Systemic versus cartilage-specific expression of a type II collagen-specific T-cell epitope determines the level of tolerance and susceptibility to arthritis

collagen-induced arthritis/T-cell tolerance/transgenic mice/autoimmunity/rheumatoid arthritis

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ABSTRACT Immunization of mice with rat type II collagen (CII), a cartilage-specific protein, leads to development of collagen-induced arthritis (CIA), a model for rheumatoid arthritis. To define the interaction between the immune system and cartilage, we produced two sets of transgenic mice. In the first we point mutated the mouse CII gene to express an earlier defined T-cell epitope, CII-(256–270), present in rat CII. In the second we mutated the mouse type I collagen gene to express the same T-cell epitope. The mice with mutated type I collagen showed no T-cell reactivity to rat CII and were resistant to CIA. Thus, the CII-(256–270) epitope is immunodominant and critical for development of CIA. In contrast, the mice with mutated CII had an intact B-cell response and had T cells which could produce γ interferon, but not proliferate, in response to CII. They developed CIA, albeit with a reduced incidence. Thus, we conclude that T cells recognize CII derived from endogenous cartilage and are partially tolerated but may still be capable of mediating CIA.

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease affecting peripheral joints. The main genetic association is to the major histocompatibility complex (MHC) class II region (HLA-DR) (1, 2), suggesting involvement of T-cell-mediated autoimmune recognition of joint-specific antigens. Known proteins within joints that can be considered joint-specific are those found in articular cartilage. These include collagens of type II (CII), type IX (CIX), and type XI (CXI), and noncollagenous proteins such as aggrecan and COMP (3–5). Some of these cartilage-specific proteins (CII, CIX, and aggrecan) have been shown to induce autoimmune arthritis in animals (6–8). The most widely used model is collagen-induced arthritis (CIA), induced with CII, the major protein component of cartilage. Immunization with CII leads to development of CIA in rats, mice, and apes (6, 9, 10). In mice, the disease has a higher incidence and severity after immunization with heterologous CII compared with after immunization with autologous CII (11). The immune recognition of CII, and the subsequent arthritis development, is surprisingly precise. The disease is associated with the expression of the MHC class II A4 molecule (12). One limited region of CII, between positions 256 and 270, is recognized after immunization with rat CII (13). This peptide is present in heterologous but not in mouse CII and can be recognized both in its posttranslational modified form (disaccharides bound to hydroxylysines) and in its nonmodified form (14). The difference is due to the presence of an aspartic acid at position 266 in mouse CII, whereas a glutamic acid is present at that position in rat CII. The lack of crossreactivity could possibly be explained by the finding that the rat CII-(256–270) binds better than the mouse peptide to the MHC class II A4 molecule (13). Still, autoreactive peptide-specific T cells can be activated, but only after immunization with mouse CII-(256–270).

In recent years there has been considerable progress in the understanding of the basic mechanisms for development of immune tolerance to self-antigens. Elimination of self-reactive T cells in thymus (central tolerance) has been clearly demonstrated (15) and autoreactive T cells which are not eliminated in the thymus may be eliminated or develop tolerance if exposed to autoantigens in the periphery (16, 17). However, in other cases T cells respond to exposed autoantigens (18). For the study of tolerance in autoimmune disease the nature of the antigen and the context by which it is presented is of critical importance. Thus, it is essential that disease-related autoantigens are studied. The expression of the CII gene is tightly controlled in a tissue-specific fashion through regulatory elements located in the promoter and first intron (19, 20). We have hypothesized that T cells specific for CII in cartilage are not eliminated but are anergized and may play an important role in the development of CIA (11). To address this question we made mice transgenic with type II and type I collagen genes which were mutated to express the immunodominant CII-(256–270) epitope. The behavior of CII-reactive T cells and arthritis susceptibility in these mice were analyzed.

MATERIALS AND METHODS

Animals. C3H.HO mice (originally from D. C. Shreffler, Washington University, St. Louis), were bred, kept, and used in environmentally controlled but conventional animal facilities.

Transgene Constructs. For cartilage-specific expression, a 40-kb genomic clone of the mouse CII gene (Col2a1) was used, containing the entire gene, including flanking sequences (21). This clone has earlier been shown to direct cartilage-specific expression in transgenic mice (22). By site-directed mutagenesis, we introduced one nucleotide mutation leading to an amino acid shift from Asp to Glu (Fig. 1A). Syngeneically fertilized C3H.HO eggs obtained from superovulated donors were subjected to pronuclear microinjections. Two founder

Abbreviations: CIA, collagen-induced arthritis; CI, type I collagen; CII, type II collagen; MHC, major histocompatibility complex; RA, rheumatoid arthritis; MMC, mutated mouse collagen; TSC, T-cell epitope in systemic collagen; RT, reverse transcriptase; LNC, lymph node cells; IFN-γ, γ interferon; ELISPOT, enzyme-linked immunospot; PPD, purified protein derivative.

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Reverse Transcriptase (RT)-PCR. Tissue was frozen in liquid nitrogen and kept at −85°C until total RNA was prepared (24). Transgene-specific primers were used in the cDNA reactions, which were followed by PCR and agarose gel electrophoresis.

Collagen Preparations. Rat CII was extracted from the Swarm chondrosarcoma (25) after pepsin digestion or from lathyritic chondrosarcoma (26) and further purified as described (27). The collagen was dissolved and stored in 0.1 M acetic acid until used. Collagen was also extracted by pepsin digestion from skin and the xiphoid process of transgenic mice and their nontransgenic littermates by the same method but without further purification. CII-(256–270) was synthesized as described (13).

Induction of Arthritis. Male mice, 7–12 weeks of age, were immunized intradermally in the base of the tail with 100 µg of CII emulsified in complete Freund’s adjuvant (Difco). They were also bled and given a boost injection with 25–50 µg of CII emulsified in incomplete Freund’s adjuvant (DIFCO) at day 35. The amount of CII-reactive antibodies in the sera was measured by a quantitative ELISA (28) and clinical scoring was performed as described (11).

Immune Response Assays of Primary Cultures. Age- and sex-matched mice were immunized in the footpads with 50 µg of lathyritic rat CII emulsified in complete adjuvant. Draining lymph nodes were collected 10 days later and single cell suspensions were prepared in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 1% fresh mouse serum, Hepes, penicillin, streptomycin, and 2-mercaptoethanol.

To measure the antigen-specific proliferative response, the lymph node cells (LNC) were cultured in flat-bottomed 96-well plates (Nunc, Roskilde, Denmark) and stimulated with antigen for 96 hr before pulsing with [3H]thymidine and were harvested 15–18 hr later in a Filtermate cell harvester (Packard). The incorporation of [3H]thymidine was determined in a Matrix 96 Direct Beta Counter (Packard); all experiments were performed in triplicate cultures.

The presence of immunoglobulin as well as γ interferon (IFN-γ)-producing cells was determined by enzyme-linked immunospot (ELISPOT) assays essentially as described (29). In the ELISPOT assay for enumeration of anti-CII immunoglobulin-secreting B cells, 96-well plates were coated with mouse CII or CII at 20 µg/ml and 5 × 10^5 LNC were added per well and incubated for 1–2 hr. After removal of the cells a peroxidase-conjugated anti-mouse immunoglobulin antibody was added. Plates were developed by using 3-amino-9-ethylcarbazole (Sigma). In the IFN-γ ELISPOT assay, 96-well nitrocellulose plates (Millipore) were coated with an anti-IFN-γ antibody. LNC from a 48-hr primary culture of 10^6 cells per well were added to the plate and incubated for 24 hr. After the cells had been removed, anti-IFN-γ rabbit immunoglobulin, biotinylated anti-rabbit immunoglobulin, and avidin-biotin-peroxidase (ExtraAvidin, Sigma) were added before developing with 3-amino-9-ethylcarbazole. Spots were counted in a microscope in both ELISPOT assays. Mean values of duplicate or triplicate cultures were used.

Assay for Detection of Transgenic Collagen. Collagen preparations from skin and the xiphoid process of the different mice were used to stimulate the CII-(256–270)-specific T-cell hybridoma HCQ.10 (14). This hybridoma is extremely sensitive to glycosylated CII and reacts at the level of 1 ng/ml; no crossreactions to nonglycosylated CII have been seen. To detect the presence of transgenic collagen containing the CII-(256–270) region in the various collagen preparations, 5 × 10^4 HCQ.10 cells were incubated with collagen and 5 × 10^5 CIIIQ splenocytes as antigen-presenting cells in 96-well plates for 24 hr, whereafter the plates were frozen. The interleukin 2 content was determined by using the cytotoxic T-lymphocyte line (CTL). In this assay 10^4 CTL cells were incubated with

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Fig. 1. (A) Gene construct for mutant CII: MMC transgenic mice. By PCR, the reverse primer 5’-TCTCCCTTGCGCGCTGTGTCGCGGTTGGGCA-3’ introduced the desired mutation, and at the same time silenced a Bcl I restriction site, which was later used for transgene identification. The mutated sequence replaced the wild-type sequence between the Sty I restriction sites. (B) Gene construct for systemic CII-(256–270) expression: TSC transgenic mice. Synthetic oligonucleotides, 113 and 122 nt, were annealed to recreate a mutant exon 43, now encoding the CII-(256–270) epitope. Restriction enzymes BstEII and Sac I were used to replace the wild-type sequence.

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lines, MMC-1 and MMC-2 were obtained (MMC, mutated mouse collagen).

To achieve systemic expression of CII-(256–270) in a chimeric type I collagen (CI), a derivative of the mouse genomic clone 10D of the Collal gene (kindly provided by Hong Wu, Whitehead Institute, Boston) was used for site-directed mutagenesis. Complementary oligonucleotides covering the CII-(256–270) coding sequence were synthesized for replacement of a cassette of wild-type sequences in exon 43 of the Collal gene (Fig. 1B). The 26.6-kb construct has been successfully expressed in 3T3 fibroblasts and the product has been shown to stimulate CII-specific T-cell hybridomas (14). This construct was similarly used for microinjection of fertilized CIIIQ eggs. Two founders were generated: TSC-1 and TSC-2 (TSC, T-cell epitope in systemic collagen).

Screening and Transgenic Expression. Genomic DNA was prepared from the tip of the tail or a toe (23). MMC DNA was screened by using PCR with CII-specific primers and a transgene-specific restriction digest (Bcl I silenced by the point mutation) followed by agarose gel electrophoresis. TSC DNA was screened by using PCR with CI-specific primers and a transgene-specific restriction digest (Sty I introduced in transgenic TSC mice). The negative, nontransgenic, littermates (CIIIQ) were used as control mice in all experiments. The copy numbers of integrated MMC constructs were determined by Southern blotting: MMC-1 has approximately six inserted copies, while MMC-2 has nine (data not shown).

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\begin{align*}
\text{Exon 19 (99bp)} & \quad \text{Exon 43 (108bp)} \\
\text{Coll2a1} & \quad \text{Col1a1} \\
\end{align*}
\]

**Fig. 1.** (A) Gene construct for mutant CII: MMC transgenic mice. By PCR, the reverse primer 5’-TCTCCCTTGCGCGCTGTGTCGCGGTTGGGCA-3’ introduced the desired mutation, and at the same time silenced a Bcl I restriction site, which was later used for transgene identification. The mutated sequence replaced the wild-type sequence between the Sty I restriction sites. (B) Gene construct for systemic CII-(256–270) expression: TSC transgenic mice. Synthetic oligonucleotides, 113 and 122 nt, were annealed to recreate a mutant exon 43, now encoding the CII-(256–270) epitope. Restriction enzymes BstEII and Sac I were used to replace the wild-type sequence.
supernatant for 24 hr before pulsing and harvesting as described for the immune response assay.

Statistics. Dichotomous variables (i.e., incidence of arthritis) were analyzed by χ² test, and nonparametric (i.e., arthritic scores) or values with a skewed distribution (e.g., stimulation indices and antibody levels) were analyzed by the Mann-Whitney U test.

RESULTS

Generation of Transgenic Mice Expressing Mutated Collagens. To express the immunodominant CII-(256–270) epitope in CII, we used a 40-kb mouse CII genomic construct. By PCR one point mutation (T to G) was created, leading to the desired amino acid change from Asp to Glu at position 266 (Fig. 1A).

To express the CII-(256–270) epitope in systemically occurring CI, a mouse Col1a1 genomic clone containing all the regulatory elements for tissue-specific expression of CI, was mutated (Fig. 1B). The replacement mutations of 9 amino acid residues remained restricted to the X and Y positions of the collagen-specific Gly-X-Y sequence, thus leaving the glycine residues in every third position unchanged as a critical structural requirement for triple helicity of the native chimeric collagen.

The above constructs were introduced as transgenes into the CIA susceptible mouse strain C3H.Q. Two transgenic lines carrying the mutated CI gene (TSC-1 and TSC-2) and two lines carrying the mutated CII gene (MMC-1 and MMC-2) were founded and maintained as heterozygotes. Only one of each (TSC-1 and MMC-1) was used, since the founders for each construct showed similar types of expression and immune responses. None of the mice have developed arthritis spontaneously and no other macroscopic disturbances in breeding or development of the mice have been noted. This is in contrast to CII-transgenic mice mutated at positions coding for glycines, or mice with truncated CII, which develop chondrodysplasias of varying severity (22, 30, 31). Similarly, CI-transgenic mice with mutated glycines develop diseases such as osteogenesis imperfecta (32).

Total RNA was prepared from newborn transgenic MMC-1 and TSC-1 mice as well as from nontransgenic littermates for the analysis of transgene-derived mRNA by RT-PCR from the following tissues: eye, thymus, liver, kidney, spleen, skin, and the sternal xiphoid process (cartilage). Transgenic mRNA could be detected only in the eye and cartilage of MMC-1 mice (Fig. 2), in accordance with the strict tissue-specific expression of the CII gene (19, 20), although illegitimate CII mRNA has been observed in many organs from normal mice by using RT-PCR under conditions apparently better than those presently used (30, 33). TSC-1 transgenic mRNA was detectable in all of the above-mentioned samples (data not shown). To analyze whether transgenic protein was actually expressed, collagen was prepared from skin and the sternal xiphoid process from the two different lines of mice. The collagen was used for stimulation of a CII-(256–270)-specific T-cell hybridoma (14) recognizing the glycosylated form of the peptide (Fig. 3). Collagen prepared from the skin and xiphoid process of TSC-1 mice and collagen prepared from the xiphoid process of MMC-1 mice contained transgenic protein. These data show that the expression of transgenic collagens is posttranslationally modified and restricted in accordance with the tissue specificity of the corresponding wild-type gene expression in the adult mouse.

TSC-1 Mice Developed no Immune Response to Rat CII. Draining lymph node cells from TSC-1 mice immunized with rat CII failed to mount a significant immune response to CII-(256–270) as measured with IFN-γ ELISPOT assays (Fig. 4) or proliferation (Table 1). This suggests that the responding T-cell population has been eliminated or at least behaves as if functionally dead. In fact, also after stimulation with the entire rat CII, no or very poor T-cell responses were recorded. In addition, T-cell help to B cells was lacking, since B cells were not, or were very poorly, activated into anti-CII immunoglobulin secretion as measured with an ELISPOT assay (Fig. 5). The relative lack of immune response to rat CII suggests that

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**FIG. 2.** Expression pattern of transgenic CII in MMC-1 mice. Expression of mutant Col2a1 mRNA in tissues of newborn MMC transgenic mice analyzed by RT-PCR and gel electrophoresis. From left to right: eye, thymus, liver, kidney, spleen, skin, and cartilage (paw). λ, Size marker Eco 47I-digested phage λ DNA. As a negative control, total RNA from a paw of a C3H.Q mouse was used. The product is 501 bp and is indicated by arrowheads.

**FIG. 3.** Detection of transgenic collagen. CTLL assay with T-cell hybridoma HCQ.10 specific for the glycosylated CII-(256–270) epitope. (A) Stimulation with CI (skin collagen) preparations from C3H.Q (□), MMC-1 (△), and TSC-1 (○). (B) Stimulation with CII (xiphoid cartilage collagen) preparations from C3H.Q (□), MMC-1 (△), and TSC-1 (○).
epitopes other than CII-(256–270) only very marginally contribute to the anti-CII immune response. In fact, this immune response is at the same level as the response to mouse CII (27), which has a cryptic CII-(256–270) peptide (13) as well as a few other weakly stimulatory peptides in other regions in the mouse CII molecule (34).

**MMC-1 Mice Mount a Partially Tolerized T-Cell Response to CII.** Draining lymph node cells from MMC-1 mice immunized with rat CII mounted a reduced but still significant response to both CII-(256–270) and rat CII compared with C3H.Q when measured with the IFN-γ ELISPOT assay (Fig. 4). In addition, mice immunized with rat CII developed normal levels of anti-CII B-cell response as quantitated with the immunoglobulin ELISPOT assay (Fig. 5). However, no significant proliferative responses to CII-(256–270) or to the entire rat CII could be found (Table 1). These findings show that the CII-(256–270)-reactive T cells were not eliminated but tolerized.

**MMC-1 Mice Develop CIA, but with a Reduced Incidence.** Immunization with rat CII induced in all groups of nontransgenic littermates (C3H.Q) a high incidence of severe arthritis (Fig. 6). None of the TSC-1 mice developed arthritis. MMC-1

### Table 1. Comparison of the stimulation index in the proliferative response after CII immunization

<table>
<thead>
<tr>
<th>Mice</th>
<th>CII-(256–270) mean ± SD</th>
<th>P</th>
<th>Rat CII mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMC-1*</td>
<td>1.87 ± 0.66</td>
<td>0.0025</td>
<td>1.71 ± 1.17</td>
<td>0.0248</td>
</tr>
<tr>
<td>C3H.Q</td>
<td>6.09 ± 4.06</td>
<td>0.65</td>
<td>6.65 ± 6.83</td>
<td></td>
</tr>
<tr>
<td>TSC-1†</td>
<td>1.01 ± 0.22</td>
<td>0.0086</td>
<td>1.16 ± 0.55</td>
<td>0.1197</td>
</tr>
<tr>
<td>C3H.Q</td>
<td>2.51 ± 1.40</td>
<td></td>
<td>1.92 ± 1.27</td>
<td></td>
</tr>
</tbody>
</table>

Proliferative response to CII-(256–270) and rat CII from MMC-1, TSC-1, and C3H.Q mice immunized with lathyrillic rat CII (±SD). The stimulation index is calculated as proliferation at the highest antigen concentration [100 μg of rat CII per ml and 50 μg of CII-(256–270) per ml, respectively] divided by the background proliferation (no antigen added). Each experiment was set up in duplicate or triplicate cultures; the methodological variation was less than 15%, and the PPD responses were comparable within the groups. The SD thus illustrates the biological variation in our mice.

*Summary of six experiments comparing 11 MMC-1 mice with 15 nontransgenic littermates.

†Summary of four experiments comparing 10 TSC-1 mice with 13 nontransgenic littermates.

**DISCUSSION**

In the present experiments we show that autoreactive CII-specific T cells behave differently, depending on whether the recognized epitope, CII-(256–270), is located in cartilage or expressed in noncartilaginous matrices. In the latter case, the CII-(256–270) epitope was incorporated in the CI gene and transgenically expressed in the TSC-1 mouse. The lack of T cells responding to CII-(256–270) in TSC-1 mice indicates that the immune system recognizes collagen located in systemically

![Fig. 4](image-url)  
**Fig. 4.** Number of IFN-γ-producing T cells after CII immunization. IFN-γ ELISPOT assay on cells from draining lymph nodes of mice immunized with rat CII. The LNC were stimulated with 100 μg of rat CII per ml, 50 μg of CII-(256–270) per ml, or 10 μg of purified protein derivative (PPD) per ml. The results are expressed as number of spots per 10⁶ LNC. The graph summarizes several experiments, each with balanced groups. In total, 23 C3H.Q, 10 MMC-1, and 9 TSC-1 mice were used. Error bars indicate SD of the biological variation.

![Fig. 5](image-url)  
**Fig. 5.** Number of anti-CII reactive B cells. ELISPOT assay of immunoglobulin on cells from draining lymph nodes of mice immunized with rat CII. The results are expressed as number of spots per 0.5 × 10⁶ LNC. The graph summarizes several experiments, each set up with balanced groups. In total, 8 C3H.Q, 6 MMC-1, and 2 TSC-1 mice were used. Error bars indicate SD of the biological variation.

![Fig. 6](image-url)  
**Fig. 6.** Incidence (%) of arthritis. All mice were immunized at day 0 and boosted at day 35. The figure represents three experiments, each with balanced groups of male mice. In total, 25 C3H.Q mice (○), 20 MMC-1 mice (△), and 6 TSC-1 mice (△) were used. The incidence of arthritis differed significantly among all groups (P = 0.0008), while the severity of disease did not (6.1 ± 2.1 for C3H.Q and 5.0 ± 2.3 for MMC-1 mice).
distributive connective tissue and therefore the responding T cells are physically or functionally eliminated. No T-cell response could be detected towards the entire rat CII either, indicating that there is no other immunodominant T-cell epitope in rat CII.

The insertion of the CII-(256–270) epitope into mouse CII required only one point mutation in the gene, and the localization of the gene product showed the predicted cartilage restriction in the transgenic MMC-1 mouse. The activation of IFN-γ secretion by the peptide-specific T cells, and the occurrence of functional help to B cells, clearly demonstrate that the CII-(256–270)-specific T cells have not been clonally eliminated and that the CII-specific T cells retain important effector functions. However, the antigen-specific proliferative response after stimulation in vitro was severely decreased. This is fully compatible with the definition of anergy as put forward by Schwartz and Jenkins: a poor proliferative capacity while effector functions are retained (35). The higher requirements to trigger activation and the failure to expand after activation could explain why the mice do not spontaneously develop arthritis. In addition, many other genetic and environmental factors may influence a possible outbreak of spontaneous arthritis. Thus, we have recently observed that an influence of stress (induced by intermale aggressiveness) in certain mouse strains, such as DBA/1, both “spontaneously” trigger arthritis (36) and lead to enhanced development of CIA (L. Jansson, personal communication). The C3H.Q strain is relatively resistant to development of stress induced arthritis (unpublished observations).

The reduction in susceptibility to arthritis in the MMC-1 mice clearly shows that the tolerant state of CII-(256–270)-reactive T cells is protective. It also shows that activation of only B-cells, with subsequent production of antibodies to native CII, is not sufficient for development of CIA. There is no doubt that antibodies play an important pathogenic role in CIA (37) but, for development of the complete disease, activation of autoreactive T cells appears to be critical.

The most pertinent but unsolved question is why 45% of the MMC-1 mice still developed severe arthritis. Is the arthritis mediated by partially tolerant T cells with retained effector functions or is it mediated by T cells which have escaped tolerance induction, perhaps by recent export from the thymus? The poor proliferative responses after secondary stimulation in vitro indicate that these T cells must be few, if any, which argues against the latter possibility. On the other hand, this possibility cannot be excluded, especially since collagens in general are problematic to handle for certain professional antigen-presenting cells and therefore usually give rise to relatively low T-cell proliferative responses in vitro (38). Another possibility is that the mutated CII is not properly posttranslationally modified, which is important because glycansated CII is more arthritogenic than the nonglycosylated CII (14). However, in the present experiment we could directly show that glycanslated peptides are presented in both types of transgenic mice, since the collagen preparations stimulated a T-cell hybridoma reactive only to glycanslated CII-(256–270). An alternative explanation could be a shift to another cytokine secretion pattern—i.e., to a TH2-like immune response. The secretion of IFN-γ by the responding T cells strongly argues against this. Moreover, the possibilities for an active anti-clonotopic regulation are limited, since CII-(256–270) is recognized by T cells using a very diverse set of T-cell receptors (A. Corthay, personal communication). Thus, an interesting remaining possibility is that it is in fact the partially tolerant T cells which mediate the arthritis. We imagine that there is a pool of CII-reactive T cells with variable degrees of tolerization depending on their antigen-interacting history. After immunization, the degree of proliferative activity ranges from full activation of T cells that have not earlier encountered the antigen to more or less tolerized T cells with limited proliferative activity, due to lack of interleukin 2 secretion.

These findings bear on the elucidation of autoimmune mechanisms in RA. The immunodominant CII-(256–270) peptide is present in human CII. In addition, the MHC class II molecule binding the CII-(256–270) peptide in the mouse, the A* molecule (12), has striking similarities (11, 44) with the DR4 class II molecules in humans thought to be associated with RA (2). Moreover, B cells producing antibodies to CII are frequently found in the joints of RA patients, and their occurrence has been shown to be associated with DR4 (39–41). In contrast, in spite of considerable efforts by many investigators, only very few examples of CII-reactive human T cell clones have been reported (42, 43). A possible explanation is that in individuals with DR4 alleles the T cells are tolerized and thereby difficult to clone. Maybe they still maintain important effector functions (such as B-cell help and IFN-γ production) if they are stimulated by some environmental challenge which will expose the CII-(256–270) peptide or a mimicking foreign peptide in an immunogenic fashion.

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