Immunization with TCR V 10 peptide reduces the frequency of type-II collagen-specific Th1 type T cells in BUB/BnJ (H-2q) mice

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Abstract

Objective

Collagen induced arthritis (CIA) in mice is mediated by synergistic T cell and humoral immune responses specific for type II collagen (CII). We have previously shown that in arthritic joints of BUB mice (TCR V ^{a} -2^{q}) the TCR repertoire is enriched for V 10 expressing T cells, and that immunization with a V 10 peptide (V 10p) prevents the phenotypic expression of disease. The objective of the present study was to understand how immunization with a synthetic TCR V peptide affected the development of the pathogenic CII-specific immune response in BUB mice.

Methods

Arthritic and protected animals were tested for V 10p- and CII-specific cytokine production by a highly specific and sensitive ELISA spot assay, and for CII-specific antibody production by standard ELISA. In adoptive transfer experiments, V 10p-specific LN cells (INF- producing) were injected into naive mice prior to immunization with type-II collagen/CFA.

Results

Immune cells from arthritic animals produced IFN- and IL-2, without IL-4 and IL-5 in response to CII and an immunodominant epitope, A2, derived from CII. Serum from these mice contained anti-CII antibodies of both IgG1 and IgG2a subtypes. Our results show for the first time that immunization with V 10p resulted in V 10p-specific IFN- and IL-2 production that was restricted to the CD4+ T cell subset. Emergence of this V 10p-specific immune response was associated with a dramatic decrease in the frequency of CII and A2-specific, cytokine producing T cells in arthritis protected mice. Protective immunity was cell mediated and could be adoptively transferred. In contrast, the protective immunization had only a marginal effect on the anti-CII antibody response indicating that the CII specific humoral immune response was not significantly affected.

Conclusion

Immunization with TCR V 10p leads to expansion of a population of V 10p- specific CD4+ T cells. This anti-TCR V 10p specific type 1 cytokine producing immune response was protective in adoptive transfer studies and appears to inhibit the expansion of the pathogenic anti-CII cellular immunity. Additionally, the anti-TCR V 10p-specific cellular immune response was mediated by CD4+ T cells and these T cells did not produce IL-4 or IL-5. Thus, our results suggest that protection against CIA in mice immunized with synthetic TCR V 10p was achieved by a specific down-regulation of the CII-specific Th1 type cellular immune response and not via immune deviation.

Key words

Arthritis, animal models, TCR, vaccination, T cell.

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Introduction

An improved understanding of the pathogenesis of autoimmune diseases over the past decade has resulted in identifying novel approaches for immunotherapy. The detection of T cell receptor (TCR) variable (V) region gene restriction in several animal models of autoimmune diseases, including experimental autoimmune encephalomyelitis (EAE) and collagen induced arthritis (CIA) among others, suggested that the TCRs expressed by pathogenic T cells themselves might serve as useful immunotherapeutic targets. This view is supported by the observation that anti-TCR V region antibodies directed at restricted populations of pathogenic T cells could prevent onset of disease through elimination of the autoreactive T cell repertoire (1). Similarly, immunization with synthetic peptides derived from the pathogenic T cell receptor repertoire prior to disease inducing immunizations have been effective in preventing the onset of the disease (2). In EAE, such protective immunization seems to result in an expansion of a pre-existing anti-idiotypic T cell population, that in turn, alters the pathogenic T cell repertoire (2-4). Although both CD4+ and CD8+ regulatory cells can participate in such a response (2, 3, 5), perhaps through direct destruction or bystander suppression of the pathogenic T cells, mechanisms of protection are only partially understood.

The appreciation of the role of cytokines produced by specific T cell subsets has provided significant insight into the pathogenesis and possible avenues for therapy of autoimmune diseases. Pro-inflammatory type 1 immunity, characterized by IFN- and IL-2 production (without IL-4 and IL-5) and complement fixing IgG2a antibodies, has been strongly associated with development of disease in several model systems, including EAE (6). Shifting of the antigen specific cytokine profile towards IL-4 and IL-5 production, while inhibiting IFN- secretion, has also been effective in protecting animals from developing EAE. EAE is unlike many human autoimmune diseases, however, in that T cells, in the absence of autoantibodies, are both necessary and sufficient to produce the full manifestations of disease (7-9). Similarly, the role that T cells play in the induction or suppression of CIA is most likely dependent on the cytokines they produce. It has been shown that Th1 cytokine IFN- can be detected in arthritic joints and that mice given IFN- parenterally develop more severe arthritis (10). In contrast, mice given IL-4 parenterally were relatively resistant to the induction of CIA as compared to controls (11).

Collagen induced arthritis (CIA) is an animal model of inflammatory polyarthritis that mimics human rheumatoid arthritis in many respects (12-14). Mice with susceptible MHC H-2q and H-2r haplotypes, when immunized with heterologous type II collagen in Complete Freunds Adjuvant (CFA), develop antibodies and T cells reactive with the autoantigen, a phenomenon which is essential for the pathogenesis of CIA (15-20). In this model system it has also been shown that for full manifestation of CIA Th1 type immune response specific for collagen is essential, and that immune deviation towards Th2 type immune response (i.e. IL-4 and IL-5 producing) can prevent the development of disease (21).

We have previously shown that BUB/BnJ mice (H-2q) have deletions of TCR V 5, 8, 9, 11, 12 and 13 gene subfamilies (TCR V ^{a} genotype) (22) but are susceptible to CIA and develop severe deforming arthritis after immunization with chicken type-II collagen in CFA and that the disease is indistinguishable both histologically and clinically from disease which develops in other susceptible strains of mice with TCR V ^{b} genotype (22-24). We have also shown that the autoreactive T cell repertoire in BUB mice with CIA is skewed towards expression of V 10 and V 3 gene families (23). We further found that immunization with a TCR V 10 peptide, prior to immunization with chicken type-II collagen can prevent the development of CIA (24). But how immunization with synthetic TCR V peptide affects the emergence of the pathogenic, autoantigen-reactive T cell and antibody repertoires is not

known. In particular, whether the protective immunization with TCR V peptide alters the frequency/cytokine profiles of the pathogenic CII specific T cells and/or alters the detectable CII specific antibody titers has not yet been determined. The present studies focused on exploring how immunization with TCR V peptides affects the development of C-II-specific cellular immune response. Understanding these effects may have important implications for devising future therapeutic strategies aimed at preventing and treating autoimmune diseases.

We used a highly sensitive cytokine ELISA spot assay to determine the frequency, antigen specificity, and cytokine profiles of the autoreactive T cell repertoire in arthritic and protected mice. Our results demonstrate that the development of CIA in BUB mice was indeed associated with a Th1 type cytokine-secreting cellular immune response specific for a single immunodominant epitope of the CII molecule (A2). A novel and highly significant finding of our studies was that the protective immunization resulted in an expansion of a population of V 10p specific IFN- and IL-2 producing, CD4+ T cells, and was associated with a dramatic reduction in the frequency of CII and A2 specific T cells in arthritis protected mice. Thus, our findings provide further evidence for the pathogenicity of CII specific Th1 type immunity in CIA, and suggest that decreasing the frequency of the autoreactive T cells, without a shift in cytokine profile, is sufficient to prevent the development of arthritis.

Materials and methods

Mice. Male BUB/BnJ mice ($H2^q$, TCR V ^{a}) 6-8 weeks old were purchased from The Jackson Laboratories (Bar Harbor, ME) and were maintained in the specific pathogen free Animal Care Facilities of the School of Medicine at Case Western Reserve University, Cleveland, OH. All experiments were carried out in accordance with the National Institutes of Health guidelines and with the approval of the Institutional Animal Care and Use Committee at Case Western Reserve University.

Antigens. Chicken type-II collagen (C-II) was obtained from Biotech Holdings Inc., Hudson, OH or Chondrex, Seattle, WA. Mouse C-II and peptide fragments from the CB11 fragment of chicken C-II were a generous gift from Dr. Marie Grifiths, Salt Lake City, UT. Synthetic peptides for V 10 (QTLDHNTMYWYKQDSKKLLKIM, amino acids 25-46 in the V 10 molecule) or control V 14 (ATGGTLQQLFYSITVGQV, residues 39-56 of the V 14 molecule) were synthesized by Tana Laboratories Houston, TX.

Immunization protocols. Immunization of mice with V 10p was performed as previously described (24). Briefly, V 10p or control V 14p were dissolved in PBS (1.5 mg/ml), and combined 1:1 with Complete Freunds Adjuvant (CFA, GIBCO-BRL). 100 l of V 10p or V 14 p mixed in CFA was injected intradermally at the tail base 10 days prior to arthritis inducing C-II/CFA immunization. CIA was induced by intradermal injection of 100 l of chicken C-II emulsified in CFA as previously described (24).

Determination of severity of arthritis. Joints were graded as 1 (edema and swelling), 2 (loss of range of motion in addition to profound edema and swelling), or 3 (ankylosis of the joint in addition to edema and swelling) as previously described (24). All affected joints in a mouse were documented. A total joint score/mouse was calculated by adding the scores for each joint of the individual animal. Thus the maximum score/mouse could be 12. Histologic analysis was carried out at the facilities of Histology and Immunohistochemistry Core of the Northeast Ohio Multipurpose Arthritis Center (NEOMAC).

Isolation of serum, lymph node cells, and splenocytes. Serum samples were prepared from whole blood specimens. Draining lymph nodes or spleen cells were dissected and single cell suspensions were prepared under sterile conditions using standard protocols. Osmotic lysis of red blood cells was performed by 1-2 min incubation in hypotonic lysis solution (150 mM NH_4Cl, 1mM $KHCO_3$, 0.1 mM Na_2EDTA) followed by washing in Hank's Balanced Salt Solution (HBSS).

ELISA assay. Immulon-4 plates (Dynatech Laboratories Inc., Chantily Va) were coated overnight at 4°C with antigen (chicken C-II, mouse C-II,A2 peptide, Hen egg white lysozyme or V 10p at a concentration of 10 g/ml (100 l/well). After washing three times with PBS + 0.5% Tween (PBST), plates were blocked for 1 hr at room temperature. Serum samples were diluted with blocking solution and then added in duplicate to the wells and incubated at 4°C overnight. Plates were washed again three times with PBST and detection antibody (Goat anti-mouse IgG1 alkaline phosphatase (Southern Biotechnology Associates Inc., Birmingham,Al) at 1:2000 or rabbit anti-mouse IgG2a alkaline phosphatase (American Qualex) at 1:500 dilution was added and incubated for 4 hours at room temperature. After washing four times with wash buffer (150 mM Tris-Cl, 450 mM NaCl, 9 mM NaN_3 pH 7.5), ELISA plates were developed with a p-nitrophenol phosphate substrate (Research Organics Inc., Cleveland, OH) for 10-60 minutes at room temperature and A_{405} OD units were quantitated.

ELISA spot assay. ELISA spot assay for the detection of IFN- , IL-2, IL-4, and IL-5 producing cells was performed essentially as previously described (25). Briefly, ELISA spot plates (Cellular Technologies Limited, Cleveland, OH) were coated overnight with capture cytokine antibodies diluted in sterile PBS: for IFN- antibody R46A2, produced in our laboratory from hybridoma cultures, was used at a concentration of at 4 g/ml; for IL-2, anti-IL-2 capture antibody JES6-1A12, (Pharmingen, San Diego, CA) was used at a concentration of 3 g/ml; for IL-4, antibody 11B11, produced in our laboratory from hybridoma cultures, was used at a concentration of 2 g/ml; and for IL-5 antibody TRFK5, produced in our laboratory from hybridoma cultures, was used at a concentration of g/ml.

After blocking the wells for 1 hour with PBS + 1%BSA, plates were washed three times with PBS and splenocytes (1×10^6) or lymph node cells (5×10^5) were added to individual antibody coat-

ed wells in HL-1 medium (BioWhittaker) with the addition of penicillin, streptomycin, and L-glutamine in the presence or absence of specific antigen. Antigen concentrations (when present) were in duplicate as follows: C-II at 10 g/ml, Hen Egg Lysozyme (HEL) at 10 g/ml, V 10p at 10 g/ml, A2 at 10 g/ml, and Conconavlin A (ConA) at 2 g/ml. The plates were incubated at 37°C for 24 hours in 5%CO_2 and then washed three times with PBS followed by four washes with PBST. Detection antibodies were: for IFN- biotinylated XMG1.2; for IL-2 biotinylated JES6-5H4; for IL-4 biotinylated BVD6-24G2; for IL-5 biotinylated TRFK4. All antibodies were purchased commercially (Pharmingen) and were diluted in PBST + 1% BSA (2 g/ml) and l solution was added to each well for the detection of cytokines and the plates were incubated overnight at 4°C.

The plates were washed three times with PBST and incubated for 90 min at room temperature with HRP-streptavidin (Dako, Denmark) at 1:2000 dilution (for IL-2 and IL-4 detection) or with anti-biotin (Vector) at 1:1000 dilution (for IFN- and IL-5 detection) followed by development with 3-Amino-9-ethyl carbazole reagent (Pierce Chemical Co., Rockford, IL) or with nitro-blue tetrazolium chloride (Biorad). The resulting spots were quantitated using a computer assisted spot image analyzer designed to detect spots using predetermined criteria based on size, shape, and colorimetric density. In some experiments blocking rat anti-mouse CD4 antibodies or blocking rat anti mouse CD8 antibodies (isolated from T cell hybridoma cultures grown in our laboratory) at 50 g/ml were added to each well.

Adoptive transfer of TCR V 10p-specific T cells. To determine whether TCR V 10p-specific T cells can protect naive mice from CIA, we immunized 5 male BUB mice with TCR V 10p/CFA. Ten days later draining LN and splenic cells were collected, T cells were purified using T cell purification columns (R & D Systems, MN) and an aliquot subjected to ELISA spot analysis. Purified T cells were suspended in PBS and 5 x 10^6 T cells/mouse were injected i.v. into 5 male BUB mice and 3 days later recipient mice were immunized for the induction of CIA and boosted 3 weeks later with C-II/CFA. Age matched male BUB mice injected i.v. with PBS only and then treated identically as the experimental group served as controls.

Results

CIA in BUB mice is associated with a Th1 type (IFN- producing) immune response specific for C-II

In initial experiments we first determined the frequency, specificity and cytokine profile of collagen specific T cells in BUB mice immunized to get CIA by the ELISA spot assay. As shown in Figure 1A, recall antigen specific immunity to C-II in these mice was characterized by IFN- and IL-2 production without IL-4 or IL-5 production, a profile which is consistent with a Th1 type immune response. C-II specific, IFN- producing cells were detectable at ~120/million cells plated, while naive mice and mice immunized with control antigens produced < 5 cytokine spots/million cells in response to C-II (data not shown).

Sera from mice immunized to get CIA contained high titers of C-II specific antibodies of the IgG1 and IgG2a subclasses (Fig. 1B). Since IFN- is known to induce an isotype switch to IgG2a production, the presence of C-II specific IgG2a antibodies is consistent with the presence of a Th1 type immune response in BUB mice immunized with C-II in CFA. This has not previously been documented in these

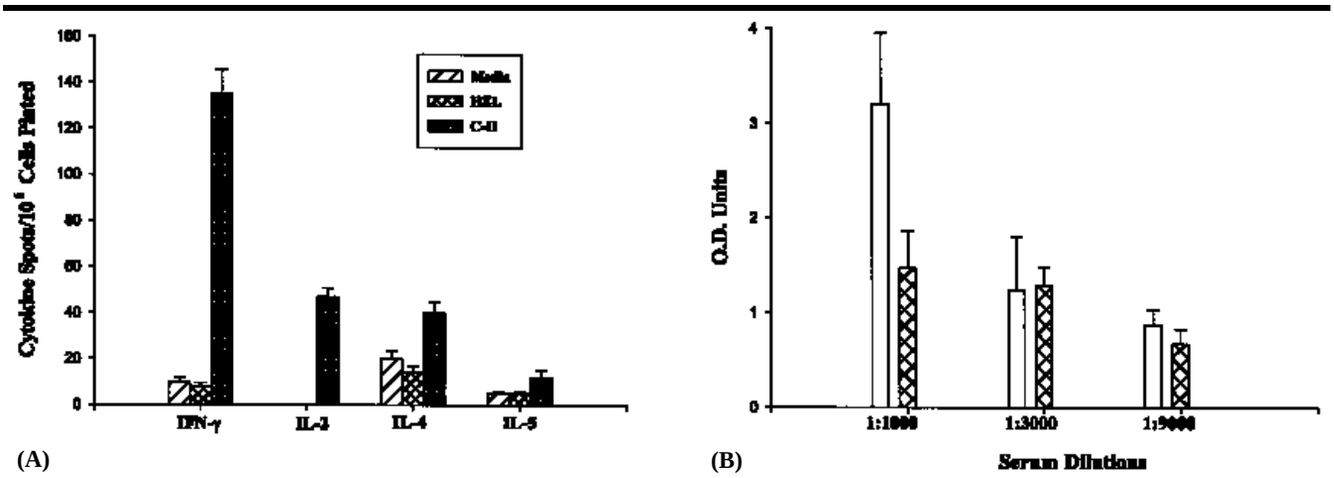

Fig. 1. C-II specific cellular and humoral immune response in BUB mice with CIA.

A: Spleen cells were isolated from mice on day 40 after CIA-inducing immunization (at least grade 2 arthritis) and tested in cytokine ELISA spot assays. The data represent mean values of three independent measurements +/- standard error calculated from duplicate wells for each animal (< 10 % variability between wells in an individual animal). Spots for culture medium alone, control antigen HEL,and C-II are shown for each cytokine. The experiment was repeated 2 times with similar results.

B: ELISA assay for C-II specific IgG2a (open bars) and IgG1 (crossed bars) was performed using serial dilutions of serum from arthritic animals obtained on day 40 after immunization. Each data point represents the mean value obtained using serum from three animals and ELISA performed in triplicate on each sample +/- standard error. C-II specific antibodies of either the IgG2a or IgG1 subtype were not detected in the serum from control animals (not shown). There was no detectable titer to control antigen HEL in any animal (data not shown). Similar results were obtained in 2 independent experiments.

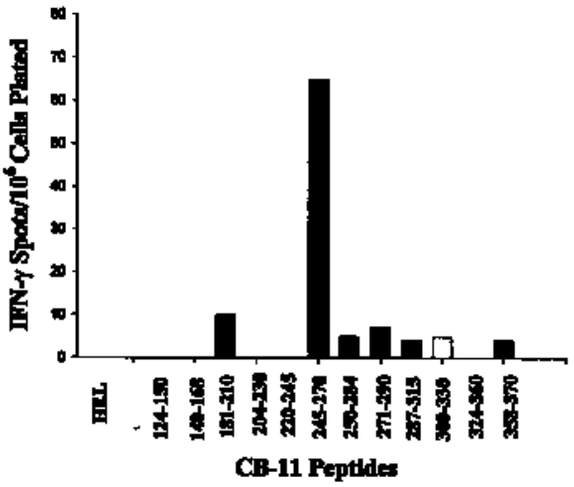

Fig. 2. Draining lymph node cells from arthritic animals respond to the C-II derived immunodominant peptide A2. $5x10^5$ lymph node cells were tested for responses to medium alone or peptide fragments spanning the entire CB11 fragment of C-II (10 g/ml) using an ELISA spot assay. Representative mean values of duplicate wells, after subtraction for non-specfic stimulation (< 12 spots per well for HEL-stimulated wells), as determined by computer assisted image analysis are shown. Similar results were obtained in 3 separate experiments using independent batches of mice (n = 6 mice/group). No IL-4 or IL-5 was detected by this method in response to any antigen (data not shown).

mice. No C-II specific antibodies were detected in naive and control mice immunized with V 10p.

In order to determine the fine specificity of the T cell response to C-II, we performed epitope mapping experiments using 12 peptide fragments spanning the cyanogen bromide fragment 11 of the C-II molecule. The CB11 fragment has previously been shown to harbor the immunodominant region of the C-II molecule for H-2^q restricted T cell immune responses (26). Representative individual IFN- ELISA spot wells are shown in Figure 2A, and the results are quantified in Figure 2B. As can be seen, the predominant T cell response was directed at peptide A2 (residues 245-270, Fig. 2). In these experiments no C-II-specific IL-4 or IL-5 spots were detected to any C-II peptide (results not shown). Importantly, the identified peptide A2 is the same as that described in the prototypic CIA susceptible DBA/1 (H-2^q) mouse strain (26). This finding is therefore consistent with our previous studies showing that the TCR V gene deletions in the BUB/BnJ mouse strain has no effect on the development of an autoreactive T cell repertoire, and that the autoreactive cellular immune response in TCR V a mice mimics the cellular response observed in other CIA susceptible H-2q strains with TCR V b genotype.

V 10p-specific cellular immune response was mediated by CD4+ Th1 type T cells

We have previously shown that pre-immunization of BUB mice with V 10p prevents the development of CIA in BUB mice (23). In an effort to gain further insight into the mechanism of this protective effect, we next sought to characterize the induced V 10p specific immune response in mice immunized with V 10p/CFA. As shown in Figure 3A, immunization with V 10p resulted in induction and expansion of IFN- and IL-2 producing T cells specific for V 10p (~80 IFN- spots/10^6 lymph node cells). The observed cytokine profile was consistent with our previously published studies showing

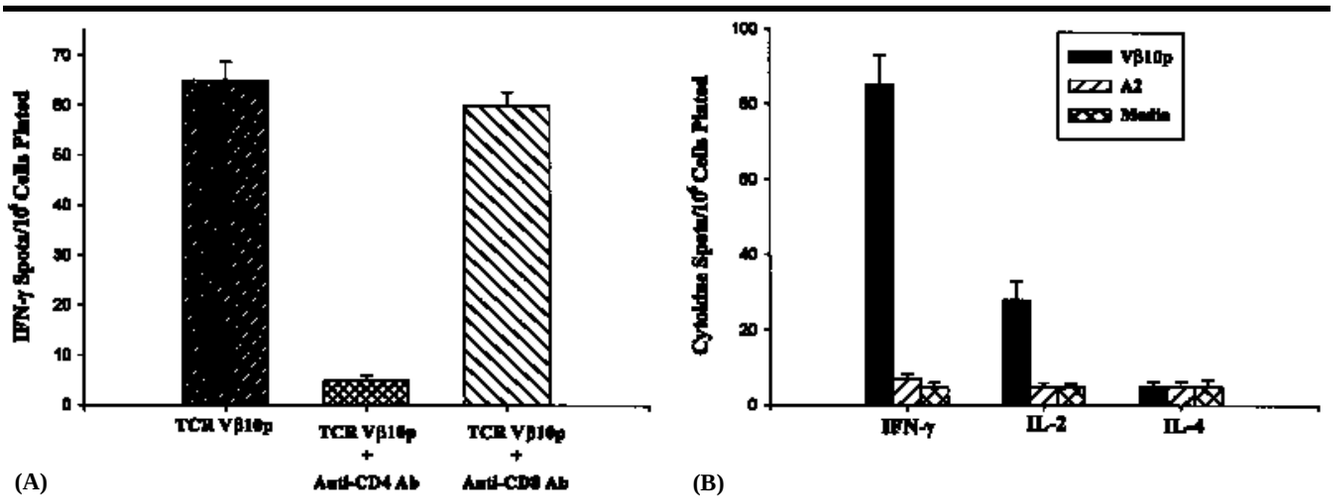

Fig. 3. Immunization with V 10p induces a CD4 restricted Th1 type cellular immune response. **A**. Draining lymph node cells obtained on day 10 after immunization with V 10p/CFA were tested using cytokine ELISA spot assays. Bars represent the mean values of duplicate wells for three individual animals tested in a single experiment. Peptide A2 served as a specificity control for these experiments. This experiments were repeated twice and similar results were obtained.

B. Draining lymph node cells obtained on day 10 after immunization with V 10p/CFA were tested for IFN- production in response to V 10p in the presence of anti-CD4 or anti-CD8 antibody (50 g/ml). Results represent mean values of duplicate determinations made from 3 mice. No response to control TCR V$14 peptide was detected (not shown). The experiment was repeated twice with similar results.

Table I. Incidence and severity of CIA in BUB mice§.

Pretreatment	Incidence	Day of onset	Joint Score
None	6/8	30, 29, 31, 30, 28,32	3,2,2,2,2,1,0,0
V 14p/CFA	3/4	29, 29, 32	6,5,2,0
V 10p/CFA	2/8	33	6,4,0,0,0,0,0,0

§Animals were either not preimmunized, or were preimmunized with V 10p or V 14p in CFA 10 days prior to immunization with C-II/CFA (day 1) to get CIA. All animals were sacrificed on day 40 for recall analysis. Clinical score (total joint score) shown here is the sum of scores for all joints of the same animal. Similar results were obtained in two other independent experiments.

that immunization with peptide in CFA induces predominantly a Th1 type immune response (25, 27). The V 10p specific immune response was also detectable in spleen cells 3-4 weeks after immunization (data not shown), indicating that the induced immune response was stable over time. No V 10p specific T cells were detected in unimmunized mice or in mice immunized with C-II to get CIA (results not shown) and is consistent with our previous findings (25). Control immunization with V 14p also resulted in a Th1 immune response (IFN- and IL-2 producing, but not IL-4 or IL-5; data not shown).

Since both CD4+ and CD8+ T cell subsets have been implicated as important mediators of protective immunity in other model systems (2, 3, 5), we next tested whether the V 10p specific response was derived from the CD4+ or CD8+ subpopulation. As shown in Figure 3B, V 10p specific cytokine production was blocked by anti-CD4, but not by anti-CD8 blocking antibody, demonstrating that the V 10p specific cytokine production was mediated by CD4+ T cells.

Protective immunization with V 10p decreases the frequency of C-II-specific T cells

We next determined how immunization with V 10p prior to disease-inducing immunization affected the development, frequency and cytokine profile of C-II-specific T cells. Groups of mice were either preimmunized with V 10p (experimental), V 14p (specificity control), or were not pre-immunized (control). Ten days later animals in all 3 groups were immunized with C-II to induce arthritis. Our results show that in mice preimmunized with V 10p, only 2 of 8 mice (25% incidence) developed clinical arthritis compared to an incidence of 75% in both the control groups. As previously described (25), clinical arthritis correlated well with the histological analysis of arthritic and arthritis protected mice (not shown). Similar results (not shown) were obtained when this experiment was repeated with a new batch of mice. The 2 mice pre-immunized with V 10p that developed arthritis after immunization with C-II/CFA had a lower frequency of V 10p specific IFN- producing T cells (10 and 35 spots/million cells plated in the arthritic mice compared to 30-100 spots/million cells plated in the arthritis protected mice, data not shown).

We also determined the frequency of C-II specific T cells in mice immunized with V 10p prior to disease inducing immunizations and these results are shown in Figure 4A. Strikingly, in these mice protection from arthritis was associated with a barely detectable C-II-specific Th1 type cellular immune response (< 10 IFN- producing cells/ 10^6 cells plated). This was in sharp contrast to the arthritic mice in the control groups where the frequency of IFN- producing T cells specific for C-II was

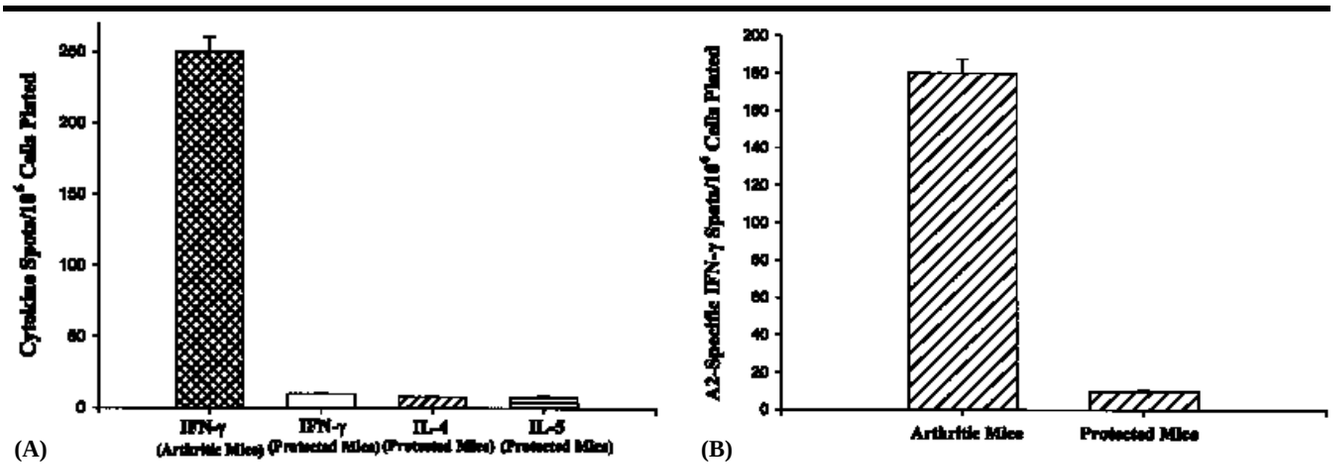

Fig. 4. TCR V 10p induced protection decreases the frequency of C-II and A2 specific T cells. ELISA spot recall assays were performed using splenocytes obtained from three independent groups of mice:Control animals (n = 6) were not pre-immunized with V 10p/CFA and developed arthritis; all animals pre-immunized with V 14p developed arthritis (peptide control,n = 6); all animals pre-immunized with V 10p were protected and did not develop arthritis (n = 6). The frequencies of C-II (panel A) and A2 (panel B) specific IFN- producing cells after subtracting nonspecific antigen induced spots are shown. There were no detectable C-II- or A2-specific, IL-4- or IL-5-producing cells in any group while positive Con A stimulated controls showed >100 spots per well (data not shown). The response to the specificity control antigen HEL was < 10 spots/million cells for all cytokines. The experiment was repeated twice with different batches of BUB mice and similar results were obtained.

* $P < 0.001$ comparing C-II specific IFN- production in arthritic and protected mice.

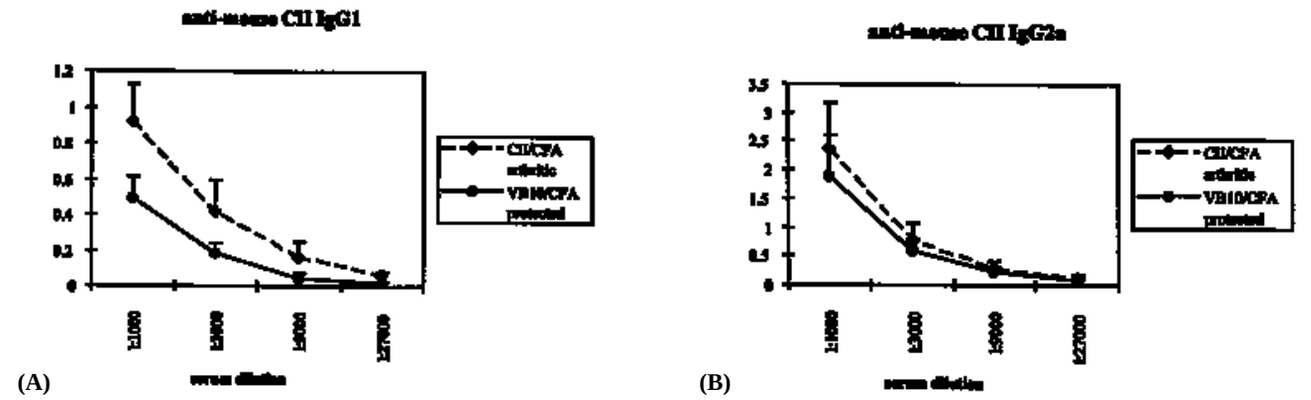

Fig. 5. Anti-mouse C-II antibody titer in protected and arthritic mice:ELISA results for IgG1 (panel A) and IgG2a (panel B) anti-mouse C-II antibody performed on serum samples from arthritic (n=6) or protected (n=3) mice. The results are expressed as the mean value of OD units measured at 405 nm after subtracting the background binding to specificity control HEL (< 0.1 units in each animal). Error bars represent 1 standard deviation from the mean.

significantly higher (30-255 IFN- producing cells/10^6 cells plated, $p < 0.01$). Remarkably, protection from arthritis was not associated with the emergence of Th2 type cytokine secreting T cells (IL-4 or IL-5 producing T cells, data not shown). Moreover, the protective immunization with V 10p was associated with a lack of detectable response to the previously defined immunodominant epitope A2 (Fig. 4B) and no alternative epitopes from the CB11 region emerged as dominant (data not shown). Antigen recall responses (not shown) from mice preimmunized with V 14p prior to disease inducing immunization that developed arthritis revealed similar frequencies of C-II specific (and A2 specific) Th1 type T cells (IFN- producing). Thus, our data demonstrate that immunization with V 10p (but not with a control TCR V peptide) protected mice from the phenotypic expression of arthritis and this protection was associated with a marked decrease in the pathogenic Th1 type T cell immune response without a shift in the cytokine profile.

In contrast to the marked effects on the C-II specific T cell immune response, protective immunization with V 10p had minimal effect on the titers or subtypes of mouse C-II-specific antibodies (Fig. 5). Both IgG1 and IgG2a antibodies specific for mouse type-II collagen were found in control arthritic mice, in mice preimmunized with V 14p and in arthritis protected mice (preimmunized with V 10p), with titer similar to those found previously in arthritic and arthritis protected mice (23). We thus conclude that the anti-autoantigen specific humoral immune response was not appreciably altered by immunization with TCR V peptides.

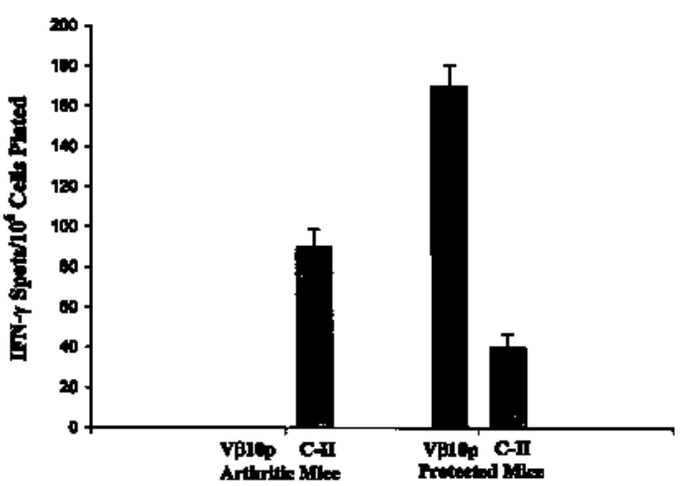

Fig. 6. Cellular immune response to TCR V 10p and C-II in arthritic and protected mice. Mice receiving TCR V 10p specific cells (IFN- producing) were protected from CIA and showed a down modulation C-II-specific Th1 type immune response. Control mice receiving only PBS were not protected from CIA and had a robust C-II-specific Th1 type immune response. Results also show that arthritic mice did not develop TCR V 10p-specific cellular immune response. Results shown are the means of two independent experiments, each performed in duplicate. Error bars represent S.D. of the mean.

Adoptive transfer of V 10p-specific T cells (IFN- producing) reduced the incidence and delayed the onset of CIA in BUB mice

T cells purified from LN and spleens of mice immunized with V 10p/CFA specifically produced IFN- (data not shown). These cells were injected i.v. into age-matched BUB mice which were then immunized with C-II/CFA for the induction of CIA. As can be seen from Table II, only 37% of the mice receiving the TCR V 10p-specific T cells developed arthritis as opposed to controls, where 100% of the mice developed CIA. Additionally, the onset of arthritis in the recipient group was significantly delayed ($p < 0.001$) when compared to controls (Table II). The severity of the disease was also mild in the affected mice that received V 10p-specific T cells prior to disease inducing immunizations (Table II) and the arthritis did not progress rapidly as is usually the case.

Discussion

We have previously shown that immunization of CIA susceptible BUB mice with TCR V 10p prior to disease inducing immunization with C-II results in protective immunity against CIA (23). Results presented in this paper

Table II. Adoptive transfer of TCR V 10p-specific T cells (IFN- producing) reduced the incidence and severity of CIA in recipient BUB mice.

Immunization Protocol	Incidence of CIA	Mean Day of Onset (Range)	Mean Arthritis Index (MAI)
PBS + V 10p-specific T cells; C-II/CFA 3 days later	3/8 (~37%)	43 (41-44)	1
PBS C-II/CFA 3 days later	8/8 (100%)	30 (27-34)	2.8

Results shown are cumulative of two experiments done independently using different batches of mice. In each experiment, mice in both groups were boosted with C-II three weeks after primary immunization with C-II/CFA.
MAI was calculated by adding the total clinical severity score of each joint in each group of mice and dividing by the total number of mice in that group. The difference in the MAI of the two groups was statistically significant ($p < 0.001$).

confirm and expand upon our previous findings and further establish that CIA in BUB mice is characterized by a C-II specific Th1 type immune response. The induced C-II-specific (and immunodominant C-II epitope-specific) T cells overwhelmingly produced IFN- and IL-2 (Fig. 1). The detection of IgG2a anti-C-II antibodies, in addition to IgG1, is also consistent with an overall Th1 type immune response (27). The proinflammatory effects of Th1 type immune responses have been well documented (28-31) and are consistent with what is known regarding the pathogenesis of CIA (12). IFN- has been shown to stimulate macrophage activation (potentially leading to the release of effector molecules TNF- and nitric oxide), upregulate MHC II and costimulatory expression on APCs (thus facilitating T cell activation), initiate chemokine release (thus attracting new T cells and monocytes into the site of inflammation), and induce antibody isotype switching to the complement fixing IgG2a subclass (32, 33). Complement fixation of anti-C-II specific IgG2a antibodies, in particular, seems to be essential (but not sufficient) for the full manifestations of CIA (12). Our results showing that the C-II specific cellular immune response in BUB mice was primarily mediated by IFN- and IL-2 producing T cells are consistent with similar findings reported by other investigators (20, 34). However, our findings are the first to characterize the cytokine profile and antigen fine specificity in a mouse strain with the TCR V ^{a} genotype. Moreover, our data provide information regarding the frequency of alloreactive T cells, and show that not a very large number of T cells are associated with the clinical expression and manifestations of arthritis in this model. Our data also reveal that the autoreactive T cell repertoire is limited (30-220 IFN- producing cells/million cells) and focused towards the A2 peptide region of C-II. As this is the same immunodominant region of C-II found in other CIA susceptible H-2q mouse strains (26), these data confirm that the large deletion of TCR V genes in BUB mice does not alter the autoimmune repertoire directed at the autoantigen C-II.

Perhaps most notably, however, our data clearly define how immunization with this TCR peptide alters the pathogenic C-II specific immunity. First, our results show that immunization with TCR V 10p induces a stable population of CD4+, IFN- and IL-2 producing T cells specific for V 10p (Figure 3). Our findings contrast with several studies of protective immunity in EAE, in which TCR V peptide immunizations led to expansion of a pre-existing population of regulatory CD8+ T cells specific for the autoreactive T cell repertoire (2, 3). Findings reported here suggest that the V 10p specific CD4+ T cells may provide a regulatory role in protection from disease.

Our results further demonstrate that the induced V 10p specific immunity in CIA-protected animals was associated with a marked decrease in the frequency of C-II- (and A2-) specific T cells without a change in their cytokine profile. As it has not been possible to readily measure the frequencies of antigen specific T cells prior to use of the ELISA spot approach, the decrease in frequencies of C-II specific T cells after protective immunization provide novel insight into the effects of this strategy. The findings are entirely consistent with the notion that the frequency of pathogenic T cells will, in part, determine whether the induced autoimmune response results in end organ damage. Interestingly, the protective immunization did not prevent arthritis in two animals, and these mice contained high frequencies of C-II specific T cells (over 100 IFN- producing cells/million cells plated, results not shown) and suggest that the induced pathogenic T cells must reach a "threshold" frequency to result in end organ damage. There is some previous experimental evidence from adoptive transfer models to suggest that the frequency of autoreactive T cells may be crucial in determining whether the autoimmune response results in end organ damage (35). Low frequencies of autoreactive T cells may not be sufficient to induce autoimmune disease despite expression of identical cytokine profiles and antigen specificities as the pathogenic T cell repertoire.

We do not yet know the mechanism(s) involved in the reduction in frequency of the C-II-specific T cells. Our data cannot distinguish T cell deletion, T cell anergy and/or T cell suppression as viable mechanisms, but definitively demonstrate that deviation towards type 2 immunity is not operative under these circumstances. As murine T cells do not express MHC II and the induced protective immunity to V 10p is CD4 restricted, it is unlikely that the protective effect is due to direct cell mediated deletion of the pathogenic T cells by the protective CD4 population. This is consistent with a form of induced bystander suppression or induced anergy, hypothesized to be the case in some experiments demonstrating protection from EAE (36-39). The specific cellular and molecular mechanisms involved in this process have yet to be established and are the focus of ongoing studies in the laboratory. It is possible that the population of CD4+ V 10p specific T cells provide help for the

induction of an anti-V 10 specific antibody response that contributes to deletion of the autoreactive T cells and we have shown that transfer of serum from protected animals can delay the onset of arthritis (but does not prevent it) (23). Thus, although we cannot rule out a role for protective antibodies induced by the V 10p immunization, our data reveal that full protection was dependent on the presence of active anti-TCR V 10p- specific T cell immunity. Consistent with this is the finding that adoptive transfer of TCR V 10p-specific T cells (IFN- producing) from V 10p immunized mice to naive recipients was sufficient to reduce the incidence and delayed the onset of CIA in recipient mice (Table II). The protected mice had anti-TCR V 10p-specific T cells (IFN- producing) indicating that the transferred immune response was stable. This was in sharp contrast to control mice where 100% of those immunized with C-II/CFA developed severe, classical arthritis (Table II) and in these mice anti-TCR V 10p-specific T cells were not detected (results not shown).

We found it intriguing that there were no detectable differences in the anti-mouse C-II IgG1 and IgG2a titers in diseased versus protected animals. The continued presence of anti-C-II specific antibodies in protected animals has two important implications. First, the finding confirms that the presence of autoreactive antibodies alone is insufficient to yield the full manifestations of CIA. This is consistent with previous observations that autoantibodies can transfer only a transient form of arthritis, and that the full manifestations of this disease require both T cell and humoral immunity specific for the autoantigen (15). Secondly, the presence of detectable autoreactive antibodies in the serum of protected animals suggests the presence of T cell help for induction of an antibody response despite our inability to detect C-II specific T cells. Such an observation is further evidence that the pathogenic autoreactive T cell repertoire is suppressed but not deleted. Immunization with V 10p prior to immunization with C-II to get disease seems to have prevented some effector functions (i.e. the ability to secrete cytokines at a time when arthritis is anticipated to occur) but have little effect on other functions, i.e. provision of help for induction of an autoantibody response.

In conclusion, our findings provide strong evidence that immunization with TCR V 10 peptide induced a stable CD4 mediated Th1 type immune response specific for TCR V 10p and this was associated with a significantly decreased frequency of autoreactive T cells in BUB mice. Further elucidating the mechanisms of this protective decrease in pathogenic T cells may result in novel approaches for the treatment of autoimmune diseases.

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