Vaccination with a Recombinant Vα Domain of a TCR Prevents the Development of Collagen-Induced Arthritis[1]

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A recombinant TCR domain, derived from a T cell hybridoma that recognizes an immunodominant type II collagen epitope, was used to vaccinate against collagen-induced arthritis in DBA/1 (H-2^q) mice. The recombinant TCR domain comprises VA11.1-JA17 gene segments and is representative of the Vα domains expressed by oligoclonal T cells in this disease model. Vaccination of mice 28 days before type II collagen (CII) immunization with this Vα11.1 domain resulted in a significantly decreased incidence of arthritis in DBA/1 mice, in contrast to vaccination with a Vα4-Jα40 domain derived from an encephalitogenic T cell hybridoma specific for MBP. Disease blockade is accompanied by a reduction in T and B cell responses to both the immunogen bovine CII and the autoantigen murine CII. Vα4 and Vα11.1 domains were found to be highly immunogenic in DBA/1 mice, inducing both T cell proliferation and the production of Vα specific Abs, indicating that the vaccination effect of Vα11.1 is specific. This is the first report of Vα-directed immunotherapy in an autoimmune disease model and demonstrates the potential use of recombinant TCR vaccines in the treatment of autoimmune diseases that involve oligoclonal autoreactive T cells. *The Journal of Immunology,* **1995, 155: 4504–4511.**

Ag-dependent stimulation of T cells involves the recognition of peptides bound to MHC proteins by the Ag-specific TCR. For many model Ag systems, it has been shown that the T cell response to cognate peptide:MHC is encoded by a limited set of V region gene segments (1–6). These observations led to the idea that the V regions of oligoclonal T cells might provide a highly specific target for the down-regulation of autoimmune responses (5, 6), and this approach has been tested successfully in animal models using both Abs specific for Vβ domains (5, 6) and by vaccination with synthetic peptides based on either CDR2 (7, 8) or the junctional region (9) of Vβ domains.

Although successful in animal models, vaccination with TCR-derived peptides is complicated by the need to identify an immunogenic peptide derived from the autoreactive TCR that will provide protection from the induction of disease. To address this limitation, we have developed a prokaryotic expression system for the production of full-length, soluble Vα domains for use as vaccines in the prevention or down-regulation of autoimmune responses (10, 11). In particular, Vα domains can be isolated in milligram quantities to a high level of purity, facilitating their use as immunomodulating reagents. In this study, we have tested the use of a recombinant Vα derived from a T cell clone associated with murine collagen-induced arthritis (CIA)[3] as a vaccine to prevent the induction of murine CIA. CIA is an experimental autoimmune disease elicited in genetically susceptible animals by immunization with type II collagen (CII) (12). In the murine CIA model, susceptibility is linked to the expression of the I-A^q and I-A^r class II molecules (13), and the immune response to CII is characterized by both a T cell proliferative response and the production of Ab (14, 15). Based on our analysis of the T cell response to an immunodominant CII peptide (16) and the analysis of the TCR repertoire responding to CII by Osman et al. (17), we have produced a Vα11.1-Jα17 recombinant TCR domain for use as a vaccine.

In this report, we demonstrate that vaccination with a full-length Vα11.1 domain can modulate the immune response to CII and subsequently prevent the development of CIA. The vaccination down-regulates the production of Abs to the immunogen bCII and the autoantigen murine CII. In addition, both a T cell and B cell response to the Vα11.1 domain can be detected as a result of vaccination. The use of a full-length Vα domain provides several advantages to TCR-specific immunotherapy over previously described modalities. These studies demonstrate that targeting the Vα domain of oligoclonal T cells can be an effective route toward the therapy of an autoimmune disease.

Materials and Methods

Collagen preparation

Native bovine type II collagen (bCII) was solubilized from articular cartilage by limited proteolysis with pepsin and purified by repeated differential salt precipitation as described by Miller (18). Native mouse type II collagen (mCII) was prepared from sternal cartilage of young mice and purified in the same manner as bCII.

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[3] Abbreviations used in this paper: CIA, collagen-induced arthritis; CII, type II collagen; bCII, native bovine type II collagen; mCII, native mouse type II collagen; MBP, myelin basic protein; DPM, decays per minute; δDPM, decays per minute, background subtracted.

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Table I. *Peptide specificity of bovine CII-specific, I-A^q-restricted T cell hybridomas*

T Cell Hybridoma	IL-2 (U/ml)[a] Ag[b] α1(II)	bCII(124–402) (CB11)	bCII(239–260)	bCII(260–270)	mCII(245–270)
qCII85.33	320	640	<20	320	320
qCII92.33	640	640	<20	640	<20

[a] Units of IL-2 produced by the T-cell hybridomas were determined using an HT-2 assay as described in *Materials and Methods*.
[b] 50 μg of Ag was added to Ag presentation cultures containing 10^5 T hybridoma cells and 4×10^5 DBA/1 spleen cells. Eighteen hours later, supernatants were analyzed for the presence of IL-2.

Expression of TCR Vα11.1 and Vα4 domains

The construction of plasmids for the expression of the TCR Vα4 and Vα11.1 domains has been described (11, 19). The cDNA encoding the Vα11.1 domain TCRAV11S1J17 was produced from mRNA isolated from an I-A^q-restricted T cell hybridoma (qCII85.33) specific for CII(260–270) (16), and the VA4 cDNA TCRAV4J40 was derived from an I-A^u-restricted T cell hybridoma (1934.4) specific for MBP Ac1–11 (20). Following expression in *Escherichia coli*, the Vα domains were purified from osmotic shock fractions of recombinant bacteria using Ni^{2+}-NTA-agarose (11).

Animals and vaccinations/immunizations

Six- to eight-week-old male DBA/1J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) for use in these experiments. In preparation for vaccination, TCR Vαs were dialyzed into 50 mM acetic acid, pH 4.6, and emulsified with equal volumes of Titermax (Vaxcel, Inc., Norcross, GA). Mice were vaccinated with 100 μg of the Vα protein s.c. at the base of the tail. Thirty days after vaccination, mice were immunized with bCII for the induction of arthritis. bCII was dissolved in 0.01 M acetic acid and emulsified at a 1:1 (v/v) ratio with CFA (Life Technologies, Grand Island, NY) as described previously (21). Mice were immunized s.c. at the base of the tail with 100 μg of bCII. Beginning at 4 wk after the bCII immunization, mice were monitored for the development of arthritis. Each paw was evaluated and scored for the degree of inflammation on a scale of 0 to 4 (13).

Proliferation assays

Inguinal and para-aortic lymph nodes were removed from immunized animals, disassociated, and washed in RPMI 1640. Lymph node cells were placed in 96-well plates at 5×10^5 cells/well in the presence of various amounts of Ag, and cultures were maintained in 300 μl of Click's medium (22) supplemented with 0.5% mouse serum at 37°C, 5% humidified CO_2 for 4 days. Eighteen hours before the termination of the cultures, 1 μCi of [^{3}H]thymidine was added to each well. Cells were harvested onto glass fiber filters and counted on a Packard Matrix 96 direct ionization β counter. Results of proliferation experiments are expressed as mean decays per minute (DPM) of quadruplicate wells with the control stimulation subtracted (ΔDPM).

T hybridoma production and Ag presentation assays

T cell hybridomas were established by polyethylene glycol-induced fusion of lymph node cells with the TCR $\alpha^-\beta^-$ thymoma line, BW5147 (23, 24). Lymph node cells were obtained from DBA/1 mice immunized with bCII emulsified with CFA and cultured in vitro with CII(245–270) for 5 days and expanded in IL-2 for 3 days before fusion. Hybridoma cells reactive to CB11 and CII(245–270) were cloned by limiting dilution to 0.3 cells/well. Ag presentation experiments were performed in 96-well microtiter plates in a total volume of 0.3 ml containing 4×10^5 syngeneic spleen cells as APC and 10^5 T hybridoma cells. Cell cultures were maintained at 37°C in 5% humidified CO_2 for 20 to 24 h, after which seven 80-μl twofold serial dilutions were made for determination of IL-2 titers. Four thousand HT-2 cells were added to each supernatant dilution, and after 16 to 20 h HT-2 cell viability was evaluated by visual inspection and the ability of the viable cells to reduce a tetrazolium dye (MTT) (25). IL-2 titers were determined by the reciprocal of the highest twofold serial dilution maintaining 90% viability of the HT-2 cells as described by Kappler et al. (26).

ELISA

Sera were collected at various time points after Vα vaccination and CII immunization, and titers were determined for individual animals. Ab titers to bCII were determined using an ELISA as described previously (27). Briefly, microtiter plates were coated with 500 ng of either bCII or mCII at 4°C overnight. After extensive washing with 0.15 M saline/0.05% Tween 20, dilutions of sera ranging from 1:4000 to 1:128,000 in normal goat sera were added to each well and incubated overnight at 4°C. After washing with saline and Tween 20, either a goat anti-mouse Ig (1:5000) or goat anti-mouse isotype-specific antisera (Southern Biotechnology Associates, Birmingham, AL) was added for 2 h. The plates were then washed and developed by the addition of *o*-phenyldiamine (Sigma Chemical Co., St. Louis, MO). After stopping the reaction with 2.5 N H_2SO_4, the degree of color development was measured at 490 nm with background absorbance of 650 nm subtracted. Data are expressed as relative mean units of activity based on a positive control anti-bCII serum. ELISA detection of Abs that bind to the recombinant Vαs were performed as described above for CII, with the exception that the plates were coated with either the Vα11.1 or Vα4 recombinant protein and that data are expressed as OD values.

Results

Production of recombinant CII-specific TCR domains

We have reported previously that the CII(260–267) sequence of the CB11 fragment of bCII contains the immunodominant T cell determinant recognized by I-A^q-restricted cells (16, 27). To analyze the T cell component of the immune response to this determinant, a panel of T cell hybridomas was produced. All of the T cell hybridomas specific for this determinant fell into one of two categories based on their ability to respond to peptides representing both the immunogen bCII(260–267) and its homologue mCII(260–267). Examples of these T cell hybridomas are shown in Table I. Whereas the T cell hybridoma qCII85.33 recognizes the antigenic determinant in both bCII and mCII(260–267), the hybridoma qCII92.33 responds only to the bCII peptide. This difference in TCR specificity is remarkable in that the only difference between these two peptides is the substitution of the aspartic acid at residue 266 in mCII with a glutamic acid residue in bCII. To date, approximately one-half of all the T cell hybridomas specific for the CII(260–267) determinant we have studied cross-react with the murine homologue.

Based on our analysis of the TCR expression by these CII(260–267)-specific T cell hybridomas, we sought to develop a vaccination protocol utilizing CII-specific TCR V domains for the prevention of CIA. We selected the Vα domain expressed by the T cell hybridoma qCII85.33, and the TCRAV11S1J17 gene segments expressed by this hybridoma were isolated and cloned into an expression vector for secretion of soluble V domains into the periplasm of *E. coli* (Table II) (10, 11). For use as a control, a Vα4 domain derived from the 1934.4 T cell hybridoma that is specific for MBP and restricted to I-A^u was also produced (11). This TCR domain comprises VA4 and JA40 gene segments (6) and shares 32% amino acid homology with Vα11.1. Both Vαs were purified by nickel affinity chromatography in yields of several milligrams

Table II. *cDNA and amino acid sequence of Vα11.1-Jα17 TCR chain expressed by CII-specific T cell hybridoma qCII85.33*[a]

	5		10	15		20		25	30		35		40	45		50		55	60
GGA	GAT	CAG	GTG	GAG	CAG	AGT	CCT	TCA	GCC	CTG	AGC	CTC	CAC	GAG	GGA	ACC	GAT	TCT	GCT
Gly	Asp	Gln	Val	Glu	Gln	Ser	Pro	Ser	Ala	Leu	Ser	Leu	His	Glu	Gly	Thr	Asp	Ser	Ala
	65		70	75		80		85	90		95		100	105		110		115	120
CTG	AGA	TGC	AAT	TTT	ACG	ACC	ACC	ATG	AGG	AGT	GTG	CAG	TGG	TTC	CGA	CAG	AAT	TCC	AGG
Leu	Arg	Cys	Asn	Phe	Thr	Thr	Thr	Met	Arg	Ser	Val	Gln	Trp	Phe	Arg	Gln	Asn	Ser	Arg
	125		130	135		140		145	150		155		160	165		170		175	180
GGC	AGC	CTC	ATC	AGT	TTG	TTC	TAC	TTG	GCT	TCA	GGA	ACA	AAG	GAG	AAT	GGG	AGG	CTA	AAG
Gly	Ser	Leu	Ile	Ser	Leu	Phe	Tyr	Leu	Ala	Ser	Gly	Thr	Lys	Glu	Asn	Gly	Arg	Leu	Lys
	185		190	195		200		205	210		215		220	225		230		235	240
TCA	GCA	TTT	GAT	TCT	AAG	GAG	CGG	CGC	TAC	AGC	ACC	CTG	CAC	ATC	AGG	GAT	GCC	CAG	CTG
Ser	Ala	Phe	Asp	Ser	Lys	Glu	Arg	Arg	Tyr	Ser	Thr	Leu	His	Ile	Arg	Asp	Ala	Gln	Leu
	245		250	255		260		265	270		275		280	285		290		295	300
GAG	GAC	TCA	GGC	ACT	TAC	TTC	TGT	GCT	GCT	GAG	GCC	TCT	TCT	GGC	AGC	TGG	CAA	CTC	ATC
Glu	Asp	Ser	Gly	Thr	Thy	Phe	Cys	Ala	Ala	Glu	Ala	Ser	Ser	Gly	Ser	Trp	Gln	Leu	Ile
	305		310	315		320		325	330										
TTT	GGA	TCT	GGA	ACC	CAA	CTG	ACA	GTT	ATG	CCG									
Phe	Gly	Ser	Gly	Thr	Gln	Leu	Thr	Val	Met	Pro									

[a] cDNA was amplified by PCR using VA11.1- and JA17-specific primers. First-strand cDNA was synthesized using mRNA isolated from the T cell hybridoma as template. Once cloned, the PCR product was sequenced from both directions for verification. The VA11.1 sequence extends through base 273, and the JA17 sequence begins at base 277. The sequence data are available from GenBank under accession number L47342.

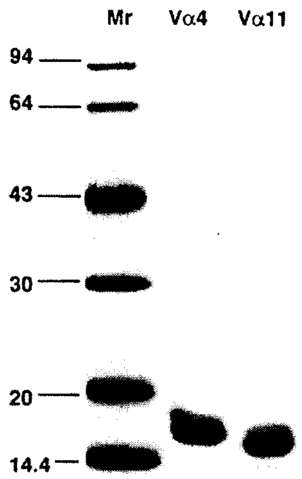

FIGURE 1. SDS-PAGE analysis of purified, recombinant Vα11.1-Jα17 and Vα4-Jα40 domains produced in bacteria. *Lane 1*, molecular weight standards; *Lane 2*, purified Vα4 domain derived from the MBP-specific T cell hybridoma 1934.4; *Lane 3*, purified Vα11.1 domain derived from the CII-specific T cell hybridoma qCII85.33.

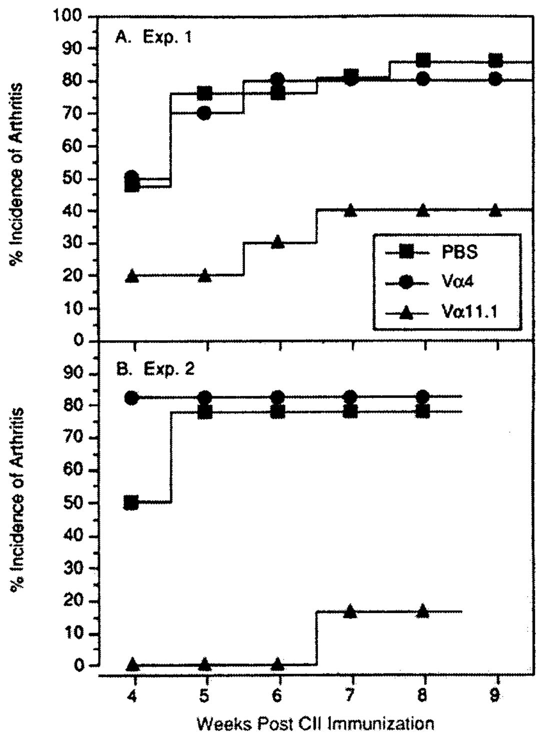

FIGURE 2. Incidence of arthritis following vaccination with recombinant Vα4 or Vα11.1. DBA/1 mice were vaccinated with Vα4, Vα11.1, or PBS and 4 wk later challenged with bovine CII. Data are expressed as percent incidence of arthritic mice. Number of mice were 10 for the Vα4 and Vα11.1 groups and 18 for the PBS group in experiment 1 (*A*) and 6 per group in experiment 2 (*B*). The incidence of arthritis for the PBS and Vα4 group in *B* were identical at weeks 5 through 8. Statistical analysis of arthritis incidence between PBS and Vα11.1 groups in *A*: week 4, $p = 0.045$; weeks 5 and 6, $p = 0.003$; and weeks 7 and 8, $p = 0.02$. For PBS group vs Vα4 group, $p > 0.2$ at all time points.

per liter of culture (Fig. 1). Amino acid composition analysis was used to confirm the identity of these proteins.

Vaccination with TCR Vα domains

To test the ability of the recombinant TCR Vα11.1 to prevent the development of CIA, the soluble TCR domain was used to vaccinate DBA/1 mice. Twenty-eight days later, these mice were immunized with bCII and monitored for the development of arthritis. In two separate experiments, mice were vaccinated with either PBS, the recombinant Vα11.1 derived from the CII-specific T cell, or the recombinant Vα4 derived from the MBP-specific T-cell hybridoma (Fig. 2). Each mouse was examined individually for both the production of Ab to bCII and mCII and the incidence and severity of arthritis. As shown in Figure 2, vaccination with Vα11.1 before CII immunization significantly reduced the

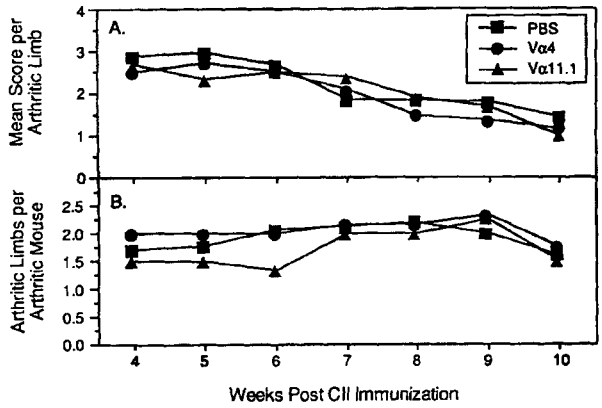

FIGURE 3. Severity of arthritis among TCR Vα domain vaccinated mice that succumbed to disease. Severity of arthritis was evaluated by assigning a score of 0 to 4 based on the degree of inflammation for each limb, with 4 indicating severe arthritis and 0 indicating no arthritis (*A*). Data are expressed as mean severity score per arthritic limb. The number of arthritic limbs per arthritic mouse was also evaluated as a second indicator of arthritis severity (*B*).

incidence of CIA in two independent experiments ($p < 0.05$ for PBS vs Vα11.1 groups at all time points). In addition, the few mice immunized with Vα11.1 that developed arthritis did so at a slower rate than Vα4- and PBS-treated groups. Mice in both experiments were observed for 10 wk, at which time no new arthritic limbs were observed and the initial arthritis had nearly resolved. Although the onset of arthritis was delayed for the few mice that developed disease in the Vα11.1-treated group, once established, there was no significant difference in severity determined by analysis of both mean score per arthritic limb and number of arthritic limbs (Fig. 3). Initially there appeared to be fewer arthritic limbs per arthritic mice in the Vα11.1 group; however, this difference was not significant and disappeared by week 7 (Fig. 3). Thus, Vα11.1 vaccination prevented disease onset in a significant proportion of mice, yet it did not affect the severity of the disease in those that succumbed to the autoimmune arthritis.

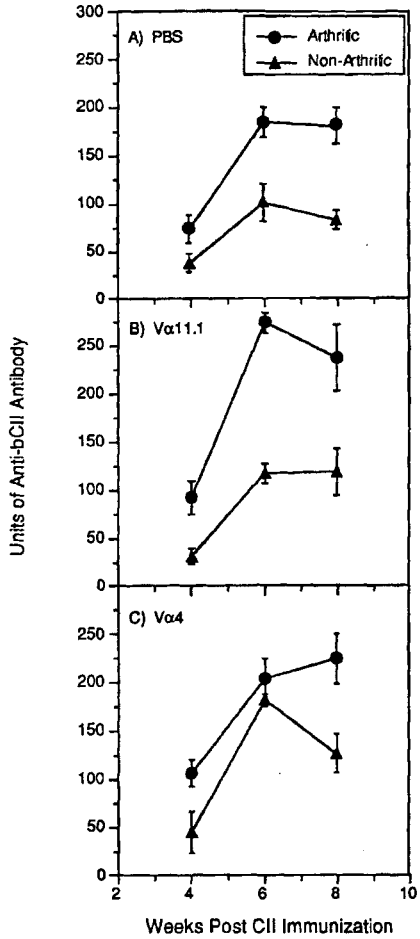

FIGURE 4. Ab titers to bovine CII following vaccination with TCR Vα domains and challenge with CII. Individual serum samples were obtained at weeks 4, 6, and 8 post-CII immunization and analyzed for quantity of Ab to native bCII. Titers were determined by ELISA and were normalized to a standard anti-bCII serum. Individual Ab calculations were made from several dilutions of each mouse serum, ranging from 1:4,000 to 1:128,000. Ab to bCII in normal DBA/1 mouse serum was undetectable. Error bars indicate SEM.

Effect of Vα11.1 vaccination on Ab response to bCII and mCII

Since the development of Ab to CII is a major factor in establishing CIA in mice (28), Ab titers to both the immunogen and the autoantigen mCII were measured. Serum samples were obtained from individual mice at several time points throughout the course of the experiments described in Figure 2. Ab titers to both bCII and mCII were determined by ELISA, and the results were normalized using a standard anti-CII serum. As shown in Figure 4, the bCII-specific Ab titers from arthritic and nonarthritic mice were very similar among all three groups. In each group, mice that developed arthritis produced higher titers of Ab to bCII in comparison with levels of Ab produced by nonarthritic mice. The decreased levels of anti-CII Ab produced by the mice vaccinated with Vα11.1 was consistent with the decreased incidence of arthritis among these mice. An analysis of the Ig subclasses specific for bCII was also consistent with these data (Fig. 5). As reported previously (29), the predominant subclasses present in the Ab response to bCII from these mice were IgG2a and IgG2b, and to a much lesser extent IgG1. In virtually every instance, the quantity of each of these Ig subclasses was lower in nonarthritic mice. The only exception was the Vα4 nonarthritic mice in which the IgG1 mean titer (Fig. 5*c*) was higher than the arthritic, although the sample size for this sub-group was very small ($n = 2$) and the SE was very large. No shifts from IgG2 to any other Ig class or subclass was detected among the Vα11.1-vaccinated mice. Little or no Ab of the IgA, IgM, or IgG3 isotypes were detected in sera from any of the experimental groups (data not shown). When Ab titers to the autoantigen mCII were measured, the results were found to be very similar to the anti-bCII titers. Again, the mice that were vaccinated with Vα11.1 and did not develop arthritis produced only low levels of Ab to mCII (Fig. 6). Taken together, these data indicate that vaccination with Vα11.1 prevented the development of arthritis by decreasing the production of bCII and mCII Abs to levels found in control animals that failed to develop arthritis.

Immune response to TCR Vα domains

To explore further the mechanism by which Vα11.1 vaccination interferes with the immune response to CII, T cell proliferative

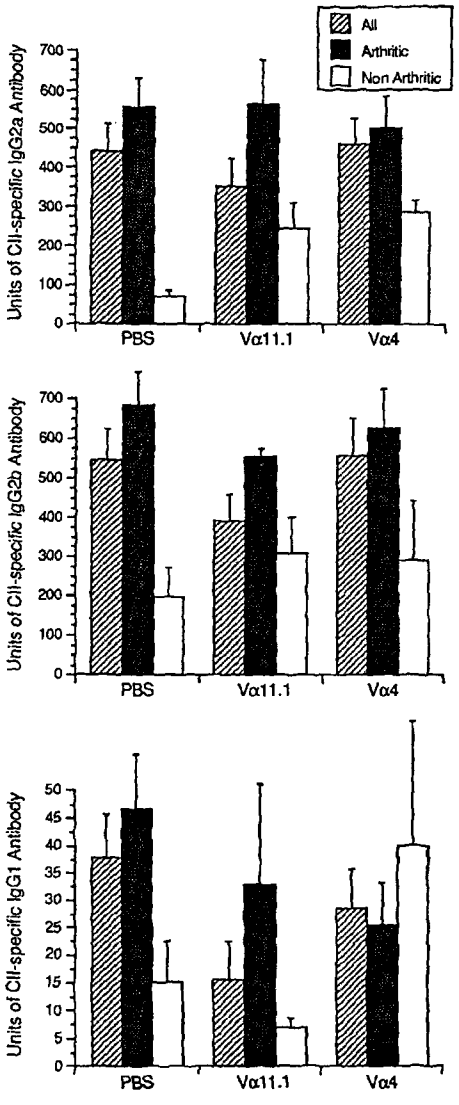

FIGURE 5. Quantity of Ig subclasses specific for CII following vaccination with TCR Vα domains and challenge with CII. The quantity of Ig subclasses specific for bovine CII was determined as described for Figure 4 with the exception that the ELISA was developed using Ig subclass-specific secondary Abs. Sera were obtained on week 6 post-CII immunization. Error bars indicate SEM.

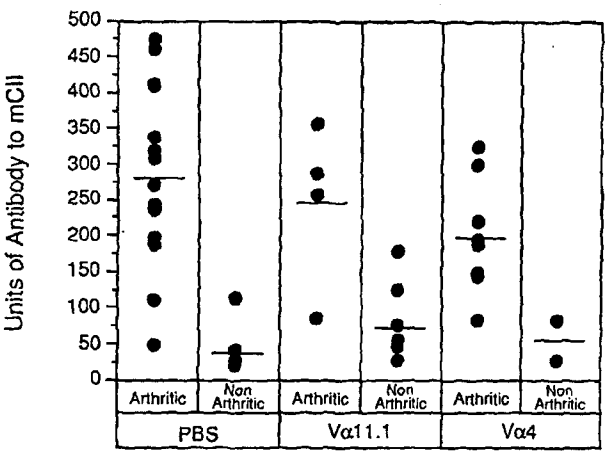

FIGURE 6. Ab titers to mCII following vaccination with TCR Vα domains and challenge with bCII. Individual serum samples obtained at week 6 post bCII immunization, the peak of arthritis, were tested for the presence of mCII-specific Abs by ELISA. Units of Ab were calculated using a standard anti-CII serum. Horizontal bars indicate the means of each group. Statistical analysis of the arthritic vs nonarthritic means within each group: PBS group, $p = 0.001$; Vα11.1 group, $p = 0.015$; and Vα4 group, $p = 0.045$.

Table III. *Proliferative responses of T cells from Vα11.1 and Vα4-immunized mice*

Antigen	Immunogen[a] Vα4[b]	Vα11.1
Vα4	98,857 ± 1,232	71,059 ± 2,747
Vα11.1	95,594 ± 2,715	149,890 ± 1,620

[a] *DBA/1* mice were immunized with 100 μg of either recombinant Vα11 or Vα4 in equal volumes of Titermax. After 10 days, draining lymph node cells were removed and tested for their ability to proliferate in response to 50 μg of the recombinant Vα chains. Proliferation was assessed by [^{3}H]thymidine incorporation.

[b] Data are expressed as mean ΔDPM of quadruplicate wells ± SEM. Mean DPM background stimulation: Vα11.1 = 12,623; Vα4 = 13,587.

responses to both recombinant Vα domains were measured. As shown in Table III, 10 days after immunization, strong T cell proliferative responses can be detected against both Vα4 and Vα11.1. Interestingly, considerable cross-reactivity is detectable among these two Ags. Although T cells from Vα4-immunized mice recognized Vα4 and Vα11.1 equally well, a higher degree of specificity was observed with Vα11.1-specific T cells. This pattern of cross-reactivity was also detected in the mice vaccinated with Vα domains and challenged with CII (from Fig. 2A). As shown in Table IV, strong T cell proliferative responses to both Vα domains were still present 14 wk after vaccination. Again, both Vαs were equally effective in stimulating T cells from the corresponding mice to proliferate, yet the specificity of the T cells differed. T cells obtained from mice vaccinated with Vα11.1 followed by CII immunization responded strongly to Vα11.1 and appeared to be highly specific, as only low level stimulation was observed with Vα4 as Ag. In contrast, the T cell response to Vα4 was much less specific. These T cells proliferated in vitro equally well to both Vα11.1 and the Vα4. As was expected, T cells from the mice that were not immunized with either TCR chain (PBS group) were not stimulated in vitro to proliferate by either Vα.

The T cell proliferative response of these mice to bCII was also measured. Unlike the responses to the Vαs, the proliferative response to CII was significantly lower 10 wk after immunization (in comparison with a standard 10-day post-immunization assay), but clearly was still detectable. In both the PBS and Vα4 groups, the proliferative responses to bCII were ~3 to 5 times higher than the Vα11.1 group, and this difference was even more evident at lower Ag concentrations (Table IV). Although these data are based on immune responses to CII 10 wk after immunization, these data suggest that the Vα11.1 vaccination prevented the in vivo priming of CII-specific T cells.

In addition to proliferative responses, serum samples were also analyzed for the presence of Ab to the Vα domains used for vaccination. As shown in Figure 7, both Vα4 and Vα11.1 induced the production of appreciable amounts of Vα-specific Ab. Interestingly, the specificity of the Abs induced by these Vαs differed and was reflective of the T cell proliferation data. Whereas vaccination with Vα11.1 produced Abs that showed little or no cross-reactivity

Table IV. *Proliferative responses of T cells from mice vaccinated with recombinant TCR domains and challenged with CII*

Antigen	μg/well	Immunogen[a] CII[b]	Vα11.1 + CII	Vα4 + CII
α1(II) collagen	100	9,137 ± 2,049	3,881 ± 985	10,738 ± 1,703
	50	9,095 ± 3,130	1,664 ± 590	8,728 ± 755
Vα11.1	100	0[c]	76,120 ± 4,532	73,208 ± 6,157
	50	NT[d]	78,859 ± 2,779	80,747 ± 8,984
Vα4	100	0	8,712 ± 2,292	81,864 ± 4,585
	50	NT	14,806 ± 4,326	90,651 ± 4,608

[a] Proliferative response of T cells from DBA/1 mice in Figure 2A. As described in Figure 2, mice were vaccinated with recombinant Vα11.1, Vα4, or PBS, followed 4 wk later with bCII. At 10 wk post-bCII immunization, lymph node cells were removed and tested for their ability to proliferate in response to bovine α1(II) collagen chains and the recombinant Vα chains. Proliferation was assessed by [^{3}H]thymidine incorporation.

[b] Data are expressed as mean ΔDPM of quadruplicate wells ± SEM. Mean DPM background stimulation: CII = 11,890; Vα11.1 = 12,906; Vα4 = 15,261.

[c] ΔDPM were slightly less than background.

[d] NT, not tested.

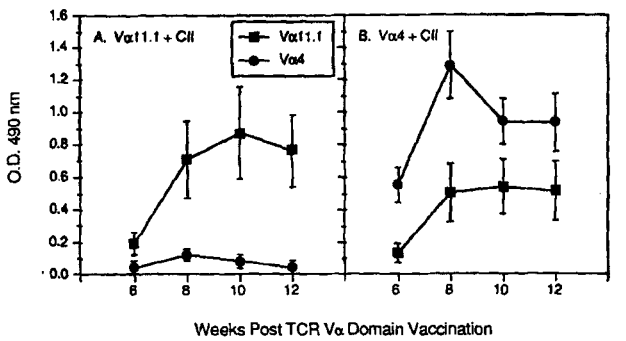

FIGURE 7. Ab titers to recombinant TCR Vα domains. Individual serum samples were collected at weeks 6, 8, 10, and 12 after TCR Vα domain vaccination and were tested by ELISA for the presence of Ab to Vα11.1 and Vα4 recombinant proteins. Ab quantity is expressed as mean OD value at 490 nm. Error bars indicate SEM.

with Vα4, Abs produced by Vα4 vaccination demonstrated a high level of cross-reactivity with Vα11.1. Although these data indicate that the Vα11.1 vaccination produced an Ab response of higher specificity, it is still not clear whether these Abs mediate the resistance to CIA. Segregation of anti-Vα11.1 titers between arthritic and nonarthritic animals within the Vα11.1 group indicated little difference in the quantity of Ab specific for the Vα11.1 protein (data not shown).

Discussion

This report describes the successful use of a recombinant TCR Vα domain to vaccinate against an experimental autoimmune disease, CIA in DBA/1 (H-2^q) mice. The Vα domain that is effective, Vα11.1-Jα17, is derived from a T cell clone that is representative, in terms of TCR usage, of the oligoclonal T cells that are associated with CIA (17) (E. Rosloniec and J. Stuart, unpublished observations). In contrast to the Vα11.1 domain, a recombinant Vα4 domain derived from an I-A^u-restricted encephalitogenic T cell clone specific for MBP (20) has no effect on the development of CIA. The data demonstrate, therefore, that it is possible to vaccinate specifically against CIA using a soluble Vα domain. To date, therapy using soluble V domains derived from TCR has been hindered by the lack of availability of systems for the production of only V domains in milligram quantities. Whereas earlier mammalian expression systems resulted in the production of TCR V segments linked to their corresponding C segments (30–32), this problem has been overcome by the recent development of *E. coli*-based expression systems (10, 11, 33, 34). Using these prokaryotic expression systems, soluble Vα domains can be produced in large quantities in *E. coli* (10, 11). Thus, this approach becomes viable for the production of soluble TCR for therapy of autoimmune diseases that are mediated by oligoclonal T cell populations.

The inhibition of CIA by vaccination of mice with the recombinant Vα11.1 is accompanied by lower B and T cell responses to the arthritogenic Ag bCII and the autoantigen mCII, as well as strong B and T cell responses against the recombinant Vαs. Whether Vα11.1 vaccination alters the quality of the anti-CII response as opposed to the quantity is unclear. Lower levels of anti-CII Ab clearly are associated with the failure to develop arthritis, yet the nonarthritic mice still produce appreciable amounts of anti-CII. Watson et al. (29) have demonstrated previously that IgG2a and -2b are the predominant isotypes mediating the pathology in murine CIA. Our analysis of the amount of these subclasses specific for CII appear to support the quantity argument, but this hypothesis has not yet been tested directly.

The immune response elicited by Vα4 as a vaccine cross-reacts strongly at both the B and T cell level with Vα11.1. The vaccination with Vα11.1, however, resulted in much higher specificity in B and T cell responses. Taken together with the observation that Vα4 does not block CIA induction, this indicates that at least for a subset of cells, the fine specificity of the immunoprotective response induced using Vα11.1 as a vaccine is distinct from that induced with Vα4. This suggests that there are "protective" epitopes within Vα11.1 that are absent or cryptic (35) in Vα4. The high level of cross-reactivity following Vα4 treatment is surprising as Vα4 and Vα11.1 share only 32% homology at the amino acid level. The observation that the immune response in Vα11.1-vaccinated mice appears to be much more specific indicates that the effect of Vα11.1 vaccination is not a result of a more general anti-Vα response and, significantly, the protective effect shows a high degree of specificity.

An obvious advantage of the use of recombinant TCR V domains is that it avoids the necessity to identify immunogenic TCR peptides that might be effective as a vaccine. This approach is also likely to be of higher specificity than vaccination with Ag-specific (36) or autoreactive T cell clones (37, 38) that may even induce anti-ergotypic responses (39). Immunization with the entire Vα domain allows all possible antigenic determinants within the V region of the TCR chain to serve as an immunogen and to provide

the protective effect. In addition, antigenicity of peptides is easier to predict for inbred strains of rodents of known MHC haplotypes, but for outbred populations this approach becomes a formidable task. If a complete Vα-Jα or Vβ-Dβ-Jβ fragment is used, the number of peptides that can be presented is much greater and, therefore, the chances of an immunogenic peptide that offers protection to be presented are increased enormously. In either approach, however, pre-existing knowledge of the TCR genes utilized in the autoimmune response is clearly required and, perhaps even more of a challenge, this requires information concerning the autoantigen(s).

The mechanism of disease blockade by Vα11.1 vaccination is not clear. It remains to be determined whether this specificity is directed against framework regions of the Vα11.1 family or if Ag specificity of the Vα11.1 domain used for vaccination is critical. It is of interest that both arthritic and nonarthritic mice within the Vα11.1-vaccinated group showed similar levels of Ab responses to Vα11.1. This suggests that there is a difference in the fine specificity of these Ab responses or, alternatively, that the protection from CIA induced by Vα11.1 vaccination is cell-mediated. The data of Osman and colleagues indicate that although Vα11.1 is preferentially utilized (54% of clones), Vα8 and Vα22 gene segments are also used by CII-specific T cell hybridomas from DBA/1 mice (17). In addition, two different J gene segments were also found to be associated with the Vα11.1 gene segment (17). Despite this diverse Vα-Jα usage, disease blockade in our study was induced with only one V-J combination. This suggests that vaccination can be an effective therapy even when only a subset of the autoreactive T cells are targeted and has obvious implications for the treatment of autoimmune diseases.

Vaccination with a TCR peptide has also been shown to be an effective therapy for EAE in Lewis rats (7–9). These TCR peptides were derived from the CDR2 (7, 8) or the junctional region (9) of the β-chain that is conserved among encephalitogenic T cells. The mechanism of protection against disease induced by these peptides has been shown to involve both B and T cells (8). Unlike CIA, which is primarily mediated by Abs to CII (15, 28), EAE is mediated by $CD4^+$ T cells (40, 41) with a lesser role of $CD8^+$ T cells (42). Nevertheless, from this study and with TCR-peptide vaccination studies in EAE (7–9), it appears that vaccination with TCR-derived (poly)peptides is an effective route toward the therapy of both EAE and CIA, despite their rather different immunopathology. Finally, the reported oligoclonality of TCR usage in human autoimmune diseases such as rheumatoid arthritis (43–45) and multiple sclerosis (46–48) suggest that vaccination with TCR V domains can be an effective therapeutic approach for autoimmune disorders.

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References

1. Fink, P. J., L. A. Matis, D. L. McElligott, M. Bookman, and S. M. Hedrick. 1986. Correlation between T cell specificity and the structure of the antigen receptor. *Nature 321:219.*
2. Hedrick, S. M., I. Engel, D. L. McElligott, P. J. Fink, M. L. Hsu, D. Hansburg, and L. A. Matis. 1988. Selection of amino acid sequences in the beta chain of the T cell antigen receptor. *Science 239:1541.*
3. Danska, J. S., A. M. Livingstone, V. Paragas, T. Ishihara, and C. G. Fathman. 1990. The presumptive CDR3 regions of both T cell receptor alpha and beta chains determine T cell specificity for myoglobin peptides. *J. Exp. Med. 172:27.*
4. Burns, F. R., X. B. Li, N. Shen, H. Offner, Y. K. Chou, A. A. Vandenbark, and K. E. Heber. 1989. Both rat and mouse T cell receptors specific for the encephalitogenic determinant of myelin basic protein use similar V alpha and V beta chain genes even though the major histocompatibility complex and encephalitogenic determinants being recognized are different. *J. Exp. Med. 169:27.*
5. Urban, J. L., V. Kumar, D. H. Kono, C. Gomez, S. J. Horvath, J. Clayton, D. G. Ando, E. E. Sercarz, and L. Hood. 1988. Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. *Cell 54:577.*
6. Acha-Orbea, H., D. J. Mitchell, L. Timmermann, D. C. Wraith, G. S. Tausch, M. K. Waldor, S. S. Zamvil, H. O. McDevitt, and L. Steinman. 1988. Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. *Cell 54:263.*
7. Vandenbark, A. A., G. Hashim, and H. Offner. 1989. Immunization with a synthetic T cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. *Nature 341:541.*
8. Offner, H., G. A. Hashim, and A. A. Vandenbark. 1991. T cell receptor peptide therapy triggers autoregulation of experimental encephalomyelitis. *Science 251: 430.*
9. Howell, M. D., S. T. Winters, T. Olee, H. C. Powell, D. J. Carlo, and S. W. Brostoff. 1989. Vaccination against experimental allergic encephalomyelitis with T cell receptor peptides [published erratum appears in *Science* 1990 Mar 9; 247(4947):1167]. *Science 246:668.*
10. Ward, E. S. 1991. Expression and secretion of T cell receptor V alpha and V beta domains using *Escherichia coli* as a host. *Scand. J. Immunol. 34:215.*
11. Ward, E. S. 1992. Secretion of T cell receptor fragments from recombinant *Escherichia coli* cells. *J. Mol. Biol. 224:885.*
12. 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.*
13. 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.*
14. Stuart, J. M., A. S. Townes, and A. H. Kang. 1982. Nature and specificity of the immune response to collagen in type II collagen-induced arthritis in mice. *J. Clin. Invest. 69:673.*
15. Stuart, J. M., and F. J. Dixon. 1983. Serum transfer of collagen-induced arthritis in mice. *J. Exp. Med. 158:378.*
16. Brand, D. D., L. K. Myers, 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 α1(II)-CB11 in $H\text{-}2^q$ mice. *J. Immunol. 152: 3088.*
17. Osman, G. E., M. Toda, O. Kanagawa, and L. E. Hood. 1993. Characterization of the T cell receptor repertoire causing collagen-induced arthritis in mice. *J. Exp. Med. 177:387.*
18. Miller, E. J. 1971. Isolation and characterization of the cyanogen bromide peptides from the α1(II) chain of chick cartilage collagen. *Biochemistry 10:3030.*
19. Ciubotaru, M., and E. S. Ward. 1995. Expression of soluble T cell receptor fragments derived from a T cell clone associated with murine collagen-induced arthritis. *Immunol. Lett. 43:139.*
20. Wraith, D. C., D. E. Smilek, D. J. Mitchell, L. Steinman, and H. O. McDevitt. 1989. Antigen recognition in autoimmune encephalomyelitis and the potential for peptide-mediated immunotherapy. *Cell 59:247.*
21. 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.*
22. Click, R. E., L. Benck, and B. J. Alter. 1972. Immune response in vitro. I. Culture conditions for antibody synthesis. *Cell. Immunol. 3:264.*
23. Marrack, P. 1982. Production of antigen-specific H-2-restricted T cell hybridomas. In *Isolation, Characterization, and Utilization of T Lymphocyte Clones.* C. G. Fathman and F. Fitch, eds. Academic Press, New York, p. 119.
24. White, J., M. Blackman, J. Bill, J. Kappler, P. Marrack, D. P. Gold, and W. Born. 1989. Two better cell lines for making hybridomas expressing specific T cell receptors. *J. Immunol. 143:1822.*
25. Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods 65:55.*
26. Kappler, J., J. White, D. Wegman, E. Mustain, and P. Marrack. 1982. Antigen presentation by Ia^+ B cell hybridomas to H-2-restricted T cell hybridomas. *Proc. Natl. Acad. Sci. USA 79:3604.*
27. Myers, L. K., J. M. Stuart, and A. H. Kang. 1989. A CD4 cell is capable of transferring suppression of collagen-induced arthritis. *J. Immunol. 143:3976.*
28. 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.*
29. Watson, W. C., and A. S. Townes. 1985. Genetic susceptibility to murine collagen II autoimmune arthritis: proposed relationship to the IgG2 autoantibody subclass response, complement C5, major histocompatibility complex (MHC) and non-MHC loci. *J. Exp. Med. 162:1878.*
30. Lin, A. Y., B. Devaux, A. Green, C. Sagerstrom, J. F. Elliott, and M. M. Davis. 1990. Expression of T cell antigen receptor heterodimers in a lipid-linked form. *Science 249:677.*
31. Gregoire, C., N. Rebai, F. Schweisguth, A. Necker, G. Mazza, N. Auphan, A. Millward, V. A. Schmitt, and B. Malissen. 1991. Engineered secreted T-cell receptor alpha beta heterodimers. *Proc. Natl. Acad. Sci. USA 88:8077.*
32. Gascoigne, N. R. 1990. Transport and secretion of truncated T cell receptor beta-chain occurs in the absence of association with CD3. *J. Biol. Chem. 265: 9296.*

33. Novotny, J., R. K. Ganju, S. T. Smiley, R. E. Hussey, M. A. Luther, M. A. Recny, R. F. Siliciano, and E. L. Reinherz. 1991. A soluble, single-chain T-cell receptor fragment endowed with antigen-combining properties. *Proc. Natl. Acad. Sci. USA 88:8646.*
34. Hoo, W. F., M. J. Lacy, L. K. Denzin, E. W. Voss, Jr., K. D. Hardman, and D. M. Kranz. 1992. Characterization of a single-chain T cell receptor expressed in *Escherichia coli. Proc. Natl. Acad. Sci. USA 89:4759.*
35. Gammon, G., E. E. Sercarz, and G. Benichou. 1991. The dominant self and the cryptic self: shaping the autoreactive T cell repertoire. *Immunol. Today 12:193.*
36. Chiocchia, G., B. Manoury-Schwartz, M. C. Boissier, H. Gahery, P. N. Marche, and C. Fournier. 1994. T cell regulation of collagen-induced arthritis in mice. III. Is T cell vaccination a valuable therapy? *Eur. J. Immunol. 24:2775.*
37. Ben-Nun, A., H. Wekerle, and I. R. Cohen. 1981. Vaccination against autoimmune encephalitis with T lymphocyte line cells reactive against myelin basic protein. *Nature 292:60.*
38. Lider, O., T. Reshef, E. Beraud, N. A. Ben, and I. R. Cohen. 1988. Anti-idiotypic network induced by T cell vaccination against experimental autoimmune encephalomyelitis. *Science 239:181.*
39. Lohse, A. W., F. Mor, N. Karin, and I. R. Cohen. 1989. Control of experimental autoimmune encephalomyelitis by T cells responding to activated T cells. *Science 244:820.*
40. Sriram, S., and L. Carroll. 1988. In vivo depletion of Lyt-2 cells fails to alter acute and relapsing EAE. *J. Neuroimmunol. 17:147.*
41. Brostoff, S. W., and D. W. Mason. 1984. Experimental allergic encephalomyelitis: successful treatment in vivo with a monoclonal antibody that recognizes T helper cells. *J. Immunol. 133:1938.*
42. Koh, D. R., W. P. Fung-Leung, A. Ho, D. Gray, H. Acha-Orbea, and T. W. Mak. 1992. Less mortality but more relapses in experimental allergic encephalomyelitis in $CD8^{-/-}$ mice. *Science 256:1210.*
43. Sottini, A., L. Imberti, R. Gorla, R. Cattaneo, and D. Primi. 1991. Restricted expression of T cell receptor V beta but not V alpha genes in rheumatoid arthritis. *Eur. J. Immunol. 21:461.*
44. Howell, M. D., J. P. Diveley, K. A. Lundeen, A. Esty, S. T. Winters, D. J. Carlo, and S. W. Brostoff. 1991. Limited T cell receptor beta-chain heterogeneity among interleukin-2 receptor-positive synovial T cells suggests a role for superantigen *in* rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA 88:10921.*
45. Paliard, X., S. G. West, J. A. Lafferty, J. R. Clements, J. W. Kappler, P. Marrack, and B. L. Kotzin. 1991. Evidence for the effects of a superantigen in rheumatoid arthritis. *Science 253:325.*
46. Oksenberg, J. R., S. Stuart, A. B. Begovich, R. B. Bell, H. A. Erlich, L. Steinman, and C. C. Bernard. 1990. Limited heterogeneity of rearranged T cell receptor V alpha transcripts in brains of multiple sclerosis patients [published erratum appears in *Nature* 1991 Sep. 5; 353(6339):94]. *Nature 345:344.*
47. Ben-Nun, A., R. S. Liblau, L. Cohen, D. Lehmann, L. E. Tournier, A. Rosenzweig, J. W. Zhang, J. C. Raus, and M. A. Bach. 1991. Restricted T cell receptor V beta gene usage by myelin basic protein-specific T cell clones in multiple sclerosis: predominant genes vary in individuals. *Proc. Natl. Acad. Sci. USA 88:2466.*
48. Wucherpfennig, K. W., K. Ota, N. Endo, J. G. Seidman, A. Rosenzweig, H. L. Weiner, and D. A. Hafler. 1990. Shared human T cell receptor V beta usage to immunodominant regions of myelin basic protein. *Science 248:1016.*