◆ REVIEW ARTICLE ◆

Role of glycopeptide-specific T cells in collagen-induced arthritis: an example how post-translational modification of proteins may be involved in autoimmune disease

Alexandre Corthay[1,2], Johan Bäcklund[1] and Rikard Holmdahl[1]

Immunization of mice with type II collagen (CII), a cartilage-restricted protein, leads to collagen-induced arthritis (CIA), a model for rheumatoid arthritis (RA). CIA symptoms consist of an erosive joint inflammation caused by an autoimmune attack, mediated by both T and B lymphocytes. CD4$^+$ αβ T cells play a central role in CIA, both by helping B cells to produce anti-CII antibodies, and by interacting with other cells in the joints, eg macrophages. In H-2^q mice, most CII-specific CD4$^+$ T cells recognize the CII(256-270) peptide presented on the major histocompatibility complex (MHC) class II A^q molecule. Post-translational modifications (hydroxylation and variable glycosylation) of the lysine residue at position 264 of CII generate at least four different T-cell determinants that are specifically recognized by distinct T-cell subsets. Most T cells recognize CII(256-270) glycosylated with the monosaccharide galactose, which is consequently immunodominant in CIA. Recent studies indicate that the arthritogenic T cells in CIA are glycopeptide-specific, suggesting that induction of self-tolerance may be rendered more difficult by glycosylation of CII. These data open the possibility that autoimmune disease may be caused by the creation of new epitopes by post-translational modification of proteins under circumstances such as trauma, inflammation or ageing.

Keywords: arthritis; autoimmunity; collagen-induced arthritis; glycosylation/glycopeptide; peptide; post-translational modification; T lymphocyte.

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From the [1]Section for Medical Inflammation Research, Lund University, Lund, Sweden; and the [2]Institute of Immunology, University of Oslo, Rikshospitalet, Oslo, Norway.

Correspondence: Alexandre Corthay, PhD, Institute of Immunology, University of Oslo, Rikshospitalet, 0027 Oslo, Norway. E-mail: Alexandre.Corthay@labmed.uio.no; Fax: +47 23073510.

Introduction

Our understanding of the cellular and molecular mechanisms leading to autoimmune disease has been greatly enhanced by the use of experimental animal models. Collagen-induced arthritis (CIA) is a model for rheumatoid arthritis (RA), which has been subjected to intensive research for more than two decades. In CIA, arthritis is induced by intradermal immunization of mice, rats or primates with type II collagen (CII), which is a major protein component of joint cartilage (1–4). The two arms of adaptative immunity, T and B cells, play a central role in the pathogenesis of CIA. The role of T cells in CIA is complex and can be divided into two main pathways that act in synergy in the development of arthritis. Firstly, T cells provide help to B cells for the production of arthritogenic anti-CII antibodies. Secondly, T cells are believed to play a triggering role in the joint inflammation through activation of other cells, eg synovial macrophages. This review focuses particularly on the importance of post-translational modification of CII in CIA. In the recent years, it was revealed that natural hydroxylation and glycosylation of CII dramatically influences T-cell recognition. The immunodominant T-cell epitope on CII in H-2^q mice was shown to be a glycosylated peptide, and there is accumulating evidence that arthritogenic T cells in CIA are glycopeptide-specific.

CD4$^+$ αβ T cells are critical in CIA

T cells that possess a T-cell antigen receptor (TCR) made of an α and a β chain (αβ T cells) are believed to play a crucial role in CIA development. The presence of αβ T cells has been reported in the joints of mice with CIA (5), and administration of anti-TCRαβ

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monoclonal antibodies (mAb) significantly reduced the incidence of CIA (5–7). Furthermore, we have recently reported that mice lacking αβ T cells as a result of a targeted genomic deletion in the tcrb locus were completely resistant to CIA (8). Histopathological examination of arthritic paws suggests that CD4+ αβ T cells may play an important role in CIA. The earliest detectable pathological change in CIA, before any macroscopic signs, is the accumulation of Mac-1+ (CD11b+) macrophage-like cells in the synovium, which show an up-regulated expression of surface major histocompatibility complex (MHC) class II molecules (9). These activated macrophage-like cells are observed close to the cartilage in foci, often in conjunction with CD4+CD25+ T cells (9). The importance of CD4+ αβ T cells in CIA is supported by experiments showing that administration of anti-CD4 mAb significantly reduced the incidence of CIA, (5, 10). Furthermore, the adoptive transfer of CIA to severe combined immunodeficient (SCID) mice by spleen cells from CII-immunized mice was suppressed by depletion of the CD4+ cells (11). Our laboratory has also shown that a mild arthritis could be transferred to normal or irradiated mice with CII-specific CD4+ T-cell lines (12). Importantly, arthritis transferred by T cells was usually not macroscopically apparent, indicating a milder disease than a typical CIA (12). However, histology sections of mice transferred with T cells revealed synovitis with extensive mononuclear cell infiltration, pannus formation and erosion of cartilage and subchondral bone (12).

The results obtained from CIA experiments with CD4-deficient mice were somewhat inconsistent, and this appears to be dependent on which mouse strain was used. In the DBA/1 strain, the incidence and severity of arthritis were similar for CD4-deficient (CD4–/–) mice compared with control CD4+/– littermates (13). In contrast, in the B10.Q strain, the CD4–/– mice were shown to be partly protected from CIA (14). These contradictory data highlight the complexity of the CIA model and invoke the importance of interacting genes that may create different diseases in different mouse strains. In addition, DBA/1 mice have a tendency to spontaneously develop a T cell-independent enthesopathy, which is characterized by joint swelling and erithema and which can be difficult to distinguish from CIA macroscopically (15). Importantly, in both DBA/1 and B10.Q strains, the proliferative response of lymph node T cells to CII was reduced in CD4-deficient (CD4–/–) mice compared with control CD4+/– littermates, thereby supporting the importance of CD4+ T cells in CIA (13, 14). The observation that CD4-deficient mice could develop arthritis is, however, of interest (13, 14). It was also shown that CD4-deficient mice contain an abnormally high percentage of double negative (CD4–CD8–) TCRαβ T cells in blood, lymph nodes and arthritic joints (13). In addition, a CII-specific T-cell line, generated from a CD4-deficient mouse, responded to CII in a MHC class II-restricted fashion, and had a CD4–CD8–TCRαβ+ phenotype. Altogether, this suggests that MHC class II-restricted, double-negative αβ T cells are mediating the anti-CII antibody production and arthritis development in CD4-deficient mice (13). Therefore, these data raise the possibility of a role for double-negative αβ T cells in CIA in normal mice.

It is believed that one of the main functions of CD4+ αβ T cells in CIA is to help B cells in the production of anti-CII antibodies. Several observations support the importance of anti-CII antibodies in the pathogenesis of CIA, anti-CII IgG in particular. CIA is associated with high levels of antibody to CII (2, 16). Furthermore, a transient arthritis could be induced by transferring either anti-CII serum (17, 18) or anti-CII IgG mAb (19). B cell-deficient mice were also shown to be resistant to CIA (20). We have recently demonstrated, by using αβ T cell-deficient mice, that the production of anti-CII IgG antibodies is strictly αβ T cell-dependent (8). However, B-cell help

Key messages

- **CD4+ αβ T cells are critical for the development of collagen-induced arthritis (CIA), a mouse model for rheumatoid arthritis.**
- **CD4+ αβ T cells in CIA predominantly recognize type II collagen peptides that have been post-translationally glycosylated.**
- **This suggests that autoimmune disease may be caused by the creation of new epitopes by post-translational modification of proteins under circumstances such as trauma, inflammation or ageing.**

Abbreviations and acronyms:

CB	cyanogen bromide
CDR3	complementarity-determining region 3
CIA	collagen-induced arthritis
CII	type II collagen
Ig	immunoglobulin
IL	interleukin
J	Joining
mAb	monoclonal antibody
MHC	major histocompatibility complex
TCR	T-cell antigen receptor
V	Variable

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is probably not the only function of $CD4^+$ αβ T cells in CIA, as transfer of anti-CII antibodies resulted in only a transient arthritis (17–19). In contrast, a potent and long-lasting arthritis occurred after anti-CII antibodies had been transferred together with anti-CII $CD4^+$ T cells to T cell-depleted mice (21) or to SCID mice (11). Altogether, this suggests that there is a synergy between B cells and $CD4^+$ αβ T cells in CIA.

Other T-cell subsets that may be involved in CIA

$CD8^+$ T cells

$CD8^+$ T cells are present in arthritic joints but at a lower frequency than $CD4^+$ T cells (5, 9). Studies with MHC recombinant B10 mouse strains revealed that susceptibility to CIA was linked to the D region of the MHC class I (22). The role of $CD8^+$ T cells in CIA is further supported by experiments in which treatment of mice with anti-CD8β mAb resulted in suppression of CIA (5). The incidence of CIA in the DBA/1 mouse strain was also significantly decreased in $CD8^{-/-}$ mice compared with $CD8^{+/-}$ mice, even though the severity of arthritis in affected mice was not altered, suggesting a role for $CD8^+$ T cells in initiating CIA (13). CII-specific T-cell hybridomas with cytotoxic function could be isolated after immunization of mice with CII (23). These $CD8^+$ hybrids specifically recognized CII in the context of the MHC class I K^q molecule (23).

γδ T cells

The number of γδ T cells is increased in the joints of mice with CIA; in arthritic mice, approximately 30% of all T cells in the joints are γδ T cells compared with 10% in normal joints (5). Interestingly, the TCR Vγ and Vδ gene segment usage is very similar in normal vs arthritic mice, suggesting that there is a local expansion of γδ T cells in the joints of mice with CIA (5). An important role for γδ T cells in CIA has been proposed as the injection of mice with an anti-TCRγδ mAb (13D5) resulted in the suppression of arthritis (24). This was successful only if the treatment was initiated before immunization with CII, whereas CIA was exacerbated if mAb was injected 40 days after immunization (24). However, in these experiments, mice injected with intact 13D5 lost weight rapidly, suggesting that 13D5 may induce a cytokine-mediated syndrome similar to that observed in mice and humans after injection of anti-CD3 mAb (24, 25). Administration of large amounts of 13D5 may consequently result in the stimulation rather than the blocking of γδ T cells. We have recently investigated CIA in mice that lacked γδ T cells as a result of targeted genomic deletion in the TCRδ locus (8). There were no significant differences observed in CIA between γδ T cell-deficient mice and control littermates, demonstrating that γδ T cells are not required for the development of arthritis (8).

CIA in lymphocyte-deficient mice

Unexpectedly, it has recently been reported that DBA/1 male mice that were lacking both mature T and B cells as a result of a targeted inactivation of the RAG-1 gene (recombination-activating gene), could develop a mild arthritis after immunization with CII (26). This suggests that the presence of lymphocytes is not absolutely required for the development of CIA (26). The above-mentioned data are difficult to explain in the light of a number of observations, in particular in that of reports showing B cell-deficient (20) or αβ T cell-deficient mice (8) to be resistant to CIA. These discrepancies suggest that $RAG\text{-}1^{-/-}$ mice, which lack all lymphocyte populations, may be responding to immunization in a manner quite different from more immunocompetent mice. However, the experiments with RAG-deficient mice also indicate that lymphocyte-independent mechanisms of disease induction may operate in the standard CIA model (26). This is a challenging view that requires further studies, preferably with other mouse strains and with types of collagen other than CII.

Post-translational glycosylation of CII dramatically influences T-cell recognition

CII is the major constituent protein of cartilage in diarthrodial joints, the predominant site of inflammation in CIA. Arthritis can be induced in mice by immunization with native CII from either homologous (27, 28) or heterologous (1, 22) origin. Immunization with homologous CII generates a milder and more chronic arthritis than with heterologous CII (27, 28). To date, for historical and technical reasons, most CIA experiments are performed with heterologous CII, usually of rat, chick or bovine origin. Immunization of mice with heterologous CII generates a strong *in vitro* T-cell proliferative response towards the immunogen (29, 30). This CII-specific T-cell proliferative response is poorly crossreactive to autologous CII, and a weak autoreactive response can be detected only in primary cultures when high concentrations of mouse CII are used (30). This implies that the relatively few amino acids that differ between mouse and heterologous CII contain the critical determinants for recognition of heterologous CII by T cells (31).

Site-specific cleavage of CII with cyanogen bromide (CB) results in the production of CII fragments of various lengths in a native form. In $H\text{-}2^q$ mice,

arthritis could be induced by immunization with the CB fragment number 11 (CB11) from bovine or chick CII (32, 33), which represents amino acids 124–402 of CII. CB11 is, in fact, the only known CB segment of CII that is able to confer arthritis in H-2^q mice (32, 33). It is interesting to note that several CB fragments of CII are immunogenic, such as CB5, 8, 9, 10, 11 and 12 (34). However, when mice are immunized with a whole heterologous CII molecule, the T-cell proliferative response of lymph node cells is strongly dominated by T cells recognizing CB11 (34). Our laboratory has shown that the T-cell proliferative response towards CB11 is restricted to amino acids 256–270 of heterologous CII (35), and this finding was later confirmed by peptide scans of bovine and human CII (33, 36). Within this area a shorter peptide, CII(260-267), was shown to induce a significant *in vitro* T-cell proliferation, defining the core residues required for T-cell stimulation (33). It should be stressed that rat, bovine and human CII are identical at position 256–270, and all types of heterologous CII that are used to induce arthritis possess the same amino acid sequence at positions 259–270 (Fig 1a). Importantly, there is a single amino acid difference between mouse and heterologous CII within that region, ie aspartic acid (mouse) vs glutamic acid (heterologous CII) at position 266 of CII (Fig 1a).

Our laboratory has reported that CD4$^+$ T-cell recognition of heterologous CII(256-270) is critically affected by post-translational modifications (hydroxylation and variable O-linked glycosylation) of the lysine residue at position 264 (37, 38). These post-translational modifications were shown to generate at least four different T-cell determinants (Fig 1b) that were each specifically recognized by a distinct T-cell subset (38). It should be noted that the four T-cell determinants resulting from various post-translational modifications of the lysine at position 264 (Fig 1b) are present on CII from all species (mouse, rat, human, bovine and chick) thus far tested (J Bäcklund, R Holmdahl, unpublished observations, 2001). Importantly, after immunization with CII, the majority of CII-reactive T cells responded to CII(256-270) glycosylated with the monosaccharide galactose (Fig 1b). We could thus conclude that this glycopeptide was the immunodominant T-cell determinant on heterologous CII in CIA (38). The critical role of epitope glycosylation in CIA was further supported by the observation that elimination of the carbohydrates rendered CII less arthritogenic (37). It has also been reported recently that a Th1 clone specific for

a) Amino acids 256–270 of type II collagen

Rat, bovine, human	G	E	P	G	**I**	**A**	**G**	**F**	**K**	**G**	**E**	**Q**	G	P	K
Chick	–	–	L	–	–	–	–	–	–	–	–	–	–	–	–
Mouse	–	–	–	–	–	–	–	–	–	–	**D**	–	–	–	–

b) Post-translational modifications of Lysine 264

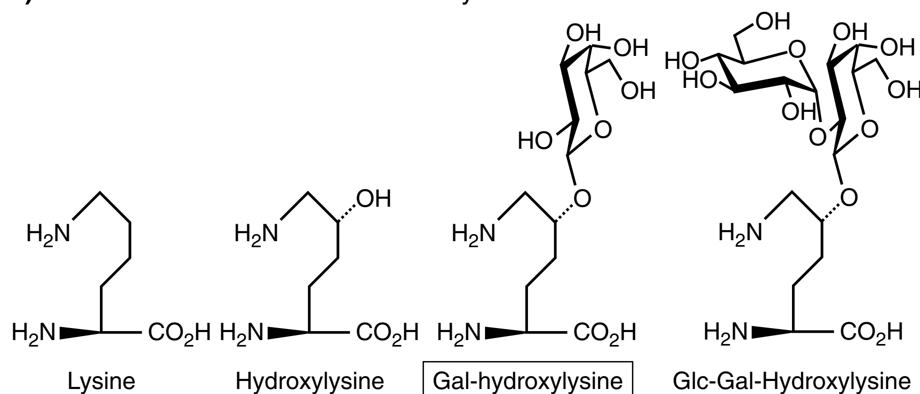

Figure 1. The T-cell determinants on heterologous type II collagen (CII). (a) The T-cell proliferative response in H-2^q mice immunized with foreign CII is mainly directed towards amino acids 256–270 of heterologous CII. The minimum peptide (core), CII(260-267), is shown in bold, and the mouse sequence is aligned for comparison. There is a single amino acid difference between mouse and rat/bovine/human CII within that region, ie aspartic acid (mouse) at position 266 vs glutamic acid (heterologous CII). (b) Post-translational modifications (hydroxylation and variable O-linked glycosylation) of the lysine at position 264 generates four different T-cell determinants that are specifically recognized by distinct T-cell subsets. The framed amino acid represents the post-translationally glycosylated form of lysine 264 that is present in the immunodominant T-cell determinant in collagen-induced arthritis. Gal: galactose; Glc-Gal: glucosylgalactose.

glycosylated CII(256-270) was able to increase the incidence of CIA when transferred to CII-immunized mice (39).

Transgenic expression of a TCRβ chain (Vβ12) derived from a mouse T-cell clone specific for human CII was shown to increase CIA incidence and severity and to result in an earlier disease onset (40). Similarly, transgenic expression of TCR α and β chains (Vβ8.2, Vα11.1) derived from a T-cell clone specific for bovine CII was shown to accelerate the onset of arthritis (41). These experiments in transgenic systems further stress the importance of T-cell recognition of heterologous CII in CIA.

Immunization of mice with heterologous CII generates a strong T-cell response towards the immunogen with only a limited crossreactivity to mouse CII (29, 30). However, in CIA induced with heterologous CII, T cell recognition of mouse CII is believed to be a key event, which would explain the full inflammatory and erosive attack on the joints. In H-2^q mice immunized with rat CII, the proliferative and cytokine (interferon, IFN-γ) responses of lymph node cells were investigated towards peptides (deprived of any post-translational modification) spanning the whole mouse CII sequence (42). More than twenty T-cell determinants were detected on mouse CII, including CII(254-268). Surprisingly, the strongest proliferative response was observed towards mouse CII(707-721), which is located on the nonarthritogenic CB10 fragment (42). The lymph node proliferative response towards CII(707-721) could be abrogated by depletion of $CD4^+$ cells, but whether this response is MHC class II-restricted remains to be shown (42).

The observation that immunization with rat CII results in a significant T-cell response towards mouse CII(254-268) is of special interest as CII(256-270) is the immunodominant T-cell determinant on heterologous CII (33, 35, 36, 38). Immunization with mouse CII(256-270) has been reported to result in a significant proliferative response to the same peptide, demonstrating that mouse CII(256-270) is immunogenic (35). It was also shown that immunization with mouse CII(256-270) results in a strong *in vitro* proliferative response to heterologous CII(256-270), suggesting crossreactivity (35). However, it should be stressed that T-cell hybridomas specific for non-glycosylated heterologous CII(256-270) are usually not crossreactive to mouse CII(256-270) (35, 43); J Bäcklund, R Holmdahl, unpublished observations, 2001). One can therefore predict that the T-cell crossreactivity between heterologous and mouse collagen mainly results from T cells recognizing post-translationally modified CII peptides. A critical issue in CIA will be to determine to what extent T cells recognizing the immunodominant heterologous CII(256-270) peptide glycosylated with a monosaccharide are crossreactive to the mouse homologue.

Role of MHC in CIA

Susceptibility to CIA is linked to genes of the MHC region, as demonstrated with the help of independent MHC haplotype mouse strains that were congenic to the C57.B10 background (2, 22). In H-2^q mice, arthritis development is associated with the MHC class II A^q molecule. This was demonstrated in experiments where CIA-resistant H-2^p mice were made susceptible to CIA by transgenic expression of an Abp gene altered to resemble the Abq gene (44). The $CD4^+$ T-cell proliferative response towards CII is MHC class II-restricted, suggesting that the critical role for MHC class II molecules in CIA is presentation of CII peptides (45). In H-2^q mice, the binding of the CII(256-270) peptide to H-2 A^q was investigated by using affinity-purified MHC molecules and alanine-substituted peptides (46, 47). The side chains of isoleucine 260 and phenylalanine 263 were shown to be critical for binding to the A^q molecule (Fig 2) (46, 47). Antigen presentation assays with analog peptides and CII-specific T-cell lines or hybridomas revealed that the TCR contacts were mainly lysine 264 and, in some cases, also glutamic acid 266 (Fig 2) (33, 46, 48, 49). The crucial role of lysine 264 as a main TCR contact is in accordance with the observations that post-translational modifications of this residue, by hydroxylation and variable glycosylation, have a dramatic influence on the TCR structures selected for recognition (38). Importantly, glycosylation at positions 264 or 270 of CII(256-270) was shown not to change the anchor positions used for binding to A^q, indicating that the presence of carbohydrates did not

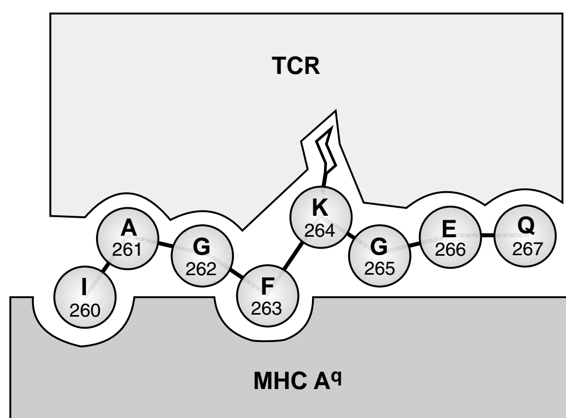

Figure 2. Major histocompatibility complex (MHC) presentation of the glycosylated type II collagen CII(256-270) peptide that is immunodominant in H-2q mice. The core residues (260–267) are shown. The side chains of isoleucine 260 and phenylalanine 263 are the main anchors to the MHC class II H-2 A^q molecule. Lysine 264, which is shown here glycosylated with a monosaccharide, is the major T-cell antigen receptor (TCR) contact residue.

affect the peptide orientation on the MHC molecule (47). The discovery that glutamic acid 266 is a TCR contact is of importance as this residue is the only one to differ between the heterologous and the mouse CII(256-270) sequence. The mouse CII(256-270) peptide (with aspartic acid at position 266) has also been reported to bind to A^q with a lower affinity compared with rat CII(256-270) (35, 47).

TCR selection for recognition of CII

The TCR repertoire used for recognition of heterologous CII in H-2^q mice is strongly influenced by post-translational modifications on CII (38). We have reported that post-translational hydroxylation and variable glycosylation of the lysine residue at position 264 of CII generate at least four different T-cell determinants (Fig 1b) that are each specifically recognized by a distinct T-cell subset (38). As represented in Figure 3, each of the four T-cell determinants appears to select for its own TCR repertoire. The TCRs used by different T-cell subsets vary significantly both in terms of gene segment usage and of amino acid conservation in the complementarity-determining regions 3 (CDR3) (38). Collectively, the TCRs selected for recognition of CII (all four determinants considered) appear to be very heterogeneous (38). To date, TCR sequences from a total of 30 T-cell hybridomas and six T-cell clones, all specific for CII

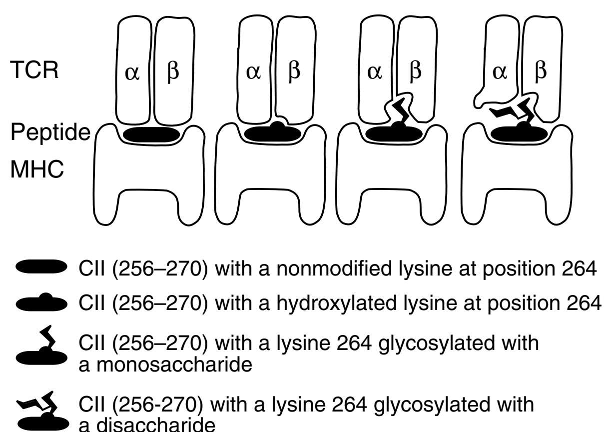

Figure 3. T-cell antigen receptor (TCR) selection for recognition of type II collagen (CII). Post-translational hydroxylation and variable glycosylation of the lysine residue at position 264 of heterologous CII generate four different T-cell determinants that are specifically recognized by distinct T-cell subsets. A specific TCR repertoire is selected for recognition of each of the four T-cell determinants. The TCRs used by various T-cell subsets are very different, both in terms of gene segment usage and of amino acid conservation in the complementarity-determining region 3. MHC, major histocompatibility complex.

and derived from H-2^q mice, have been published (38, 39, 50, 51). These sequence data indicate that as many as seven different TCRVα gene segments (Vα 2, 4, 8, 11, 17, 19, 22) and 10 different TCRVβ gene segments (Vβ 1, 4, 6, 8.1, 8.2, 8.3, 10, 11, 12 and 20) may be used for recognition of CII (38, 39, 50, 51). Altogether, this suggests that the creation of new T-cell determinants by post-translational modification generates a highly diverse TCR repertoire for recognition of CII.

In contrast to the overall extreme diversity, separate examination of the TCR repertoire, selected for recognition of each of the four T-cell determinants on heterologous CII, revealed evidences for recurrent TCR structures. For instance, the TCR α chain sequences from three T-cell hybridomas of DBA/1 origin, which all were specific for the nonmodified CII(256-270) peptide, were remarkably conserved in the CDR3s and all used the Vα2 gene segments (38). The physiological relevance of this observation was shown by *in vivo* antibody-driven depletion of TCR Vα2-positive T cells, which resulted in an inhibition of the *in vitro* T-cell proliferative response towards the nonmodified CII(256-270) but not towards a glycosylated epitope (38). Similarly, we observed recurrent TCR structures amongst T-cell hybridomas specific for the immunodominant CII(256-270) peptide glycosylated with a monosaccharide. For example, two hybridomas specific for the glycosylated peptide had very conserved α chains (Vα4-Jα17), and two other hybridomas had similar TCRs made of Vα17 (named Vα16 in the original paper, according to a difference in nomenclature), Jα20, Vβ10, and Jβ2.5 segments (38). Interestingly, the above-mentioned conserved TCR structure (Vα17/Ja20, Vβ10/Jβ2.5) has recently been found by another laboratory in several T-cell clones that recognized the immunodominant glycosylated CII(256-270) (39). Furthermore, the presence of mRNA transcripts specific for the TCRβ CDR3 of this clonotype was shown in the lymph nodes of the majority of DBA/1 mice with CIA, whereas it was never detected in control animals (39). These data confirm that some recurrent TCR structures are used *in vivo* for recognition of CII, and further support the immunodominance of CII(256-270) glycosylated with a monosaccharide in CIA in H-2^q mice.

The TCR repertoire in CIA has also been investigated with the help of mice harbouring genomic deletions of some tcrb genes. For instance, BUB/BnJ mice, which have the tcrb-a haplotype and consequently lack expression of TCRVβ 5, 8, 9, 11, 12 and 13 as the result of a genomic deletion, were susceptible to CIA (52). Similarly, B10.Q mice congenic for the tcrb-a haplotype developed CIA to the same extent as B10.Q wild-types (tcrb-b) (53). Furthermore, B10.Q mice congenic for the tcrb-c

haplotype, and therefore lacking the TCRVβ 5, 6, 8, 9, 11, 12, 15, 17 and 19, were also susceptible to CIA, but the incidence of arthritis was reduced to 30% (wild-types 70%) and the disease was less severe (53). In contrast, in a F2 cross between B10.RIII (tcrb-b) and RIIIS/J (tcrb-c) there was no linkage of arthritis or of the immune response to the tcrb locus (54). Altogether, these reports demonstrate that none of the TCRVβ 5, 6, 8, 9, 11, 12, 13, 15, 17 and 19 segments is absolutely required for the development of arthritis.

It has been proposed that T cells using the Vβ8.2 domain in their TCRs would play a critical role in CIA (50, 55). This hypothesis is most strongly supported by experiments in which mice were treated with large amounts of mAb. Thus, administration of mAb anti-TCRVβ8 (KJ16, a rat IgG anti-Vβ8.1/2, or F23.2, a mouse IgG1 anti-Vβ8.2) significantly reduced the incidence of CIA in DBA/1 mice (H-2^q) (50, 55). In these experiments, the antibody treatment was shown to decrease the number of Vβ8$^+$ T cells dramatically, suggesting that the cells had been deleted (50, 55). These data are obviously in conflict with the above-mentioned reports showing that mice lacking Vβ8 as a result of a genomic deletion were as susceptible to CIA as wild-types (52, 53). This raises questions about the significance of the experiments based on treatment with anti-TCRVβ8 antibodies. Vβ8$^+$ T cells are known to account for a large portion (12–18%) of the normal T-cell repertoire, and Vβ8.2$^+$ T cells by themselves represent 8–12% of peripheral T cells (50, 55). Therefore, it is possible that antibody-driven depletion of such a large portion of the peripheral T-cell pool may have unpredictable effects, such as the release of cytokines, which in turn may affect the course of CIA.

T-cell tolerance in CIA

Several methods have been used to induce tolerance towards heterologous CII and subsequent protection against induction of arthritis. Thus, intragastric (56, 57), nasal (57–59), or intravenous (60, 61) administration of heterologous CII could suppress CIA. Intravenous injection of CII has been suggested to induce a specific Th2 response (ie secretion of interleukin (IL)-4 and IL-10) towards the antigen (62). Intranasal vaccination has been reported to induce a reduction of the Th1 response (IFN-γ secretion), with (57, 58) or without (59, 63) any corresponding raise in the Th2 response. Any treatment of CIA with CII glycopeptides has not yet been reported. However, in the light of the immunodominance of a glycosylated CII(256-270) peptide in H-2^q mice it is somewhat surprising that CIA could be suppressed by intragastric (64), nasal (58, 59, 63), or intravenous (61) administration of nonglycosylated CII(256-270). It would be of great interest to compare the tolerogenic effect of glycosylated vs nonglycosylated CII(256-270). Complete resistance to CIA was obtained by systemic expression of a transgenic type I collagen construct that had been mutated to contain the heterologous CII(256-270) sequence (65). These transgenic mice were unable to mount a T-cell response towards CII(256-270) after immunization with rat CII (65). Importantly, both T- and B-cell responses towards the whole CII molecule were abrogated in CII(256-270) transgenic mice, demonstrating the immunodominance of this peptide in CIA in H-2^q mice (65).

A critical issue in CIA concerns the nature of T-cell tolerance towards the presumed autoantigen, ie mouse CII. CII has a highly restricted tissue expression as it is only present in cartilage and in the vitreous humour of the eye. As cartilage is a tissue lacking both blood and lymph drainage, this raises the possibility that CII may be a sequestered antigen. Thus, T-cell tolerance towards mouse CII would be achieved by immunological ignorance. Several observations suggest, however, that this is not the case and that mouse CII is to some extent available for the immune system. Immunization of mice with heterologous CII results in a strong *in vitro* T-cell proliferative response towards the immunogen but only in a poor proliferative response towards mouse CII (30). As CII is a phylogenetically very conserved protein, it is striking that foreign T-cell epitopes are predominantly selected after immunization with heterologous CII (31). This strongly suggests that the T cells specific for mouse CII have been tolerized prior to the immunization with heterologous CII.

T-cell tolerance towards CII has been examined in H-2^q mice made transgenic for a mouse CII construct mutated to contain the heterologous rat CII(256-270) sequence (65). In these mice, the immunodominant heterologous CII(256-270) epitope is expressed in a cartilage-specific fashion and is thereby made autologous (65). Immunization of CII(256-270) transgenic mice with rat CII revealed the presence of T cells that could produce IFN-γ and provide B cell help but that proliferated poorly in response to rat CII (65, 66). It was therefore concluded that T cells recognize CII derived from endogenous cartilage and are partially tolerized (65). This tolerance induction was further shown to be epitope-specific (31). Importantly, induction of T-cell tolerance in the transgenic mice was more pronounced towards the nonmodified CII(256-270) than towards the glycosylated form of the epitope (66). The CII(256-270) transgenic mice developed CIA, albeit with a reduced incidence, and thymectomy did not render the mice completely resistant to CIA (65, 66). These data suggest that CIA is mediated by T cells that are partially tolerized to CII rather than by new thymic emigrants that have not yet encountered their antigen (65, 66).

It has been reported that some mature T cells bear two different TCR α chains and thereby two distinct TCRs at the cell surface (67, 68). We have recently reported that in normal mice as much as 8% of total peripheral T cells expressed two surface α chains (69). Dual α T cells have been suggested to play a critical role in autoimmunity. Any dual α T cell may be positively selected by using one TCR-αβ pair. However, it may prove autoreactive via its second αβ combination, particularly if the escape is facilitated by the second receptor being expressed at low density on emergent cells (70). We have recently investigated the importance of dual α T cells in CIA by using mice that are hemizygous for a disrupted TCRα locus ($tcra^{+/-}$) and consequently unable to express two rearranged tcra genes. When we compared $tcra^{+/-}$ mice and wild-type ($tcra^{+/+}$) littermates for the development of CIA we did not observe any statistically significant difference between the groups in the incidence of arthritis, mean day of disease onset, disease course, and mean maximum arthritic score. We could thus conclude that dual α T cells are not required for the development of CIA (69).

Conclusion

$CD4^+$ αβ T cells are crucial for the development of CIA, and it is now clear that post-translational modifications of CII, by hydroxylation and variable glycosylation, are predominantly recognized in $H\text{-}2^q$ mice. The immunodominant T-cell determinant in CIA in $H\text{-}2^q$ mice is a CII glycopeptide, and there is accumulating evidence that arthritogenic T cells in CIA are glycopeptide-specific. Interestingly, in the recent years, post-translational modification of proteins has been implicated in another immunological disorder, ie coeliac disease in humans. In coeliac disease patients, T cells have been shown to recognize gluten peptides that have been deamidated through post-translational modification (reviewed in (71)). There are indications that deamidation *in vivo* is mediated by the enzyme tissue transglutaminase. Notably, tissue transglutaminase can also cross-link glutamine residues of peptides to lysine residues in other proteins including the enzyme itself, which could explain the occurrence of gluten-dependent tissue transglutaminase autoantibodies in coeliac disease (71). These observations in both CIA and coeliac disease suggest that induction of self-tolerance may be rendered more difficult by post-translational modifications of proteins. This raises the possibility that autoimmune disease may be caused by the creation of new epitopes by post-translational modification of proteins under circumstances such as trauma, inflammation or ageing.

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