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Inhibition of human T-cell responses to house dust mite allergens by a T-cell receptor peptide

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Abstract

Recent analysis of the usage of T-cell receptor (TcR) β chain variable region ($V\beta$) gene elements by house dust mite (HDM)–reactive T cells from an atopic donor suggested that TcR- $V\beta 3$ gene products may form a major component of the human T-cell repertoire reactive to this common allergen. In this study a peptide analog of the TcR- $V\beta 3$ complementarity determining region 2 (CDR2) is shown to inhibit the polyclonal human T-cell response to HDM; this effect is specific because inhibition is dependent on the presence of $V\beta 3$ + T cells. This experimental approach has been used to determine whether the pattern seen in T-cell clones derived from one atopic donor reflects TcR- $V\beta$ usage in the polyclonal response to allergen in the general population. Inhibition of more than 50% of the polyclonal response to allergen by $V\beta 3$ -CDR2 peptide was observed in 16 of 21 donors tested, suggesting that TcR- $V\beta 3$ gene usage may form a major component of the human HDM repertoire and as such offer a suitable target for T cell–directed specific immunotherapy in HDM-allergic individuals. Depletion of CD8 + T cells abolishes peptide-mediated inhibition of CD4 + T-cell proliferation to HDM, suggesting that induction of a CD8 + regulatory T-cell subset by the CDR2 peptide may modulate HDM-specific allergic T-cell responses. (J ALLERGY CLIN IMMUNOL 1994;94:844-52.)

CDR2	Complementarity determining region 2
EAE	Experimental allergic ancephalomy-elitis
HDM	House dust mite
IL-2	Interleukin-2

MHC	Major histocompatibility complex
MTSE	<i>Mycobacterium tuberculosis</i> soluble extract
PBMCs	Peripheral blood mononuclear cells
TeR	T cell-specific antigen receptor
Vβ	Variable region of the β chain of the T-cell receptor

Keywords: [House dust mite](#), [T cells](#), [TcR-CDR2 peptide](#)

CD4 + T lymphocytes from atopic individuals recognize processed fragments of allergens from house dust mite (HDM) bound to major histocompatibility complex (MHC) class II molecules. These T cells become activated to induce and regulate the effector mechanisms, including the production of IgE and the activation of inflammatory cells, of the allergic immune response.^{1,2} The capacity to specifically downregulate antigen-mediated stimulation of allergen-reactive CD4 + T cells from atopic individuals may provide a means to alleviate the symptoms of allergic disease without the undesirable and long-term side effects associated with the nonspecific immunosuppressive therapies currently in use. Evidence for the recognition of a limited array of HDM epitopes or the recruitment to the disease site of T cells expressing T cell-specific antigen receptor (TcR) with shared characteristics would allow these cells to be specifically targeted and facilitate the development of TcR-based immunotherapy.

Recent experiments in our laboratory have demonstrated that T lymphocytes from an atopic individual with allergy to HDM show a bias in usage of TcR-V β gene elements. This was demonstrated with anchor polymerase chain reaction analysis of T-cell clones derived from peripheral blood that were reactive with HDM. Of 10 clones analyzed, six different TcR $\alpha\beta$ chain combinations were identified of which three, or 50%, used V β 3 and the others used V β 6.7a, V β 9, and V β 21. This TcR-V β gene usage appears to persist with time because when the same individual was tested over a period of several years, the HDM-reactive TcR repertoire appeared unchanged,³ and dominant long-lived clones could still be detected in the periphery 6 years later. If the same pattern of restricted V β gene usage observed in T-cell clones derived from a single atopic individual is representative of the polyclonal response observed in the majority of HDM-allergic individuals, it may be possible to design specific immunotherapy for HDM-allergic individuals by targeting allergen-reactive T cells with restricted V β usage. In an earlier study analysis of TcR-V β usage by HDM-reactive T cells at the population level was limited by the requirement to clone, confirm antigen specificity, and perform anchor polymerase chain reaction and sequence analysis of the TcR repertoire of multiple T-cell clones derived from a large number of individuals. We have circumvented this approach by using an experimental design that permits analysis of V β 3 usage in the polyclonal response to HDM.

The T cell-specific antigen receptor is a member of the immunoglobulin superfamily of molecules, and the variable regions of both the α (V α) and β (V β) chains of the TcR have four hypervariable loops, also known as complementarity determining regions (CDR1, 2, 3, and 4), which form the antigen-binding regions.⁴ CDR3 appears to be the most diverse of these regions and is believed to be involved in antigen contact. The CDR1 and CDR2 regions are implicated in MHC binding.⁵ The fourth hypervariable region, CDR4, appears to participate in superantigen binding.⁶ Recent experiments in rodent models suggest that these regions of the TcR not only serve to promote antigen and MHC binding but may also (in particular the V β -CDR2 region) serve as an antigenic determinant or recognition site for regulatory T cells.⁷⁻⁹

"Disease-associated" T cells that preferentially use selected TcR gene products have been identified in animal models of experimental allergic encephalomyelitis (EAE). Encephalitogenic T cells in both rat and mouse EAE express predominantly TcR-V β 8.2 genes, and immunization with a synthetic peptide corresponding to the CDR2 of TcR-V β 8.2 prevents clinical signs of disease.⁷⁻¹² We have tested the capacity of a CDR2 peptide derived from the human TcR-V β 3 to modulate the polyclonal human T-cell response to HDM allergen. We demonstrate that this peptide specifically inhibits the antigen responsiveness of V β 3-expressing T cells, and we have used this observation to study the contribution of V β 3 T cells to the HDM response in the population as a whole. A panel of HDM-primed, genetically unrelated individuals was chosen

for this study. Inhibition of more than 50% of the polyclonal response to HDM by CDR2 peptide was observed in 75% of all individuals tested; less than 10% of donors showed no inhibition of CDR2. These data suggest that TcR-V β 3 usage may form a major component within the HDM-reactive T-cell population and may thus provide a target for immunotherapy. Studies into the mechanism of CDR2 inhibition are also presented.

Methods

Patients

The atopic status of patients with HDM allergy was defined by the presence of a positive skin prick test response ^{13, 14} (wheal diameter ≥ 3 mm) to *Dermatophagoides pteronyssinus* and *D. farinae* extracts in the presence of a negative saline control. Sixteen HDM-allergic atopic subjects, aged 15 to 60 years, were included in the study. All of these individuals had clinical symptoms of HDM allergy (perennial rhinitis and/or asthma) and had grade III or IV serum-specific IgE for HDM as measured by RAST.

Nonatopic status was defined in a subject who was free of symptoms by the absence of positive skin prick test responses to a panel of common environmental allergens (*D. pteronyssinus*, *D. farinae*, mixed grass pollen, birch pollen, plane tree, nettle, *Aspergillus fumigatus*, *Cladosporium herbarium*) in the presence of a positive wheal response to histamine hydrochloride (1 mg/ml) of 3 mm or greater and a negative response to a saline control. Five normal healthy nonatopic individuals were used for this study. All had negative skin prick test results and normal total IgE levels.

Antigens

Lyophilized, unfractionated extracts of *D. pteronyssinus* and *D. farinae* were generously provided by Dr. A. Wheeler (SmithKline Beecham PLC, Brentford, Middlesex, U.K.) and Dr. H. Lowenstein (ALK Laboratories, Horsholm, Denmark), respectively. *Mycobacterium tuberculosis* soluble extract (MTSE) was a kind gift from the TB and Related Infections Unit at Hammersmith Hospital, London.

Peptides

The CDR2 40-61 (GLGLRLIYFSYDVKMKEKGDI) peptide used overlaps the predicted region for the second hypervariable region of the TcR-V β 3 expressed by the CD4 + T-cell clone HA1.7. Influenza virus hemagglutinin peptide HA 307-319 (PKYVKQNTLKLAT), keratin peptide K1-9 (QRQRP AEIK), and CDR2 40-61 were synthesized by use of standard solid-phase methods, purified by high-performance liquid chromatography, and identified by amino acid sequence analysis. ¹⁵

Antibodies

Jovi 1.3 cells, which secrete a murine monoclonal antibody specific for human TcR-V β 3, ¹⁶ were a kind gift of Dr. J. Viney (Imperial Cancer Research Fund, London, U.K.). Antibodies for cell staining and population analysis were all purchased from Becton-Dickinson (Oxford, U.K.).

T-cell proliferation assays: Clonal

The T-cell clones used in these experiments were isolated as previously described. ¹⁷ HA1.7 was reactive to HA 307-319 peptide and expressed the V β 3 TcR, and the two HDM-reactive (responsive to both *D. pteronyssinus* and *D. farinae*) clones DE26 and DE9 were TcR-V β 3 and TcR-V β 6.7a, respectively. For proliferation assays the cells were cultured at 2×10^4 per well in round-bottom 96-well microtiter plates in complete RPMI-1640 medium (Gibco, Paisley, Scotland) supplemented with 2 mmol/L L-glutamine, 100 U/ml penicillin/streptomycin and 5% screened, heat-inactivated, A human serum (North London Blood Transfusion Service). An equal number of autologous Epstein-Barr virus-transformed B cells (5000 rad) or peripheral blood mononuclear cells (PBMCs) (2500 rad) of the correct haplotype were used as antigen-presenting cells. HA 307-319 or *D. farinae* and CDR2 40-61 were added at the concentrations indicated to the appropriate wells to produce a final volume of 200 μ l. As a control for toxicity, cells were incubated with CDR2 40-61 and

10% interleukin-2 (IL-2) (Lymphocult-T; Biotest Folex, Frankfurt, Germany) or in wells coated with anti-CD3 (10 µg/ml for 30 minutes at 37° C) and IL-2. After 72 hours of incubation, tritiated methylthymidine (1 µCi/well; Amersham International Inc., Amersham, U.K.) was added to cultures, which were harvested onto glass fiber filters 8 to 16 hours later. Proliferation was measured as tritiated methylthymidine incorporation per culture by means of liquid scintillation spectroscopy with the results expressed as mean counts per minute for 3 to 9 replicate wells. Standard deviation rarely exceeds 20%.

T-cell proliferation assays: Polyclonal

PBMCs from nonatopic and atopic donors were separated by discontinuous density centrifugation over Ficoll-Hypaque (Pharmacia Ltd., Milton Keynes, U.K.). PBMCs (1×10^5) were incubated in 96-well round-bottom microtiter plates with various combinations of HDM antigen (*D. pteronyssinus* extract) and CDR2 or K1-9 peptides at the concentrations indicated. PBMCs were also cultured with 10% IL-2, on plates coated with anti-CD3 antibody and IL-2, with the T-cell mitogen phytohemagglutinin protein (10 µg/ml; Sigma Chemical Co., St. Louis, Mo.), MTSE, or the bacterial superantigen *Staphylococcus aureus* enterotoxin B (at 0.01 to 1 µg/ml, Sigma) in the presence or absence of CDR2 peptide. Complete RPMI-1640 medium was used at a final volume of 200 µl. After 6 days, cells were pulsed with 1 µCi/well of tritiated methylthymidine for 8 to 16 hours, after which the cells were harvested and proliferation measured as described above for T-cell clones.

Vβ3 +, CD4 +, and CD8 + T-cell depletions

PBMCs were depleted of Vβ3 +, CD4 +, or CD8 + T cells, respectively by immunomagnetic separation with Dynabeads (Dyna, New Ferry, U.K.) used according to the manufacturer's instructions. Briefly, to deplete PBMCs of Vβ3 + cells, they were incubated with Jovi 1.3 culture supernatant on ice for 30 minutes. Cells were then washed in the cold, and sheep anti-mouse Ig-conjugated magnetic beads were added at the approximate ratio of 10:1 beads to Vβ3 + T cells. The beads were washed three times before use to ensure complete removal of the azide in which the beads are stored. The mixture was rotated in the cold for an additional 30 minutes, and then Vβ3 + T cells were removed by a magnet. PBMCs were depleted of more than 90% CD4 + or CD8 + T cells in a similar fashion with commercially available Dynabeads coated with monoclonal antibodies binding the CD4 or CD8 antigens on human T cells. For CD8 depletion, two rounds of magnetic depletion were performed to optimize elimination of contaminating CD8 + T cells. The cells were analyzed by flow cytometry (Coulter EPICS Profile II; Coulter Corp., Hialeah, Fla.) to ensure purity.

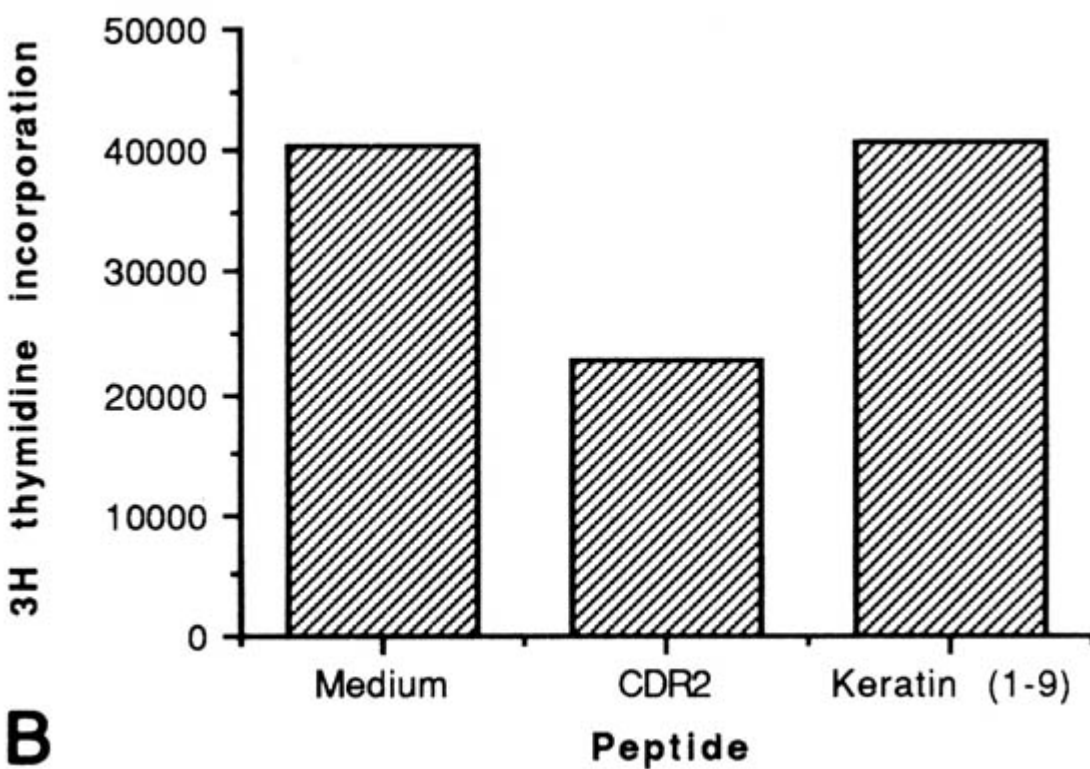
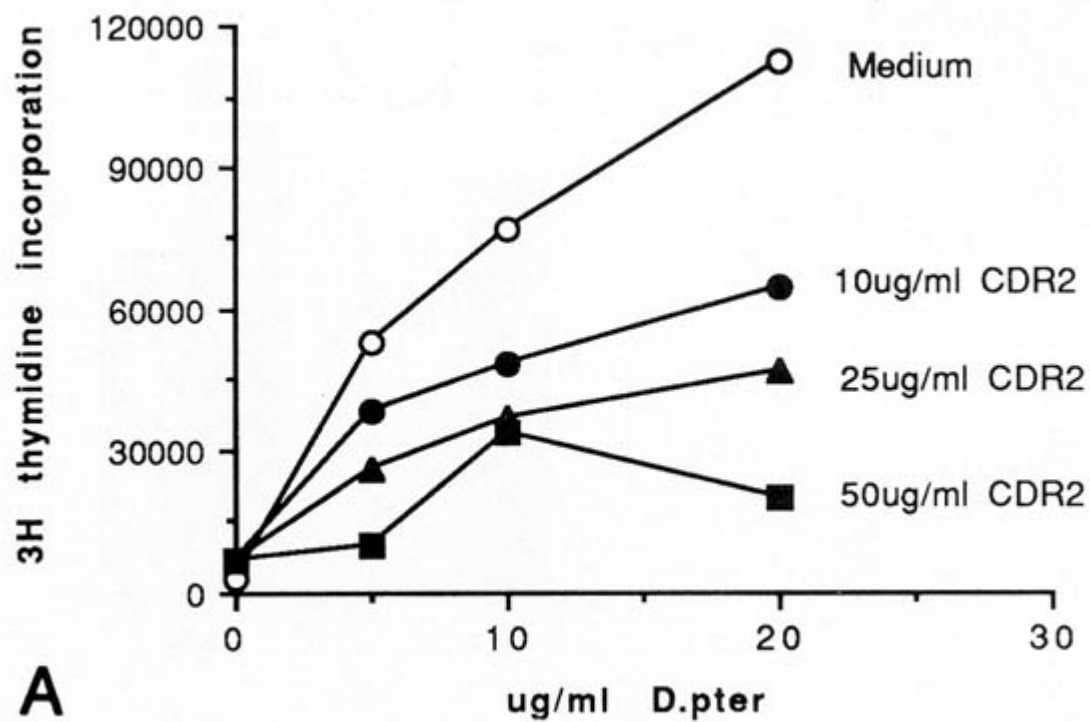
Results

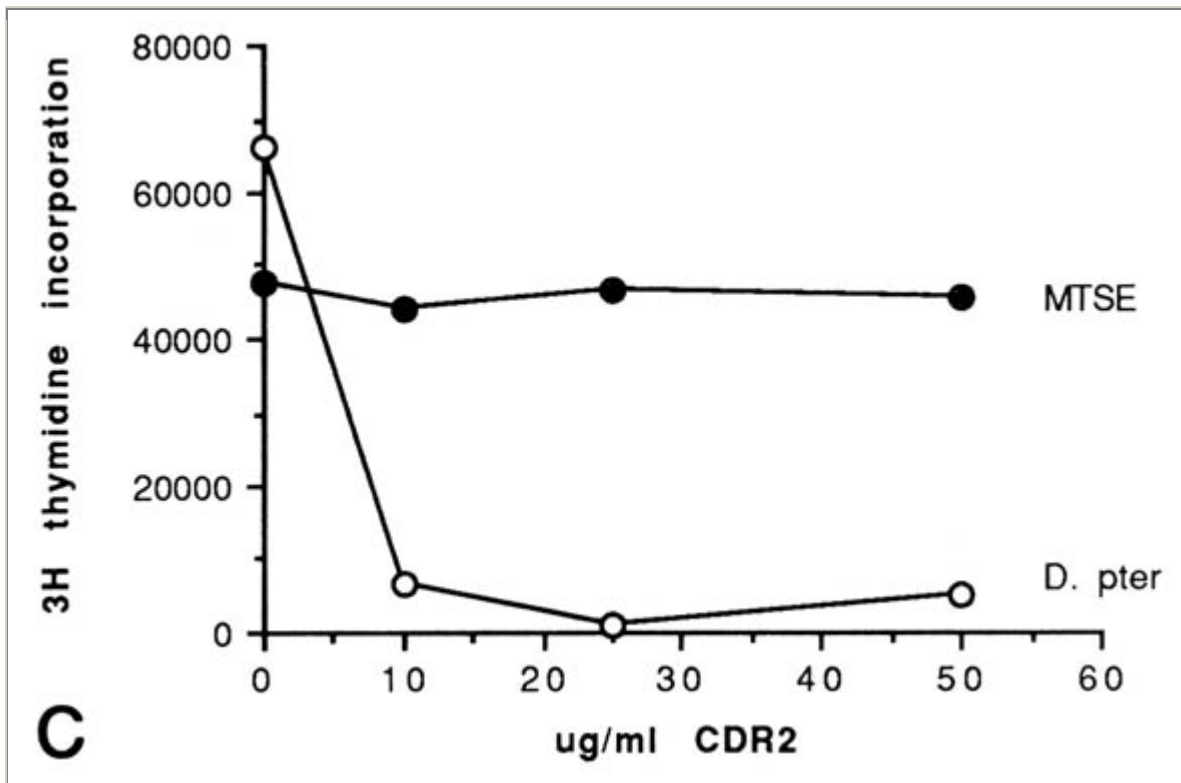
Inhibition of polyclonal T-cell responses to HDM by Vβ3-CDR2 peptide

A peptide derived from the CDR2 region of the TcR-Vβ3 has been tested for its capacity to selectively inhibit the response of Vβ3 +, HDM-reactive human T cells. PBMCs from an atopic individual were stimulated with HDM antigen, *D. pteronyssinus* extract over a concentration range of 5 to 20 µg/ml in the presence or absence of CDR2 peptide, residues 40-61, derived from the human TcR-Vβ3 sequence. Addition of the Vβ3-CDR2 peptide resulted in a dose-dependent inhibition of polyclonal T-cell responses to *D. pteronyssinus* (■ Fig. 1, A). In a second donor substantial inhibition of the polyclonal response to HDM by the CDR2 peptide, but not by a control peptide derived from keratin (residues 1-9; ■ Fig. 1, B), was observed. A second control peptide, overlapping the CDR2 region of a murine TcR-Vβ8 gene failed to inhibit the HDM response in five individuals: mean inhibition values in the presence of murine CDR2 TcR-Vβ8 and human CDR2 TcR-Vβ3 peptides were 4.4 ± 8.6 versus 52.0 ± 22.0 , respectively.

FIG. 1. Modulation of antigen-dependent polyclonal human T-cell responses by a synthetic peptide corresponding to the CDR2 region of the human TcR-Vβ3. PBMCs (1×10^5 /well) were stimulated in 96-well microtiter plates with (A) 5 to 20 µg/ml HDM allergen (*D. pteronyssinus*) alone (open circles) or with 10 to 50 µg/ml CDR2 peptide (filled symbols), (B) 20 µg/ml HDM in medium or together with 25 µg/ml CDR2 or keratin (K1-9) peptides, or (C) µg/ml HDM or MTSE control antigen in medium or with 20 CDR2 peptide. The experiments shown in panels A to C were performed with PBMCs derived from three separate donors. T-cell responses were assessed as tritiated methylthymidine uptake as described in the Methods section. Data are presented as

mean tritiated methylthymidine incorporation from replicate (3 to 9) wells with a standard deviation of less than 20%.



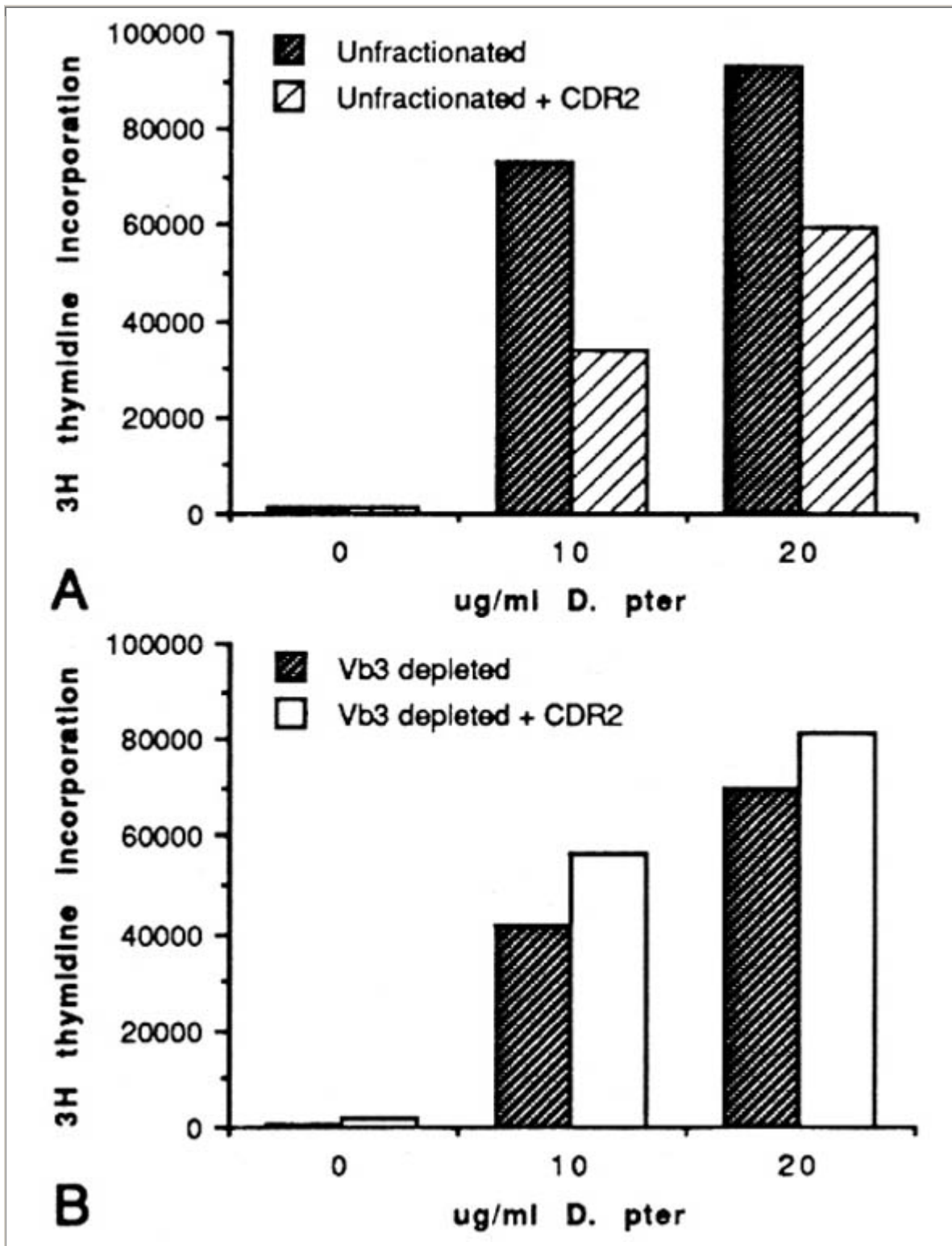


The effect of the CDR2 peptide on peripheral T cells stimulated with either HDM or an irrelevant antigen, MTSE was also compared, and in contrast to HDM, the response to MTSE was not inhibited by the CDR2 peptide (■Fig. 1, C). Furthermore, when investigated at concentrations of up to 300 μ g/ml, the CDR2 peptide was not mitogenic and unable to inhibit T-cell proliferation stimulated by exogenous IL-2, by mitogens such as phytohemagglutinin protein, by cross-linked anti-CD3 antibody plus IL-2, or by the bacterial superantigen *S. aureus* enterotoxin B (data not shown).

Specificity of V β 3-CDR2 peptide action

To demonstrate that CDR2 peptide inhibition of the HDM response is V β 3-specific, the effects on V β 3-depleted and unfractionated PBMCs were compared. After greater than 90% depletion of V β 3-expressing T cells from PBMCs with a V β 3-specific monoclonal antibody, a vigorous response to antigen was still observed, presumably reflecting the contribution of other TcR-V β regions, including V β 6.7a and V β 21, to the HDM response.³ However, after V β 3 + T-cell depletion, the HDM response was completely refractory to CDR2 peptide inhibition (■Fig. 2, A and B). These experiments confirm that inhibition by TcR-V β 3 CDR2 peptide of the polyclonal human T-cell response to HDM is dependent on the presence of V β 3 + T cells.

FIG. 2. V β 3 + T-cell depletion leads to loss of CDR2 inhibition of the HDM response. Unfractionated (A) or V β 3-depleted (B) PBMCs were cultured with HDM in the presence or absence of 25 μ g/ml CDR2 peptide as described in the legend to ■Fig. 1 and in the Methods section.



Population study

Because V β 3-CDR2 peptide inhibition of the polyclonal response to HDM appears to be specific, we have used this experimental approach to determine what percentage of HDM-reactive individuals have HDM responses inhibited by this peptide. A population of HDM-reactive individuals who were first seen with a wide range of clinical symptoms, ranging from mild atopy to severe asthma, was selected at random from the allergy clinic. These individuals were unrelated and assumed to show no skewing or bias in HLA or other genetic haplotypes. Included in this study were five unrelated, nonatopic individuals who showed in vitro responsiveness to HDM. The earlier data obtained in studies with a single individual suggested that TcR-V β

3-expressing cells may be dominant in the HDM-reactive T-cell repertoire, ³ and this study of 21 donors suggests that this may be true within the population as a whole. Of 16 atopic donors tested, only two failed to show substantial inhibition of the HDM response by the CDR2 peptide. The magnitude of modulation varied considerably between donors (range 9% to 92%), presumably reflecting differences in the relative contribution of TcR-V β 3 to the HDM response between individuals. It seems unlikely that when very high inhibition (>90%) of the HDM response occurs, the T-cell response of that individual is solely represented by V β 3-expressing T cells. Rather, it seems probable that regulatory mechanisms induced by the CDR2 peptide in culture may have bystander effects on the polyclonal response to allergen.

There was no marked difference in the ability of the CDR2 peptide to inhibit the HDM response of either atopic (mean inhibition, 53.4% $\pm$ 25.0%) or nonatopic individuals (mean inhibition, 59.6% $\pm$ 9.3%) (■Table I). Therefore although the same antigen appears to activate peripheral T cells from both atopic and nonatopic individuals, the functional consequences of T-cell activation differ between the two groups. ¹⁸

TABLE I. Inhibition of HDM allergen-dependent T-cell responses of HDM-allergic individuals

	<u>Tritiated thymidine incorporation (cpm)</u>			Inhibition (%)
	Medium	<i>D. pter</i>	<i>D. pter</i> + CDR2	
Donor				
A1	3,004	14,568	6,681	54
A2	1,031	32,216	21,454	33
A3	428	37,208	20,265	46
A4	3,037	112,067	20,306	82
A5	324	25,400	7,956	69
A6	251	13,589	4,320	68
A7	486	27,886	24,165	13
A8	265	22,294	7,784	65
A9	881	63,035	51,201	9
A10	5,988	16,246	7,993	51
A11	679	23,363	7,610	67
A12	1,327	73,281	33,716	54
A13	634	22,117	13,241	41
A14	397	66,297	5,162	92
A15	947	45,351	15,964	65
A16	3,798	27,538	9,359	66
NA1	356	5,575	1,321	76
NA2	467	71,003	32,354	55
NA3	759	15,629	6,731	57
NA4	378	26,703	12,483	53
NA5	4,744	32,415	13,763	57
T-cell clones				
HA1.7 (V β 3; HA)	70	12,800	13,283	0

DE26 (V β 3; HDM)	2,954	15,365	15,365	0
DE9 (V β 6.7a; HDM)	2,275	27,788	27,179	2

PBMCs from 16 atopic (A1-16) and five nonatopic (NA1-5) donors were cultured as indicated in Fig. 1 in medium alone or with 20 μ g/ml HDM in the presence or absence of CDR2 peptide (data shown are for the concentration of CDR2 producing maximal inhibition of the HDM response for each donor: A2 10 μ g/ml, the rest 25 or 50 μ g/ml). T-cell clones (HA 1.7, DE26 and DE9) were cultured at 2×10^4 cells/well in the presence of an equal number of irradiated (2500 rad) histocompatible PBMCs in medium, 1 μ g/ml HA 307-319, or 20 μ g/ml *D. farinae*, respectively in the presence or absence of 25 μ g/ml CDR2 peptide; and proliferation was measured on day 3 as described in the Methods section.

D. pter, *D. pteronyssinus*.

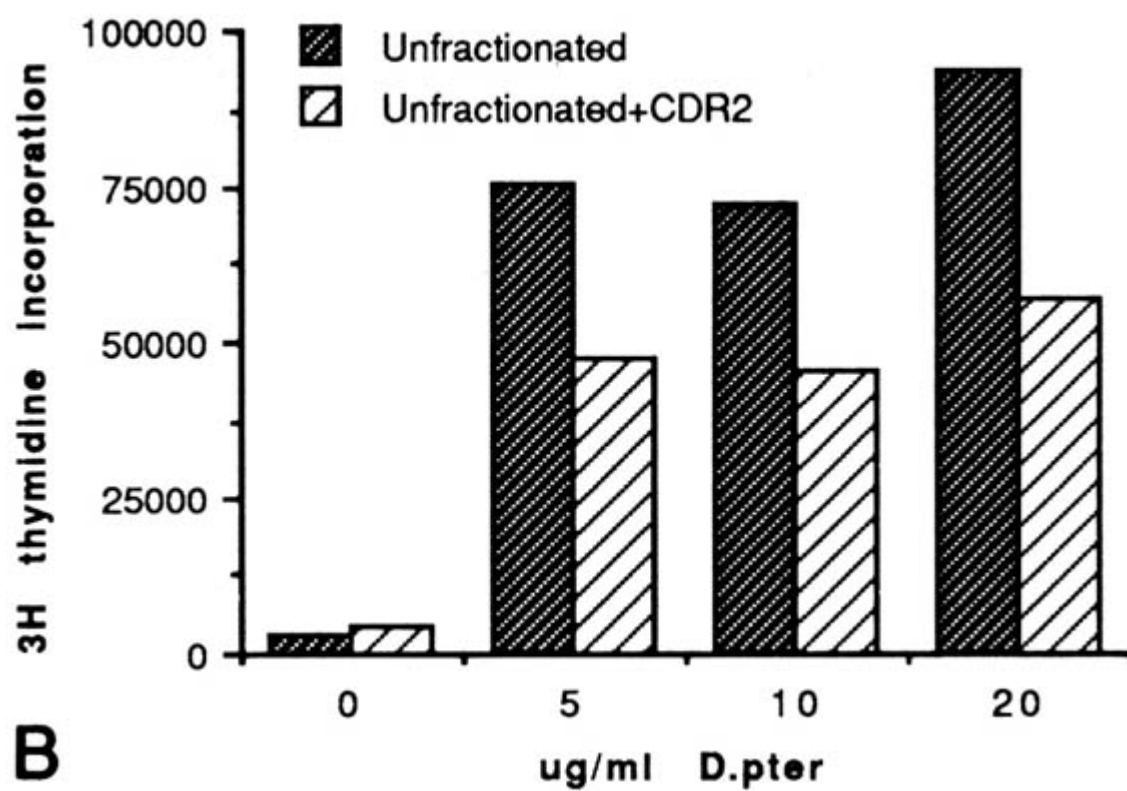
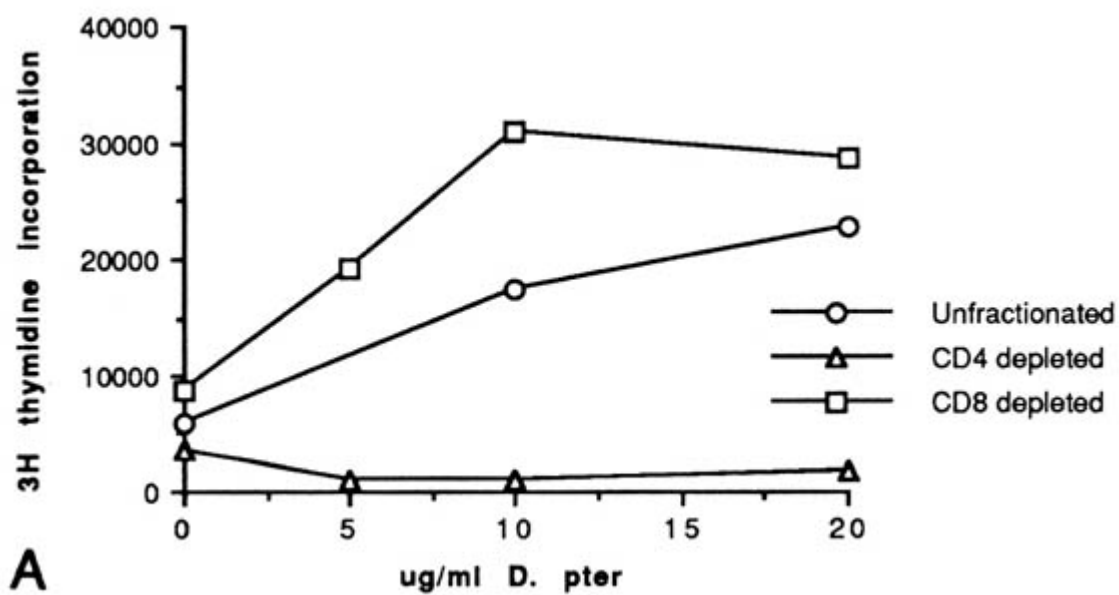
Effect of CDR2 peptide on the response of HDM-specific T-cell clones

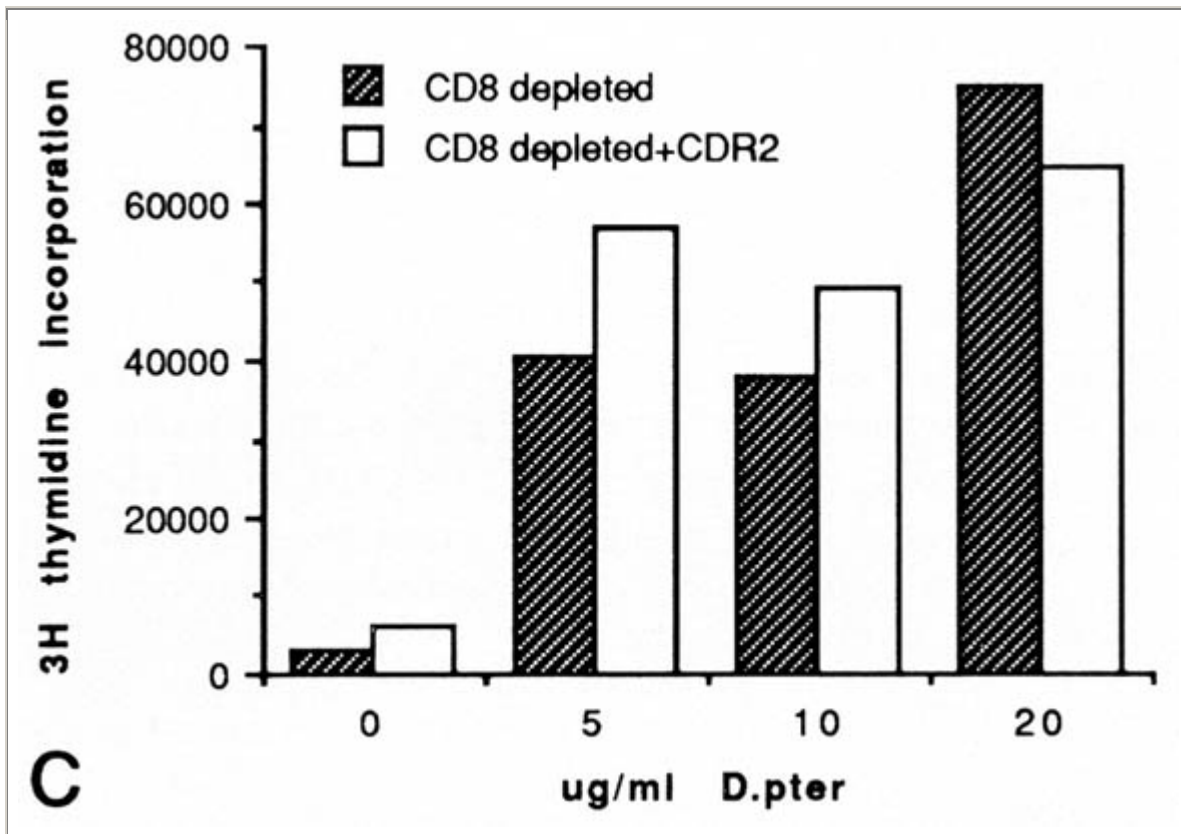
Two CD4 + V β 3 + human T-cell clones, one reactive with a peptide derived from influenza virus hemagglutinin (HA 307-319), the other with HDM, and a third V β 6.7a +, HDM-reactive T-cell clone were all resistant to CDR2 peptide-induced inhibition (Table I). This finding suggests that the modulatory effect of the TcR peptide may not simply be explained by a direct effect on the CD4 + V β 3 + antigen-responsive T-cell population.

Role of CD8 + T cells

In animal studies it has been proposed that CDR2 inhibition of antigen-induced EAE acts through the induction of a CD8 + regulatory T-cell population.⁸ To assess evidence for a similar regulatory population in human beings, the capacity of CDR2 peptide to inhibit the HDM response in unfractionated or CD8 + -depleted human PBMCs was compared. With the use of antibody-conjugated magnetic beads, depletion of CD4 or CD8 populations by more than 90% was routinely achieved. The response to antigen was only observed in the CD4 + T cell-enriched population (Fig. 3, A). Nevertheless, after depletion of the CD8 + cells, the capacity to modulate the response by TcR peptide was lost (Fig. 3, B and C). CD8 + T-cell depletion resulted not only in loss of inhibition of the HDM response by the CDR2 peptide, but led to an increased response in the presence of the peptide. A similar increase was also observed after depletion of V β 3 + T cells from PBMCs (Fig. 2). The reason for this is as yet unclear, but it might reflect the presence of a subpopulation of CD4 + T cells that are also CDR2-reactive (but non inhibitory). It seems likely that, as in the animal studies, a CD8 + T-cell regulatory subset is induced by the CDR2 peptide that modulates the allergen responsive CD4 + T-cell population.

FIG. 3. Effect of CD8 + cell depletion on CDR2 modulation of the HDM response. **A**, PMBCs were depleted of either CD4 + or CD8 + cells with specific antibody directly conjugated to magnetic beads, and the proliferative response to HDM of unfractionated, CD4-depleted, or CD8-depleted PBMCs was compared. **B**, Unfractionated or **(C)** CD8-depleted PBMCs were cultured with HDM and 25 μ g/ml CDR2 peptide.





Discussion

In this report we demonstrate that a TcR peptide, based on the sequence of the CDR2 region of the TcR-V β 3 gene segment, downregulates the human polyclonal T-cell response to HDM. This effect is specific because it is dependent on the presence of V β 3 + cells in the stimulation culture. In an earlier study we demonstrated, using anchor polymerase chain reaction analysis, that T-cell clones specific for HDM, derived from a single atopic individual, showed a bias towards TcR-V β 3 usage. In this study inhibition of the polyclonal response to HDM by CDR2 TcR-V β 3 peptide in the majority (16 of 21) of donors examined has been used to demonstrate that a bias toward V β 3 usage occurs not only in clones but, more relevantly, among the polyclonal responses of peripheral blood derived, HDM-responsive T cells within the population as a whole. Although T cells expressing TcR other than V β 3 clearly contribute to the overall HDM-reactive repertoire,³ our results demonstrate that V β 3 + T cells are a major component of the HDM-reactive repertoire. Although the relative contribution of V β 3 + T cells to the HDM response will vary between individuals, because inhibition of up to 90% is achievable, it is possible that once the regulatory mechanism has been activated, nonspecific bystander mechanisms may operate to dampen the polyclonal response to antigen.¹⁹⁻²¹ An example of this has been described in the model of murine responses to the group I allergen of *D. pteronyssinus* in which intranasal administration of an immunodominant peptide was able to tolerize mice to subsequent challenge with the intact protein.²¹

The goal in the treatment of immunologically mediated diseases has been to design therapies that specifically target T lymphocytes that induce or regulate the disease process and to avoid nonspecific immunosuppressive regimens and the long-term problems associated with their use. To design such therapies, it is necessary to define the dominant T-cell epitope, the presence of limited TcR usage or HLA restriction involved in the disease inducing immune response, which can be specifically targeted. The design of this study demonstrates that it is possible to use peptide analogs of the appropriate TcR-V β region to specifically target and significantly inhibit the proliferative response of allergen-reactive T cells. Although these studies have as yet only demonstrated inhibition of the human T-cell response to allergen in vitro, it is noteworthy that similar studies have been performed in vivo in both rat and mouse models of EAE, in which restricted V β 8.2 usage by encephalitogenic T cells was observed. In these studies the appropriate CDR2 peptides not only prevented the onset of clinical disease but also, more practically, caused regression of ongoing EAE.¹²

Initial studies to address the mechanism whereby CDR2 peptides modulate the polyclonal response to allergen suggest that induction of a CD8 + regulatory T-cell subset by the peptide, which modulates the responsiveness of HDM-responsive T cells, may occur. In experiments with T-cell clones we find no evidence for a direct effect of the peptide on HDM-reactive T cells, which suggests that the CDR2 peptide is not directly binding the TcR, nor is it competing with antigen at the level of MHC binding. Failure to block phytohemagglutinin or anti-CD3–induced T-cell proliferation suggests that the CDR2 peptide does not act through CD2 or the T cell–specific antigen receptor. More direct evidence comes from studies in which depletion of CD8 + T cells from the HDM stimulation cultures leads to loss of CDR2 peptide–mediated inhibition. The antigenic peptide recognized by the putative “regulatory” CD8 + T-cell subset, which is presumably derived from the sequence of TcR expressed on V β 3 + HDM-reactive CD4 + T cells, and the mechanism or mechanisms by which the CD8 + T cells modulate the response of these cells remain to be clarified in future experiments.

TcR peptide–based therapy may have broader applications in allergic and autoimmune diseases in which TcR gene usage is reported to be restricted (e.g., multiple sclerosis and myasthenia gravis in which V β 5.2 and V β 12, respectively, are implicated).²²⁻²⁴ In certain diseases, however, such as rheumatoid arthritis, the observed TcR expression of cells infiltrating disease sites appears to be patient-specific rather than disease-specific, and involves multiple V β families.²⁵⁻²⁸ Nevertheless, specific therapy based on TcR structure may have a practical application in allergic disease. The inactivation of selected components of the specific T-cell repertoire, such as those T cells that express the dominant TcR-V β specificity and represent long-lived allergen reactive clones,³ may be sufficient to downregulate the severity of the clinical phenotype.

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