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Research paper

# Efficient promotion of collagen antibody induced arthritis (CAIA) using four monoclonal antibodies specific for the major epitopes recognized in both collagen induced arthritis and rheumatoid arthritis

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## Abstract

An antibody response to defined epitopes located on the triple helical portion of type II collagen (CII) is associated with the development of collagen-induced arthritis (CIA) and rheumatoid arthritis (RA). Monoclonal antibodies to epitopes associated with arthritis, but not antibodies specific for epitopes not associated with arthritis, induce arthritis in mice, the so-called collagen antibody induced arthritis (CAIA) model. We have selected monoclonal IgG antibodies specific for four well-defined major epitopes on triple helical CII, the C1, J1, D3 and U1 epitopes. These antibodies bind the epitopes specifically as determined using recombinant or synthetic triple helical epitopes. They are encoded from somatically mutated V genes. They all bind cartilage *in vivo* in normal mice. All of the antibodies induce mild arthritis after injection intravenously and if injected as a cocktail they induce severe clinical arthritis. Intravenous injection of a total of 4 mg antibodies (0.5 mg antibodies per clone) induced arthritis in several different mouse strains without any secondary immune stimulus and intraperitoneal injection of LPS 7 days later dramatically raised the severity. Thus, this method is recommended as a new protocol for the induction of CAIA. © 2005 Elsevier B.V. All rights reserved.

*Keywords:* Collagen type II; Monoclonal antibodies; Arthritis

*Abbreviations:* CII, collagen type II; CIA, collagen induced arthritis; CAIA, collagen antibody induced arthritis; ECM, extra cellular matrix; EU, endotoxin unit; LPS, lipopolysaccharide; Mabs, monoclonal antibodies; PBS, phosphate buffered saline; RA, rheumatoid arthritis; TLR, toll-like receptor.

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## 1. Introduction

The most commonly used animal model for rheumatoid arthritis (RA) is the collagen-induced arthritis (CIA). Collagen type II (CII) is one of the major constituents of the articular cartilage matrix proteins and immunization with native CII in adjuvant induces autoimmune polyarthritis by a cross-reactive autoimmune

response to CII in joint cartilage. As in RA, susceptibility to CIA is linked to the expression of certain class II MHC alleles (Wooley et al., 1981; Brunsberg et al., 1994) thus explaining the crucial role of T cells. The predominant role played by T cells in the initiation of CIA was demonstrated by using T cell deficient mice (Corthay et al., 1999). However, T cell reactivity alone could not explain the disease pathology in CIA. Both the cellular and humoral immune mechanisms act in concert to mediate the progression of disease in CIA (Seki et al., 1988). A requirement for the generation of CII specific antibodies in the progression of CIA is well documented. B-cell deficient mice with highly CIA susceptible genetic background are resistant to the development of CIA (Svensson et al., 1998). A significant part of the inflammatory attack on the joints is mediated by pathogenic antibodies and was emphasized using collagen specific polyclonal sera both in rats (Stuart et al., 1982) and mice (Stuart and Dixon, 1983). Purified monoclonal antibodies induced arthritis in DBA/1 mice but the arthritis was very mild (Holmdahl et al., 1986). A possible reason for this was the use of too little of such single specificity antibodies. Later, a mixture of anti-collagen monoclonal antibodies purified from ascites (Terato et al., 1992, 1995) was shown to induce more severe arthritis. Furthermore, transfer of a combination of two CII specific monoclonal antibodies, CIIC1 and M2139, induced arthritis (collagen type II antibody induced arthritis, CAIA) in naïve mice (Johansson et al., 2001; Svensson et al., 2002; Nandakumar et al., 2003a,b, 2004; Hietala et al., 2004; Nandakumar and Holmdahl, 2005). Similarly, both polyclonal and monoclonal antibodies against the ubiquitously expressed self-antigen glucose-6-phosphate isomerase (GPI) induced arthritis in mice (Korganow et al., 1999; Maccioni et al., 2002). These studies collectively demonstrate the pathogenic nature of the auto-antibodies directly in vivo.

There is, however, emerging evidence that the epitope-specificity of the antibodies is of critical importance for their pathogenicity. Thus, we could show that co-injection of the CIIF4 antibody, specific for the CII triple helical epitope F4, reduced the induction of arthritis (Burkhardt et al., 2002). The protective effect was difficult to explain as this antibody bound equally well to the cartilage surface in vivo and also shared the same Fc isotype. Furthermore, recent evidence also suggests that an antibody

response to certain epitopes is better associated with arthritis than a response to other epitopes. Interestingly, antibodies to the protective F4 epitope were in fact negatively associated with RA whereas antibodies to other epitopes, C1, J1 and U1, were positively associated (Burkhardt et al., 2002). In the CIA model in the mouse the antibody response that correlated with arthritis was mainly associated with binding to the epitopes C1, J1 and U1 and this association was maintained throughout the chronic relapsing disease course (Bajtner, Nandakumar, Engström and Holmdahl, unpublished observations). To test whether a combination of antibodies to the most dominant epitopes associated with arthritis were arthritogenic and have reduced threshold levels for the induction of arthritis, we have used combinations of the CIIC1 (C1 epitope), M2139 (J1 epitope), CIIC2 (D3 epitope) and the UL1 (U1 epitope) antibodies. These epitopes are all major epitopes in mice (Schulte et al., 1998) and rats immunized with CII and developing arthritis postpartum in mice (Hultqvist et al., 2004) as well as in rheumatoid arthritis (RA) (Burkhardt et al., 2002). These epitopes have been conserved during evolution and are shared between mouse, rat and human.

The **CIIC1 clone** was isolated from a chick CII immunized DBA/1 mouse and produces IgG2a antibodies specific for triple helical CII binding the C1 epitope (Holmdahl et al., 1986). The C1 epitope has been found to be a major epitope in the antibody response to CII and the various antibodies recognize different parts of the epitope; the C1<sup>I</sup> epitope 359–363, the C1<sup>II</sup> epitope 359–366 and the C1<sup>III</sup> epitope 359–369 (Schulte et al., 1998). All antibodies are, however, dependent on the first part of the epitope where the C1 antibody binds. The C1 epitope has been described in detail and the amino acid side chains used for binding of CIIC1 have been determined (Schulte et al., 1998). The CIIC1 antibodies bind mouse cartilage in vivo (Jonsson et al., 1989; Holmdahl et al., 1991; Mo et al., 1994). The CIIC1 antibodies induced sub clinical arthritis if injected at low (0.5 mg) doses (Holmdahl et al., 1986) and clinical arthritis in mice if injected at higher doses and in combination with M2139 antibodies (Johansson et al., 2001; Svensson et al., 2002; Nandakumar et al., 2003a,b; Hietala et al., 2004). The C1 epitope is a major epitope in the immune response to CII and dominates the response in collagen-induced arthritis

in both mice (Bajtner, Nandakumar, Engström and Holmdahl, unpublished observations) and rats (Wernhoff et al., 2001). These antibodies also impaired cartilage formation by cultured chondrocytes (Amirahmadi et al., 2004), strongly inhibited the self-assembly of CII in vitro (Gray et al., 2004) and caused disorganization of CII fibrils in the ECM without affecting chondrocyte morphology along with increased matrix synthesis (Amirahmadi et al., 2005).

The **M2139 clone** (Mo and Holmdahl, 1996) was raised from a DBA/1 mouse and produces IgG2b antibodies specific for the triple helical type II collagen J1 epitope (Mo and Holmdahl, 1996). The M2139 antibodies induced arthritis when injected alone in DBA/1 mice (Holmdahl et al., 1986) and clinically severe arthritis in mice if injected in higher concentrations and together in combination with CIIC1 antibodies (Johansson et al., 2001; Svensson et al., 2002; Nandakumar et al., 2003a,b; Hietala et al., 2004). These antibodies also strongly inhibited collagen fibrillogenesis in vitro (Gray et al., 2004) and caused thickening and aggregation of CII fibrils in the ECM and abnormal chondrocyte morphology whilst matrix synthesis was unaffected (Amirahmadi et al., 2005).

The **CIIC2 clone** was derived from CII immunized DBA/1 mice (Holmdahl et al., 1986) and produces IgG2b antibodies specific for triple helical type II collagen binding the D3 epitope (Holmdahl et al., 1986). The genes used for the CIIC2 antibodies are somatically mutated and unique (Holmdahl et al., 1989; Mo and Holmdahl, 1996) and the CIIC2 antibodies induced sub clinical arthritis if injected at low (0.5 mg) doses (Holmdahl et al., 1986).

The **UL1 clone** was derived from the B10.Q mouse (Bajtner, Nandakumar, Engström and Holmdahl, unpublished observations) and produces IgG2b antibodies specific for a defined triple helical epitope, the U1 epitope. The U1 epitope was described as a major epitope for B cells in patients with rheumatoid arthritis (Kraetsch et al., 2001; Burkhardt et al., 2002) as well as in mice (Bajtner, Nandakumar, Engström and Holmdahl, unpublished observations) and rats (Wernhoff et al., 2001) with collagen induced arthritis. Injection of UL1 antibodies induced mild but significant arthritis (Nandakumar, Bajtner and Holmdahl, unpublished observations).

The goal with the present study was to combine these well-described monoclonal antibodies and

develop a new and efficient protocol for the induction of collagen antibody induced arthritis (CAIA) in mice.

## 2. Materials and methods

### 2.1. Animals

DBA/1, BALB/c and C57Bl/6 mice were obtained from Jackson laboratories (Bar Harbor, ME). The B10.Q and B10.RIII strains originated from Professor Jan Klein (Tubingen, Germany). [(BALB/c × B10.Q)] F1 mice (short named QB) were bred in the medical inflammation research animal house facility in Lund. Eight- to ten-week-old male mice were used in all the experiments. All the animals were kept in a conventional barrier animal facility (as defined in <http://www.net.inflam.lu.se>) with a climate controlled environment having 12-h light/dark cycles in polystyrene cages containing wood shavings, fed standard rodent chow and water ad libitum. Local animal welfare authorities permitted all the animal experiments.

### 2.2. Purification of CII specific monoclonal antibodies

The CII specific hybridomas were generated and characterized as described in detail elsewhere (Holmdahl et al., 1986; Karlsson et al., 1995; Schulte et al., 1998). The anti-CII antibody producing hybridomas, M2139, C1, C2 and UL-1 were cultured in ultra low bovine IgG containing DMEM Glutamax-I culture medium (Gibco BRL, Invitrogen AB, Sweden) with 100 mg/l of Kanamycin monosulfate (Sigma, USA). Monoclonal antibodies were generated on a large scale as culture supernatants using Integra cell line 1000 flasks (Integra biosciences, Switzerland). Antibodies were purified using  $\gamma$ -bind plus affinity gel matrix (Pharmacia, Sweden) and the Äkta purification system. Briefly, culture supernatants were centrifuged at 12500 rpm for 30 min, filtered and degassed before applying to the gel matrix. Antibodies were eluted using acetic acid buffer at pH 3.0 and neutralized with 1 M Tris-HCl, pH 9.0. The peak fractions were pooled and dialyzed extensively against PBS, pH 7.0, with or without azide. The IgG content was determined after freeze-drying. The antibody solutions were filter sterilized using 0.2  $\mu$ m syringe filters (Dynagard,

Spectrum Laboratories, CA, USA), aliquoted and stored at  $-70^{\circ}\text{C}$  until used. The amount of endotoxin content in the antibody solutions prepared was found to be in the range of 0.02–0.08 EU/mg of protein as analyzed by limulus amoebocyte lysate (Pyrochrome) method (Cape Cod Inc., MA, USA).

### 2.3. Passive transfer of antibodies

The cocktail of CIIC1, M2139, CIIC2 and UL1 monoclonal antibodies was prepared by mixing equal concentrations of each of the sterile filtered antibody solutions. Mice were injected intravenously with 0.5–0.6 ml volumes of antibody solutions as a single dose. As internal controls, mice received equal volumes of PBS. On day 7, lipopolysaccharide (25  $\mu\text{g}/\text{mouse}$ ) was injected intraperitoneally into all the mice. None of the control mice developed arthritis.

### 2.4. Clinical evaluation of arthritis

Mice were examined daily for the development of arthritis before and after LPS treatment for a minimum of 21 days or until the inflammation disappeared. Scoring of animals was done blindly using a scoring system based on the number of inflamed joints in each paw, inflammation being defined by swelling and redness as described previously (Holmdahl et al., 1998). Scoring was recorded in the phalangeal joints (maximum of 1 point per digit, 5 points per paw), the metacarpus or metatarsus (5 points), and in the wrist and ankle joints (5 points). Thus, the maximum score was 15/paw resulting in a peak of 60 for the total joint count.

### 2.5. Histological preparations

On day 15, paws were dissected from both arthritic and control mice (3 mice in each group), fixed in 4% paraformaldehyde solution for 24 h, decalcified for 3–4 weeks in an solution containing EDTA, polyvinylpyrrolidone and Tris-HCl, followed by dehydration and embedding in paraffin. Sections of 6  $\mu\text{m}$  were stained with hematoxylin and eosin. For immuno-histochemistry, 2–5-day-old mice were injected with 100  $\mu\text{g}$  of anti-CII antibodies in 100  $\mu\text{l}$  volume i.p. After 24 h, paws were immediately dissected and frozen in OCT compound using isopentane on dry ice. The samples were stored at  $-70^{\circ}\text{C}$  until cryosectioned at 10  $\mu\text{m}$  at

$-30^{\circ}\text{C}$ . HRP conjugated goat anti-mouse IgG antibodies were used for detection. DAB staining was performed according to established procedures.

### 2.6. Statistical analyses

All the mice were included for the calculation of arthritis susceptibility and severity. The severity of arthritis was analyzed by Mann-Whitney *U* tests and disease incidence by Chi Square tests using the Statview 5.0.1 version.

## 3. Results

### 3.1. Preparation and characterization of the CII specific monoclonal antibodies

All the monoclonal antibodies CIIC1, M2139, CIIC2 and UL1 were purified from hybridoma culture supernatant and the concentrations were determined by weight-determination after freeze-drying. The various characteristics of monoclonal antibodies such as epitope specificity, isotype and V gene usage are depicted in Table 1.

### 3.2. Binding of Mabs in vivo

Various monoclonal antibodies generated from mice immunized with CII bind specifically to the cartilage in vivo demonstrating that the collagen type II epitopes are available for antibody binding. However, a monoclonal antibody (CIIB1) binds non-specifically to CII (Holmdahl et al., 1991). Hence, to confirm that all the four monoclonal antibodies used in this study were binding to the cartilage specifically, we injected the monoclonal antibodies separately into the 2–5-day-old mice and the binding of antibodies after 24 h was detected using cryosections. As shown in Fig. 1, all these monoclonal antibodies bound to cartilage specifically in vivo.

### 3.3. A cocktail of CII specific monoclonal antibodies induces severe arthritis at low doses

The monoclonal antibodies CIIC1, M2139, CIIC2 and UL1 purified from hybridoma supernatants were mixed at equal concentrations in PBS and injected

Table 1  
Characteristic features of the anti-CII monoclonal antibodies

Antibody	Epitope	Epitope sequence of the $\alpha$ -chain of the triple helical epitope <sup>a</sup>	Isotypes	V genes
CIIC1	C1 <sup>lb</sup>	359–363: ARGLT	IgG2a $\kappa$	J558/Vk21C
M2139	J1	551–564: GERGAAGIAGPK	IgG2b $\kappa$	J5582c/Vk21G
CIIC2	D3	687–698: RGAQGGPGATGF	IgG2b $\kappa$	X24/Vk21E
UL1	U1	494–504: GLVGPRGERGF	IgG2b $\kappa$	not done

<sup>a</sup> All epitopes are in native triple helical form. Amino acids are abbreviated as; G=glycine, P=proline or hydroxyproline, E=glutamic acid, R=arginine, K=lysine, H=histidine, F=phenylalanine, A=alanine, L=leucine, T=threonine, Y=tyrosine.

<sup>b</sup> The C1 epitope consists of three distinct epitopes (C1<sup>I</sup> ; C1<sup>II</sup> ; C1<sup>III</sup> ). CIIC1 binds the shortest part of the epitope that is critical for binding of all C1 specific antibodies.

intravenously in QB mice at standardized volumes. A dose titration (2, 4, 8 and 12 mg) showed that the lowest dose (2 mg) induced arthritis in 40% of the

mice (Fig. 2). The frequency, but not the severity, increased with the dose. Intravenous injection of LPS led to development of arthritis in most mice

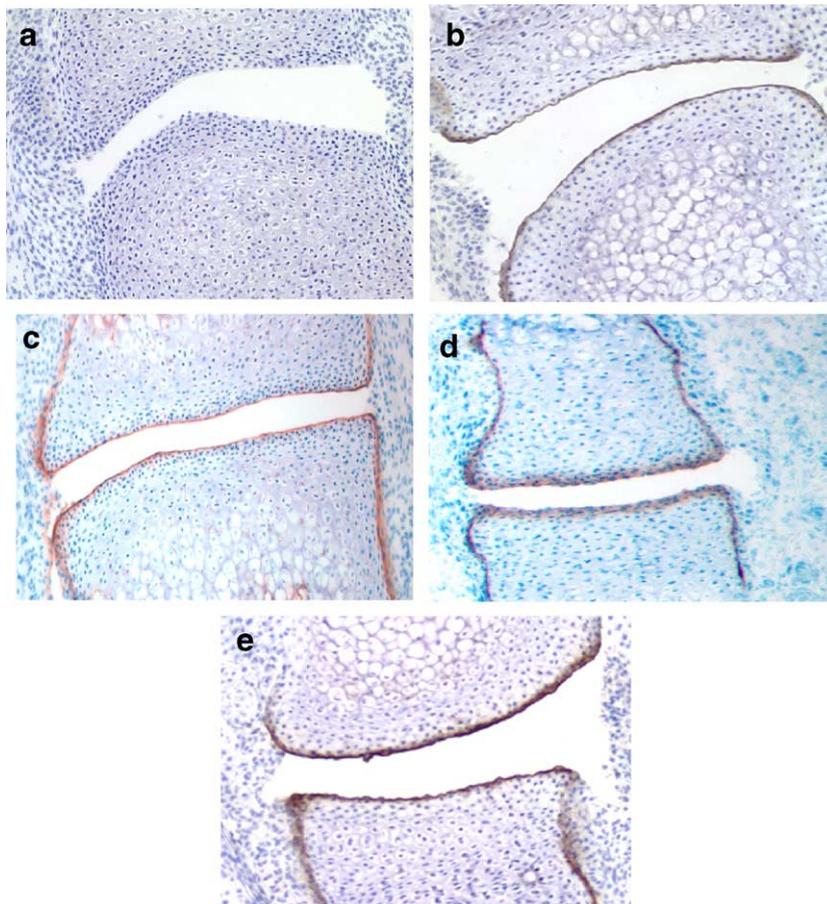


Fig. 1. Anti-CII antibody binding in vivo. All the four monoclonal antibodies used in this study were injected in vivo into young mice and paws were collected after 24 h. Cryosections were stained with DAB using peroxidase conjugated goat anti-mouse IgG as detection system. Joint sections from PBS (a), M2139 (b), CIIC1 (c), CIIC2 (d) or UL-1 (e) antibodies injected animals were depicted. Results shown are representative of those obtained from three to four mice in each group. Original magnifications,  $\times 10$ .

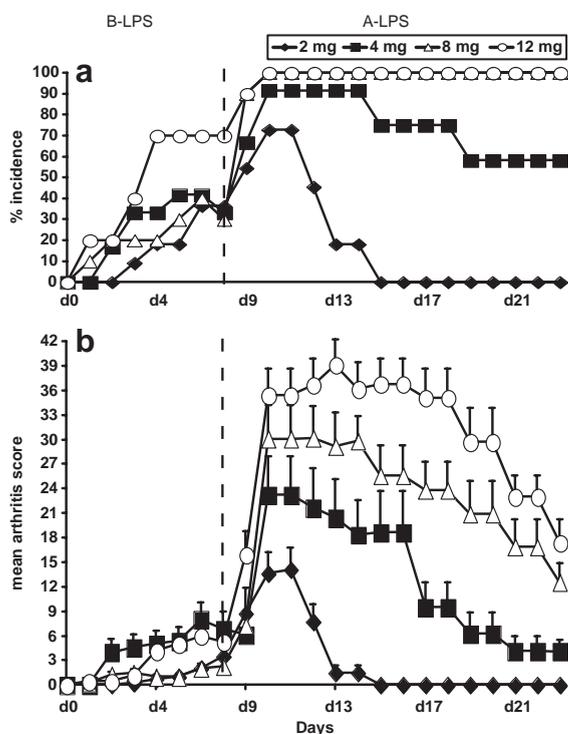


Fig. 2. Collagen antibody induced arthritis (CAIA) using four monoclonal antibodies. Arthritis incidence (a) and mean arthritis score (b) are indicated. Groups of mice were treated (by i.v. transfer) with four collagen specific monoclonal antibodies (M2139, C1C1, UL-1 and C1C2) recognizing J1, C1<sup>I</sup>, U1 and D3 epitopes. Different concentrations of the four monoclonal antibody cocktail, 2 mg ( $n=11$ ), 4 mg ( $n=12$ ), 8 mg ( $n=10$ ) and 12 mg ( $n=10$ ) were used. Equal concentrations of individual monoclonal antibodies were mixed to achieve the total indicated concentrations. LPS (25  $\mu\text{g}/\text{mouse}$ ) was injected i.p. on day 7. Mice were monitored for arthritis development on the indicated days. Arthritic mice were used for calculations.  $n$ —indicates number of mice used in each group. B-LPS: before LPS, A-LPS: after LPS.

(70–100%) with a dramatic enhancement of arthritis severity. The frequency (90%–100%) and severity was almost the same at 4 mg and higher. Therefore, a dose of 4 mg is recommended for the induction of CAIA using the cocktail of these four antibodies.

#### 3.4. Induction of arthritis in different strains of mice using the four Mabs

To confirm the above observation of 4 monoclonal antibodies inducing arthritis in mice, we used different mouse strains and tested the arthritogenicity of this cocktail. The cocktail of four monoclonal antibodies

induced arthritis in all of the mouse strains tested. However, there is genetic heterogeneity in the induction of antibody-induced arthritis as shown in Fig. 3a and b. As reported earlier, BALB/c genes contributed significantly to the inflammatory phase of arthritis in (BALB/c  $\times$  B10.Q) F1 mice (Nandakumar et al., 2003b).

#### 3.5. Induction of arthritis with single antibodies

We have shown earlier that single monoclonal antibodies to CII can induce arthritis. To test whether the monoclonal antibodies used in this study can induce arthritis on their own, we injected 9 mg of single antibodies intravenously into the mice. As shown in Table 2, all of the monoclonal antibodies can induce arthritis. However, the severity, duration and incidence of arthritis were low compared to the combination of monoclonal antibodies (Fig. 4).

#### 3.6. CAIA induced with the cocktails of two and four monoclonal antibodies: a comparison

As shown in Table 3, we have compared the antibody-mediated disease induced with two and four monoclonal antibody cocktails. Although we did not foresee any possible differences in the mechanisms of the antibody-mediated disease using these two different protocols, there are obviously several advantages in using the four monoclonal antibodies as a standard protocol for the induction of arthritis. The major advantage in using the current protocol of four monoclonal antibodies is the requirement for small amounts of antibody to induce severe arthritis and the usage of younger animals in the experiments. Furthermore, DBA/1 mice developed arthritis even before LPS injection with the four but not with the two Mabs cocktail, thus providing an opportunity to study the TLR-4 independent pathway in the mouse strain commonly used for arthritis experiments.

## 4. Discussion

The role of antibodies in the pathogenesis of arthritis has recently been highlighted. Although the arthritogenic potential of antibodies to CII has been well known since the 1980s the use of well characterized

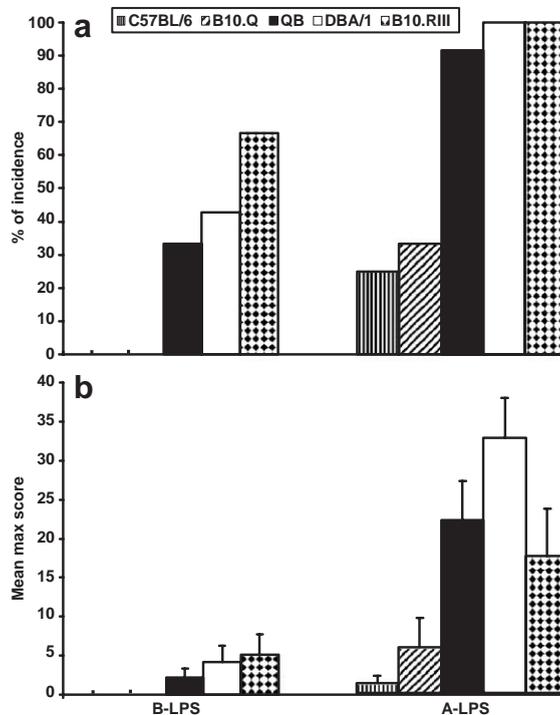


Fig. 3. Collagen antibody induced arthritis (CAIA) in different strains of mice. CAIA was induced by i.v. transfer of four monoclonal antibodies (M2139, CIIC1, UL-1 and CIIC2) in C57BL/6 ( $n=8$ ), B10.Q ( $n=6$ ), QB=(BALB/cXB10.Q)F1 ( $n=12$ ), DBA/1 ( $n=7$ ) and B10.RIII ( $n=6$ ) mice. Arthritis incidence (a) and mean arthritis score (b) are indicated on different days. Equal concentrations of individual monoclonal antibodies were mixed to achieve the total indicated concentrations. LPS (25  $\mu\text{g}/\text{mouse}$ ) was injected i.p. on day 7. Mice were monitored for arthritis development on the indicated days. All the mice were used in the calculations.  $n$ —indicates number of mice used in each group. B-LPS: before LPS, A-LPS: after LPS.

monoclonal antibodies as tools for studies involving the understanding of disease pathways in arthritis pathogenesis is still not fully explored. Hence, we have characterized a cocktail of 4 monoclonal antibodies, selected for their epitope specificity, and describe a new efficient protocol for the induction of collagen antibody induced arthritis (CAIA).

Antibodies, as a constituent of immune complexes, may contribute significantly in triggering inflammation in a number of autoimmune diseases. Thus, the initial triggering event in this antibody transfer model could be the formation and deposition of collagen-IgG immune complexes in the joint spaces. Complement

fragments binding to these immune complexes, tissue damage, and/or Fc $\gamma$ R cross-linking can activate local mononuclear cells which in turn could release pro-inflammatory cytokines in or near the joints inducing neutrophil and macrophage recruitment. Release of granules containing many tissue-degrading enzymes by these cells can amplify their responses, thereby perpetuating the ensuing inflammatory response. In this context, it is of interest to note that the plasminogen deficient mice were completely resistant to antibody mediated disease (Li et al., 2005).

Similarly, in rheumatoid arthritis (RA) patients, activated B cells and plasma cells are present in the

Table 2  
CAIA with single monoclonal antibodies

Mab clone	Incidence	Max score (mean $\pm$ SEM)
M2139	5/5	13.4 $\pm$ 2.1
CIIC1	2/5	5.0 $\pm$ 3.9
CIIC2	1/3	2.0 $\pm$ 2.0
UL-1	1/5	1.2 $\pm$ 1.2

Groups of 6-month-old male BALB/c or (BALB/c  $\times$  B10.Q) F1 mice were injected with 9 mg of single antibodies on day 0 and LPS (25  $\mu$ g/mouse) was injected on day 5. Mice were scored for arthritis for 21 days. Results shown above were observed after LPS injection. Before LPS injection, none of the antibodies used alone induced arthritis. All the animals are included in the calculations.

inflamed synovium. These differentiate locally into antibody producing plasma cells and in the established disease, lymphoid follicles with germinal centers may be present as well. This results in high levels of local immunoglobulin production and the deposition of immune complexes (which may include IgG and IgM rheumatoid factors) in the synovium and articular cartilage (Schroder et al., 1996; Kim et al., 1999). Immune complexes in the joints have been proposed to play a role in the generation of invasive pannus and

Table 3  
Comparison of arthritis induction using two and four monoclonal antibody cocktails

Characteristics	Two monoclonals	Four monoclonals
Arthritis induction	Before LPS in B10.RIII and QB mice	Before LPS in B10.RIII DBA/1 and QB mice
Minimum age of animals	4 months	2 months
Max arthritis score <sup>a</sup>		
DBA/1	15.1 $\pm$ 3	32.7 $\pm$ 5
B10.RIII	13 $\pm$ 1	17.5 $\pm$ 6
B10.Q	4.5 $\pm$ 1	5.8 $\pm$ 4
QB	20.3 $\pm$ 2	22.2 $\pm$ 5
C57BL/6	0.5 $\pm$ 0.3	1.3 $\pm$ 0.9

<sup>a</sup> Maximum score for arthritis was compared after LPS injection between 9 mg of a two monoclonal antibodies cocktail and 4 mg of a four monoclonal antibodies cocktail. Only male animals were tested with both the cocktails.

in irreversible cartilage matrix degeneration. Indeed, B cells and immune complexes are often located adjacent to and within sites of tissue destruction in RA joints. Antibodies sequestered within cartilage layers have been detected in more than 80% of cartilage

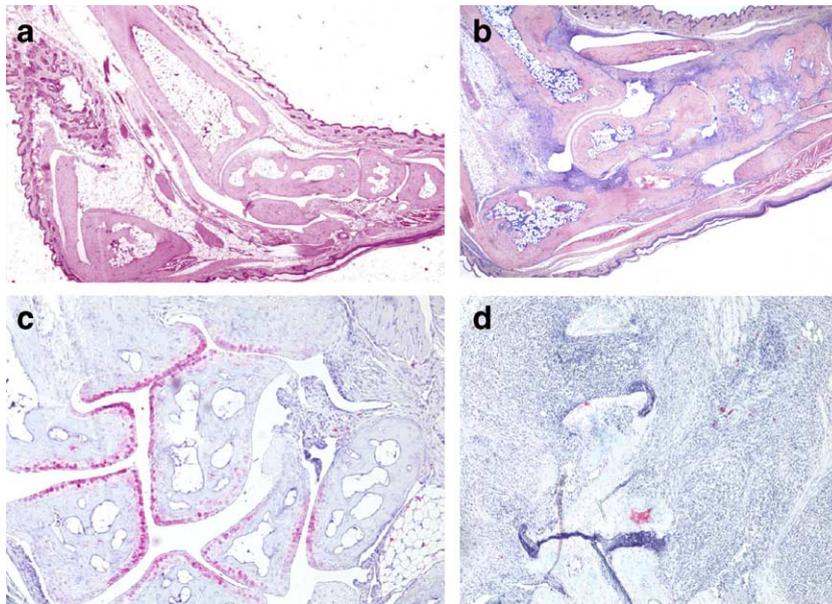


Fig. 4. Histology of normal and arthritis joint sections in four monoclonal antibody induced arthritis. Paws were taken on day 15 after antibody transfer (8 days after LPS injection), paraformaldehyde fixed and decalcified before sectioning. Top: Hematoxylin staining of normal (a) and arthritis hind paw (b) of B10.RIII mice. Original magnifications,  $\times$  2.5. Bottom: Safranin staining of normal (c) and inflamed front paw (d) of DBA/1 mice. Original magnifications,  $\times$  10. Results shown are representative of those obtained from three mice in each group.

samples from RA patients. Intravenous injection of antibodies to CII, purified from the plasma of an RA patient, induced arthritis in normal mice (Wooley et al., 1984). These data suggest that the threshold level, epitope specificity, affinity and isotype of the accumulated antibodies on the joint cartilage surface and the presence of inducers of pro-inflammatory cytokines such as LPS from intestinal flora might contribute to the ultimate pathogenicity of the antibodies.

Several groups have reported the transfer of arthritis using anti-CII antibodies with LPS (Wallace et al., 1999; Johansson et al., 2001; Han et al., 2002; Itoh et al., 2002; Kagari et al., 2002; Svensson et al., 2002; Yumoto et al., 2002; Kato et al., 2003; Murata et al., 2003; Nandakumar et al., 2003b, 2004; Ichiyama et al., 2004; Lange et al., 2005; Sehnert et al., 2004). In the present study, we could transfer arthritis with low doses of pure IgG monoclonal antibodies specific for defined epitopes on CII without LPS, which opens up the field for understanding the molecular parameters of the antibodies responsible for their arthritogenicity and also facilitates the use of the CAIA model. LPS has been previously shown to exacerbate arthritis both in CIA and an antibody transfer model (Terato et al., 1995, 1996; Yoshino et al., 2000; Nandakumar et al., 2003b). However, LPS is not essential for antibody induced arthritis as the disease can be induced in non-LPS responder mice (Nandakumar et al., 2003b). The arthritis induced with antibodies only is a different pathogenic process as compared with the arthritis induced by the addition of LPS since the former phase of the disease is TLR4 independent. Moreover, there was a substantial difference in the type of infiltrating granulocytes in disease mediated with antibody alone compared to LPS driven disease (Nandakumar et al., 2003b). It is most likely that the antibodies act as a qualitative trigger and LPS enhances an already ongoing inflammatory response. Thus, LPS could act as a secondary immune stimulus to the ensuing immune activation in this model. It has also been shown that LPS decreased the threshold level of antibodies required to induce arthritis apart from bypassing the epitope specificity of antibodies (Terato et al., 1995). These data could also be interpreted as follows: LPS is able to enhance the severity of arthritis which aids in macroscopic visualization of the mild arthritis seen microscopically with small amounts of antibodies (Holmdahl et al., 1986).

The CAIA model is rapid and reproducible and easy to standardize. It should be mentioned that our monoclonal antibody cocktail differs from that previously described (Terato et al., 1992, 1995). Our monoclonals bind to well defined triple helical epitopes spread over the entire CII (CB8, CB10 and CB11 fragments), possibly encouraging better immune complex formation on the cartilage surface for the initiation of arthritis. Furthermore, our antibodies induce arthritis both with and without LPS, thereby providing an opportunity to study both TLR independent and dependent pathways leading to arthritis. Thus it is suitable as one of the models for RA not only for the dissection of molecular mechanisms but also for drug screening to contain the inflammatory phase of arthritis in RA patients.

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