunnen van groot belang zijn voor ontwikkelingstrategieën die moeten leiden tot een adequate therapie voor arthritis patiënten.

CURRICULUM VITAE

Naam : **Nicolaas Petrus Maria Bakker**
Geboren : **30 juli 1958**
Plaats : **Bovenkarspel**

1970 - 1975 : **M.A.V.O. te Lutjebroek**

1975 - 1978 : **M.B.O. opleiding tot chemisch analist aan het Bakhuis Roozenboom Instituut te Beverwijk**

1978 - 1980 : **H.B.O. opleiding tot botanisch analist aan het Ir. W. van den Broek Instituut te Amsterdam**

1980 - 1987 : **Studie Biologie aan de Universiteit van Amsterdam**

1987 - heden : **Verbonden als wetenschappelijk medewerker aan het Instituut voor Toegepaste Radiobiologie en Immunologie - TNO te Rijswijk**

LIST OF PUBLICATIONS

1. *N.P.M. Bakker, W.M.C. Eling, A. de Groot, E.J. Sinkeldam, and R. Luyken. The influence of quantity and quality of protein on rodent malaria. *Proc. Nutrition Soc.* **49**:160 (1988).
2. *R. Luyken, N.P.M. Bakker, W.M.C. Eling, A. de Groot, and E.J. Sinkeldam. De invloed van de hoeveelheid en aard van het eiwit in de voeding op het verloop van malaria bij proefdieren. *Voeding* **49**:160 (1988).
3. N.P.M. Bakker, M.G.M. van Erck, C. Zurcher, P. Faaber, A. Lemmens, M. Hazenberg, R.E. Bontrop, and M. Jonker. Experimental immune mediated arthritis in rhesus monkeys. A model for human rheumatoid arthritis? *Rheumatol. Int.* **10**:21 (1990).
4. *B.A.'t Hart, J.M. Simons, S. Knaan-Shanzer, N.P.M. Bakker, and R.P. Labadie. Anti-arthritic activity of the newly developed neutrophil oxidative burst antagonist apocynin. *Free Rad. Biol. Med.* **9**:127 (1990).
5. N.P.M. Bakker, M.G.M. van Erck, C.A.D. Botman, M. Jonker, and B.A. 't Hart. Collagen-induced arthritis in an outbred group of rhesus monkeys comprising responder and nonresponder animals. *Arthritis Rheum.* **34**:616 (1991).
6. *N.P.M. Bakker, M.G.M. van Erck, M. Jonker, and B.A. 't Hart. Resistance to collagen-induced arthritis in rhesus monkeys: a role of CD8+ cells and IL2 in T cell low-responsiveness. *J. Cell. Biochem. suppl.* **15**:161 (1991).
7. B.A.'t Hart, C.A.D. Botman, and N.P.M. Bakker. Induction of type II collagen-specific antibody production in blood lymphocyte cultures of rhesus monkeys (*Macaca mulatta*) with collagen-induced arthritis using the immobilized native antigen. *Clin. exp. Immunol.* **83**:375 (1991).
8. *Jonker M., Bakker K., Slierendregt B., 't Hart B., Bontrop R. Autoimmunity in non-human primates: the role of major histocompatibility complex and T cells and implications for therapy. *Hum. Immunol.* **32**:31 (1991).

9. N.P.M. Bakker, M.G.M. van Erck, L.A. 't Hart, and M. Jonker. Acquired resistance to type II collagen-induced arthritis in rhesus monkeys is reflected by a T cell low-responsiveness to the antigen. *Clin. exp. Immunol.* **86**: 219 (1991).
10. N.P.M. Bakker, M.G.M. van Erck, N. Otting, N.M. Lardy, R.C. Noort, B.A. 't Hart, M. Jonker, and R.E. Bontrop. Resistance to collagen-induced arthritis in a nonhuman primate species maps to the major histocompatibility complex class I region. *J. Exp. Med.* **175**: 933 (1992).
11. *N.P.M. Bakker, W.M.C. Eling, A.M.Th. de Groot, E.J. Sinkeldam, and R. Luyken. Suppression of malaria infection and prevention of paralysis and lesions in the central nervous system by low protein diets in rats. *Acta Tropica* **50**: 285 (1992).
12. *Lohse, A.W., Bakker, N.P.M., Hermann, E., Poralla, T., Jonker, M., Meyer zum Buschenfelde K.H. Induction of an anti-vaccine response by T cell vaccination in nonhuman primates and humans. In press (1992).
13. *Margreet Jonker, Wim Slingerland, George Treacy, Peer van Eerd, Koon Yan Pak, Eileen Wilson, Susan Tam, Peter E Dadonna, Koos Bakker, Albert F LoBuglio, Gert Reithmuller and John Iuliucci. In vivo treatment with a monoclonal chimeric anti CD4 antibody results in prolonged depletion of circulating CD4⁺ cells in chimpanzees. Submitted (1992).
14. *Nicole van Besouw, Peter van der Meide and Koos Bakker. The frequency of interferon-gamma producing peripheral blood mononuclear cells in rhesus monkeys is age-dependent. Submitted (1992).
15. *L.A. 't Hart, N.P.M. Bakker, M. Jonker, R.E. Bontrop. Resistance to autoimmune-arthritis by immunization with attenuated antigen. Submitted (1992).

*Not incorporated in this thesis.

NAWOORD

Dit proefschrift is tot stand gekomen dankzij de inzet en de ondersteuning van zeer velen. Een aantal mensen wil ik daarbij met name noemen.

In de eerste plaats wil ik alle medewerkers van de afdeling Chronische Ziekten van het ITRI-TNO bedanken. In het bijzonder wil ik Margreet Jonker, Bert 't Hart en Ronald Bontrop bedanken, zonder hun bijdragen was dit proefschrift er niet gekomen. Dank voor de stimulerende discussies en voor de steun in perioden waarin de resultaten uitbleven. Monique van Erck wil ik bedanken voor haar technische ondersteuning gedurende de eerste jaren van het onderzoek.

Mea van der Sman wil ik bedanken voor de lay-out en het drukklaar maken van dit proefschrift en Henk van Westbroek voor de verzorging van de figuren en het ontwerpen van de omslag.

Alle mensen van de afdeling biotechniek dank ik voor hun inzet en toewijding bij de verzorging van de apen en bij de assistentie van het dierexperimentele werk. Peer van Eerd wil ik bedanken voor zijn veterinaire ondersteuning.

Mijn ouders wil ik bedanken omdat zij mij de gelegenheid hebben gegeven te studeren.

De paranimfen Angelos Avgoustidis en Ben Verstegen wil ik danken voor hun activiteiten rond de promotie.

En, geheel onterecht maar op een bijzondere laatste plaats, Corrie, zonder jouw morele en liefdevolle steun was het schrijven van dit proefschrift nooit zo snel gelukt.