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A Transient Post-Translationally Modified Form of Cartilage Type II Collagen Is Ignored by Self-Reactive T Cells

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Lysine residues in type II collagen (CII) are normally hydroxylated and subsequently glycosylated in the chondrocyte. The immunodominant T cell epitope of CII involves such post-translationally modified lysine at position 264 that has been shown to be critical in the pathogenesis of murine collagen-induced arthritis and also in human rheumatoid arthritis. In this study we identified a line of transgenic mice expressing a TCR specific for hydroxylated rat CII epitope. They were crossed with transgenic mice expressing the rat CII epitope, either specifically in cartilage (MMC mice) or systemically (TSC mice), to analyze T cell tolerance to a post-translationally modified form of self-CII. The mechanism of T cell tolerance to the hydroxylated CII epitope in TSC mice was found to involve intrathymic deletion and induction of peripheral tolerance. In contrast, we did not observe T cell tolerance in the MMC mice. Analysis of CII prepared from rat or human joint cartilage revealed that most of the lysine 264 is glycosylated rather than remaining hydroxylated. Therefore, we conclude that the transient post-translationally modified form of cartilage CII does not induce T cell tolerance. This lack of T cell tolerance could increase the risk of developing autoimmune arthritis.


Rheumatoid arthritis (RA) is a chronic inflammatory disease, primarily attacking joints containing a synovial joint capsule and hyaline cartilage. The cause of the chronic inflammatory attack is unknown, but there is strong evidence that the immune system and specific immune recognition of joint-related Ags are important factors. Type II collagen (CII), a major protein component of hyaline cartilage, is one of the self-Ags involved in the pathogenesis of RA. Abs against CII are detected in sera of a subset of RA patients (1, 2). Immunization with CII induces chronic arthritis in animals (collagen-induced arthritis (CIA)) that has many similarities with human RA (3). Furthermore, RA has an association with MHC class II molecules that have a shared peptide binding pocket (the shared epitope, e.g., DRB1*0401 and DRB1*0101). Similarly, the development of CIA in mice is associated with an MHC class II haplotype, I-Aβ (4–7). The T cell response in H-2q mice is directed toward the dominant epitope, 256–270 of CII (8). This epitope overlaps with the T cell epitope in DR4 or DR1 transgenic mice (9–11). We have recently found that T cells in RA patients with DR4 or DR1 alleles also respond to the same epitope (12), suggesting involvement of T cell recognition of the dominant CII peptide in the pathogenesis of human RA as well as mouse CIA.

Importantly, CII 256–270 involves a lysine residue at position 264 that can be post-translationally modified. The post-translational modification of CII is a unique process in which lysines are first hydroxylated and thereafter galactosylated (13, 14). These modifications occur in the chondrocyte before the triple helical structure is formed and are dependent on the functional state of the chondrocyte. The efficiency of this process is not known, and it is possible that each lysine, including the lysine at position 264, contains different levels of the various post-translational forms of the side chain. In support of this view, we were able to establish T cell clones with different specificities for the post-translationally modified form of lysine 264 from mice immunized with CII (15, 16). This also indicates that the in vivo T cell response to CII is polyclonal, although the response is restricted to the single dominant peptide in the amino acid sequence. We have found that the T cell reactivity to CII is predominantly directed toward the glycosylated form of the CII peptide in both mice and humans (12, 17). However, the influence of such various modifications of lysine 264 on T cell tolerance to self-CII is not well known.

In mouse CIA, the disease has higher incidence and severity after immunization with heterologous CII (e.g., rat CII) than homologous CII (18). The T cell response to mouse CII is hardly detected, suggesting tolerance of self-CII specific T cells (19). The difference between homologous and heterologous CII within the dominant T cell epitope is only a single amino acid at position 266, i.e., an aspartic acid in homologous CII and a glutamic acid in heterologous CII (e.g., rat and human). Similar to the case of whole CII molecules, T cells respond well to the heterologous CII peptide 256–270, but not to the mouse peptide, suggesting T cell tolerance to self-CII even at peptide levels (20). However, we also found that the mouse peptide has lower binding affinity to I-Aβ molecules. This is an alternative explanation for the hyporesponsiveness of T cells to mouse CII peptide. To test T cell tolerance to self-CII and avoid this complication, we generated a line of transgenic mice expressing mutated CII with the dominant epitope.
of heterologous CII (MMC mice) (21). In these mice the heterologous CII epitope is expressed as a cartilage-specific self-Ag. MMC mice have a lower incidence of arthritis after immunization with rat CII than normal mice. The T cell response to rat CII was also reduced, but not completely diminished, in MMC mice. The T cell tolerance in MMC mice was found to be independent of the presence of the thymus (22). These results suggest that T cell tolerance to CII is indeed induced by the self-Ags expressed in the cartilage, although we have only limited information on the mechanism of the T cell tolerance.

We also generated another line of transgenic mice expressing mutated type I collagen (CI) with the heterologous CII epitope (TSC mice) (21). Consequently, in these mice the CII epitope is expressed systemically (i.e., skin). In contrast to MMC mice, none of the TSC mice developed arthritis after immunization with heterologous CII. There was virtually no T cell response to rat CII in vitro. The result indicates that T cells specific for the heterologous CII epitope are completely tolerized when the epitope is expressed systemically. However, as in the case of MMC mice, the mechanism of T cell tolerance in TSC mice is also unclear.

Studies using TCR transgenic mice have contributed greatly to revealing the mechanisms of T cell tolerance to various self-Ags. The tolerance mechanisms include clonal deletion, clonal anergy, and active suppression (23–28). Self-ignorance is an alternative mechanism preventing the development of autoimmune, even though the self-reactive T cells are not tolerized and maintain their potential to induce the disease in this case (29). In this study we used a line of CII-specific TCR transgenic mice (30) to clarify the mechanism of T cell tolerance to the heterologous CII epitope in either MMC mice or TSC mice. It was revealed that the post-translational modification of CII influences T cell tolerance to self-CII.

Materials and Methods

Mice

The B10.Q mice originated from Dr. J. Klein (University of Tübingen, Tübingen, Germany) and have been maintained as an inbred strain for many years in our animal facility. The MMC-1 (in this work referred to as MMC) and TSC transgenic mice have previously been described (21). 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 heterologous CII256–270 epitope in a CII-restricted fashion. TSC mice express the heterologous CII256–270 within mouse CII sequence. MMC and TSC transgenic mice were backcrossed 10 generations onto the B10.Q background. The anti-CII TCR transgenic founder mouse was provided by Dr. W. C. Ladiges (University of Washington, Seattle, WA) (30). Mice were originally of the DBA/1 background, but were backcrossed eight generations onto the B10.Q background. The TCR transgenic mice were also crossed with MMC or TSC background, but were backcrossed eight generations onto the B10.Q background. The TCR transgenic mouse originated from a TCR Vα/Vβ double-transgenic founder and was backcrossed to B10.Q mice. The TCR genes were derived from a DBA/1 T cell specific for bovine CII (30). However, the epitope specificity and its potent cross-reactivity to mouse CII were originally not determined. Therefore, we first examined the response of T cells in the TCR transgenic mice to rat or mouse CII (Fig. 1a). LN cells from the naive TCR transgenic mice responded to heterologous rat CII; at the same time they showed no cross-reactivity with mouse CII. Because the CII256–270 peptide is the immunodominant peptide in the I-A<sup>q</sup>-restricted T cell response, we investigated the response to the various modified forms of this peptide (depicted in Fig. 2). As shown in Fig. 1b, T cells in the TCR transgenic mice recognized the CII256–270 peptide when the lysine residue at position 264 is hydroxylated. They did not respond to the peptide when the hydroxylated lysine was further glycosylated. Thus, it was revealed that the transgenic TCR is specific for heterologous CII peptide 256–270 with hydroxylated lysine at 264. These results indicate that the TCR transgenic mice can be used as a suitable tool for analyzing T cell tolerance in MMC or TSC mice that express the T cell epitope of heterologous CII. To avoid polyclonal T cell responses to various post-translationally modified forms of CII, we used the hydroxylated CII256–270 peptide instead of whole CII molecules for stimulation of the T cells.

Flow cytometry

FITC-conjugated V<sub>β</sub>8.2 mAb (F23.2) and PE-conjugated anti-TCR β-chain mAb (H57-597; BD Pharmingen, San Diego, CA) were used. The cells were stained with mAbs and analyzed by FACSort flow cytometer with the CellQuest Pro program (BD Bioscience, Mountain View, CA). We added propidium iodide (1 μg/ml) to the cell suspensions before running them on the cytometer to exclude dead cells.

T cell transfer experiment

LN cells were prepared from naive TCR single transgenic mice and pooled. The cells were labeled with a cytoplasmic dye, CFSE (Molecular Probes, Leiden, The Netherlands). Briefly, the cells were incubated 10 min with 5 μM/CFSE in PBS at 37°C. After stopping the reaction by adding an equal volume of FBS, the cells were washed three times with PBS. Then the CFSE-labeled cells (1 × 10<sup>6</sup>) were injected i.v. into naive normal BQ mice, MMC transgenic mice, or TSC transgenic mice. In some experiments, the recipient mice were immunized with 100 μg of CII peptide 256–270 with hydroxylsine at 264 emulsified with an equal volume of IFA (Difco, Detroit, MI) 1 wk after the transfer. Inguinal LN cells of the recipients were harvested and stained with PE-conjugated anti-TCR β-chain mAb (H57-597). Then the cells were analyzed with a flow cytometer. The rest of the LN cells were tested for in vitro response to the CII peptide.

T cell hybridoma assays

T cell hybridoma cell clones specific for a different post-translationally modified form of CH256–270 were generated as described previously (16). The hybridoma cells (5 × 10<sup>4</sup>) were stimulated with corresponding CII peptides, rat chondrosarcoma-derived CII, rat joint cartilage-derived CII, or human joint cartilage-derived CII in the presence of irradiated syngeneic spleen cells (5 × 10<sup>5</sup>) in flat-bottom, 96-well plates. The responses of the hybridoma cells were determined by measuring IL-2 production in the supernatants by ELISA.

Results

Fine specificity of the CII-specific transgenic TCR

The TCR transgenic mouse originated from a TCR V<sub>β</sub>8.2<sup>11.1a</sup>cDNA double-transgenic founder and was backcrossed to B10.Q mice. The TCR genes were derived from a DBA/1 T cell specific for bovine CII (30). However, the epitope specificity and its potent cross-reactivity to mouse CII were originally not determined. Therefore, we first examined the response of T cells in the TCR transgenic mice to rat or mouse CII (Fig. 1a). LN cells from the naive TCR transgenic mice responded to heterologous rat CII; at the same time they showed no cross-reactivity with mouse CII. Because the CII256–270 peptide is the immunodominant peptide in the I-A<sup>q</sup>-restricted T cell response, we investigated the response to the various modified forms of this peptide (depicted in Fig. 2). As shown in Fig. 1b, T cells in the TCR transgenic mice recognized the CII256–270 peptide when the lysine residue at position 264 is hydroxylated. They did not respond to the peptide when the hydroxylated lysine was further glycosylated. Thus, it was revealed that the transgenic TCR is specific for heterologous CII peptide 256–270 with hydroxylated lysine at 264. These results indicate that the TCR transgenic mice can be used as a suitable tool for analyzing T cell tolerance in MMC or TSC mice that express the T cell epitope of heterologous CII. To avoid polyclonal T cell responses to various post-translationally modified forms of CII, we used the hydroxylated CII256–270 peptide instead of whole CII molecules for stimulation of the T cells.

Microcurie of [3H]Thymidine was added to each well for an additional 15–18 h. Cell proliferation was measured by counting the incorporation of [3H]Thymidine. The amount of IFN-γ in the culture supernatant was measured by ELISA, as described previously (32).
arthritis or, in fact, any other autoimmune disease. IFN-

cant difference in proliferation or fi

complete tolerance of T cells to the systemically expressed self-Ag.

We measured the number of transgenic T cells in lymphoid organs

for the transgenic TCR

nor TCR

were only able to measure the number of T cells expressing the

expressed systemically (TSC mice) or in a cartilage-speci

c transgenic TCR.

Thus, central deletion may be involved in the mechanisms of T cell
tolerance in TSC mice, but such a deletional mechanism does not
operate in MMC mice.

No evidence of in vivo recognition of CII by the TCR transgenic
T cells in MMC mice

Because the T cell tolerance observed in MMC single-transgenic
mice is not complete (21), it is possible that a too high frequency
of Ag-specific T cells in TCR transgenic mice overcomes the rela-
tively weak tolerance induction in MMC mice. To test this pos-
sibility, we transferred a limited number of T cells from naive TCR
single-transgenic mice to MMC or TSC single-transgenic mice.
We then analyzed the proliferative response of the transferred T
cells to the CII peptide in vitro and in vivo. To detect the trans-
ferred T cells and also to visualize in vivo division, cells were
labeled with a cytoplasmic dye, CFSE, before the transfer.

After the transfer into TSC mice, TCR transgenic T cells were
clearly detected, because they had spontaneously divided in vivo
(Fig. 5a). Subsequently, they lost their ability to respond to re-
stimulation with the Ag in vitro (Fig. 5b). This indicates that the T
cell epitope is expressed in the peripheral part of the immune sys-
tem of TSC mice as well as in the thymus. It also reveals that
induction of anergy may work as a T cell tolerance mechanism in
these mice. In contrast, the T cells transferred to MMC mice
showed no in vivo division similar to those transferred to normal
mice. In addition, the T cells in MMC recipient mice clearly re-
sponded to the CH peptide in vitro. There was no significant dif-
ference in either the proliferation or the number of transferred T
cells between MMC and normal recipient mice (0.63 ± 0.09 and
0.71 ± 0.14% in TCRαβ+ cells, respectively).

In an additional experiment, we immunized the recipients with
the hydroxylated CII peptide 1 wk after transfer and subsequently
determined the number of dividing T cells in the draining LNs
(Fig. 6a). However, we found no differences between MMC and
normal mice in either dividing or nondividing cells (Fig. 6b). The

Tolerance of the TCR transgenic T cells in TSC mice, but not in
MMC mice

To analyze the T cell tolerance to the heterologous CII epitope
expressed systemically (TSC mice) or in a cartilage-specific man-
ner (MMC mice), we crossed the TCR transgenic mice with TSC
or MMC mice on B10.Q (H-2q) background. Neither TCR × TSC
nor TCR × MMC double-transgenic mice developed spontaneous
arthritis or, in fact, any other autoimmune disease.

We compared the responses of LN cells in naive TCR single-
transgenic mice and TCR × MMC or TCR × TSC double-trans-
genic mice to the CII peptide (Fig. 3). The LN cells of TCR × TSC
double-transgenic mice showed no proliferative response or cyto-
kine production to the heterologous CII peptide, indicating com-
plete tolerance of T cells to the systemically expressed self-Ag.
Furthermore, there was no significant difference in proliferation or
IFN-γ production between TCR single-transgenic mice and TCR ×
MMC double-transgenic mice. We also tested the T cell response
after in vivo immunization with the CII peptide, but again there
was no difference in the response of T cells in TCR single-trans-
genic mice and TCR × MMC double-transgenic mice (data not
shown). T cells in TCR × TSC mice did not respond to CII peptide
even after immunization (data not shown). Thus, the heterologous
CII-specific TCR transgenic T cell did not show any tolerance to
the T cell epitope expressed specifically in the cartilage.

Involvement of central deletion as a mechanism of T cell
tolerance in TSC mice

We measured the number of transgenic T cells in lymphoid organs
of naive mice by FACS analysis. Unfortunately, as mAb specific
for the transgenic TCR α-chain (Vα11.1a) is not yet available, we
were only able to measure the number of T cells expressing the
transgenic TCR β-chain (Vβ8.2). Consistent with the data of T cell
response, we observed no significant difference in the number of
Vβ8.2-expressing T cells from lymph nodes between the TCR
single-transgenic mice and TCR × MMC double-transgenic mice
(Fig. 4a). In contrast, there was a significant reduction of Vβ8.2-
expressing T cells in the LN of TCR × TSC double-transgenic
mice compared with TCR single-transgenic mice (p < 0.05). The
reduction of Vβ8.2+ T cells in TSC mice was also observed in the
thymus, but there was no difference between TCR single-trans-
genic mice and TCR × MMC double-transgenic mice (Fig. 4b).
Thus, central deletion may be involved in the mechanisms of T cell
tolerance in TSC mice, but such a deletional mechanism does not
operate in MMC mice.

No immunodominant CII 256–270 epitope. The lysine residue
at position 264 (K, nonmodified; HK, hydroxylated; Gal-HK, hydroxylated
and galactosylated). Cell proliferation was measured as described in

FIGURE 1. Ag specificity of the CII-specific transgenic TCR. a, LN
cells of naive TCR transgenic mice were cultured with various concen-
trations of rat (○) or mouse (●) CII for 96 h. Cell proliferation was measured
by counting the incorporation of [3H]thymidine pulsed for the final 18 h.
Data are shown as the stimulation index (cpm with Ag/cpm without Ag).

b, LN cells of naive TCR transgenic mice were cultured with various con-
centrations of rat CII peptide with different modi-
fications of lysine residue
at position 264 (K, nonmodified; HK, hydroxylated; Gal-HK, hydroxylated
and galactosylated). Cell proliferation was measured as described in a.

FIGURE 2. The immunodominant CII 256–270 epitope. The lysine residue
at position 264 of CII is variably post-translationally modified in the chondro-
cyte by hydroxlation, followed by galactosylation of hydroxysine (de-
picted with the O-linked β-galactose). Mouse CII expresses aspartic acid
at position 266, whereas heterologous CII (e.g., rat and human) expresses
glutamic acid at this position.
Lack of hydroxylated lysine in the dominant epitope of CII in joint cartilage

From these data we concluded that T cells specific for hydroxylated CII are immunologically ignorant of CII in cartilage. However, this is incompatible with previous data obtained from MMC single-transgenic mice, where T cells responded poorly to heterologous CII (21). One of the differences between these systems is that the CII-specific T cells are monoclonal in TCR × MMC double-transgenic mice, whereas the responding T cells are more polyclonal in MMC single-transgenic mice. As shown in Fig. 1, the transgenic TCR is specific for hydroxylated CII peptide. One possible explanation for the discrepancy is a selective lack of hydroxylated CII in cartilage. Because the rat CII used in the previous experiment was prepared from a rat chondrosarcoma, it was of interest to examine whether natural joint cartilage-derived CII also stimulates T cells for hydroxylated CII peptide. However, it is difficult to collect enough joint cartilage CII from MMC mice. Therefore, we compared various T cell hybridoma clones with different specificities for post-translational modification of CII in their responses to rat CII derived from joint cartilage or chondrosarcoma. T cell clones specific for the glycosylated peptide responded to both chondrosarcoma-derived and cartilage-derived rat CII (Fig. 7c). However, the hydroxylated peptide-specific T cells responded only to chondrosarcoma-derived, not to cartilage-derived, rat CII (Fig. 7b). Therefore, it is likely that the lack of T cell tolerance in the TCR × MMC double-transgenic mice is explained by the absence of hydroxylated lysine 264 in cartilage CII in vivo. We also tested CI prepared from the skin of TSC mice using a similar assay. In this case, hydroxylated peptide-specific clone responded to CI from TSC mice (data not shown), which is consistent with the induction of tolerance to the TCR transgenic T cells in TSC mice.

We also prepared human CII from the joint cartilage of six different individuals and tested the responses of T cell hybridomas to these samples. In this experiment we used heterologous CII peptides with different modifications of lysine 264 as controls. It should be noted that there is no difference in the amino acid sequence between human and rat CII within this T cell epitope. All the T cell clones responded well to the corresponding CII peptides, indicating that there is almost no difference in the binding capacities of different peptides to class II molecules. However, there was a large difference in the response to human cartilage CII between the T cell clones. Nonmodified peptide-specific clone responded weakly to human CII (Fig. 8a), whereas the glycosylated peptide-specific clone responded vigorously to all human CII samples (Fig. 8c). In striking contrast, T cells specific for the hydroxylated peptide did not respond to human CII (Fig. 8b). All six different human cartilage samples showed the same pattern, indicating an invariable glycosylation at position 264. These data indicate that most of the lysine residue at 264 of CII, even in human joint cartilage, is glycosylated, whereas there are almost no CII molecules with hydroxylated lysine at 264.

Discussion

We have previously reported (21) that transgenic mice expressing heterologous CII epitope specifically in cartilage (MMC mice) or

![FIGURE 3. T cell responses in TCR MMC or TCR TSC double-transgenic mice. Inguinal LN cells from naive TCR single-transgenic (n = 5), TCR MMC double-transgenic (n = 5), or TCR TSC double-transgenic (n = 5) mice were cultured with or without 10 μg/ml rat CII256–270 peptide with hydroxyllysine at position 264. a, Cell proliferation was measured as described in Fig. 1. b, IFN-γ in the culture supernatants was measured by ELISA.](http://www.jimmunol.org)
systemic (TSC mice) display T cell tolerance to the immunodominant CII epitope. In this study we used heterologous CII-specific, TCR transgenic mice to clarify the mechanism of T cell tolerance in these mice.

We found that the T cell tolerance in TSC mice involves central tolerance (intrathymic deletion) as well as peripheral tolerance (induction of anergy). The latter may serve as a failsafe mechanism of self-tolerance for T cells that escape the central tolerance. The results seem reasonable, because in TSC mice the heterologous CII epitope is expressed on CI, which is a major component in connective tissues, and is expressed systemically, thus including the thymus. Previously, we had confirmed the expression of mRNA of the transgenic TSC CI in the thymus (21), although we did not know where the CII epitope was actually presented to T cells and, therefore, did not know the mechanism of T cell tolerance in these mice. To our knowledge, this is the first report showing in vivo presentation of the transgenic CII epitope in the thymus, as evidenced by the deletion of Ag-specific T cells in the thymus. This is possible through the use of transgenic TCR mice, because these enabled visualization of Ag-specific T cells. We also observed the expression of TSC CI mRNA in other organs, such as spleen and skin, that may be involved in the induction of peripheral anergy in vivo. These results are of importance in helping to understand the generation of T cell tolerance to systemically expressed extracellular matrix proteins.

In contrast, we did not observe any evidence of T cell tolerance in TCR × MMC double-transgenic mice. Tolerance of the TCR transgenic T cells was not observed in a T cell transfer experiment. This transfer of cells is a widely accepted method to reduce the unnaturally high frequency of Ag-specific T cells in TCR transgenic mice (33). These data are in contrast with the results from MMC single-transgenic mice, in which T cell tolerance to the CII epitope was observed (21, 22). The T cell tolerance in MMC single-transgenic mice indicates that the heterologous CII epitope is actually presented to T cells in vivo. Similar to MMC mice, we have also observed T cell tolerance to heterologous CII in human CII transgenic mice (34). One of the major differences between these studies is the clonality of the responding T cells; in the TCR × MMC double-transgenic system the responding T cells are monoclonal, whereas there is a polyclonal T cell response to CII in MMC single-transgenic mice or human CII transgenic mice. At this point, post-translational modification of CII molecules would play an important role. T cell clones with different specificities for the post-translational modification of the immunodominant epitope respond to CII in MMC single-transgenic mice (15, 16), whereas the TCR transgenic mice that we used in this study are specific only for the hydroxylated CII epitope (Figs. 5 and 6) and consequently are not tolerized. In addition, we have observed, using another line
of TCR transgenic mice, that glycosylated CII-specific T cells are tolerized in MMC mice (35). This suggests that the glycosylated CII epitope is indeed expressed in vivo. Even though the variable post-translational modification of lysine residues in CII molecule is known (13, 14), it is difficult to establish by biochemical methods the extent of modification of a lysine residue at a particular position. The use of T cell clones specific for different post-translationally modified forms of rat CII256–270 makes it easy to solve this problem (Figs. 7 and 8). We revealed that most of the lysine in rodent as well as human cartilage CII is glycosylated in vivo. In our view this is the first study to show the predominance of a particular form of post-translational modification of CII molecule in natural cartilage.

This result provides important information on the role of post-translational modification of CII in the pathogenesis of autoimmune arthritis. Firstly, glycosylated forms of CII have been suggested to be the major target of immune responses to cartilage CII. Neonatal treatment with glycosylated CII peptides was superior to CII peptides with other modifications in protecting mice from CIA development (17). The T cell response to CII is skewed toward the glycosylated form of CII peptide in “humanized” transgenic mice that express DR4, human CD4, and human CII (12). These results seem reasonable, because most of the cartilage CII is in glycosylated form. Importantly, this is not only observable in murine models of arthritis, but also in human RA. We have recently shown that in RA patients with the arthritis-susceptible DR4 (DRB1*0401) or DR1 (DRB1*0101) alleles, T cells recognize mainly glycosylated CII and that DR4-restricted T cell clones predominantly recognize the side chain of lysine 264 (12). The predominance of glycosylated lysine at position 264 of human cartilage-derived CII (Fig. 8) is consistent with these data. Secondly, the lack of hydroxylsine in the T cell dominant epitope of CII molecules and the resulting lack of T cell tolerance suggest that T cells are immunologically ignorant of the presence of the self-Ag transiently expressed on cartilage. Consequently, these T cells are easily activated and may induce an autoimmune attack on joint cartilage once the epitope is generated and exposed at a certain level. Immunological self-ignorance was first demonstrated more than a decade ago in a study using transgenic mice expressing a viral Ag in the pancreas (29). The mice suffered from autoimmune insulitis after an infection with the virus, followed by activation of the neo-self-Ag-specific T cells. This clearly indicates a possible development of autoimmune disease by self-ignorant T cells. It is of note that such T cell ignorance is observed only when self-Ags are expressed at lower than certain levels (36). Higher dose of self-Ags induces T cell
tolerance. Lysine residues in CII molecule are first hydroxylated, thereafter galactosylated (13, 14). These modifications occur in the chondrocyte before the triple helical structure is formed. At present, we do not know under what conditions the levels of post-translational modification of CII changes in vivo and the resulting outcomes. Additional studies are required on the role of changes in post-translational modification of CII in T cell tolerance and the development of arthritis.

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