

In vivo clonal dominance and limited T-cell receptor usage in human CD4⁺ T-cell recognition of house dust mite allergens

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ABSTRACT Sensitivity to house dust mite antigens in atopic individuals is a major cause of allergic diseases, ranging from asthma to rhinitis and dermatitis. We have studied the T-cell receptor (TCR) usage of house-dust-mite-specific CD4⁺ T-cell clones isolated from an atopic individual, by using the anchored polymerase chain reaction, and have analyzed the peripheral TCR repertoire of the same individual. Several T-cell clones had identical TCRs at the sequence level, despite the fact that they had been independently isolated, in some cases, in different years. These data suggest the presence *in vivo* of long-lived T-cell clones. We have also shown that junctional sequences identical to these clones are present in peripheral blood T cells taken 6 years after the isolation of the T-cell clones. The analysis of TCR genes used by the panel of clones reveals oligoclonality, with the variable (V) region gene segments V α 8 and V β 3 being dominant, although there is minimal conservation of junctional sequences. The results have implications for understanding the TCR recognition of an environmental aeroallergen and the life span of T-cell clones *in vivo* during a chronic immune response.

The production of specific IgE by atopic individuals after exposure to common environmental aeroallergens represents an aberrant and potentially pathogenic immune response. One-third of the population is atopic, whereas allergic diseases affect $\approx 10\%$ of the population. CD4⁺ T lymphocytes play a central role in the regulation of IgE production (1, 2). Human house-dust-mite (HDM)-specific CD4⁺ T-cell clones isolated from atopic individuals show an "interleukin (IL) 4-dominant" (Th2) functional phenotype (3, 4) and promote specific IgE synthesis *in vitro* from autologous B cells (3). In contrast, HDM-specific T-cell clones from nonatopic individuals express an "interferon γ -dominant" (Th1) phenotype and fail to support IgE production *in vitro*. The two major species of HDM, *Dermaphagoides pteronyssinus* and *Dermaphagoides farinae*, produce ubiquitous allergens that are derived from the fecal pellets and bodies of the mites. There are at least four major groups of HDM antigens (groups I–IV). The cloning and sequencing of some of these allergens (5) have facilitated the study of their major T-cell epitopes; however, to our knowledge, the T-cell receptor (TCR) gene segments that recognize these antigens have not been previously investigated.

The TCR $\alpha\beta$ heterodimer confers antigen specificity through the use of different variable [α chain V region (V α) and β chain V region (V β)], diversity (D β), and joining (J α and J β) gene segments that undergo somatic rearrangement during thymocyte development (6). The insertion or deletion of junctional nucleotides creates further diversity (for review, see ref. 7). Studies of TCRs recognizing defined peptide-major histocompatibility complex (MHC) complexes have

shown restriction of V gene usage and/or conservation of junctional sequences (8–10). Other groups have demonstrated oligoclonality of V gene usage by T cells isolated directly from a disease site, where the antigen specificity is unknown (11, 12). There have been few detailed studies of TCR sequences from human T cells whose central role in a chronic immune response is well documented and, to our knowledge, no such previous studies in the field of allergy.

We have analyzed the TCR sequences of a panel of HDM-specific T-cell clones from an individual with perennial rhinitis, using the anchored PCR.^{||} These clones are heterogeneous in both antigen and HLA class II restriction specificities, yet analysis of TCR gene usage indicates limited diversity. The unexpected finding of identical TCR sequences in clones isolated in different years led us to screen peripheral blood lymphocytes (PBLs) isolated 6 years later for the same junctional sequences.

MATERIALS AND METHODS

Antigens. Lyophilized unfractionated extracts of *D. pteronyssinus* and *D. farinae* HDM were generously provided by Bencard (Brentford, Middlesex, U.K.) and H. Lowenstein, (ALK Laboratories, Copenhagen).

PBL and T-Cell Clones. PBLs were isolated by Ficoll density gradient centrifugation. HDM-specific T-cell clones were isolated from an atopic donor with perennial rhinitis as described (13). Serological typing showed the haplotype of this donor to be A2, 24(9); B50(21), 27; DR11(5), 7; DR52, 53; and DQ2, 7. Briefly, PBLs were stimulated for 7 days with unfractionated *D. farinae* extract, and activated T cells were then cloned by limiting dilution (0.3 cells per well) in the presence of specific antigen, irradiated autologous PBLs as accessory cells, and IL-2. To determine MHC restriction specificity, T-cell clones (1×10^5 cells per ml) were stimulated with HDM (1–30 $\mu\text{g}/\text{ml}$) in the presence of an equal number of irradiated (2500 rads; 1 rad = 0.01 Gy) autologous PBLs or mitomycin C-treated murine fibroblasts expressing HLA-D region gene products as antigen-presenting cells. Murine fibroblasts (DAP3), transfected with the following HLA-D region genes, were used: *DRB1*0101* (from J. Trowsdale, ICRF), *DRB1*1201* and *DRB4*0101* (from S. Rosen-Bronson, Georgetown University, Washington, DC), and *DRB3*0101* and *DRB3*0201* (from B. Mach, University of Geneva, Geneva). After a 60-hr incubation, the cultures were pulse-labeled with [³H]thymidine (1 μCi per well; 1 Ci = 37 GBq; Amersham) and harvested 8–16 hr later. Proliferation

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Abbreviations: TCR, T-cell receptor; V, variable; D, diversity; C, constant; J, joining; IL, interleukin; MHC, major histocompatibility complex; PBL, peripheral blood lymphocyte; V β , C α , etc., β chain V region, α chain C region, etc.; HDM, house dust mite.

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^{||}The sequences reported in this paper have been deposited in the GenBank data base (accession nos. Z22965–7 and Z23039–47).

as correlated with [³H]thymidine incorporation was determined by liquid scintillation spectroscopy.

Isolation of RNA, cDNA Synthesis, and PCR. Total RNA was isolated from T-cell clones and PBLs by the acid guanidinium thiocyanate/phenol method (14). Total RNA (5 µg) was used in first-strand cDNA synthesis, using a dT₍₁₂₋₁₈₎ primer and avian myeloblastosis virus reverse transcriptase. The products were precipitated three times using 2 M ammonium acetate and 3 vol of ethanol and then tailed with dGTP by using terminal deoxynucleotidyltransferase. Five percent of the cDNA product was used as template in the PCR with a 5' polydeoxycytosine primer (5'-CTATCTAGAGAGCTCGCGGCCGCCCCCCCCCCCCC) and a 3' primer for the β chain constant region (Cβ) (5'-CGGAATTCAGATCTCTGCTTCTGATG), or the α chain constant region (Cα) (5'-TGACCGCAGTCGACAGACTTGTCAC-TGGATT). Reaction conditions were as follows: one cycle of 94°C for 5 min, 55°C for 1 min, and 72°C for 5 min, followed by 29 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, with a final extension period of 10 min. These reactions produced specific products of 600–650 bp.

Cloning and Sequencing of Amplified TCR Gene Products. After treatment with proteinase K, the amplified products were digested with *Not* I and *Bgl* II (TCRβ chain products) or *Not* I and *Sal* I (TCRα chain products) and cloned into a modified form of the M13mp18 bacteriophage vector (kindly supplied by P. Moss and J. Bell, Oxford). DNA sequencing was by the dideoxynucleotide chain-termination method (15), using T7 DNA polymerase. For T-cell clones, between 10 and 20 M13 clones from each amplification were sequenced. From the products of PBL cDNA amplification, 50 TCRα and 50 TCRβ in-frame sequences were determined and assigned to previously published V gene families (7, 16–23). Out-of-frame or aberrant rearrangements were not included in the 50 sequences but were noted.

Oligonucleotide Probing of PBL Transcripts. For the screening of PBLs for TCR sequences that were identical to those of the T-cell clones, venous blood was taken 6 years after the original cloning work, and cDNA was prepared. PCR was performed using the 3' Cβ primer as above and a 5' Vβ primer for Vβ21 (5'-TGCTGGGCGGCCGCTGTC-TCCCTGGGAG) or Vβ6 (5'-GGATGTAGAGCTCAGGTGT-GATCCAATTTCAG) families with conditions as follows: 94°C for 5 min, followed by 29 cycles of 94°C for 1 min, 63°C for 1 min, and 72°C for 2 min. PCR products were digested, cloned into modified M13mp18, and transferred to Hybond-N+ nylon filters. Oligonucleotides were labeled using T4 polynucleotide kinase and [³²P]ATP (5000 Ci/mmol, Amersham). Filters were probed with oligonucleotides complementary to the N region of the clones of interest, (DH15N, 5'-CTCTCCTCGACTGCTCCCGCTAG; DD11N, 5'-GGC-TGGTTTCCCCCCTATCTAA), and internal Vβ-specific oligonucleotides (Vβ6.7a, 5'-GAGCTCAGGTGTGATC-CAATTTCA; Vβ 21.5, 5'-TGTGGCTTTTGGTG-CAATCCTAT). Membranes were prehybridized in 6× standard saline citrate (SSC)/5× Denhardt's solution/0.1% SDS and hybridized at 58°C for 16 hr. They were then washed at 45°C in 3× SSC/0.1% SDS for 10 min before exposure to x-ray film for 4 hr at –70°C. Positive plaques were picked and the DNA sequences of their inserts were determined.

RESULTS

Recognition Patterns of T-Cell Clones. The antigen specificity of the panel of 10 T-cell clones was examined using *D. farinae*, the inducing antigen, and a closely related HDM species, *D. pteronyssinus*. Six of the clones were species specific to *D. farinae*, and the remaining T-cell clones were cross-reactive on *D. farinae* and *D. pteronyssinus* (Table 1). Further analysis of these cross-reactive clones showed that

Table 1. Antigen specificity and MHC restriction of HDM-induced T-cell clones

Clone	Proliferation to		MHC restriction
	<i>D. farinae</i>	<i>D. pteronyssinus</i>	
DE41	+	–	DR52
DE47	+	–	DR52
DE12	+	–	DR52
DD11	+	–	DR52
DH15	+	+	DR11(5)
DE9	+	+	DR11(5)
DE5	+	–	DR53
DE26	+	+	DR52
DH12	+	+	DR11(5)
DE49	+	–	ND

ND, not determined.

they were specific for the group I allergen (data not shown). The MHC class II restriction specificities of the T-cell clones, as determined using transfected murine fibroblasts expressing HLA-D region gene products, are shown in Table 1.

TCRα and TCRβ Gene Rearrangements in T-Cell Clones. The Vα and Vβ regions used by the 10 T-cell clones analyzed are summarized in Table 2. The nucleotide and predicted protein sequences spanning the junctional V(D)J regions of the TCRα and TCRβ chains are shown in Fig. 1. Only one in-frame TCRβ rearrangement was detected in each of the T-cell clones. However, in several T-cell clones, two distinct in-frame TCRα sequences were detected. In each case, one TCRα transcript was always found at a much higher frequency than the other in the PCR amplification products. Therefore, it is most probable that the products of the major TCRα transcript are expressed in association with the TCRβ chain, at the cell surface. Six T-cell clones utilized unique TCR sequences, whereas four clones were found to be identical at the sequence level (including the sequence across the VDJ or VJ junctions) to others within the panel. Thus, DE12, DE41, and DE47 were identical to DD11, and DE9 was identical to DH15, although all clones had been isolated separately, and clones with different letters (DD, DE, or DH) originated from different blood samples. Specifically, clone DD11 was established in the year prior to the DE clones, whereas DE9 and DH15 were isolated 10 months apart.

Oligoclonality of V Gene Usage. A restricted number of TCR V genes were used by the panel of T-cell clones. Three clones, DH12, DE26, and DE49, used Vβ3.1, whereas members of the Vα8 family were used by three clones, DD11 (itself identical to three other clones), DH12, and DH15 (itself identical to one other clone) (Table 2). The Vβ sequence of DD11 (and DE12, DE41, and DE47) is identical at the protein level to the cDNA clone VbIW10 (22), where it is named Vβ21.5, and differs by only 1 nt, which is silent at the protein level, from a sequence (cDNA clone IGRb01) classified

Table 2. Summary of TCR V gene segments used by the panel of T-cell clones

Clone(s)	TCRβ				TCRα	
	V	D	J	C	V	J
DE49	3.1	1	1.2	1	15.1	AB19
DE26	3.1	2	2.5	2	1.2	JaG
DH12	3.1	1	1.1	1	8.2	IGRJα04
DD11, DE12	21.5	1	1.5	1	8.1	AB22
DE41, DE47						
DH15, DE9	6.7a	2	2.7	2	8.1	JaC
DE5	9.1	1	1.4	1	Vδ5	JaN

TCR α and β sequences were assigned according to previously described families, by using refs. 7, 16, 18, 21, and 22 for TCRβ chains and refs. 7, 17, 20, 24, and 25 for TCRα chains.

a					
Clone	V α	N		J α	C α
DE49	TACTTCTGTGCA Y F C A	GAGAGAATCACGGGCAGGAGA E R I T G R R		GCACCTACTTTTGGGAGTGGAAACAAGACTCCAAGTGCAACCA A L T F F G S G T R L Q V Q P	AATATCCAGAAC N I Q N
DE26	TACTTCTGT Y F C	GTTGTGAGTGACCTTCATGGCTCTAGCAACACAGGC V V S D L H G S S N T G		AAACTAATCTTTGGGCAAGGGACAACCTTTACAAGTAAACCA K L I F G Q G T T L Q V K P	GATATCCAGAAC D I Q N
DH12	TACTTTTGTGCA Y F C A	GTTGGGGCTGGAGGCTTC V G A G G F		AAACTATCTTTGGAGCAGGAACAAGACTATTGTAAAGCA K T I F G A G T R L F V K A	AATATCCAGAAC N I Q N
DD11	TACTTCTGTGCC Y F C A	TACAAGAACACAGGCTTTCAG Y K N T G F Q		AAACTGTATTTTGGAACCTGGCACCAGCTCTGGTCAGTCCA K L V F G T G T R L L V S P	AATATCCAGAAC N I Q N
DH15	TACTTC Y F	TCGCCAGGGGTTCTGCAAGG S P R G S A R		CAACTGACCTTTGGATCTGGGACACAATTGACTGTTTTCACCT Q L T F G S G T Q L T V L P	GATATCCAGAAC D I Q N
DE5	TACTTCTGTGCA Y F C A	GCAAGCGCTAACGACTAC A S A N D Y		AAGCTCAGCTTTGGAGCCGGAACACAGTAAGTGAAGAGCA K L S F G A G T T V T V R A	AATATCCAGAAC N I Q N

b						
Clone	V β	N1	D	N2	J β	C β
DE49	TGTGCCAGCACT C A S S	TTATATGCGCGGACC L Y G A T			TATGGCTACACCTTCGGTTCGGGGACCAAGTTAACCGTTGTA Y G Y T F F G S G T R L L T V V	GAGGACCTGAAC E D L N
DE26	TGTGCCAGCACT C A S S	TTATTCAATAGCGGGTAC L F N S G Y			CAAGAGACCCAGTACTTCGGGCCAGGCACGGGCTCCTGGTGCTC Q E T Q Y F G P G T R L L V L	GAGGACCTGAAA E D L K
DH12	TGTGCCAGCACT C A S S	TTAGTTGAACGGGTC L V E R V			ACTGAAGCTTTCTTTGGACAAGGCACCAAGTCTCAGTGTGTA T E A F F F G Q G T R L L T V V	GAGGACCTGAAC E D L N
DD11	TGTGCCAGCAGC C A S S	TTAGATAGGGGGGA L D R G G			AACGAGCCCCAGCATTTTGGTGATGGGACTCGACTCTCCATCCTA N Q P Q H F G D G T R L S I L	GAGGACCTGAAC E D L N
DH15	TGTGCCAGCAGC C A S S	CCTCCTAGCGGGAGCAGTCGAGGA P P S G S S R G			GAGCAGTACTTCGGGCCGGGCACCAAGGCTCAGCGTCA E Q Y F G P G T R L L T V T	GAGGACCTGAAA E D L K
DE5	TGTGCCAGCAGC C A S S	CAAGGTCCCACTGGT Q G P T G			GAAAACTGTTTTTTGGCAGTGGAAACCCAGCTCTCTGTCTTG E K L F F G S G T Q L S V L	GAGGACCTGAAC E D L N

FIG. 1. Sequence analysis of HDM-reactive T-cell clones. DNA sequences encoding TCR α (a) and TCR β (b) chains spanning the junctional regions are shown. Predicted amino acid sequences are also given. Spaces have been introduced to allow alignment of conserved sequences. The nucleotides contributed by germ-line D β sequences are underlined. Those T-cell clones with identical junctional sequences (i.e., DE9, identical to DH15; DE12, DE41, and DE47, all identical to DD11) are not shown.

within the V β 21 family (19). The V α segment of DE5 is classified as a V δ gene (17) but has rearranged to a J α and the C α segment. This phenomenon is now well recognized (26, 27) and has been estimated to occur in $\approx 1\%$ of all productive TCR α rearrangements (23). However, we believe this to be the first demonstration of the use of V δ in a TCR α chain of a well-characterized human T-cell clone. There was no detectable conserved motif in the V(D)J junctional region of either the TCR α or TCR β chains, although four of the six TCR β chains have a leucine residue at position 96 (28), the first coded for by N region nucleotides.

Screening of PBLs for Persistent Clones. To study the possible *in vivo* life span of T-cell clones that appear to persist in this chronic response, venous blood was taken 6 years after the original isolation of these clones. cDNA was prepared and screened for the TCR β sequences of interest by using a V β -specific family PCR and oligonucleotide probing. In both cases, sequences identical to the VDJ region of the clones were demonstrated in full-length in-frame transcripts from unstimulated PBLs (Fig. 2).

Peripheral Repertoire Studies. The peripheral blood TCR V α and V β gene usage of the individual from whom the T-cell clones were established was analyzed by anchored PCR. The repertoire of this individual is shown in Fig. 3. Each of the 50 V α J or V β DJ in-frame productive sequences was unique. The frequency of V α and V β usage was in broad agreement with previously published repertoire studies, although there is considerable interindividual variation (29). Three TCR α chains used a V δ gene segment. In addition to the productive TCR sequences, several sequences could not code for a functional TCR chain. Thus, 10% of V α and 5% of V β rearrangements had no open reading frame for VDJ translation. The changes in translational reading frame were always found within the junctional regions, consistent with out-of-frame rearrangements, rather than errors in the PCR ampli-

fication. In addition, 5% of both TCR α and TCR β transcripts contained only D and/or J sequences 5' of C α or C β or contained germ-line sequence 5' to the C gene segments. PBLs from this individual were also stained with a panel of antibodies specific for V β gene products, within the CD3-, CD4-, and CD8-positive populations and analyzed by immunofluorescence. These data were in broad agreement with those obtained by PCR analysis (data not shown).

DISCUSSION

In this study, designed to investigate the diversity of TCR usage by CD4 $^{+}$ T cells in atopic individuals sensitized with allergens of *Dermatophagoides* spp. (HDM), we have observed dominant expression of TCR V β 3 and V α 8 genes. Furthermore, the TCR sequence data suggest the presence of dominant T-cell clones that are long lived *in vivo*, presumably maintained by chronic allergen exposure. The analysis of the HDM-specific CD4 $^{+}$ T-cell response in atopic individuals has attracted considerable attention (13, 30). The results of such studies have established that T cells expressing an IL-4-dominant phenotype are predominantly activated. From our investigation of the antigen and HLA class II restriction specificity, it appears that even within one individual the HDM T-cell response is heterogeneous. Several epitopes derived from both the group I and II major allergens are known to be recognized by "disease-associated" T cells, namely, those that produce high levels of IL-4 and support the production of HDM-specific IgE. It has also been demonstrated that HLA-DRB1, -3, -4, and -5 gene products and DP class II molecules may restrict recognition of HDM-derived T-cell determinants (31).

It is remarkable, therefore, that a considerable degree of oligoclonality was observed in this panel of HDM-reactive clones. A further unexpected observation was that two

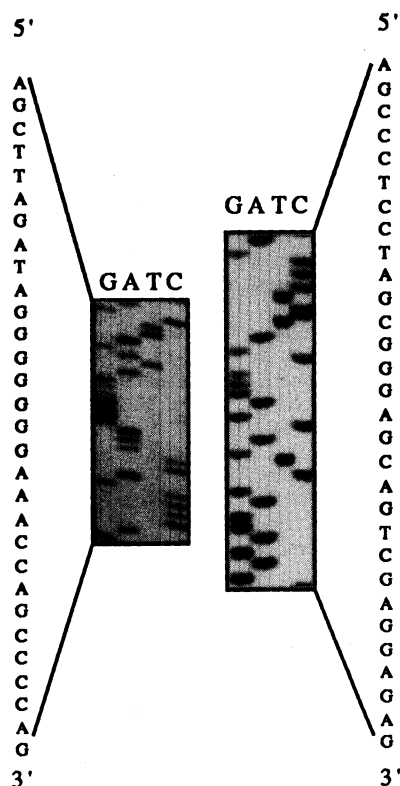


FIG. 2. Autoradiographs from sequencing gels of PBL TCR β transcripts showing sequences corresponding to the N-region nucleotides of the DD11 (Left) and DH15 (Right) T-cell clones (for comparison of these sequences with those in the T-cell clones, see Fig. 1).

groups of T-cell clones used identical TCRs. Each of these groups must represent cells that originated from one precursor because the TCR sequences were identical even across the N regions. We have also demonstrated sequences iden-

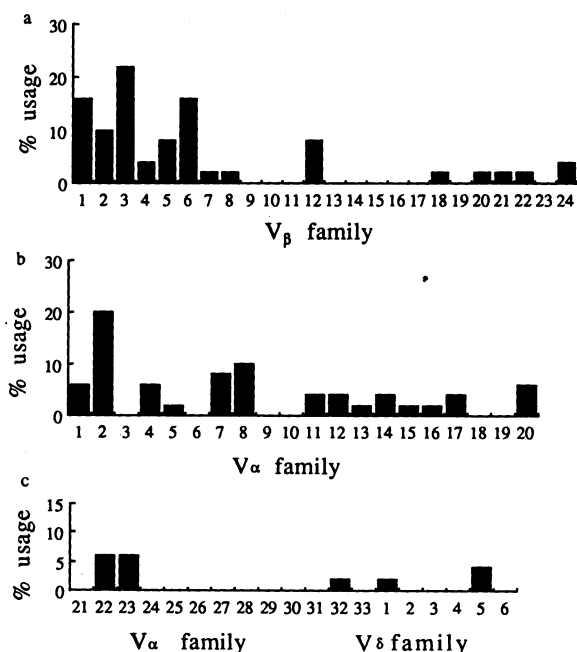


FIG. 3. V β and V α repertoire analysis. The percent usage of V β 1-24 (a), V α 1-20 (b), and V α 21-33 and V δ 1-6 (where a V δ has rearranged to the C α gene) (c) in 50 PBL TCR α and 50 TCR β sequences analyzed is shown.

tical to the TCR β N regions in unstimulated PBLs from 6 years later. It is possible that different peripheral blood T cells possess TCR β sequences identical at the nucleotide level even across the CDR3 junctional region but use different TCR α chains. However, we believe that these data provide strong evidence for the existence of the same long-lived clone over 6 years. This is to our knowledge the first report of such long-lived T-cell clones *in vivo*. One explanation for the existence of long-lived clones in this individual is that disease-specific T cells are constantly stimulated to divide by chronic exposure to HDM antigens. In this context, it is interesting that the CD45RO "memory" T-cell population has been shown to have a short intermitotic time *in vivo* (32).

The anchored PCR has now been used by several groups to study TCR use by T-cell clones and lines and PBLs. It has the theoretical advantage of being able to amplify all possible TCR sequences equally, since the two primers are the same for any transcript. The results of our repertoire study are in many ways comparable to other studies, although the high representation of V β 3 in PBL transcripts from this individual was unusual. In other reports, V β 3 usage is generally between 1 and 6% of TCR β in PBLs (ref. 29 and L.R.W., unpublished observations). Many factors could account for this, such as MHC or other genetic differences and environmental influences, all of which will have affected thymic selection.

The potential to inhibit allergic inflammation using components of specific antigen is the underlying goal of allergen-based desensitization. There is evidence to suggest the presence of dominant antigenic regions in the major allergens of HDM, but overall both the array of epitopes recognized and the number of restriction elements used in the T-cell response of atopic individuals are diverse. Therefore, while it has been reported that the administration of immunodominant T-cell epitopes, as peptides, *in vivo* can induce antigen-specific nonresponsiveness (33), peptide-based hyposensitization may be difficult to achieve for allergic responses to HDM. Furthermore, in some of the autoimmune diseases, the specificity of the "disease-associated" T-cell repertoire may shift as illustrated in murine experimental allergic encephalomyelitis (34). During the induction phase of disease, the T-cell response is directed toward an immunodominant NH₂-terminal region of myelin basic protein, but as the disease becomes chronic, additional determinants that are cryptic after the initial immunization then become immunogenic (34).

From TCR sequence analysis of cloned T cells and PBLs, we suggest that the *in vivo* T-cell response to HDM is dominated by, and limited to, a small number of long-lived T-cell clones, which use a restricted number of TCR gene segments. Therefore, even if responses to minor T-cell determinants are retained, inactivation of the dominant HDM-reactive T cells might allow a decrease in clinical symptoms. In the experimental allergic encephalomyelitis model, it has been reported that the "pathogenic" T cells predominantly express the V β 8⁺ TCR and immunization with CDR2 peptides derived from V β 8 prevents disease (35). This would support the potential application of TCR-based therapy as an alternative approach for regulating HDM allergic immune responses.

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1. Okumura, K. & Tada, T. (1971) *J. Immunol.* **106**, 1019-1025.

2. Ishizaka, K. (1984) *Annu. Rev. Immunol.* **2**, 159–182.
3. O'Hehir, R. E., Bal, V., Quint, D., Moqbel, R., Kay, A. B., Zanders, E. D. & Lamb, J. R. (1989) *Immunology* **66**, 499–504.
4. Wierenga, E. A., Snoek, M., Jansen, H. M., Bos, J. D., Van Lier, R. A. W. & Kapsenberg, M. L. (1991) *J. Immunol.* **147**, 2942–2949.
5. Stewart, G. A., Ward, L. D., Simpson, R. J. & Thompson, P. J. (1992) *Immunology* **75**, 29–35.
6. Davis, M. M. & Bjorkman, P. J. (1988) *Nature (London)* **334**, 395–402.
7. Wilson, R. K., Lai, E., Concannon, P., Barth, R. K. & Hood, L. E. (1988) *Immunol. Rev.* **101**, 149–172.
8. Hedrick, S. M., Engel, I., McElligott, D. L., Fink, P. J., Hsu, M. L., Hansburg, D. & Matis, L. A. (1988) *Science* **239**, 1541–1544.
9. Acha-Orbea, H., Mitchell, D., Timmermann, L., Wraith, D. C., Tausch, G. S., Waldor, M. K., Zamvil, S. S., McDevitt, H. O. & Steinman, L. (1988) *Cell* **54**, 263–273.
10. Moss, P. A. H., Moots, R. J., Rosenberg, W. M. C., Rowland-Jones, S. J., Bodmer, H. C., McMichael, A. & Bell, J. I. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 8987–8990.
11. Sottini, A., Imberti, L., Gorla, R., Cattaneo, R. & Primi, D. (1991) *Eur. J. Immunol.* **21**, 461–466.
12. Davies, T. F., Martin, A., Concepcion, E. S., Graves, P., Cohen, L. & Ben-Nun, A. (1991) *N. Engl. J. Med.* **325**, 238–244.
13. O'Hehir, R. E., Young, D. B., Kay, A. B. & Lamb, J. R. (1987) *Immunology* **62**, 635–640.
14. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
15. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
16. Toyonaga, B., Yoshikai, Y., Vadasz, V., Chin, B. & Mak, T. W. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8624–8628.
17. Takihara, Y., Reimann, J., Michalopoulos, E., Ciccone, E., Moretta, L. & Mak, T. W. (1989) *J. Exp. Med.* **169**, 393–405.
18. Li, X., Szabo, P., Robinson, M. A., Dong, B. & Posnett, D. N. (1990) *J. Exp. Med.* **171**, 221–230.
19. Ferradini, L., Roman-Roman, S., Azocar, J., Michalaki, H., Triebel, F. & Hercend, T. (1991) *Eur. J. Immunol.* **21**, 935–942.
20. Roman-Roman, S., Ferradini, L., Azocar, J., Genevee, C., Hercend, T. & Triebel, F. (1991) *Eur. J. Immunol.* **21**, 927–933.
21. Wilson, R. K., Lai, E., Kim, L. D. H. & Hood, L. (1990) *Immunogenetics* **32**, 406–412.
22. Hansen, T., Qvigstad, E., Lundin, K. & Thorsby, E. (1991) *Tissue Antigens* **38**, 99–103.
23. Moss, P. A. H., Rosenberg, W. M. C., Zintzaras, E. & Bell, J. I. (1993) *Eur. J. Immunol.* **23**, 1153–1159.
24. Klein, M. H., Concannon, P., Everett, M., Kim, L. D. H., Hunkapiller, T. & Hood, L. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6884–6888.
25. Kimura, N., Toyonaga, B., Yoshikai, Y., Du, R. P. & Mak, T. W. (1987) *Eur. J. Immunol.* **17**, 375–383.
26. Miossec, C., Caignard, A., Ferradini, L., Roman-Roman, S., Faure, F., Michalaki, H., Triebel, F. & Hercend, T. (1991) *Eur. J. Immunol.* **21**, 1061–1064.
27. Sottini, A., Imberti, L., Fiordalisi, G. & Primi, D. (1991) *Eur. J. Immunol.* **21**, 2455–2459.
28. Kabat, E. A., Wu, T. T., Reid-Miller, M., Perry, H. M. & Gottesman, K. S. (1991) in *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD), 5th Ed.
29. Rosenberg, W. M. C., Moss, P. A. H. & Bell, J. I. (1992) *Eur. J. Immunol.* **22**, 541–549.
30. Rawle, F. C., Mitchell, E. B. & Platts-Mills, T. A. (1984) *J. Immunol.* **133**, 195–201.
31. O'Hehir, R. E., Mach, B., Berte, C., Greenlaw, R., Tiercy, J. M., Bal, V., Lechler, R. I., Trowsdale, J. & Lamb, J. R. (1990) *Int. Immunol.* **2**, 885–892.
32. Michie, C. A., McLean, A., Alcock, C. & Beverley, P. C. L. (1992) *Nature (London)* **360**, 264–265.
33. Wraith, D. C., Smilek, D. E., Mitchell, D. J., Steinman, L. & McDevitt, H. O. (1989) *Cell* **59**, 247–255.
34. Lehmann, P. V., Forsthuber, T., Miller, A. & Sercarz, E. E. (1992) *Nature (London)* **358**, 155–157.
35. Vandenbark, A. A., Hashim, G. & Offner, H. (1989) *Nature (London)* **341**, 541–544.