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Genetic Control of Tolerance to Type II Collagen and Development of Arthritis in an Autologous Collagen-Induced Arthritis Model

Johan Bäcklund,* Kutty Selva Nandakumar,* Robert Bockermann,* Lucia Mori,† and Rikard Holmdahl2*

T cell recognition of the type II collagen (CII) 260–270 peptide is a bottleneck for the development of collagen-induced arthritis (CIA), an animal model of rheumatoid arthritis. We have earlier made C3H.Q mice expressing CII with glutamic acid instead of aspartic acid at position 266 (the MMC-C3H.Q mouse), similar to the rat and human CII epitope, which increases binding to MHC class II and leads to effective presentation of the peptide in vivo. These mice show T cell tolerance to CII, but also develop severe arthritis. The present investigation shows that non-MHC genes play a decisive role in determining tolerance and arthritis susceptibility. We bred MMC into B10.Q mice, which display similar susceptibility to CIA induced with rat CII as the C3H.Q mice. In contrast to MMC-C3H.Q mice, MMC-B10.Q mice were completely resistant to arthritis. Nontransgenic (B10.Q × C3H.Q)F1 mice were more susceptible to CIA than either of the parental strains, but introduction of the MMC transgene leads to CIA resistance, showing that the protection is dominantly inherited from B10.Q. In an attempt to break the B10-mediated CIA protection in MMC-transgenic mice, we introduced a transgenic, CII-specific, TCR β-chain specific for the CII 260–270 glycopeptide, in the highly CIA-susceptible (B10.Q × DBA/1)F1 mice. The magnification of the autoreactive CII-specific T cell repertoire led to increased CIA susceptibility, but the disease was less severe than in mice lacking the MMC transgene. This finding is important for understanding CIA and perhaps also rheumatoid arthritis, as in both diseases MHC class II-restricted T cell recognition of the glycosylated CII peptide occurs. The Journal of Immunology, 2003, 171: 3493–3499.

The chronic inflammatory process in rheumatoid arthritis (RA) involves an erosive destruction of bones and cartilage in peripheral joints. The initiation and self-perpetuation of this pathologic process are largely unknown, and we have only a few significant landmarks for its deeper understanding. One of the most important finding is the known association to immune response genes in the MHC class II region. The occurrence of RA is associated with a certain set of MHC class II molecules that share a common peptide binding pocket, such as the DR4 molecule DB1*0401 and the DRB1*0101 (1). However, the nature of the bound peptides and the precise role of the class II molecule in the disease process are not known (2–5). Another important observation is that autoimmune responses are often found in RA. Some of these responses are directed toward cartilage-derived molecules such as the major protein of cartilage, type II collagen (CII). It has been known for a long time that some, but not all, RA patients have increased levels of Abs to native (triple helical) CII (6–9). B cells producing these Abs are found in the joints, and the titers are higher in synovial fluid than in blood. Despite the observation that most of these Abs are of the IgG isotype, indicating that CII-specific B cells have acquired T cell help for isotype switching, the identification of CII-reactive T cells in RA patients has been difficult.

As a matter of fact, these findings in human RA stem from observations made in the most commonly used animal model for RA, collagen-induced arthritis (CIA). Mice of the H-2d haplotype are susceptible to CIA, and the disease-associated MHC class II molecule has been identified to be Aα (10, 11). Furthermore, the Aα molecule has been found to present and predominantly activate T cells specific for a glycosylated immunodominant peptide from CII located between positions 260 and 270. Interestingly, a peptide from the same region, at positions 261–271, binds to RA-associated DR4 and DR1 molecules, and transgenic expression of these class II molecules in mice renders them susceptible to CIA (12–16). In addition, in both mice and men, T cells are exposed to cartilage-derived CII (17, 18) and recognize the same post-translational modified O-linked carbohydrates on this peptide (19).

Thus, the observations mentioned above indicate that the structural recognition patterns are shared to a large extent between mouse and man. However, mice need to be immunized with CII to develop arthritis, and humans develop RA as a result of strong, but unknown, environmental influences. The next question is therefore why individuals expressing CII peptide-binding class II molecules do not spontaneously develop arthritis. This question is strongly linked to tolerance, and we addressed it by taking advantage of the observation that the amino acid sequence of mouse CII differs at position 266, i.e., within the immunodominant 260–270 peptide, in which the mouse has an aspartic acid, whereas other species have a glutamic acid. The expression of CII with this position mutated in the mouse, the transgenic MMC mouse, induces partial

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2 Address correspondence and reprint requests to Dr. Rikard Holmdahl, Section of Medical Immunology Research, 111, BMC, Lund University, 221 84 Lund, Sweden. E-mail address: rikard.holmdahl@inflamm.lu.se

3 Abbreviations used in this paper: RA, rheumatoid arthritis; CIA, collagen-induced arthritis; CII, type II collagen.
tolerance with anergized T cells and reduced frequency of arthritis (17). We have now investigated the importance of the non-MHC genetic background for induction of tolerance and arthritis susceptibility. This has been done by comparing C3H.Q, B10.Q and DBA/1 mice and has been confirmed using mice with a biased TCR repertoire (20) specific for the glycosylated CII(260–270) epitope.

Materials and Methods

**Mice**

Male and female C3H.Q mice, B10.Q, (B10.Q × C3H.Q)F1, and (B10.Q × DBA/1)F1 mice (H-2) were used in the experiments. C3H.Q mice were originally obtained from Dr. D. C. Shreffler (St. Louis, MO). The transgenic mouse, MMC-1 (in this work referred to as MMC), have previously been described (17). Briefly, the MMC transgene is a mutated mouse CII gene in which position 266 has been changed from an aspartic acid (D) to a glutamic acid (E), thereby expressing the rat CII(260–270) epitope in a CII-restricted fashion. MMC transgenic mice were generated and maintained in the C3H.Q strain and also backcrossed eight generations onto the B10.Q background. The MMC transgene was kept and used as a heterozygote. MMC transgenic B10.Q mice were also crossed with DBA/1 mice, expressing a transgenic TCR β-chain (Vβ12) obtained from a CII-specific T cell clone, originally derived from a CII-immunized DBA/1 mouse (20), to generate [B10.Q × DBA/1]F1 mice transgenic for Vβ12, MMC, or both. All animals were between 7 and 12 wk of age and were weaned at the start of the experiments. Animals were bred and kept in our animal facility (http://net.inflamm.lu.se), and all animal experiments were permitted by local animal welfare authorities.

**Antigen**

Rat CII was prepared from the SWARM chondrosarcoma by pepsin digestion or from lethally irradiated chondrosarcoma and was further purified as described previously (21). Pepsin-digested CII was used for immunization of mice, whereas lethally irradiated CII was used for in vitro restimulation of lymph node cells to eliminate the T cell response to pepsin. The following CII peptides, containing the 260–270 sequence of rat CII with or without modifications of the K264 residue, were used: K peptide (CII259–273) with a nonmodified lysine at position 264), HyK peptide (CII266–270 with (SR)-5-hydroxy-l-lysine at position 264), and Gal peptide (CII259–273 with a β-α-galactopyranosyl residue on l-lysine at position 264). The CII peptides were synthesized, purified, and characterized as previously described (22–24). Both collagen and collagen peptides were dissolved and stored in 0.1 M acetic acid at 4°C.

**Immunization**

For induction of arthritis, mice were immunized with 100 µg of rat CII, emulsified 1/1 in CFA (Difco, Detroit, MI) at the base of the tail in a total volume of 100 µl. Five weeks later mice were also given a boost injection of 5 µg of CII inIFA (Difco) in a total volume of 50 µl. For in vitro experiments, mice were immunized with 60 µg of rat CII in CFA in the tailbase and in each hind paw in a total volume of 180 µl.

**Arthritis development and anti-CII Ab response**

The development of clinical arthritis was followed through visual scoring of the mice, starting 2 wk after immunization and continuing until the end of the experiment. Arthritis was scored using an extended scoring protocol (25) ranging from 1–15 for each paw, with a maximum score of 60 per mouse. Each arthritic toe and knuckle was scored as 1, with a maximum of 10/paw, and an arthritic ankle or midpaw was given a score of 5. During arthritis experiments, blood samples were taken at the time of boost immunization for analysis of the CII-specific Ab response. The amounts of total anti-CII IgG as well as IgG1 and IgG2a/IgG2c isotypes were determined through quantitative ELISA, as previously described (26). It should be noted that the C57BL strains and C3H/DBA/1 strains express different, but related, IgG2 alleles: IgG2c and IgG2a, respectively (27). Sera from nonimmunized syngenic mice were used as a negative control, but do not contain any detectable amounts of CII-specific IgG Abs.

**In vitro assays**

Mice were immunized with CII or CII peptides in CFA, and 10 days later cells from draining lymph nodes were prepared and restimulated in vitro for determination of CD4-specific proliferation and IFN-γ production as previously described (28).

**Results**

B10 haplotype influences tolerance to self-CII and susceptibility to autoimmune arthritis

B10.Q (BQ) and C3H.Q (CQ) mice, either with or without transgenic expression of the rat CII epitope in cartilage, were immunized with rat CII in adjuvants and monitored for arthritis development (Table I and Fig. 1, A and B). Nontransgenic BQ and CQ mice developed arthritis with similar frequency, and disease severity as well as disease onset were comparable between the two strains. In contrast, a distinct difference in CIA susceptibility between these strains was observed after introduction of the MMC transgene. MMC-BQ mice were fully resistant to CIA, whereas MMC-CQ mice had as severe arthritis as nontransgenic littermates. We also immunized (B10.Q × C3H.Q)F1 (BC-F1) mice, either positive or negative for MMC, with CII for arthritis induction (Table I and Fig. 1C). Nontransgenic BC-F1 mice developed arthritis with a higher frequency and had an earlier onset of disease with more severe arthritis compared with either of the two parental strains. Despite the more frequent and severe arthritis in nontransgenic BC-F1 mice, MMC-BC-F1 mice had a lower frequency of CIA susceptibility. Therefore, the MMC-mediated protection in BQ mice appeared to be an additive to dominant trait. In accordance with earlier results, transgenic expression of the rat epitope in cartilage leads to tolerance and partial protection from autoimmune arthritis (17, 28–30). However, here we additionally show that the degree of protection in MMC mice is strongly influenced by the genetic background. Furthermore, the relative susceptibility to arthritis after immunization with rat CII among CQ, BQ, and BC-F1 mice differs remarkably between transgenic and nontransgenic mice, showing that the genetic background influences the susceptibility to arthritis induced by autologous and heterologous CII differently.

Sera were investigated for anti-CII Abs 5 wk after immunization in mice of the BQ-, CQ-, and BC-F1 backgrounds. In line with our observations on arthritis susceptibility, arthritis-resistant MMC-BQ mice had low levels of anti-CII IgG Abs compared with nontransgenic littermates and MMC-CQ mice (Fig. 2). Fig. 2 shows the results with IgG2a/c Abs, but similar results were obtained with IgG1 and total IgG (data not shown). In contrast, IgG levels in MMC-CQ mice did not differ from those in nontransgenic littermates. Furthermore,

<table>
<thead>
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<th>Table I.</th>
<th>Arthritis development in MMC-transgenic mice</th>
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<td>MMC</td>
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<td>2/21</td>
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**a** Tg, transgene; CI, cumulative incidence; CS, cumulative score; MDO, mean day of onset; MMS, mean maximum score; BQ, B10.Q; CQ, C3H.Q; BC-F1, (B10.Q × C3H.Q)F1; n.a., not applicable.

**b** Including all animals.

**c** Including arthritic animals only.

**d** Statistical significance differences between nontransgenic and MMC-transgenic mice (p < 0.001).
anti-CII IgG levels were higher in MMC-BC-F1 mice than in MMC-BQ mice, but were lower than those in MMC-CQ mice, thus correlating with the moderate arthritis susceptibility in MMC-BC-F1 mice. To investigate the influence of the C3H and B10 genetic background on T cell tolerance to self-CII, MMC-transgenic mice on BQ-, CQ-, and BC-F1 backgrounds were immunized with rat CII. Ten days later lymph node cells were rechallenged in vitro against modified CII peptides, and IFN-γ production was measured (Fig. 3). Cells from CII-primed, transgene-negative littermates responded to nonmodified (K264), hydroxylated (HyK264), as well as galactosylated (Gal264) CII peptides, but recall responses in BQ mice were weak compared with those in CQ- or BC-F1 mice. Introduction of the MMC transgene on these backgrounds resulted in an overall reduction of the CII-specific recall response, indicating self-CII-specific tolerization of T cells. However, T cells derived from MMC-CQ mice still mounted a strong response to CII and all CII peptides compared with MMC-BQ and MMC-BC-F1 mice and had an almost intact response to the galactosylated CII peptide compared with nontransgenic CQ mice. Conversely, BQ genes seem to have a profound impact on the level of T cell tolerance to self-CII that correlates with a reduced B cell response to CII and a reduced susceptibility to autoimmune arthritis.

Increased frequency of CII-specific T cells mediates susceptibility, but not severity, of autoimmune arthritis

MMC transgenic mice on the C3H background had a relatively stronger CII-specific response in vitro compared with MMC-BQ or MMC-BC-F1 mice. This observation was particularly prominent when the autoimmune response against the galactosylated CII peptide was compared. We were therefore interested to determine whether enhancement of the glycopeptide-specific immune response could influence arthritis susceptibility in mice carrying the B10 background. To do this, we crossed MMC-BQ mice with DBA/1 mice expressing a transgenic TCR β-chain (Vβ12) derived from a CII-specific, Aα-restricted, T cell clone originating from a CII-immunized, DBA/1 mouse (20) to generate (B10.Q × DBA/1)F1 (QD-F1), (MMC-QD-F1), or both (MMC/Vβ-QD-F1). Such mice were then immunized with rat CII, and both specific proliferation and IFN-γ release from cells draining lymph nodes were assessed in vitro with CII and CII peptides (Fig. 4). Compared with that in QD-F1 mice, the transgenic expression of the CII-specific Vβ12-chain lead to a greatly enhanced recall response to Gal peptide as well as to CII. This fine specificity was expected, since we have shown in parallel work the transgenic TCR to be specific for the galactosylated CII260–270 epitope (R. Bockermann and R. Holmdahl, manuscript in preparation). In contrast, due to the biased T cell repertoire in Vβ12-transgenic mice, Vβ-QD-F1 mice had a reduced response to the K and HyK peptides compared with QD-F1 mice. As expected from previous results with MMC-BQ mice (Fig. 3), MMC-QD-F1 mice responded poorly to CII and CII peptides compared with nontransgenic QD-F1 mice. Compared with MMC-QD-F1 and nontransgenic QD-F1 mice, introduction of the CII-specific Vβ-chain in MMC/Vβ-QD-F1 mice resulted in a profoundly enhanced response to the Gal peptide, but not to the K and HyK peptides. However, despite the strong response to the Gal peptide in MMC/Vβ-QD-F1 mice, tolerance to self-CII was evident in these mice, as the response was reduced compared with that observed in Vβ-QD-F1 mice. Interestingly, CII-specific tolerance in MMC/Vβ-QD-F1 mice was skewed, primarily resulting in a reduced proliferative response, but with less influence on Gal peptide-stimulated IFN-γ production. This kind of partial tolerance to self-CII is in agreement with our earlier observations and thus again shows that cartilage-Ags indeed are available for immune recognition under normal circumstances, but that these interactions only lead to incomplete tolerance (17, 19, 28).

We next wanted to investigate whether an enhanced immune response to CII would influence arthritis susceptibility in CII-tolerized mice. Consequently, QD-F1 mice expressing MMC, Vβ12...
transgene, or both, were immunized with rat CII and monitored for development of arthritis (Fig. 5 and Table II). Similar to that observed for BC-F1 and MMC-BC-F1 mice (Table I), QD-F1 mice were highly susceptible to CIA (85%), but endogenous expression of the heterologous CII epitope in cartilage resulted in a pronounced resistance to arthritis, as only 10% of MMC-QD-F1 mice developed arthritis. On the other hand, while MMC-BC-F1 mice tended to develop arthritis with similar severity as their nontransgenic littermates (Table I), the few arthritic MMC-QD-F1 mice appeared to develop a milder disease compared with nontransgenic QD-F1 mice. However, due to the low number of arthritic MMC-QD-F1 mice, no significant difference in disease severity was obtained. Interestingly, expression of the transgenic CII-specific Vβ12-chain resulted in a dramatically increased frequency of arthritis in MMC/Vβ-QD-F1 mice (Table II). However, although arthritis onset was earlier or similar to that in nontransgenic QD-F1 and Vβ-QD-F1 mice, respectively, arthritic MMC/Vβ-QD-F1 mice displayed a relatively mild disease. Thus, these results are in agreement with those obtained from mice of the BQ- and BC-F1 backgrounds, as nontransgenic QD-F1 mice were highly susceptible to CIA after immunization with rat CII, but transgenic expression of the immunodominant rat-CII260–270 epitope in cartilage induces strong tolerance and protection against CIA after immunization with rat CII. Introduction of the transgenic CII-specific Vβ12-chain led to an increased susceptibility to

FIGURE 3. Reduced T cell response to CII and CII-peptides in MMC-transgenic mice. Nontransgenic (WT; n = 6) or MMC-transgenic (MMC; n = 6–7) mice on the C3H.Q (CQ), B10.Q (BQ), and (B10.Q × C3H.Q)F1 (BC-F1) background were immunized with rat CII (CII), and 10 days later cells from draining lymph nodes were restimulated with 50 μg/ml of denatured CII or 25 μg/ml of the following CII peptides: CII259–273 with a nonmodified lysine at position 264 (K), CII256–270 with a 5-hydroxy-L-lysine at position 264 (HyK), or CII259–273 with a β-1,4-galactopyranosyl-5-hydroxy-L-lysine at position 264 (Gal). After 4 days of restimulation the culture supernatants were investigated for IFN-γ content. Δresponse, response in the presence of Ag–response in the absence of Ag. Asterisks indicate significant differences in the response between WT and MMC-transgenic mice: *, p < 0.05; **, p < 0.01 (by Mann-Whitney U test).

FIGURE 4. Transgenic expression of a CII-specific Vβ12-chain enhances the response to the galactosylated CII peptide. Mice on the (B10.Q × DBA/1)F1 background, either nontransgenic (QD-F1; n = 4), Vβ12-transgenic (Vβ-QD-F1; n = 10), MMC-transgenic (MMC-QD-F1; n = 4), or MMC- and Vβ12-double transgenic (MMC/Vβ-QD-F1), were immunized with CII and 10 days later cells from draining lymph nodes were restimulated with titrated amounts of denatured CII or CII peptides (see Fig. 3) for 4 days before Ag-specific proliferation and IFN-γ production were determined.
arthritis in both wild-type and MMC mice, again showing that a biased CII-specific TCR repertoire can enhance predisposition to CIA as previously shown (20, 31). However, a skewed TCR repertoire seemed to primarily affect the incidence and time of arthritis onset, whereas the severity of disease was not influenced to the same extent. This was especially noted when MMC/Vβ-QD-F1 were compared with MMC-QD-F1 mice.

Investigation of anti-CII Ab levels in sera 5 wk after immunization revealed that the cartilage-specific expression of the heterologous T cell epitope in MMC-QD-F1 mice lead to a reduced B cell response to CII compared with nontransgenic QD-F1 mice (Fig. 6). MMC/Vβ-QD-F1 mice, on the other hand, had a significantly increased B cell response to CII compared with MMC-QD-F1 mice. This is not surprising, since the production of anti-CII IgGs is dependent on CII-specific T cells. Furthermore, comparison of Ab levels between Vβ-QD-F1 and MMC/Vβ-QD-F1 or QD-F1 mice suggest that differences in CII-specific IgG2a levels could explain the relatively milder disease observed in mice with a naive anti-CII TCR repertoire. The development of CIA, as classically induced with a heterologous CII (human, chick, bovine, or rat) CII in H-2b mice (10), is highly dependent on the activation of T cells recognizing the foreign peptide 260–270 (i.e., with 266E as in the CII used for immunization) (17, 22, 38). These T cells will subsequently activate B cells to produce arthritogenic Abs (39). Interestingly, nontransgenic BC-F1 mice developed arthritis with a higher incidence and increased severity compared with nontransgenic CQ mice, although both strains showed comparable B and T cell responses to CII. Hence, the increased severity observed in BC-F1 mice needs to be explained by other factors, such as an increase in TNF-α, IL-1, IL-6, and/or IL-12 or a decrease in anti-inflammatory processes. The clarification of these differences was beyond the scope of this study, but would certainly be interesting to determine. The role for autoreactive CII-specific T cells in the heterologous CIA model is elusive. However, in the MMC mouse, which is expressing CII with 266E in the cartilage (17), only self-reactive T cells are primed. These will subsequently also trigger B cells to produce anti-CII Abs, but may also play other disease-promoting or regulatory roles. These T cells are physiologically passively or dominantly tolerized through exposure of cartilage-derived CII, and their possibility of being activated is critical for the subsequent outcome of disease. Because of the striking structural similarities between mouse and man in terms of T cell recognition

**FIGURE 5.** Transgenic expression of a CII-specific Vβ12-chain breaks tolerance to self-CII in MMC-transgenic mice. The Vβ12 and MMC transgenes were bred on the (B10 × DBA/1)F1 background (QD-F1). The mean scores of arthritis (severity) in (QD-F1), Vβ12-transgenic (Vβ-QD-F1), MMC-transgenic (MMC-QD-F1), and MMC- and Vβ12-double transgenic (MMC/Vβ-QD-F1) mice after immunization with rat CII in adjuvant are shown. Curves include only mice that developed arthritis before termination of the experiment (AAO), and the numbers of arthritic mice over the total number of mice per group are indicated in parentheses.

### Discussion

The MHC region plays an important role in susceptibility to CIA, as first shown in congenic mouse strains (10), and H-2q mouse susceptibility was later linked to the Aβ-chain of the q-variant (11). However, there is also an important contribution from non-MHC genes, as shown by the fact that several Aq-expressing mouse strains are more or less resistant to CIA, and the involvement of genes related to TCR repertoire and complement as well as other non-MHC regions has been suggested (20, 32–37). The present investigation also examined the role of non-MHC genes in susceptibility to CIA, but here we were interested in addressing the genetic influence on tolerance to self-CII and what impact this would have on autoimmune arthritis. To accomplish this we used different H-2q congenic MMC transgenic mice, which express the rat version of the immunodominant CII260–270 glycoprotein in the joints, and immunized these with rat CII. This way we could compare CIA susceptibility of MMC transgenic mice of different genetic backgrounds as well as with nontransgenic littermates, thus enabling us to separate the genetic influence on the heteroreactive immune response to CII (i.e., immune response to rat CII in nontransgenic mice) and the autoreactive immune response to CII (i.e., immune response to rat CII in MMC transgenic mice). The threshold determining the degree of tolerization of CII-reactive T cells to cartilage-derived CII is most likely a critical factor in CIA. Here we could show that this threshold for autologous CII is under genetic control and differs remarkably from an immunization with heterologous CII. We found that resistance to autoimmune collagen-induced arthritis due to tolerance to self-CII, i.e., the immunodominant galactosylated CII260–270 epitope, is more pronounced in mice with B10 compared with C3H and DBA/1 backgrounds, and immunized these with rat CII. This way we could compare CIA susceptibility of MMC transgenic mice of different genetic backgrounds as well as with nontransgenic littermates, thus enabling us to separate the genetic influence on the heteroreactive immune response to CII (i.e., immune response to rat CII in nontransgenic mice) and the autoreactive immune response to CII (i.e., immune response to rat CII in MMC transgenic mice). The threshold determining the degree of tolerization of CII-reactive T cells to cartilage-derived CII is most likely a critical factor in CIA. Here we could show that this threshold for autologous CII is under genetic control and differs remarkably from an immunization with heterologous CII. We found that resistance to autoimmune collagen-induced arthritis due to tolerance to self-CII, i.e., the immunodominant galactosylated CII260–270 epitope, is more pronounced in mice with B10 compared with C3H and DBA/1 backgrounds, and that the protective phenotype is dominantly inherited (Table III).

### Table II. Arthritis development in MMC- and Vβ12-transgenic mice

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<td>52(9)</td>
<td>132</td>
<td>27</td>
<td>102</td>
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* Tg, transgene; CI, cumulative incidence; CS, cumulative score; MDO, mean day of onset; MMS, mean maximum score; QD-F1, (B10.Q × DBA/1)F1.
* Including all animals.
* Including arthritic animals only.
* Statistical significance differences between nontransgenic and MMC transgenic mice (p < 0.001).
* p < 0.01.
MHC presentation of the CII_260–270 peptide, it is likely that the same considerations on T cell tolerance observed in the MMC mouse will also be of importance in RA (12, 14, 16, 19). With the experimental system characterized herein and previously, this should open the possibility of addressing the crucial events in explaining the role of T cell tolerance in human disease. We know to date that CII-reactive T cells in the MMC mouse are most likely not tolerized in the thymus, but meet their Ag shortly after leaving the thymus, presumably in lymph nodes draining the joints (28). After meeting Ag, not all T cells are deleted, and the remaining T cells are still capable of becoming activated if CII is presented together with adjuvant stimuli. They will then have the capacity to help B cells and perhaps also perform other types of effector functions.

There is a diverging effect of both T cell tolerance induction and arthritis susceptibility, most likely through the influence of several genes. Interestingly, in the C57BL/10 background, tolerance seems to primarily affect T cells specific for nonmodified peptides rather than T cells specific for the glycosylated variants of the epitope, confirming experiments performed with mice expressing human CII in the joints (19). Arthritis resistance in MMC mice with the B10 background correlates with a reduced T and B cell response to CII and the galactosylated CI260–270 epitope. An increased frequency of T cells specific for glycosylated CII can break arthritis resistance in B10-positive MMC transgenic mice, but the resulting arthritis differs from that observed in MMC transgenic mice on the C3H background in terms of the onset and severity of disease.

The observed differences between MMC mice on the B10 and C3H backgrounds in terms of tolerance and disease phenotypes argues that some genes influence Ag specificity or, rather, the likelihood of responding to neoantigens such as the various glycosylated forms, while other genes control tolerance induction and also effector mechanisms leading to arthritis. It is possible to identify

<table>
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Notes:

- Simplified overview of obtained result describing susceptibility to CIA (Inc), severity of disease, onset of disease, in vitro response to CII and CII peptides, and anti-CII IgG levels, +, positive, ++, moderate, ++++, high; +++++, very high.
- Strongly biased to the CII_260–270 glycopeptide.
- Biased reduction in anti-CII IgG2a levels.
gene regions containing genes responsible for the different phenotypes through genetic segregation experiments (40). We have already identified a number of loci using crosses between B10.Q and DBA/1 mice (37) and isolated them in congenic strains (to be published), opening the possibility to investigate whether genes within these loci will play a role in the outcome of T cell tolerance to CII. Thus, the established MHC and TCR transgenic models expressed on different genetic backgrounds will now allow identification of the naturally selected genes controlling tolerance development and thereby the crucial events leading to T cell-mediated autoimmune arthritis.

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References


