

IDENTIFICATION OF AN IMMUNOSUPPRESSIVE EPITOPE OF TYPE II COLLAGEN THAT CONFERS PROTECTION AGAINST COLLAGEN-INDUCED ARTHRITIS

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The immune response to normal joint proteins probably plays a critical role in the joint destruction of rheumatoid arthritis (for review, see references 1 and 2). Many patients have detectable immunity to various connective tissue components (3-5), and arthritis can be induced in rodents by immunizing them either with collagen or proteoglycans isolated from cartilage (6, 7). Localizing determinants of connective tissue with the capacity to generate immunogenic responses may give us a better understanding of the way in which arthritis is initiated and regulated.

Our laboratory has previously reported (8) that a cyanogen bromide (CB)¹ peptide of type II collagen (CII) (CB11) carried important epitopes for the induction of arthritis in mice. We and others (9-11) have also found that injection of CII but not type I collagen intravenously would induce resistance to arthritis in rodents. Oral feeding of native but not denatured CII prevented arthritis in mice (12). However, delineation of determinants important for resistance to arthritis had not yet been reported.

The present experiments were undertaken to determine if specific regions of CII capable of inducing resistance could be identified. CII exists *in vivo* as a homotrimer with a rather rigid helical conformation. We first determined that, under the conditions used, either native or denatured CII (obtained by thermal denaturation) would suppress collagen-induced arthritis. We then cleaved CII into smaller fragments, isolated the resulting peptides, and tested the major ones for suppressive activity. Finally, we generated synthetic peptides, 21-26 amino acids in length, corresponding to selected regions of CB11 and injected them into either adult or neonatal mice before immunization in order to define a suppressive epitope of CII.

Materials and Methods

Animals. DBA/1 mice were obtained from The Jackson Laboratory (Bar Harbor, ME),

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¹ *Abbreviations used in this paper:* CB, cyanogen bromide peptide; CII, type II collagen.

maintained in groups of six in polycarbonate cages, and fed standard rodent chow (Ralston Purina Co., St. Louis, MO) and water ad libitum. The environment was specific pathogen free, and sentinel mice were tested routinely for mouse hepatitis and Sendai viruses. Neonatal mice were obtained by breeding mice from The Jackson Laboratory in our facility. Mice were immunized at 8–12 wk of age.

Preparation of CII and CB Peptides. Native CII was solubilized from the sternae of adult chickens by limited pepsin digestion, as described earlier (13). Purified $\alpha 1(\text{II})$ chains, obtained by thermally denaturing the CII, were subjected to nonenzymatic cleavage with CB and the resulting peptides isolated as described by Miller (14). Studies describing the isolation, criteria of purity, and physicochemical properties of each of the CB peptides have been reported in detail elsewhere (15).

Chemical Synthesis of Oligopeptides of CB11. Oligopeptides representing sequences of chick CII were chemically synthesized by a solid-phase procedure described previously (16) using a peptide synthesizer (990; Beckman Instruments, Inc., Fullerton, CA). Protected tBoc amino acids were purchased from Peninsula Laboratories, Inc. (Belmont, CA) and coupled sequentially to a benzhydrylamine resin. Deprotection was achieved with trifluoroacetic acid (25% in dichloromethane), and coupling was obtained in the presence of dicyclohexyl-carbodiimide. The completed peptide was cleaved from the resin, and the side chain protecting groups were removed by treatment with liquid HF at 0°C. The desired peptide was initially purified by filtration through a Sephadex G-25 column (4.0 × 60 cm) previously equilibrated with 0.1 M acetic acid. The effluent was collected in 10-ml fractions, and aliquots were taken for fluorescamine analysis. The remaining peptide fraction was pooled, lyophilized, and further purified by reverse-phase HPLC. Separation was obtained using an HPLC system (Beckman Instruments, Inc.) and an ODS-3 (1 × 25 cm) semipreparative column (Whatman Inc., Clifton, NJ). Buffer A was 0.05% trifluoroacetic acid and buffer B was 0.05% trifluoroacetic acid in acetonitrile. The gradient consisted of 20–30% B in 30 min at 2.0 ml/min. The elution was monitored at 230 nm and confirmed by reaction with fluorescamine. Amino acid composition of the final peptide was determined with an automatic amino acid analyzer (121 MB; Beckman Instruments, Inc.), and amino acid sequences were confirmed by automatic Edman degradation (890 M; Beckman Instruments, Inc.). The amino acid content found was ± 10% theoretical, and amino acid sequence analysis confirmed the peptide structure.

Tolerization. When native CII was used for tolerization, it was first dissolved at a concentration of 1 mg/ml in 0.1 M acetic acid by stirring overnight at 4°C, then dialyzed against 0.15 M NaCl, 0.02 M sodium phosphate, pH 7.4 (PBS). CB peptides and synthetic peptides were solubilized directly in PBS at a concentration of 1 mg/ml. In some experiments, CII was thermally denatured by placing native CII dissolved in PBS in a boiling water bath for 10 min immediately before use. To tolerize adult mice to CII, CB, or synthetic peptides, each animal was given 1 mg of the antigen intravenously either as one dose or as daily doses of 0.33 mg each for three consecutive days. In some experiments a larger dose of antigen, 5 mg/mouse, was given as 1.66 mg daily for three consecutive days. Mice were immunized 3 d after the last intravenous injection. Neonatal mice were tolerized using a protocol described by Gammon et al. (17), in which antigen emulsified with IFA was injected intraperitoneally. Each mouse received 100 µg of antigen in 0.1 ml of emulsion. The mice were injected within 24 h of birth. When they were 8 wk of age, they were immunized with CII and observed for arthritis as described below.

Immunization. For routine immunization, antigen (normally chick CII) was dissolved in 0.01 N acetic acid and emulsified with an equal volume of CFA. CFA was made by grinding heat-killed, freeze-dried *Mycobacterium tuberculosis* (Fisheries and Food Central Veterinary Lab., Weybridge Surrey, UK) with a mortar and pestle and then adding the ground *M. tuberculosis* to IFA (Difco Laboratories, Detroit, MI) at a ratio of 4 mg/ml. The antigen solution was added dropwise to the CFA while continuously mixing with a homogenizer (model 23; Virtis, Gardner, NY). The temperature was maintained by an ice bath. The resulting emulsion was injected intradermally into the base of the tail. Each mouse received a total volume of 0.05 ml containing 100 µg of *M. tuberculosis* and 100 µg of antigen.

Measurement of the Incidence of Arthritis. The presence of arthritis was determined by examining and scoring each of the forepaws and hindpaws on a scale of 0–4, as described else-

where (18). There were two separate examiners, one of whom was unaware of the identity of the treatment groups. Each mouse was scored three times a week by visual examination beginning 3 wk post-immunization and continuing through 8 wk post-immunization. In some experiments, the data were expressed as the percentage of arthritic limbs per group of mice, plotted either at various time points or at 6 wk post-immunization. Analyzing the number of limbs involved rather than the number of mice permitted use of smaller groups. It assumed that the occurrence of arthritis in a limb was random and not related to whether other limbs in the same mouse were involved. To confirm the validity of this assumption, the incidence of arthritis (number of animals with one or more arthritic limbs) was also analyzed at one time point, usually 6 wk post-immunization.

Measurement of Serum Antibody Titers. Mice were bled at 4 and 8 wk after immunization to test for antibodies reactive with native CII or CB peptides of collagen using a modification of an ELISA, previously described (19). Briefly, native CII or the antigen to be tested was dissolved in 0.01 M acetic acid at 4 mg/ml and diluted to a final concentration of 5 μ g/ml in 0.1 M NaHCO₃ immediately before use. Microtiter plates (Nunc, Roskilde, Denmark) were coated by adding 100- μ l aliquots of each collagen preparation to the wells, incubating overnight at 4°C, and washing the plates to remove unbound collagen. Sera for analysis were serially diluted with 0.15 M NaCl, 0.05 M Tris-HCl, pH 7.4, containing 0.05% Tween 20, and added to the collagen-coated microtiter plates in 100- μ l aliquots. After incubation overnight, the wells were washed and 100 μ l of peroxidase-conjugated goat anti-mouse IgG (Cappel Laboratories, Cochranville, PA) was added. Plates were incubated for 4 h, washed, and developed using 40 mg of orthophenylenediamine dissolved in 100 ml of citrate phosphate buffer, pH 5.5, to which 40 μ l of 30% H₂O₂ was added immediately before use. After 15 min, the absorbance at 490 nm was measured using an automated reader (Vmax; Molecular Devices, Menlo Park, CA). A standard serum was added to each plate in serial dilutions. From these values, a standard curve was derived by computer analysis using a four-parameter logistic curve. Results are reported as units of activity, derived by comparison of test sera with the standard serum, which was arbitrarily defined as having 50 U of activity.

Statistical Analysis. The incidences of arthritis in various groups of mice were compared using either the Kruskal Wallis analysis of variance or χ^2 analysis with Yates' correction. Antibody levels were compared using the student's *t* test.

Results

Suppression of Arthritis with Intact CII. Mice injected intravenously with native or denatured CII before immunization were resistant to arthritis. By 6 wk post-immunization, the control groups, given OVA or type I collagen, had an arthritis incidence of 17/18 (94%) or 5/5 (100%), respectively; animals given denatured CII had an incidence of 1/18 (6%); and animals given native CII had no arthritis (Table I). The incidence of arthritis in groups receiving either native or denatured CII were both significantly different from OVA controls, with $p \leq 0.004$ by Kruskal Wallis analysis of variance. Mice were also evaluated according to the mean percentage of arthritic limbs per group of six mice (Table I). By 6 wk post-immunization, the control group injected with OVA had 56% arthritic limbs and the group given type I collagen had 46% arthritic limbs. Animals injected with denatured CII had 3% arthritic limbs and animals injected with CII had no arthritis. Both groups administered CII were significantly different from OVA controls using χ^2 with Yates' analysis ($p \leq 0.0001$).

All of the animals were bled at 4 and 8 wk post-immunization and tested for antibody response to native CII by ELISA. Animals given a tolerizing injection of either native or denatured CII before immunization had significantly lower antibody levels to CII at both 4 and 8 wk post-immunization, in comparison with control mice in-

TABLE I
Suppression of Arthritis with Soluble Collagen

Antigen injected	Arthritic mice	Arthritic limbs	Mean antibody levels to CII 4 wk post-immunization
OVA	17/18 (94%)	40/72 (56%)	60.5 ± 26
CII (denatured)	1/18 (6%)*	2/72 (3%)‡	14.0 ± 16 [§]
CII (native)	0/18 (0%)*	0/72 (0%)‡	5.3 ± 2 [§]
Collagen type I	5/5 (100%)	11/24 (46%)	58.4 ± 28

Female DBA/1 mice, 8 wk of age, were tolerized by intravenous injection of 1 mg of OVA, denatured CII, native CII, or type I collagen, 3 d before immunization with 100 µg of native CII. Data is expressed as the number of mice with arthritis and the number of arthritic limbs at 6 wk post-immunization. The data were analyzed statistically using Kruskal Wallis analysis of variance and student's *t* test. Mice were bled 4 wk post-immunization and tested for antibody to CII using ELISA. Each serum was diluted 1:73,000, and values are expressed as mean units of activity. Statistical analysis compared the experimental (CII-injected animals) with control (OVA-injected animals).

* $p \leq 0.004$ using Kruskal Wallis analysis of variance.

‡ $p \leq 0.0001$ using student's *t* test.

§ $p \leq 0.005$ using student's *t* test.

jected with OVA (Table I). Animals given denatured CII had antibody levels intermediate to those given native CII and controls that correlated well with the incidence of disease.

The onset of arthritis was also delayed in animals given denatured CII (Fig. 1). Control animals given OVA had the onset of arthritis beginning day 21, followed by a rapid increase in the number of arthritic limbs. A plateau occurred ~6 wk after immunization, but the animals continued to develop newly affected limbs until the termination of the study at 8 wk post-immunization. Animals given native CII before immunization had no arthritis develop throughout this study, but animals given denatured CII had the onset of disease beginning around day 35 post-immunization. The suppressive effect began to fade by 8 wk after immunization, as evidenced by an increasing frequency of arthritis and by smaller differences in antibody levels.

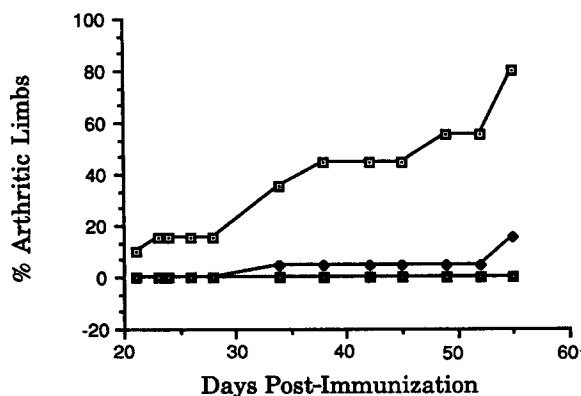


FIGURE 1. Suppression of arthritis with intact CII. Female DBA/1 mice, 8 wk of age, were tolerized by intravenous injection of 1 mg ova (□), denatured CII (◆), or native CII (■) 3 d before immunization with 100 µg of native CII. There were six mice in each group. Arthritis was first evident at day 20, and mice were evaluated three times weekly for the presence of arthritis. Data are expressed as the percentage of limbs that were arthritic.

Suppression of Arthritis with Fragments of CII. To localize regions of the molecule responsible for inducing tolerance, CII was cleaved by treatment with CB, and the resulting peptides were individually isolated. CB peptides 11, 10, and 8 were selected for further experimentation because they comprise 75% of the entire molecule and because our earlier work (8) showed that sera from arthritic animals immunized with CII contain antibodies that recognize these peptides selectively. We injected mice with the peptides 11, 10, or 8 intravenously and challenged them 3 d later by immunization with intact CII. By 6 wk post-immunization, 92% of the animals given OVA, 69% of the animals given CB10, and 83% of the mice given CB8 were arthritic (Table II). In contrast, only 25% of the mice given CB11 were arthritic ($p < 0.002$). Both native CII and CB11 effectively suppressed the overall antibody responses to intact CII and to each of the CB peptides tested, while neither CB8 nor CB10 did so (Table III).

Suppression of Arthritis in Adult Mice with a Synthetic Peptide Representing Sequences of Chick $\alpha 1(II)CB11$. To further localize the most important determinant of suppression, five synthetic peptides, each 21–26 amino acids in length, were generated. These peptides corresponded to sequences of CB11 selected by comparison of CII to type I collagen and identifying areas with the greatest differences. CII and type I collagen are 70% homologous within the CB11 region, excluding glycine at every third residue. The greatest differences are confined to two areas, the first 126–140 amino acids from the NH_2 terminal, and the second 240–257 from the amino terminal of $\alpha 1(II)CB11$. (20). Of the five peptides tested, only CB11 122–147 had suppressive activity (Table IV). Mice receiving CB11 122–147 had an incidence of 9/18 (50%) arthritis and 16/72 (22%) arthritic limbs, when compared with animals given OVA, which had 17/18 (94%) arthritic mice and 39/72 (54%) arthritic limbs ($p = 0.05$ and $p = 0.01$, respectively). A larger dose (5 mg/mouse) of CB11 122–147 was even more suppressive, giving 2/6 (33%) arthritic mice and 4/24 (17%) arthritic limbs,

TABLE II
Suppression of Arthritis with Collagen Fragments

Antigen injected	Arthritic mice	Arthritic limbs
OVA	11/12 (92%)	27/48 (56%)
CII	0/12 (0%)*	0/48 (0%) [†]
CB8	5/6 (83%)	10/24 (42%)
CB10	11/16 (69%)	22/64 (34%) [§]
CB11	3/12 (25%)*	5/48 (10%) [‡]

Female DBA/1 mice, 8 wk of age, were randomly allocated into groups of from 6 to 16 mice, and each group was injected intravenously with 250 μ g of OVA, CII, CB8, CB10, or CB11 daily for 3 d. 3 d after the last injection, they were immunized with 100 μ g of CII. Data are expressed as the number of arthritic mice and the number of arthritic limbs per group at 6 wk post-immunization. The number of arthritic animals given CB11 and CII was significantly different from controls given OVA.

* $p \leq 0.002$ using Kruskal Wallis analysis of variance.

[†] $p \leq 0.0001$ ($\chi^2 = 19.3$ for mice injected with native CII; $\chi^2 = 20.7$ for mice injected with CB11; both had Yates' correction applied).

[§] $p \leq 0.03$ (χ^2 with Yates correction = 4.5).

TABLE III
Suppression of Antibodies to Collagen Using Collagen Fragments

wk post-immunization	Antigen injected	Mean antibody titers to collagen fragments			
		CII	CB8	CB10	CB11
4	OVA	60 ± 12	13 ± 8	5 ± 4	40 ± 15
	CII	5 ± 2*	1 ± 0.7 [‡]	1 ± 0.6 [‡]	3 ± 3 [‡]
	CB8	58 ± 5	17 ± 7	13 ± 6	31 ± 15
	CB10	61 ± 10	4 ± 3	4 ± 2	43 ± 17
	CB11	28 ± 7 [‡]	2 ± 1 [‡]	2 ± 0.4	6 ± 2 [‡]
8	OVA	55 ± 13	18 ± 13	18 ± 12	37 ± 11
	CII	5 ± 4*	1 ± 1 [‡]	1 ± 0.4 [‡]	3 ± 2*
	CB8	57 ± 6	14 ± 4	10 ± 5	30 ± 8
	CB10	61 ± 9	18 ± 11	13 ± 8	35 ± 16
	CB11	32 ± 5 [‡]	3 ± 2 [§]	3 ± 2 [§]	6 ± 2*

DBA/1 female mice, 8 wk of age, were injected intravenously with 250 µg of either OVA, CII, CB8, CB10, or CB11 daily for 3 d. 3 d after the last injection, they were immunized with 100 µg of native CII. Antibody levels to CII and CB peptides 8, 10, and 11 were determined by ELISA at 4 and 8 wk post-immunization. Results are expressed as mean units of activity ± SD. Each result is compared with the result obtained in the mice injected with OVA using student's *t* test.

* $p \leq 0.005$.

[‡] $p \leq 0.01$.

[§] $p \leq 0.05$.

$p = 0.009$ and $p = 0.003$, respectively. Of note is that CB11 116–137 overlaps CB11 122–147, suggesting the region from 137 to 147 is critical.

Suppression of Arthritis by Neonatal Injection of a Synthetic Peptide of CII. Because neonatal tolerance may be due to a different mechanism than adult tolerance, we determined which epitopes of CII were important in suppressing arthritis when the antigens were given neonatally. Using a protocol for inducing neonatal tolerance described in Materials and Methods, CB11 and three of its synthetic peptides were injected

TABLE IV
Suppression of Arthritis in Adult Mice with Synthetic Peptides of CB11

Antigen injected	Mice with arthritis	Limbs with arthritis
OVA	17/18 (94%)	39/72 (54%)
Chick CB11	3/12 (25%)*	5/48 (10%)*
Synthetic CB11 122–147	9/18 (50%) [‡]	16/72 (22%)*
Synthetic CB11 239–263	6/6 (100%)	12/24 (50%)
Synthetic CB11 116–137	12/12 (100%)	23/48 (48%)
Synthetic CB11 97–122	6/6 (100%)	13/24 (54%)
Synthetic CB11 148–173	6/6 (100%)	10/24 (42%)

Female DBA/1 mice, 8 wk of age, were randomly allocated into groups of 6–18 mice and injected intravenously with 0.33 mg of OVA, CII, or one of the synthetic peptides daily for 3 d. 3 d after the last injection, they were immunized with 100 µg of CII. Data are expressed as the number of arthritic mice and the number of arthritic limbs per group at 6 wk post-immunization. Results were analyzed by χ^2 with Yates' correction.

* $p \leq 0.01$.

[‡] $p \leq 0.05$.

TABLE V
Suppression of Arthritis in Neonatal Mice with Synthetic Peptides of CB11

Antigen injected	Mice with arthritis	Limbs with arthritis	Mean antibody levels to CII
No antigen	5/6 (83%)	12/24 (50%)	60.5 ± 22.8
Chick CB11	0/6 (0%)*	0/24 (0%)†	6 ± 0.6†
Synthetic CB11 122-147	2/7 (29%)	2/28 (7%)*	18.5 ± 7.8†
Synthetic CB11 239-263	5/6 (83%)	12/24 (50%)	58.7 ± 10.5
Synthetic CB11 116-137	5/6 (83%)	13/24 (54%)	79.5 ± 38.5

Neonatal DBA/1 mice were injected intraperitoneally with 0.1 mg of CB11 or one of three synthetic peptides emulsified with IFA. At 8 wk of age, mice were immunized with 100 µg of CII. Data are expressed as the number of arthritic mice and the number of arthritic limbs per group at 6 wk post-immunization. Results were analyzed by χ^2 with Yates' correction for non-parametric data and by student's *t* test for antibody levels.

* $p \leq 0.02$.

† $p \leq 0.001$.

into neonatal mice that were subsequently immunized with CII at 8 wk of age. Both CB11 and the synthetic peptide CB11 122-147 were suppressive. None of the six mice given CB11 neonatally developed arthritis and 2/7 (29%) of the mice given CB11 122-147 developed arthritis, compared with 83% of mice given CB11 239-263 or CB11 116-137 (Table V). The structure of the synthetic peptide CB11 122-147 is shown in Fig. 2.

Discussion

In this study, we have identified an epitope of CII that is of critical importance for regulation of arthritis. It has long been felt that autoimmunity to CII plays a major role in the pathogenesis of collagen-induced arthritis and rheumatoid arthritis as well (1-7). Therefore, identification of critical determinants of CII that trigger immune responses can lead to better understanding of the mechanisms behind their immunopathogenicity.

Our laboratory has previously shown (8) that the CB11 contained critical determinants for induction of disease in mice. In addition, other investigators have shown that intravenous administration of soluble CII (10), collagen-coupled spleen cells (9), and partially purified collagenous peptides (11) are capable of suppressing collagen-induced arthritis in rats. Yet localization of critical determinants for the suppressive phenomenon remained to be determined. We have confirmed that resistance to collagen-induced arthritis could also be induced in DBA/1 mice by intravenous injection of CII. This resistance was associated with tolerance to the immunizing antigen as measured by decreased antibody levels. Furthermore, although tolerance

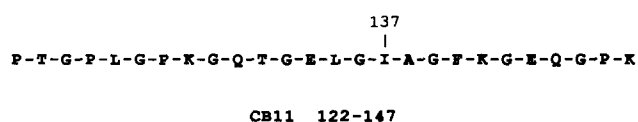


FIGURE 2. Structure of a synthetic peptide that confers protection against arthritis. The synthetic oligopeptide of CB11 was chemically synthesized by a solid-phase procedure as described in Materials and Methods.

induced by native collagen was profound, the phenomenon was not dependent upon native antigen. Both denatured collagen and peptide fragments derived by CB digestion were effective, although slightly less potent. The finding that denatured collagen fragments were effective is similar to that of Englert et al. (11), who found that partially purified CB peptides enriched for CB10-induced resistance in rats. However, in the DBA/1 mouse, the most potent and consistently suppressive fragment was CB11. CB10 showed a trend toward suppressing arthritis but this was not significant with the number of mice tested. It is possible that inclusion of additional animals might have identified another, perhaps less potent, tolerogenic site on CB10. It may also be that species differences exist in the recognition of tolerogenic peptides.

Of particular importance, however, was the finding that a 26-amino acid synthetic peptide correlating with CB11 122–147 was capable of suppressing arthritis, whether given to adult animals or neonates. Additionally, because an overlapping peptide CB11 116–137 did not suppress arthritis, the critical determinant resides within the region CB11 137–147, an 11-amino acid stretch.

Distinct immunosuppressive epitopes have been demonstrated with several non-collagenous protein antigens. Hen egg-white lysozyme (21, 22), insulin (23), β galactosidase (24), and BSA (25) have all been shown to have nonoverlapping immunogenic and suppressive epitopes on the same molecule. Studies done in experimental allergic encephalomyelitis, an animal model for an autoimmune demyelinating disease, have revealed that the same epitope on myelin basic protein carries the ability to either suppress or cause disease (26), and an additional inhibitory determinant could be found, distant from the encephalitogenic site (27). In addition, a synthetic peptide composed of the NH₂-terminal nine amino acids of myelin basic protein, when given neonatally, rendered mice resistant to subsequent disease induced by immunization with the same peptide, although such treatment did not make them resistant to disease induced by immunization with the whole rat myelin basic protein (28).

The superiority of a particular peptide over others of similar size in initiating immune responses is not completely understood. Recent studies of Grey, Geftter, and Unanue, and their coworkers (29–31), have suggested that the immunodominant peptide may bind with greater affinity to the MHC antigen on the APC than do other peptides that have no effect on the immune response. Alternately, investigators have proposed that certain protein determinants trigger a greater diversity of T cells to respond with a wider range of fine specificities than other determinants can induce (32). These two hypothetical mechanisms may both be playing a role in the case of the immune response to collagen.

In addition, mechanisms have been proposed to explain the phenomenon that intravenous injection of a protein can lead to profound suppression of the subsequent immune response to that antigen. Suppression may be the property of a particular cell, or an intrinsic property of the immune system, or carried out by known components that become suppressive only under particular circumstances (33). Possibly, prior exposure to exogenous peptide causes inactivation or deletion of certain T cell clones, as has been proposed in neonatal tolerance to cytochrome and lysozyme peptides (28, 34). The induction of T cells capable of suppressing Th cells, either by secretion of lymphokines or via cytotoxicity, has also been proposed (33).

In addition, the display of antigen on inappropriate APC may result in the inactivation, rather than the activation, of specific T cells (35). The suppression of collagen-induced arthritis with intravenous injection of CII has been reported to be antigen specific and transferrable with cells to naive animals (36–38). Recent studies showed abrogation of suppression of CIA by cyclophosphamide treatment (39) and suppression of CIA by a T cell hybridoma (40). This suggested that T cells mediated the suppression of CIA. We have found that CD4⁺ cells play a role in the suppressive phenomenon induced by intravenous injection of CII (41), but a role for each of the other mechanisms that have been proposed cannot be ruled out.

We have successfully suppressed arthritis by peptide-induced tolerization. Further studies are currently underway to determine which amino acids of CB11 122–147 are pivotal for its immunological activities in the DBA/1 mouse. A better definition of determinants critical for regulation of arthritis may lead to improved therapies exploiting the observation that tolerance to certain immunogenic epitopes of autoimmune proteins can result in a downregulation of the immune response and ultimately of the autoimmune disease.

Summary

We have previously reported that collagen-induced arthritis can be suppressed by intravenous injection of native type II (CII) but not type I collagen. We have now identified denatured fragments of CII capable of suppressing collagen-induced arthritis and inducing tolerance. Purified CII was cleaved with cyanogen bromide (CB), and the major resulting peptides were isolated. Female DBA/1 mice were administered OVA, native CII, or one of the CB peptides, intravenously, before immunization with native CII. 6 wk after immunization, mice tolerized with CII or CB11 had a markedly lower incidence of arthritis compared with controls. There was a correlation between the overall antibody response and the incidence of arthritis. In addition, animals tolerized with either CII or CB11 had a decreased antibody response not only to CII, but also to each of the other CB peptides tested. To identify the epitope involved in suppression of arthritis, five synthetic peptides, 21–26 amino acids in length, corresponding to selected regions of CB11, were generated. Each of the peptides was injected intravenously into mice before immunization. Only one of these, CB11 122–147, was capable of suppressing arthritis. In addition, mice given the synthetic peptide CB11 122–147 neonatally were suppressed for arthritis and antibody responsiveness when immunized with CII at 8 wk of age. Thus, we have identified CB11 122–147 as an epitope of CII important in induction of tolerance and suppression of disease. Further experiments narrowing down the pivotal amino acids for the immunogenicity of this epitope and the role this epitope plays in induction and regulation of disease will enhance our understanding of how the immune response to collagen affects autoimmune arthritis.

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