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T Cell Immunity to Type II Collagen in the Biobreeding Rat: The Identification and Characterization of RT1^u-Restricted T Cell Epitopes on α1(II)[1]

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Susceptibility to experimental collagen-induced arthritis in rodents is dependent on MHC class II elements to bind peptides from the type II collagen (CII) molecule. Although a substantial body of data has been reported in mice defining these peptide Ags, little has been reported in rats. In this study, we investigate the locations and sequences of CII peptides, which are bound by RT1^u molecules, expressed by diabetic-resistant, arthritis-susceptible Biobreeding rats, and, in turn, stimulate CII-specific T cells. By using overlapping and substituted peptide homologues of CII, we have identified and characterized an immunodominant and five subdominant epitopes on CII, which stimulate RT1^u-restricted T cell proliferation. The immunodominant epitope, CII (186–192), contains a QGPRG core sequence, which was found in a subdominant epitope CII (906–916). Similar sequences containing single conservative substitutions were identified in three other epitopes. One, CII (263–272), contained a conservatively substituted R→K substitution, whereas CII (880–889) and CII (906–916) contained nonconservative substitutions, i.e., P→D and R→M, respectively. Homologue peptides containing these sequences stimulated T cell proliferative responses, although less intensely than peptides containing CII (186–192). Substituting QGR residues in the QGPRG core with alanine, isoleucine, or proline reduced proliferation, as did substituting flanking E and G residues at the N terminus and E at the C terminus. Collectively, these data indicate that RT1^u-restricted immunodominant and several subdominant epitopes on CII often share a QGPRG-like motif, with conservative substitutions present at either P or R positions. This motif is similar to one recognized by collagen-induced arthritis-susceptible HLA-DR1- and HLA-DR4-transgenic mice. ***The Journal of Immunology*****, 2004, 173: 1795–1801.**

Type II collagen (CII),[3] the major constituent protein of articular cartilage, is a potent immunogen and arthritogen both in rodents and nonhuman primates, and has been implicated as an autoantigen in the pathogenesis of rheumatoid arthritis (1–4). Studies performed in rodents show that susceptibility to collagen-induced arthritis (CIA) is linked to the expression of certain MHC class II alleles (2, 5–9). The importance of B cell immunity in the pathogenesis of CIA has been clearly demonstrated by the passive transfer of erosive arthritis to normal rats and mice with mAbs specific for CII (10, 11). Thus, while the adoptive transfer of T cells has been largely unsuccessful in transferring CIA, T cells are nonetheless critical to the production of arthritogenic Abs, given that CII is a T-dependent Ag, and may help perpetuate disease once arthritis has been initiated by Ab (12).

Compared with inbred mice and mice bearing human class II DR1 and DR4 transgenes, little is known about rat MHC and T cell recognition of CII, even though the number of susceptible rat strains is substantially greater (13, 14). To determine the number, location, and primary structure of T cell determinants present on the CII molecule, we have studied immunity to CII in the Biobreeding (BB) rat (RT1^u), which is highly susceptible to CIA. This rat also expresses the same shared epitope found in DRB1*0101, DRB1*0401, DRB1*0404, and DRB1*0405 alleles, which are associated with increased risk for rheumatoid arthritis (9, 15, 16). Data presented in this work show that human, bovine, and rat CII contain a common immunodominant determinant, CII (186–192), found in cyanogen bromide (CB) peptide fragment 11 (CB11). Moreover, we identified five other subdominant epitopes present in CB12, 11, 10, 5, and 9. The minimal essential and core sequences needed to stimulate T cell proliferation were ascertained using overlapping 15-mer oligopeptides, which advanced by a single amino acid. Key residues, within these regions, were identified using substituted peptides in which successive residues were replaced with alanine or isoleucine. These data show that the BB rat recognizes a number of unique T cell determinants on CII important in the development of immunity to CII and, consequently, the induction of autoimmune arthritis. Finally, several of the epitopes characterized share a QGPRG-like motif, which is similar to epitopes described in CIA-susceptible DR1- and DR4-transgenic (Tg) mice, as well as a subdominant epitope overlapping with one recognized by DBA/1 mice.

Materials and Methods

Animals

Diabetic-resistant BB male and female rats were obtained from the University of Massachusetts (Worchester, MA) and have been maintained at this facility for ~6 years. Rats in groups of three to four are housed in polystyrene cages in a controlled environment with 12-h cycles of light and

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[3] Abbreviations used in this paper: CII, type II collagen; BB, Biobreeding; CB, cyanogen bromide-generated peptide; CI, type I collagen; CIA, collagen-induced arthritis; Tg, transgenic; WT, wild type.

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dark. Standard rodent chow and water are provided ad libitum; all studies described in this work used rats weighing ≥125 g. Housing and animal studies were conducted according to American Association of Laboratory Animal Care Guidelines. Previous studies by our laboratories show that male and female BB rats are equally susceptible to CII-induced arthritis and develop very severe disease (our unpublished observations).

Preparation of collagens, CB peptides, and synthetic oligopeptides

CII was prepared from fetal bovine articular cartilage, rat articular cartilage, and human costochondral cartilage by pepsin digestion, as reported earlier (17); CII peptides were isolated and purified after cyanogen bromide digestion by ion-exchange and molecular-sieve chromatography (17). CII homologue peptides 24–28 residues long were synthesized in bulk using F-moc chemistry and a solid-phase procedure (Applied Biosystems, Foster City, CA; model 430), as reported elsewhere (18). The 15-mer peptides of human CII were prepared by Mimotope peptide synthesis so that each peptide overlapped with the preceding one by either 12 or 14 aa (9). The published sequence of human CII reported by Ala-Kokko and Prockop (19) was used for this purpose; rat CII sequences were obtained from reports by Michaelsson et al. (20) and Urabe et al. (21). Alanine, isoleucine, and type I collagen (CI) residue-substituted 16-mer CII peptides were prepared by Chiron (Raleigh, NC).

Immunization protocol

Native CII was dissolved in 0.01 M acetic acid (4 mg/ml) at 4°C and emulsified in IFA (Difco, Detroit, MI) (17). Each rat was injected once intradermally at several sites with 300 μg of CII at the base of the tail; arthritis typically appeared 9–16 days later. Virtually 100% of rats developed arthritis; with exception to those studied at day 7, all others were arthritic.

Production of long-term T cell lines

Draining inguinal lymph nodes were excised 14 days after immunization, disbursed gently into single cell suspensions, washed in HBSS, and resuspended in DMEM containing 1% fresh rat serum, 5×10^{-5} M 2-ME, 4 mM L-glutamine, nonessential amino acids, 10 mM HEPES, and penicillin/streptomycin. The cells were cultured with heat-denatured bovine CII (50 μg/ml) in 24-well microtiter plates in an environment of humidified air at 37°C in 5% CO_2. Seventy-two hours later, activated lymphoblasts were isolated on a discontinuous gradient of Histopaque (Sigma-Aldrich, St. Louis, MO) by centrifugation and expanded in propagation medium at a concentration of 5×10^5 cells/ml. The latter medium consists of DMEM supplemented with 2-ME, 10 mM HEPES, L-glutamine, antibiotics, nonessential amino acids, 10% FBS, and 15% (v/v) of a IL-2-enriched DMEM, which was produced by stimulating normal rat spleen cells (5×10^6/ml) with Con A (2 μg/ml) in DMEM containing 10% FBS (Invitrogen Life Technologies, Gaithersburg, MD) for 48 h. The supernatant was passed through a Sepharose column twice before use. After 10 days' culture in propagation medium, T cell blasts were recovered, as described, adjusted to a 5×10^5 cells/ml concentration, and cocultured with a 40-fold excess of γ-irradiated (2000 rad) normal syngeneic thymus APC in medium containing denatured CII. Three days later, the T cell lymphoblasts were recovered and cultured in medium containing IL-2. The T cell line was maintained by repeating this cycle every 2 wk. Later, the parent line BB1 was cyclically stimulated with oligopeptide CII (181–210) and IL-2 to produce T cell sublines. The phenotype of line BB1 was solely $CD4^+$ as determined by flow cytometry analysis using fluorescein-conjugated mouse anti-rat CD4 and CD8 mAb (Invitrogen Life Technologies).

T cell proliferation assay

Draining lymph nodes were excised and gently disrupted, and the cells passed through a nylon wool column (22). T cells recovered from the filtrate were cultured for 72 h in 96-well microtiter plates in a humidified atmosphere containing 5% CO_2. A total of 5×10^5 cells was added per well along with a 4-fold excess of γ-irradiated APC (2×10^6 cells/well) and 25 μg of CII or synthetic peptide. Fifteen- and 16-mer Mimotope peptides were dissolved in 600 μl of 0.05 M HEPES and acetonitrile, respectively; 5–20 μl of these solutions was added to microtiter wells, as determined by preliminary studies. Proliferation studies used DMEM supplemented with 1% fresh rat serum, 5×10^{-5} M 2-ME, L-glutamine, and amino acids, as previously reported (22). Aliquots of 0.05 μCi of [^{3}H]thymidine were added to the wells 72 h later; the cells were harvested 16 h later onto fiberglass filters. DNA-incorporated [^{3}H]thymidine was measured using a Matrix 96 direct beta ionization counter (Packard Instrument, Meriden, CT). These data are expressed as mean dpm. All assays using single-well cultures were confirmed by a second study.

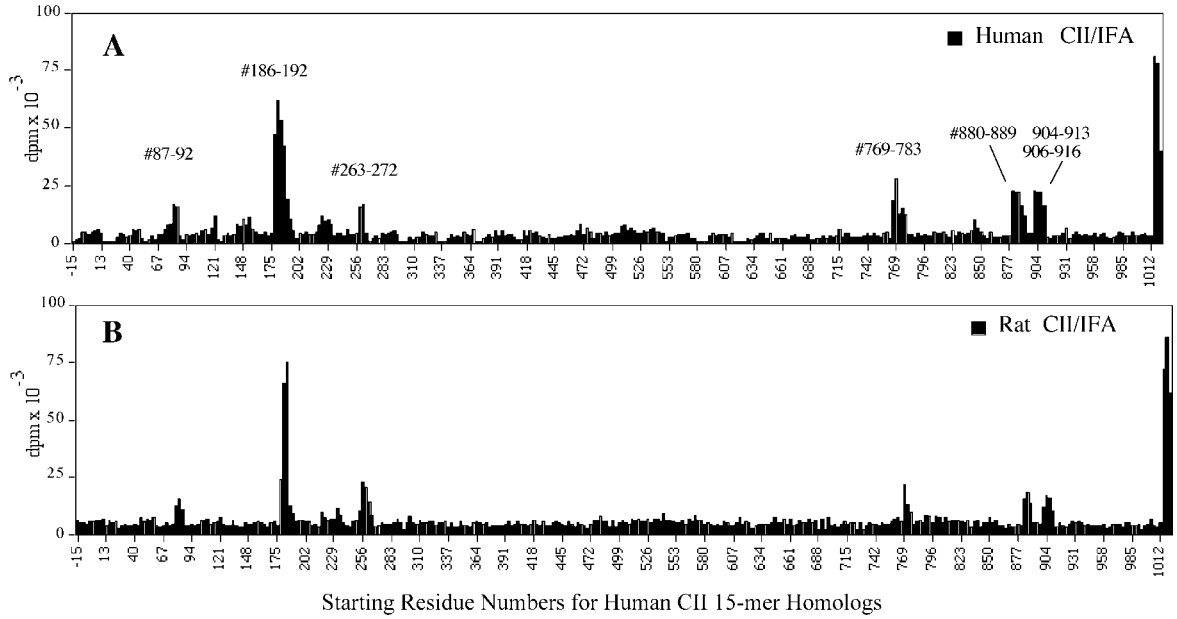

FIGURE 1. Identification of immunodominant and subdominant epitopes of human α1(II) presented to RT1^u. T cells were obtained from BB rats immunized 14 days earlier with human CII (*A*) or rat CII (*B*) and tested for their ability to proliferate on culture with a panel of 15-mer Mimotope peptides, which overlap by 12 residues. The *x*-axis indicates the N-terminal starting position of each peptide homologue shifting by increments of three residues. The [^{3}H]thymidine incorporation of each peptide is shown on the *y*-axis. A background value of 1200 dpm was obtained when medium was added in place of 10 μl of solution containing peptide. The numbers shown above selected peaks indicate the location of 15-mer peptides, which yielded the greatest stimulation. Virtually identical results were obtained with BB rats immunized with bovine CII (data not shown). The three bars to the *extreme right end* of the figure show T cell proliferative responses to native human, bovine, and rat CII, respectively.

Table I. *Mimotype peptides that evoke T cell proliferation, location, and deduced epitope sequence using human α1 (II) sequence*

Parent 15-mer CII Peptide No.	CNBr Region[a]	15-mer Peptide Sequences[b]	Deduced Epitope	≈ Core Length	Proliferation Response[c]
Human CII (82–96)	CB12	GLBGV**KGHRG**YBGLD	87–92	6	15,500
(Rat)		----------D---			
Human CII (181–195)	CB11	GARGP**EGAQGPR**GEB[d]	186–192	7	42,200
(Rat)		----------S-----			
Human CII (259–273)	CB11	GIAG**FKGEQGPKGE**B[e]	263–272	10	20,300
(Rat)		---------------T			
Human CII (880–894)	CB10	**GPTGKQGDRG**EAGAQ	880–889	10	22,900
(Rat)		---------------			
Human CII (889–903)	CB10–5	GE**AGEQGPMGPSGPA**	892–903	13	18,300
(Rat)		---------------			
Human CII (904–918)	CB5–9	**GARGIQGPQGPRG**DK	904–916	13	17,400
(Rat)		-----A---------			

[a] CB peptide regions are ordered as they appear from the N to the C terminus of the CII molecule.

[b] The epitope in CII (769–873) is not shown, because attempts to define it using single-residue-shifted Mimotope peptides were not successful. However, 15-mer peptides containing GQRGIV stimulated T cell proliferation responses two to three times above background values. The seventh epitope that could not be characterized, CII (775–786), is not shown above; however, the 15-mer peptide (based on human CII) that produced the greatest response was GPBGPQGLAGQRGIV. This sequence is the same as reported in the rat (21).

[c] Proliferation responses to selected 15-mer peptides (Fig. 1) using T cells from BB rats immunized 14 days earlier with rat CII. Deduced epitope core sequences are shown in boldface type; regions of potential commonality are underlined and aligned for comparison. Background values using medium in place of Ag ranged between 2600 and 5400 dpm, and were subtracted from the response to the corresponding peptide.

[d] Sequence 181–195 is identical for human, bovine, and rat CII; chick differs in the substitution of hydroxyproline (B) with serine.[4]

[e] Sequence 259–272 is identical for human, bovine, and chick CII.[4] The rat CII sequence is identical, except that "B" (hydroxyproline) is replaced by a threonine residue.

Results

Localization of T cell determinants on the α1(II) chain of CII

T cell determinants within the CII sequence were identified by measuring the proliferative response of lymphocytes cultured with a panel of Mimotope peptide homologues spanning the entire length of human α1(II) chain as it is expressed in cartilage. Each 15-mer Mimotope peptide overlapped with the previous one by 12 residues and advanced from N to C terminus by three residue shifts. Lymphocytes were isolated from arthritic BB rats immunized 14 days earlier with human or rat CII in IFA. As shown in Fig. 1, an immunodominant peak is prominent in peptide CII (181–195) found in CB11, while at least five other smaller reproducible peaks are also shown. Notably, responses in rats immunized with rat CII closely mirrored those of human CII-immunized animals, indicating remarkable cross-reactivity between the two collagens and a lack of tolerance to rat CII. The sequences of the 15-mer peptides, which produced the greatest proliferative response for each T cell determinant, are shown in Table I. An additional peak was found in peptide CII (769–783), but proved to be too variable to be confirmed as a seventh determinant, particularly when rat CII was used as an immunogen. With this exception, T cell responses to the other peptides were consistent and easily detected at days 14, 21, and 28. Evidence of epitope spreading was not noted during the time frame of these studies. Not unexpectedly, day 7 responses were weaker than those of later dates; however, CII (181–195) responses were easily detected (data not shown). Additional studies using bovine CII-immunized BB rats yielded results virtually identical with those shown in Fig. 1, as did limited studies with Wistar-Furth (RT1^u) rats.

Characterization of the determinant core sequences essential for T cell proliferation

To identify the core sequences of CII determinants identified in the previous study, T cell proliferation studies were repeated in BB rats using overlapping 15-mer Mimotope peptides, which advanced by a single residue. Second, to investigate the cross-reactivity between rat and human CII determinants, rat CII was used as an immunogen and human CII peptide homologues as Ags. Data presented in Fig. 2 show the result of these studies, which are also summarized in Table I. The sequence EGAQGPRG, CII (186–192), proved essential for the immunodominant determinant to stimulate T cell proliferation. Overall, the primary sequences of core regions of each epitope were unique, except for QGPRG, which was shared by CII (186–192) and CII (906–916). The QGPR sequence is also found at CII (72–75) and CII (393–396); however, peptides representing these sites did not stimulate T cell proliferation. Two conservatively substituted sequences, both similar to QGPR, were also identified: QGPK is present in epitope CII (263–272), and QGDR in CII (880–889). Epitopes CII (894–903) and CII (904–918) were in such close proximity to each other that single-shift peptides were required to identify them as separate entities. The one epitope found in CB12, KGHRGY, CII (87–92), was the smallest and arguably the most unique; peptides CII (904–916) and CII (892–903) contained the largest epitopes.

Identification of key residues within core determinants CII (186–192) and CII (263–272) essential for T cell activation

To identify the key residues within the core regions of CII (186–192) and CII (263–272) essential for T cell stimulation, panels of alanine-substituted 15- and 16-mer peptides were prepared. Because peptide CII (181–196) contains two naturally occurring alanine resides, they were replaced with isoleucine. Additional CII (181–196) substituted peptides were generated using residues that correspond to those found at the same position in nonarthritogenic CI. A CII-specific T cell line to epitope CII (181–196) was used to measure the effect of each amino acid substitution (see Fig. 3). This line responds robustly to native bovine, human, and chick CII as well as synthetic CII peptides. Peptide analogues of CII (259–274) were tested using CII-immune lymph node cells, because a stable T cell line could not be established to this epitope. This determinant was selected for further investigation, because of its similarity with the immunodominant epitope recognized by arthritis-susceptible DBA/1 (23) and HLA-DR1- and HLA-DR4-Tg mice (9, 24), as shown in Table II.

Data shown in Fig. 3*A* illustrate that substituting wild-type (WT) residues at positions 186E, 187G, 189Q, 190G, 192R, and 194E

[4] Amino acid sequences for bovine, human, and mouse CII, in addition to different species sources of α1(I) and α2(I) may be viewed at: www.utmem.edu/ctr/RDRCC_CollagenCore/

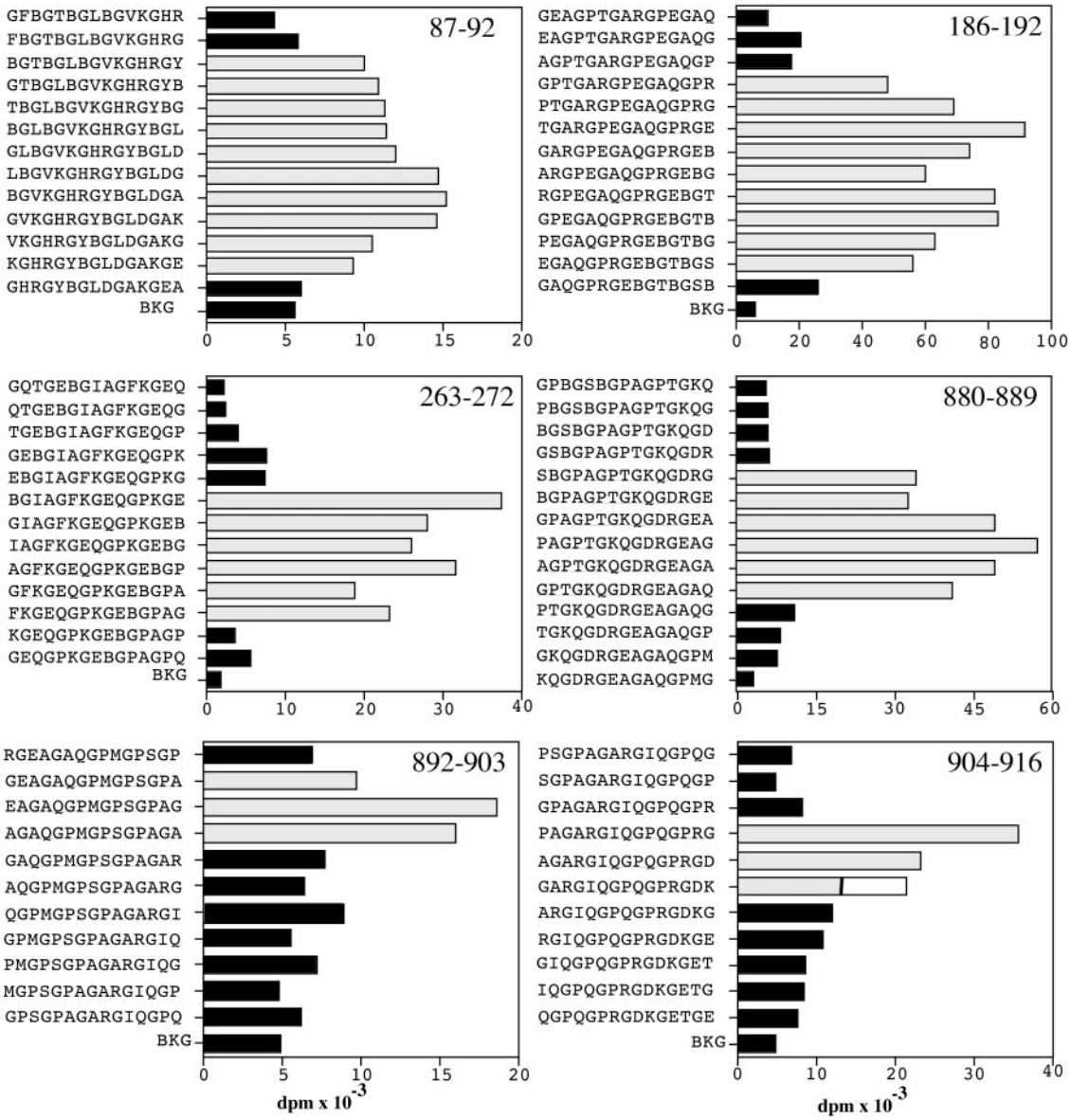

FIGURE 2. Identification of core sequences of human α1(II) presented by RT1^u. Studies essentially the same as those shown and described in Fig. 1 were performed, except that BB rats were immunized with rat CII instead of bovine, and 15-mer peptide homologues advancing by single residues were used in place of those advancing by three residues. The peptides showing the greatest response, used to define core determinants, are cross-hatched. One bar shown in the *lower right panel* CII (904–916) has an open extension, which indicates the response when bovine CII was used as the immunogen. The remaining two cross-hatched bars were not altered, because responses elicited by bovine CII were so similar to rat. Numbers shown at the *top* of each graph represent the core sequence of each peptide homologue deduced from the experiment. Data shown on the *x*-axis represent [^{3}H]thymidine incorporation expressed as dpm $\times$ 10^{-3}.

with alanine, and isoleucine at 188A, greatly reduced the ability of the substituted peptides to stimulate the T cell line specific for CII (181–196). Comparable results were obtained with CII-immune lymph node cells (data not shown). The importance of residue 188A was further demonstrated by substitution at this position, and others nearby, with amino acids found at the same position in CI (Fig. 3*B*). All peptides containing 188A→P failed to stimulated proliferation, unlike peptides containing single, double, or triple 182A→P, 185P→S, or 191P→V substitutions, which produced responses similar to WT peptide. The negative effect of 188A→P substitution on proliferation was greater than the isoleucine substitution. Data shown in Fig. 4 demonstrate that residues within CII (266–271) (EQGPKG) are critical for T cell stimulation. There was substantial loss of proliferative activity when residues with substitutions were made in any one of these residues, in addition to a modest loss when 272E was substituted. Modest changes were noted when 260I→A and 262G→A substitutions were made.

Discussion

Our studies reported in this work demonstrate that the BB rat (RT1^u) is capable of recognizing at least six definable epitopes on human and rat CII, as determined by the ability of synthetic CII peptides to stimulate T cell proliferation. Of the six epitopes detected, CII (186–192) proved immunodominant and is located within α1(II)-CB11. This finding expands an earlier finding by Ku et al. (25), who studied Louvain rats (RT1^u) and reported an epitope that we have identified and characterized as CII (186–192). Of the five other epitopes, only one, CII (263–272), was also found in CB11, whereas the others were located in CB12, CB10,

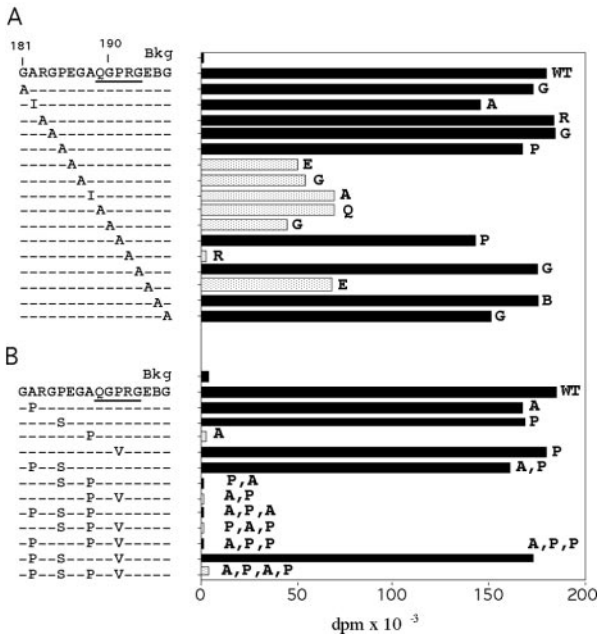

FIGURE 3. Identification of key residues in the immunodominant epitope present in peptide CII (181–196) for RT1^u-restricted T cell proliferation. Two panels of 16-mer peptide homologues were synthesized using either an alanine or isoleucine residue to sequentially replace existing amino acids (*A*) or the corresponding amino acid residue found in CI (*B*). The unaltered WT peptide sequence of CII (181–195) is shown at the *top* of both panels; Bkg indicates background stimulation in the absence of antigenic peptide; and each residue substituted is shown at the end of the respective bar. The peptides tested were added to separate cultures containing APC and T cells from the BB1 line, which reacts specifically with native CII and CII (181–196) peptide. The values shown in this figure and in Fig. 4 represent assays in which 10 μl of Ag solution was added to cultures; virtually identical results were obtained with 20 μl, and weaker responses with 5 μl. Cellular incorporation of [^{3}H]thymidine was measured, as previously described. Cross-hatched bars indicate those peptides, which were most affected by the substitution of normally occurring residues with alanine or amino acids found in CI at the corresponding location. The amino acid(s) substituted is shown at *right* (*top*) of each bar, and the one used for substitution shown at the *left*. Comparable results were obtained when unfractionated lymphocytes recovered from CII-immune lymph nodes were used in place of BB1 T cells (data not shown); however, BB1 cells yielded more definitive results.

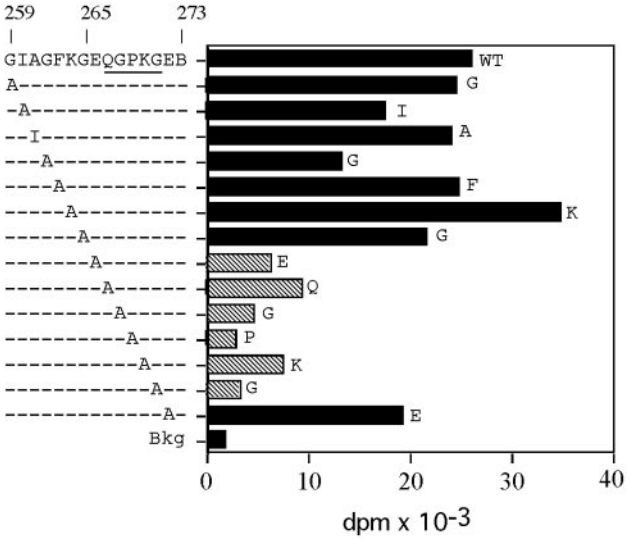

FIGURE 4. Identification of key residues in the subdominant epitope in peptide CII (259–273) for RT1^u-restricted T cell proliferation. A panel of 15-mer peptide homologues was synthesized using alanine to sequentially replace existing amino acids. The unaltered WT peptide sequence of CII (259–273) is shown at the *top* of the panel; Bkg indicates background stimulation in the absence of antigenic peptide; and each residue substituted is shown at the end of the respective bar. As described in Fig. 3, cross-hatched bars indicate those substitutions, which most affected the stimulation of unfractionated CII-immune lymph node cells. [^{3}H]Thymidine incorporation results are shown on the *x*-axis and are expressed as dpm $\times$ 10^{-3}.

CB5, and CB9 (Table I), thus spanning much of the CII molecule. Importantly, the identification of all epitopes proved reproducible among multiple studies in which either three- or single-residue-shifted 15- or 16-mer peptide homologues of CII were tested. The length of the core sequences of these epitopes, deduced by cell proliferation assays, varied from 6 to 13 residues. The core regions of two epitopes, i.e., CII (186–192) (five residues) and CII (87–92) (six residues), proved smaller than murine CII determinants reported by others (9, 24, 26). The variance of core length might reflect the method by which the data were obtained. The use of overlapping 15-mer peptides, advancing by single residues, should allow for the resolution of the core determinant to a single amino acid. This approach, however, does not account for: 1) the concentration of peptide tested, which is dependent on the efficiency of each peptide's synthesis; 2) the stability of peptide homologues in culture; 3) the role of posttranslational modifications (glycosylation, hydroxylation, or citrullination), typically absent on synthetically produced homologues (27, 28); nor 4) the effects of flanking residues on class II MHC/TCR binding, which might be the most significant in our model. An example of the flanking residues affecting binding might be epitope CII (263–272), in which alanine substitutions at residues 260 and 262, both outside of the core region, substantially reduced T cell proliferation. Also possibly

Table II. *Summary of T cell core determinants in CII (259–273) recognized by inbred BB rats and inbred mice*[a]

	259					266						273		
Human, chick, bovine CII	G	I	A	G	F	K	G	E	Q	G	P	K	G	E	B[b]	Ref.
Rat CII sequence														T		
BB rat					F	K	G	**E**	**Q**	**G**	**P**	**K**	**G**	E		
DBA/1 and B10.Q mice	**I**	**A**	**G**	**F**	**K**	**G**	**E**	**Q**								23
B.10-DR4-Tg mouse				**F**	**K**	G	**E**	**Q**	G	P	**K**					24
B.10-DR1-Tg mouse				**F**	**K**	G	**E**	**Q**	G	P	**K**					34

[a] This table shows the amino acid sequence of CII 259–273 (top line) and deduced CII core determinants (below) capable of stimulating T lymphocyte proliferation in the strains of CIA-susceptible rodents shown. Boldface letters represent amino acids whose substitution with alanine or isoleucine reduces T cell proliferation. The sequence shown above is identical for human, bovine, and chick CII. The sequence of rat CII is identical, except for the threonine substitution of hydroxyproline at 273.

[b] B, Hydroxyproline.

relevant to epitope size are the observations of Sercarz and Maverakis (29). These investigators have shown that peptide fragments eluted from class II molecules can be smaller than their predicted size. This observation has led to the hypothesis that some Ags might be bound first as larger, unfolded peptides, which are subsequently trimmed to smaller fragments. Hence, glycine-X-Y-rich CII peptides might be less likely to form stable folds than peptides derived from globular proteins. This difference, plus the presence of proline or hydroxyproline residues, which limit flexibility, could allow some smaller, less flexible, CII-peptide fragments to bind to rat class II molecules and be trimmed with greater efficiency, particularly if the peptides bind with high affinity. The testing of this hypothesis in the BB rat model, however, is beyond the scope of this current report and will require the use of purified class II molecules for binding studies.

The possibility of a sequence homology being shared by the core regions of several epitopes warrants mention. Two peptides, CII (181–195) and CII (904–918), both capable of stimulating T cell proliferation, share an identical QGPRG sequence. This sequence occurs at only one other site, CII (516–519), in addition to a conservatively substituted variation, i.e., QGARG, which is present at CII (72–76) and CII (393–397). None of the 15-mer peptides containing these sequences stimulated proliferation, indicating that differences in flanking residues were important to recognition. This explanation perhaps applies best to CII (516–519), which is flanked by two positively charged lysine residues, thus differing from the same sequence found at position CII (186–192), or similar sequences at CII (263–272), CII (880–889), and CII (894–903), which are flanked by a single positively charged residue on the carboxyl end. The importance of the proline residue in QGPRG is shown by P→A- and P→V-substituted peptides, which do not stimulate T cells to divide. These substitutions were based on corresponding sequences found in nonarthritogenic CI. Three other determinants shared a QGPRG-like motif. One, CII (263–272), contains a conservatively substituted QGPR/KG sequence, whereas the others, CII (880–889) and CII (906–916), contained nonconservative substitutions, i.e., QGP/DRG and QGPR/MG, respectively. Peptide homologues containing these sequences generated proliferative responses, although weaker than CII (186–192). The importance of the positive charge on arginine or lysine residues in CII (186–192) and CII (263–272) is shown by studies in which R/K→A substitutions resulted in a marked loss in T cell proliferation activity. The presence of a small nonpolar residue on the amino side of R also appears essential, because substitution of alanine with a larger nonpolar amino acid such as proline (as found in CI) abrogated proliferation. Another feature shared by five of the six epitopes was a glutamine residue found in the core determinant, often in the context of the QGPR/KG motif. A substantial, but smaller, reduction in T cell proliferation was seen when this residue was substituted with alanine in homologues CII (181–196) and CII (259–274). Finally, epitope CII (87–92) (KGHRGY) remains potentially the most unique sequence; however, even here a XGXRG core persists along with relatively conservative substitutions at X, i.e., Q→K and P→H.

Our findings also suggest a high degree of immunological cross-reactivity between human and rat CII. The epitopes identified in this study could be mapped using human CII-peptide homologues as Ags in rats immunized with rat CII. Our unpublished studies (M.A.C. and X.J.Y.) also demonstrate that substantial cross-reactivity occurs between $RT1^u$-restricted epitopes on rat and heterologous CII. We found that T cells from BB rats immunized with 18- to 21-mer bovine CII homologues of the six epitopes described proliferated on culture with tissue-derived rat CII. A better understanding of the true degree of cross-reactivity between rat and heterologous CII, however, can best be determined by using peptide homologues based on the rat CII sequence, which has only been recently reported in its entirety (20, 21). It should be noted that human and bovine share a common sequence through the immunodominant CII (181–196) region (19, 20, 30), whereas rat and chick CII are identical with human, except for a serine substitution for proline and hydroxyproline at position 188 or 195, respectively (21, 31). These differences, however, do not appear to significantly affect cross-reactive responses between rat and heterologous CII, or vice versa, in the BB rat.

Interestingly, two CII epitopes recognized by the BB rat share some commonality with those recognized by strains of CII-high responder, CIA-susceptible mice. The immunodominant epitope CII (186–192) of the BB rat overlaps with a subdominant epitope within peptide CII (184–198) recognized by DBA/1 ($I\text{-}A^q$) and B.10Q mice (26, 32). Studies by others in our group, however, have shown that residues at positions 183–185 are critical for a response in DBA/1 mice, but not the BB rat (unpublished observations: D.D.B., L.K.M., and E.F.R.). Second, data presented in Table II show that peptide CII (259–273) contains sequences serving as a subdominant epitope for the BB rat and an immunodominant epitope for DBA/1, B10.Q, HLA-DR1-, and HLA-DR4-Tg B10.M mice (9, 24, 26, 33, 34). Nonetheless, even though the core determinants recognized by these mice are similar to those of BB rats, they only overlap, and alanine substitution studies clearly demonstrate that different residues are again critical for T cell stimulation. Notably, alteration of the phenylalanine residue at position 263 or the lysine at 264 abrogated the T cell responses of DR1/DR4-Tg mice (34), but only modestly affected BB rat responses. Finally, T cell epitope mapping has been completed in CIA-susceptible B10.RIII ($I\text{-}A^r$) mice with the characterization of two T cell epitopes, CII (445–453) and CII (610–618). Neither of these epitopes bears resemblance to those described in the BB rat or the aforementioned mouse strains (35, 36).

In summary, BB rats are capable of mounting a robust T cell response to CII, which involves the recognition of at least six epitopes. Interestingly, the majority of these epitopes share a QGPRG-like motif, which bears resemblance to an epitope recognized by HLA-DR1- and HLA-DR4-Tg mice. The data described in this study provide a foundation for additional studies comparing T cell responses of other rat and mouse strains. A clearer understanding of the interaction between these epitopes and APC and T cells mediating immunity should facilitate the development of specific interventions to suppress autoimmunity to CII and the destructive polyarthritis that it initiates.

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References

1. Trentham, D. E., A. S. Townes, and A. H. Kang. 1977. Autoimmunity to type II collagen an experimental model of arthritis. *J. Exp. Med. 146:857*.
2. Courtenay, J. S., M. J. Dallman, A. D. Dayan, A. Martin, and B. Mosedale. 1980. Immunization against heterologous type II collagen induces arthritis in mice. *Nature 283:666*.
3. Cathcart, E. S., K. C. Hayes, W. A. Gonnerman, A. A. Lazzari, and C. Franzblau. 1986. Experimental arthritis in a nonhuman primate. I. Induction by bovine type II collagen. *Lab. Invest. 54:26*.
4. Watson, W. C., R. E. Tooms, P. G. Carnesale, and J. P. Dutkowsky. 1994. A case of germinal center formation by CD45RO T and CD20 B lymphocytes in rheumatoid arthritic subchondral bone: proposal for a two-compartment model of immune-mediated disease with implications for immunotherapeutic strategies. *Clin. Immunol. Immunopathol. 73:27*.
5. Wooley, P. H., H. S. Luthra, J. M. Stuart, and C. S. David. 1981. Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I region) linkage and antibody correlates. *J. Exp. Med. 154:688*.

6. Griffiths, M. M., and C. W. DeWitt. 1984. Genetic control of collagen-induced arthritis in rats: the immune response to type II collagen among susceptible and resistant strains and evidence for multiple gene control. *J. Immunol. 132:2830*.
7. Griffiths, M. M., and G. W. Cannon. 1993. Background genes confer protection against homologous rat type II collagen-induced arthritis in ACI (RT1a) rats. *Transplant. Proc. 25:2829*.
8. Griffiths, M. M., M. A. Cremer, D. S. Harper, S. McCall, and G. W. Cannon. 1992. Immunogenetics of collagen-induced arthritis in rats: both MHC and non-MHC gene products determine the epitope specificity of immune response to bovine and chick type II collagens. *J. Immunol. 149:309*.
9. Rosloniec, E. F., D. D. Brand, L. K. Myers, K. B. Whittington, M. Gumanovskaya, D. M. Zaller, A. Woods, D. M. Altmann, J. M. Stuart, and A. H. Kang. 1997. An HLA-DR1 transgene confers susceptibility to collagen-induced arthritis elicited with human type II collagen. *J. Exp. Med. 185:1113*.
10. Stuart, J. M., M. A. Cremer, A. S. Townes, and A. H. Kang. 1982. Type II collagen-induced arthritis in rats: passive transfer with serum and evidence that IgG anticollagen antibodies can cause arthritis. *J. Exp. Med. 155:1*.
11. Terato, K., K. A. Hasty, R. A. Reife, M. A. Cremer, A. H. Kang, and J. M. Stuart. 1992. Induction of arthritis with monoclonal antibodies to collagen. *J. Immunol. 148:2103*.
12. Takagishi, K., N. Kaibara, T. Hotokebuchi, C. Arita, M. Morinaga, and K. Arai. 1985. Serum transfer of collagen arthritis in congenitally athymic nude rats. *J. Immunol. 134:3864*.
13. Griffiths, M. M., E. J. Eichwald, J. H. Martin, C. B. Smith, and C. W. DeWitt. 1981. Immunogenetic control of experimental type II collagen-induced arthritis. I. Susceptibility and resistance among inbred strains of rats. *Arthritis Rheum. 24:781*.
14. Cremer, M. A., M. M. Griffiths, K. Terato, and A. H. Kang. 1995. Type XI and II collagen-induced arthritis in rats: characterization of inbred strains of rats for arthritis-susceptibility and immune-responsiveness to type XI and II collagen. *Autoimmunity 20:153*.
15. Watson, W. C., J. P. Thompson, K. Terato, M. A. Cremer, and A. H. Kang. 1990. Human HLA-DR β gene hypervariable region homology in the biobreeding BB rat: selection of the diabetic-resistant subline as a rheumatoid arthritis research tool to characterize the immunopathologic response to human type II collagen. *J. Exp. Med. 172:1331*.
16. Weyand, C. M., and J. J. Goronzy. 1997. The molecular basis of rheumatoid arthritis. *J. Mol. Med. 75:772*.
17. Rosloniec, E. F., A. H. Kang, L. K. Myers, and M. A. Cremer. 1996. Collagen-induced arthritis. In *Current Protocols in Immunology*. R. Coico and E. Shevach, eds. Weily & Sons, New York, p. 15.5.1.
18. Myers, L. K., K. Terato, J. M. Seyer, J. M. Stuart, and A. H. Kang. 1992. Characterization of a tolerogenic T cell epitope of type II collagen and its relevance to collagen-induced arthritis. *J. Immunol. 149:1439*.
19. Ala-Kokko, L., and D. J. Prockop. 1990. Completion of the intron-exon structure of the gene for human type II procollagen (COL2A1): variations in the nucleotide sequences of the alleles from three chromosomes. *Genomics 8:454*.
20. Michaelsson, E., V. Malmstrom, S. Reis, A. Engstrom, H. Burkhardt, and R. Holmdahl. 1994. T cell recognition of carbohydrates on type II collagen. *J. Exp. Med. 180:745*.
21. Urabe, K., H. J. Kim, G. Sarkar, J. T. Bronk, and M. E. Bolander. 2003. Determination of the complete cDNA sequence of rat type II collagen and evaluation of distinct expression patterns of types IIA and IIB procollagen mRNAs during fracture repair in rats. *J. Orthop. Sci. 8:585*.
22. Cremer, M. A., X. J. Ye, K. Terato, S. W. Owens, J. M. Seyer, and A. H. Kang. 1994. Type XI collagen-induced arthritis in the Lewis rat: characterization of cellular and humoral immune responses to native types XI, V, and II collagen and constituent α-chains. *J. Immunol. 153:824*.
23. Rosloniec, E. F., K. B. Whittington, D. D. Brand, L. K. Myers, and J. M. Stuart. 1996. Identification of MHC class II and TCR binding residues in the type II collagen immunodominant determinant mediating collagen-induced arthritis. *Cell. Immunol. 172:21*.
24. Rosloniec, E. F., D. D. Brand, L. K. Myers, Y. Esaki, K. B. Whittington, D. M. Zaller, A. Woods, J. M. Stuart, and A. H. Kang. 1998. Induction of autoimmune arthritis in HLA-DR4 (DRB1*0401) transgenic mice by immunization with human and bovine type II collagen. *J. Immunol. 160:2573*.
25. Ku, G., M. Kronenberg, D. J. Peacock, P. Tempst, M. L. Banquerigo, B. S. Braun, J. R. Reeve, Jr., and E. Brahn. 1993. Prevention of experimental autoimmune arthritis with a peptide fragment of type II collagen. *Eur. J. Immunol. 23:591*.
26. Brand, D. D., L. K. Myers, K. Terato, K. B. Whittington, J. M. Stuart, A. H. Kang, and E. F. Rosloniec. 1994. Characterization of the T cell determinants in the induction of autoimmune arthritis by bovine α 1(II)-CB11 in H-2q mice. *J. Immunol. 152:3088*.
27. Kjellen, P., U. Brunsberg, J. Broddefalk, B. Hansen, M. Vestberg, I. Ivarsson, A. Engstrom, A. Svejgaard, J. Kihlberg, L. Fugger, and R. Holmdahl. 1998. The structural basis of MHC control of collagen-induced arthritis; binding of the immunodominant type II collagen 256–270 glycopeptide to H-2Aq and H-2Ap molecules. *Eur. J. Immunol. 28:755*.
28. Vossenaar, E. R., S. Nijenhuis, M. M. Helsen, A. van der Heijden, T. Senshu, W. B. van den Berg, W. J. van Venrooij, and L. A. Joosten. 2003. Citrullination of synovial proteins in murine models of rheumatoid arthritis. *Arthritis Rheum. 48:2489*.
29. Sercarz, E. E., and E. Maverakis. 2003. MHC-guided processing: binding of large antigen fragments. *Nat. Rev. Immunol. 3:621*.
30. Seyer, J. M., K. A. Hasty, and A. H. Kang. 1989. Covalent structure of collagen: amino acid sequence of an arthritogenic cyanogen bromide peptide from type II collagen of bovine cartilage. *Eur. J. Biochem. 181:159*.
31. Kim, O.-H., L. J. Santell, Q. A. Shakey, M. Kreschmer, E. W. Kuhn, W. B. Upholt, W. Fowler, and C. E. Costello. 1998. ESI MS and MALDI-TOF MS confirmation of the primary structure and determination of posttranslational modifications of chicken type II collagen. In *Proceedings of 46th American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topic*. p. 236.
32. Myers, L. K., J. M. Seyer, J. M. Stuart, K. Terato, C. S. David, and A. H. Kang. 1993. T cell epitopes of type II collagen that regulate murine collagen-induced arthritis. *J. Immunol. 151:500*.
33. Myers, L. K., E. F. Rosloniec, J. M. Seyer, J. M. Stuart, and A. H. Kang. 1993. A synthetic peptide analogue of a determinant of type II collagen prevents the onset of collagen-induced arthritis. *J. Immunol. 150:4652*.
34. Rosloniec, E. F., K. B. Whittington, D. M. Zaller, and A. H. Kang. 2002. HLA-DR1 (DRB1*0101) and DR4 (DRB1*0401) use the same anchor residues for binding an immunodominant peptide derived from human type II collagen. *J. Immunol. 168:253*.
35. Myers, L. K., H. Miyahara, K. Terato, J. M. Seyer, J. M. Stuart, and A. H. Kang. 1995. Collagen-induced arthritis in B10.RIII mice (H-2r): identification of an arthritogenic T-cell determinant. *Immunology 84:509*.
36. Miyahara, H., L. K. Myers, E. F. Rosloniec, D. D. Brand, J. M. Seyer, J. M. Stuart, and A. H. Kang. 1995. Identification and characterization of a major tolerogenic T-cell epitope of type II collagen that suppresses arthritis in B10.RIII mice. *Immunology 86:110*.