Structure–function relationship among T-cell receptors specific for lysozyme peptides bound to A^b or A^{bm-12} molecules

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ABSTRACT   The $\alpha\beta$ T-cell receptor (TCR) recognizes antigenic peptides bound to major histocompatibility complex (MHC) molecules. In contrast to the antibody combining site, for which the antigen contact or complementarity-determining residues (CDRs) have been precisely defined, the location and function of the corresponding CDR regions of the α and β TCR chains are not known. To develop a model system for systematic analysis of the CDRs of the $\alpha\beta$ TCR, we isolated a panel of murine T-cell clones that recognize a lysozyme peptide containing residues 74–88 bound to either A^b or A^{bm-12} MHC class II molecules. Although these two MHC molecules differ by only three amino acid residues within the $A\beta$ chain, each of the T-cell clones was specific for peptide bound to the self-MHC molecule and did not recognize the same peptide bound to the other MHC molecule. The structural basis for this exquisite ligand specificity of the TCRs was analyzed by isolation and characterization of α and β chain genes from five closely related T-cell clones. Comparison of predicted amino acid sequences mapped the ligand specificity differences to residues present within the α chain variable region segment and the α and β chain variable–joining region junction regions. Thus with current models of TCR–ligand interactions, the results suggest that residues 26–30 of the α chain variable region may constitute one of the CDR regions of the TCR.

The unique ligand specificity of the T-cell receptor (TCR) for peptides bound to major histocompatibility complex (MHC) molecules is the basis for selection of self-MHC-restricted T cells in the thymus and for MHC-restricted antigen recognition by mature T cells (1–3). Comparison of the α and β TCR chains with their antibody heavy and light chain counterparts has revealed similarities in genomic organization of gene segments encoding these polypeptides, in genetic mechanisms that give rise to structural diversity, and in conservation of many amino acid residues at identical positions (4, 5). These common features suggest that the TCR and the antibody combining sites are closely related in structure and function. However, unlike the antibody molecule, the TCR does not directly bind to the antigen and its ligand combining site is likely to contact residues of both antigenic peptides and the MHC molecule. The precise location of these complementarity-determining residues (CDRs) of the TCR is not known.

Direct gene transfer studies as well as comparison of primary amino acid sequences of α and β chains derived from TCRs with defined antigenic specificities have suggested that variable (V) regions of both chains contribute to ligand specificity (for review, see ref. 6). Several exceptions are known, however, where ligand specificity strongly correlates with the utilization of a particular TCR β chain V region (Vβ) and, occasionally, of α chain V region (Vα) segments (7–12). An essentially similar model has been proposed indepen-

dently by Davis and Bjorkman (13) and by Chothia et al. (5) to explain the structural relationship between the $\alpha\beta$ TCR combining site with the antigen–MHC ligand recognition function. This model was prompted by solution of the crystal structure of the class I MHC molecule that revealed the location of the polymorphic residues within the MHC molecules as lining the antigen binding groove on the top surface of the molecule (14). By extrapolating the known structure of the antigen combining site of antibody molecules to the TCR combining site, the model suggests that residues within the V segments contact the MHC and residues within the CDR3 regions contact the antigen. Whereas changes in the ligand specificity of TCRs do correlate with amino acid substitutions within the V(D)J regions (where D is diversity and J is joining) of the α and β chains, the role of the Vα/Vβ segments and of the MHC molecules in determining these effects is not known (6, 15).

In this report, we describe the development of a model system to systematically test the structure–function relationship of the $\alpha\beta$ TCR. We have generated a panel of T-cell clones that are specific for lysozyme peptides bound to either the A^b or the A^{bm-12} class II MHC molecules. These two MHC molecules differ only in three amino acids at positions 67, 70, and 71 of their $A\beta$ subunits (16). We have identified minimal peptide determinants recognized by these TCRs and have determined the sequences of five pairs of closely related Vα and Vβ regions.‡ Comparison among these sequences demonstrates that differences in TCR ligand specificity correlate with amino acid substitutions within the Vα segment and within the Vα–Jα and Vβ–Jβ junction regions.

MATERIALS AND METHODS

Animals and Peptides. C57BL/6 (B6, A^b) and B6-C-H-2^{bm-12} (bm-12, A^{bm-12}) mice were obtained from The Jackson Laboratory. Mice of age 2–12 months old of either sex were used. Mice were immunized with 7 nmol of synthetic peptides in complete Freund's adjuvant (17). Hen egg white lysozyme peptides corresponding to residues 74–96 (p74–96), NH$_2$-Asn-Leu-Cys-Asn-Ile-Pro-Cys-Ser-Ala-Leu-Leu-Ser-Ser-Asp-Ile-Thr-Ala-Ser-Val-Asn-Cys-Ala-Lys-OH were prepared by solid-phase synthesis (17).

Cell Culture. Methods for generation and maintenance of antigen-specific long-term T-cell lines or T-cell hybrids have been described (17). Mouse L-cell fibroblasts expressing the A^b (FT7.1) or the A^{bm-12} (FT7.2) MHC molecules (18) were kindly provided by F. Ronchese and R. Germain.

Abbreviations: APC, antigen-presenting cell; CDR, complementarity-determining residue; MHC, major histocompatibility complex; TCR, T-cell receptor; V, D, J, and C, variable, diversity, joining, and constant, respectively; Vα, Jβ, etc., α chain V region, β chain J region, etc.; IL-2, interleukin 2; p(74–96), p(74–90), etc., lysozyme peptides containing residues 74–96, 74–90, etc.
‡The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M87844–M87853).

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Specificity Assays. T-cell specificity assays were carried out as described (17). Briefly, 1×10^4 long-term T cells or 1×10^5 T-cell hybrids were cocultured with antigen-presenting cells (APCs) in the presence of medium alone or the indicated peptides at 7 μM in 0.2 ml in 96-well tissue culture plates. APCs were either 5×10^5 syngeneic spleen cells treated with mitomycin C (Sigma, 50 μg/ml) at 37°C for 30 min or 1×10^5 untreated L-cell transfectants. The specific T-cell response of lymph node cells or long-term T-cell lines was determined by incorporation of [³H]thymidine (Amersham) directly into proliferating T cells. For T-cell hybrids, 50 μl of supernatant from 18- to 24-h cultures was tested for interleukin 2 (IL-2) by using a secondary culture containing the IL-2-dependent cell line HT-2 (17).

Isolation and Sequence Analysis of TCR β and α Chain Genes. DNA rearrangements encoding the functional VDJβ-Cβ (where Cβ is β chain constant region) chains were identified as either EcoRI or HindIII fragments by Southern blot analysis of DNA isolated from the T-cell hybrids (19) and cloned with size-selected DNA (including the rearranged fragment) isolated from agarose gels as described (20). The isolated clones were subjected to restriction enzyme analysis and appropriate fragments containing the VDJβ sequences were subcloned into M13mp18 vector and sequenced by the dideoxynucleotide chain-termination method (21). Specific Jβ oligonucleotide primers and the universal M13 vector primer were used to sequence both strands of the clones.

Total cellular RNA was prepared from the T-cell hybrids and poly(A)+ RNA was isolated by two passes over oligo(dT)-cellulose (Collaborative Research, type 3). cDNA was synthesized from the poly(A)+ RNA using avian myeloblastosis virus reverse transcriptase, RNase H, and Escherichia coli DNA polymerase I as described (22). The cDNA fragments >1 kilobase were inserted into λgt10 vector after addition of EcoRI linkers. The cDNA libraries were screened with murine Cα and Cβ probes, and positive clones were probed with Vα1 and Vβ1 DNA fragments to eliminate clones derived from the BW5147 fusion partner (23, 24). The EcoRI inserts of the λgt10 cDNA clones were isolated, subcloned into M13mp18 vector, and sequenced as for β chain genes above with oligonucleotides specific for the 5' ends of Cα or Cβ and the universal M13 primers (23, 24).

RESULTS AND DISCUSSION

Responsiveness of bm-12 Mice to p74–96. In our earlier studies with p74–96-specific T cells in C57BL/6 (B6) mice, two distinct lysozyme peptides containing residues 74–90

(p74–90) and 81–96 (p81–96) were identified as the major antigenic determinants recognized in the context of the A^b MHC molecule (25, 26). B6-C-H-2^{bm-12} (bm-12) mice express the A^{bm-12} MHC molecule, which differs from the A^b MHC molecule in only three amino acid residues at positions 67, 70, and 71 of the Aβ chain. To assess the influence of the amino acid differences among A^{bm-12} and A^b molecules toward the antigen-specific T-cell response, bm-12 mice were immunized with p74–96. The antigen-specific proliferative response of draining lymph node cells and long-term T-cell lines derived from the B6 and bm-12 mice was tested with the immunogen and with smaller peptides (Table 1). Similar to B6 T cells, bm-12 T cells responded strongly to p74–96 and to p74–90. Furthermore, the minimal peptide determinant recognized by both B6 and bm-12 mice within p74–90 was similar in that truncation of the four C-terminal residues 87–90 in p74–86 abolished the antigen-specific response. However, unlike B6 mice, which also elicited T cells specific for p81–96, T cells with this specificity were not detected in bm-12 mice. Thus, the three amino acid substitutions between the two class II MHC molecules did not alter the ability of bm-12 mice to respond to the p74–90 but were, however, associated with the absence of p81–96-specific T cells in bm-12 mice.

Both B6 and bm-12 T-Cell Clones Recognize Similar Minimal Peptide Determinants. The finding that T cells with similar antigen specificity were induced in both B6 and bm-12 mice is in marked contrast to our earlier studies (17). Comparison of T-cell responses induced by p74–96 had shown dramatic differences in the minimal peptide determinants recognized in the context of A^k (p74–82) or E^k (p85–96) class II MHC molecules. These differences were attributed to the extensive structural differences between the polymorphic MHC molecules (17). To identify possible differences in the minimal peptide determinant(s) within p74–90 recognized by individual B6 and bm-12 T cells, additional synthetic peptides were tested with individual T-cell clones. Each of the four B6 and five bm-12 T-cell hybrids recognized p74–90, p74–89, and p74–88, demonstrating that the minimal antigenic determinants were contained within p74–88 (Table 2). Because each of the clones failed to recognize p74–86, residues 87 and/or 88 were required for antigen recognition. The requirement for both residues 87 and 88 was, however, not absolute because BO4T27.6, bm1T5.1, and bm4T17.2 T cells could also recognize p74–87. Furthermore, truncation of a single amino acid from the N-terminal end (p75–96) completely abolished peptide recognition by either B6 or bm-12 T-cell clones (data not shown). Thus, in contrast to the nonoverlapping peptides p74–82 vs. p85–96, which were presented

Table 1. Response of B6 and bm-12 mice to p74–96

| Cells | [³H]Thymidine incorporation per culture, Δcpm $\times 10^{-3}$ | | | | |
	Medium	p74–96	p74–90	p74–86	p81–96
B6 LNC	(1.0 ± 0.1)	**83.7 ± 3.8**	ND	0.7 ± 0.9	**34.8 ± 4.9**
B6 LNC	(7.0 ± 0.3)	**127.0 ± 6.4**	**70.5 ± 2.1**	1.7 ± 3.1	**24.3 ± 6.3**
BO4T	(5.5 ± 4.2)	**65.9 ± 1.8**	**84.6 ± 3.4**	0.2 ± 2.9	**57.8 ± 3.4**
bm-12 LNC	(1.7 ± 0.2)	**54.3 ± 6.8**	ND	4.1 ± 3.9	0.5 ± 0.6
bm-12 LNC	(10.1 ± 3.8)	**89.8 ± 4.6**	**40.3 ± 2.3**	1.2 ± 0.8	2.3 ± 1.0
bm1T	(0.7 ± 0.3)	**48.6 ± 2.1**	**82.6 ± 3.0**	ND	0.5 ± 0.0
bm2T	(0.9 ± 0.0)	**200.9 ± 7.2**	**269.1 ± 18.6**	ND	0.3 ± 0.1

Lymph node cells (LNCs) were obtained from either B6 or bm-12 mice (pool of three animals) immunized 10 days earlier with p74–96. BO4T, bm1T, and bm2T are bulk cultures of long-term T-cell lines established from B6 (BO4T) or bm-12 (bm1T and bm2T) mice. Approximately 4×10^5 LNCs alone or 1×10^4 T cells plus 5×10^5 mitomycin C-treated normal syngeneic spleen cells were cultured in triplicate for 4 days in medium alone or with the indicated peptides at 7 μM. Incorporation during the final 18-h incubation of cultures pulse-labeled with 1 μCi of [³H]thymidine is shown (1 Ci = 37 GBq); data are mean ± SD. Background cpm with medium alone are shown in parentheses and have been subtracted to show antigen-specific cpm. Boldface type emphasizes positive responses. ND, not determined.

Table 2. Minimal peptide determinants recognized by p74–96-specific B6 and bm-12 T-cell clones

	[^{3}H]Thymidine incorporation per culture, Δcpm $\times$ 10^{-3}					
T cells	Medium	p74–90	p74–89	p74–88	p74–87	p74–86
BO4H9.1	(1.9 ± 0.3)	93.0 ± 5.1	69.8 ± 2.2	38.8 ± 1.0	1.7 ± 0.4	0.1 ± 0.0
BO3H.25	(1.8 ± 0.1)	9.2 ± 0.2	6.8 ± 1.8	10.6 ± 2.0	3.1 ± 0.2	0.2 ± 0.1
BO4T8.3	(8.5 ± 0.5)	44.1 ± 8.3	82.2 ± 4.5	35.7 ± 2.4	0.2 ± 1.5	0.1 ± 0.6
BO4T27.6	(1.0 ± 0.1)	80.1 ± 2.1	68.7 ± 4.1	53.4 ± 7.7	43.7 ± 0.9	2.1 ± 1.0
bm2T3.1	(10.8 ± 1.5)	55.4 ± 2.6	59.3 ± 4.2	38.2 ± 3.1	7.3 ± 1.1	1.4 ± 0.6
bm4T6.2	(1.1 ± 0.3)	64.0 ± 4.8	89.1 ± 5.0	55.0 ± 1.4	2.6 ± 1.3	ND
bm1T7.6	(3.9 ± 0.8)	194.8 ± 0.8	125.6 ± 18.2	86.0 ± 3.0	6.5 ± 1.2	0.3 ± 1.1
bm1T5.1	(1.5 ± 0.4)	80.9 ± 2.8	76.1 ± 6.2	81.7 ± 4.6	92.2 ± 4.2	0.5 ± 0.2
bm4T17.2	(1.1 ± 0.3)	36.0 ± 0.8	40.1 ± 3.6	66.1 ± 2.1	70.9 ± 3.6	2.1 ± 1.1

Prefix B or bm indicates the B6 or bm-12 origin of T-cell hybrids. Cells were tested with parental spleen cells of the appropriate strain. T cells (1 $\times$ 10^5 cells) were cultured with 5 $\times$ 10^5 mitomycin C-treated normal B6 or bm-12 spleen cells in medium alone or with the indicated peptides at 7 μM. After 24 h, culture supernatants were tested for presence of IL-2 in a secondary culture with the IL-2-dependent HT-2 cells. Incorporation in HT-2 cells pulse-labeled with [^{3}H]thymidine at 1 μCi per culture during the final 4 h of an 18-h culture is shown. Other details are as in Table 1.

exclusively by A^k and E^k molecules (17), the A^b and A^{bm-12} MHC molecules apparently presented the same p74–88 to T cells.

The B6 and bm-12 TCRs Distinguish Between the Peptide-A^b or –A^{bm-12} Complexes. The close similarity in structures of the A^b and A^{bm-12} class II MHC molecules and in the peptide specificity of the B6 and bm-12 T cells suggested that these TCRs may cross-react with either antigen–MHC complex. To assess this possibility, we tested the ability of A^b and A^{bm-12} APCs to stimulate T cells with the same antigen. For this experiment, mouse L cells that had been transfected with the $A\alpha^b$ and either $A\beta^b$ (FT7.1) or $A\beta^{bm-12}$ (FT7.2) genes were used as APCs (18). The data in Table 3 demonstrate that each of these T cells recognized antigen presented only by self A^b or A^{bm-12} molecules. Thus, the TCRs expressed by these T cells, despite their similar antigen specificity, clearly distinguish between the two class II MHC molecules.

The three amino acid substitutions between the A^b and A^{bm-12} are located within one of the two top α-helices in the hypothetical model of the class II MHC molecule (27). In this location, one or more of the side chains could directly affect interactions of the MHC molecules with the peptides and/or with the TCR. The absence of p81–96-specific T cells in bm-12, but not in B6 mice (Table 1), could be due to the inability of this peptide to bind to the A^{bm-12} molecule.

Table 3. B6 and bm-12 T cells recognize antigen only with self-MHC molecules

	[^{3}H]Thymidine incorporation per culture, Δcpm $\times$ 10^{-3}			
T cells/ APCs	FT7.1 (A^b)		FT7.2 (A^{bm-12})	
	Medium	Antigen	Medium	Antigen
BO4H9.1	(10.6 ± 1.0)	117.9 ± 6.4	(1.8 ± 0.6)	0.8 ± 0.8
BO3H.25	(0.4 ± 0.1)	6.8 ± 0.2	(1.1 ± 1.0)	0.2 ± 0.1
BO4T27.6	(0.6 ± 0.0)	86.8 ± 9.0	(0.8 ± 0.1)	0.2 ± 0.1
BO4T8.3	(6.6 ± 1.6)	11.6 ± 1.2	(1.6 ± 2.5)	0.1 ± 0.7
bm2T3.1	(1.9 ± 0.5)	0.3 ± 0.4	(1.8 ± 0.6)	39.5 ± 0.7
bm4T6.2	(1.8 ± 0.1)	0.8 ± 0.1	(1.6 ± 0.2)	13.2 ± 0.1
bm1T5.1	(7.6 ± 0.6)	0.8 ± 0.6	(6.4 ± 0.4)	86.7 ± 1.0
bm1T7.6	(2.4 ± 2.9)	0.9 ± 3.1	(1.7 ± 4.8)	44.1 ± 1.6
bm4T17.2	(1.1 ± 0.1)	0.1 ± 0.1	(1.3 ± 0.2)	23.6 ± 2.4

Culture conditions were identical to those described in Table 2, except that 1 $\times$ 10^5 FT7.1 or FT7.2 L cells expressing the A^b or A^{bm-12} class II MHC molecules were used as APCs instead of normal spleen cells. The response to antigen was tested at 7 μM p74–96. Other details are as in Table 1.

Alternatively, the absence of p81–96-specific T cells could be due to an influence of these three residues on positive or negative selection of self-MHC reactive TCRs in the thymus (3). Nevertheless, existence of p74–88-specific T cells in both B6 and bm-12 mice demonstrates that both A^b and A^{bm-12} molecules can both bind to the same peptide. Furthermore, the fact that each of the two sets of TCRs fails to cross-react between the peptide-bound A^b and A^{bm-12} molecules shows that these three residues do affect TCR–MHC interactions. The simplest interpretation of these results is that, for TCRs specific for p74–88, the interactions between the TCR and MHC include contacts with one or more of the three amino acid substitutions. However, more complex interpretations in which the lack of cross-reaction may be due to differences in conformation of p74–88 when bound to A^b vs. A^{bm-12} MHC molecules cannot be dismissed.

Characterization of the $\alpha\beta$ TCRs of B6 and bm-12 T-Cell Clones. To determine the structural basis for the ligand specificity of these T-cell clones, we analyzed their TCR α and β chains. We first isolated the rearranged genomic fragments containing the functional VαJα and VDJβ exons from T-cell hybrid BO4H9.1. The nucleotide sequence of these V regions showed that the β and α chains, respectively, contained the Vβ3 and a member of the Vα11 subfamily (Figs. 1 and 2). This Vα segment, referred to as Vα11.b, is identical to the Vα11.3 segment derived from B10.A mice (31), except for nine nucleotide substitutions that result in two amino acid replacements at positions -20 and 43 of the precursor polypeptide. Because these TCRs were derived from the B6 strain, it is not clear whether this Vα segment is allelic to Vα11.3 of B10 mice or whether it is a member of the Vα11 subfamily (39). The Vβ3 and Vα11.b fragments were used to screen Northern blots of nine B6 and eight bm-12 T-cell clones to identify other TCRs that also expressed these two V gene segments. From this analysis, one additional B6 (BO3H.25) and two bm-12 (bm2T3.1 and bm4T6.2) T-cell clones were identified that expressed transcripts hybridizing with both the Vβ3 and Vα11.b probes (see Fig. 2). By sequence analysis of rearranged genomic fragments, we also determined that BO4T27.6, bm1T7.6, and bm4T17.2 TCRs contained the Vβ6, Vβ2, and Vβ3 gene segments, respectively (Fig. 2 and data not shown). Interestingly, the bm4T17.2 TCR contained the Vα3 gene segment showing that the Vβ3-bearing β chains could alternatively pair with α chains bearing either Vα11.bm or Vα3 segments (Fig. 2). To facilitate identification of functionally significant residues, we characterized the subset of TCRs that shared identical Vβ3 and closely related Vα11 segments in detail.
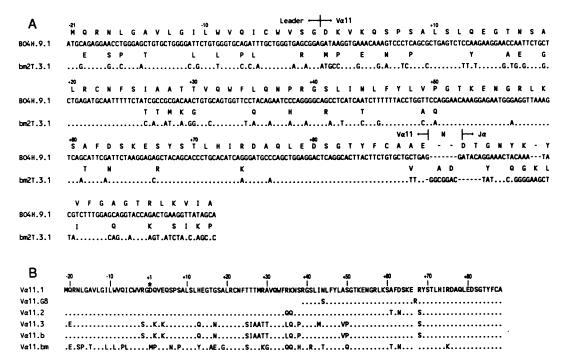
A

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                                        Leader ——|—→ Vα11
    -21                           -10              •1                        •10
    M Q R N L G A V L G I L W V Q I C W V S G D K V K Q S P S A L S L Q E G T N S A
BO4H.9.1  ATGCAGAGGAACCTGGGAGCTGTGCTGGGGATTCTGTGGGTGCAGATTTGCTGGGTGAGCGGAGATAAGGTGAAACAAAGTCCCTCAGCGCTGAGTCTCCAAGAAGGAACCAATTCTGCT

          E     S P     T         L     L     PL        R     M P     E     N     P         Y     A E     G
bm2T.3.1  ...G......G..C....A..........C.G....T.....C.C.A........A..A...ATGCC....G....G.A...TC....C........TT.T......G.TG.G....G.

          •20                      •30                   •40                           •50
          L R C N F S I A A T T V Q W F L Q N P R G S L I N L F Y L V P G T K E N G R L K
BO4H.9.1  CTGAGATGCAATTTTTCTATCGCCGCGACAACTGTGCAGTGGTTCCTACAGAATCCCAGGGGCAGCCTCATCAATCTTTTTTACCTGGTTCCAGGAACAAAGGAGAATGGGAGGTTAAAG

                  T T M K G               Q       H     R     T         A Q
bm2T.3.1  .................C.A..AT..A.GG...C........T.A...A....A...A.....A..T....C...G........C..A................A.........

          •60                 •70                   •80                        Vα11 —|   N   |— Jα
          S A F D S K E S Y S T L H I R D A Q L E D S G T Y F C A A E - - D T G N Y K - Y
BO4H.9.1  TCAGCATTCGATTCTAAGGAGAGCTACAGCACCCTGCACATCAGGGATGCCCAGCTGGAGGACTCAGGCACTTACTTCTGTGCTGCTGAG-------GATACAGGAAACTACAAA---TA

             T   N     R             K                                            V   A D     Y   Q G K L
bm2T.3.1  ...A.....A...........C..................A......A.................................TT..-GGCGGAC------TAT...C.GGGGAAGCT


          V F G A G T R L K V I A
BO4H.9.1  CGTCTTTGGAGCAGGTACCAGACTGAAGGTTATAGCA

             I     Q         K     S I K P
bm2T.3.1  TA........CAG..A....AGT.ATCTA.C.AGC.C
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B

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          -20        -10        •1        •10       •20       •30       •40       •50       •60       •70       •80
          |          |          *|        |         |         |         |         |         |         |         |
Vα11.1    MQRNLGAVLGILWVQICWVRGDQVEQSPSALSLHEGTGSALRCNFTTTMRAVQWFRKNSRGSLINLFYLASGTKENGRLKSAFDSKE RYSTLHIRDAQLEDSGTYFCA
Vα11.G8                                                                                 .....S.............R...........
Vα11.2    ......................................................QQ......................T.N... S....................
Vα11.3    .E.................S..K.K........Q...N.......SIAATT....LQ.P....M.....VP................ S....................
Vα11.b    ..................S..K.K........Q...N.......SIAATT....LQ.P...........VP................ S....................
Vα11.bm   .E.SP.T...L.L.PL.....MP...N.P....Y..AE.G.....S...KG....QQ.H..R..T.....Q............T.N... ........K...........
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FIG. 1. (*A*) Nucleotide and deduced amino acid sequences of coding regions from TCR α chain genes of BO4H9.1 and bm2T3.1 T cells. The boundary between the N region and Jα is based on germ-line sequences of Jα33 (28) and Jα18 (B. Koop, R. Wilson, and L.H., unpublished data) for BO4H9.1 and bm2T3.1 cell lines, respectively. (*B*) Members of the Vα11 subfamily. The predicted location of the cleavage site (*) of the leader peptide is based on the rules of von Heijne (29). In *A* and *B*, dots indicate sequence identity to the top sequence and dashes indicate lack of that residue in either sequence. Sequences of Vα11.1 and Vα11.2 (9, 10, 30), Vα11.3 (31), and Vα11.G8 (32) are from the indicated reference.

Sequence data of functional TCR α and β chain genes isolated from BO4H9.1, BO3H.25, bm2T3.1, bm4T6.2, and bm4T17.2 cells are shown in Figs. 1*A* and 2. Comparison of the nucleotide and deduced primary sequence of α chain cDNA clones isolated from bm2T3.1 and bm4T6.2 T cells showed that both TCRs utilized an identical Vα gene segment (Fig. 1*B*, sequence Vα11.bm). Each of the independently isolated Vα11.b and Vα11.bm members was identical in their nucleotide sequences, consistent with lack of somatic hypermutation among TCR genes. Comparison of the Vα11.bm sequence with other members of the Vα11 subfamily shows that this segment shares 70–80% similarity with either Vα11.1/.2 or Vα11.3/.b segments at either nucleotide or amino acid levels (Fig. 1*B*).

Comparison of nucleotide and predicted amino acid sequences encoding Vα and Vβ chains expressed by the B6 and bm-12 clones showed that these TCRs are closely related (Figs. 1*A* and 2). Despite the fact that each of these T cells was isolated from different mice, BO3H.25 and BO4H9.1 TCRs contained identical Vα11.b segments joined to Jα33, with only a single Ala → Gly substitution at the Vα–Jα

junction. Similarly, bm2T3.1 and bm4T6.2 TCRs used the same Vα11.bm and Jα18 segments with only three residue differences (Glu-Ala-Asp to Asp-Met-Asn) at the Vα–Jα junction. Each TCR contained the same Vβ3 segment with differences localized only to the Jβ and VDJβ junctions. Again, both B6- and bm-12-derived TCRs used either the Jβ1.2 or the Jβ2.6 gene segments. Furthermore, both bm-12 TCRs used identical β chain polypeptides despite nucleotide sequence differences at the V–J junction. All three Vβ3+ bm-12 TCRs used the same Jβ2.6 segment. The repeated usage of the same Jβ segments suggests that they serve an important function in determining the ligand specificity of these TCRs.

How do these αβ TCRs recognize the peptide–MHC complex? Among these two B6 and bm-12 sets of T cells, each specific for the same antigen p74–88 bound to either A^b or A^{bm-12} MHC molecules, at least five pairs of α and β chains were expressed. Distinct TCR α and β chain pairs that recognize the same peptide–MHC complex can be thought to bind the antigen–MHC ligand by three mechanisms. (*i*) Despite their apparent similarity, the antigen–MHC ligands

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          ---Vα--  -----N-----  ------Jα------    -----Vβ----  -----Dβ----  ----Jβ---

          A   E              D   T   G   N      S   S   L   M   G   N     S   D
BO4H9.1    GCT GAG         G AT ACA GGA AAC    AGC AGT CTG ATG GGG AA  C TCC GAC    Vα11.b-Jα33/Vβ3-Jβ1.2
          G   E              D   T   G   N      S   S   L   F   G   T     S   D
BO3H25     GGG GAG         G AT ACA GGA AAC    AGC AGT CTG TTC GGG AC  C TCC GAC    Vα11.b-Jα33/Vβ3-Jβ1.2
          V   E   A   D          Y   N          S   S       R   D   F     Y   E
bm2T3.1    GTT GA  G GCG GAC         TAT AAC    AGC AGT C    GG GAC TTT    TAT GAA    Vα11.bm-Jα18/Vβ3-Jβ2.6
          V   D              M   N   Y   N      S   S       R   D   F     Y   E
bm4T6.2    GTT GA  T       A TG AAT TAT AAC    AGC AGT C    GG GAC TT  C TAT GAA    Vα11.bm-Jα18/Vβ3-Jβ2.6
          V   S          A   H   G   N   E      S   S       R   G   S     S   Y
bm4T17.2   GTG AGC         GCG C AT GGA AAT GAG  AGC AGT C    GG GGG AG  C TCC TAT    Vα3-Jα40/Vβ3-Jβ2.6
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FIG. 2. B6 and bm-12 TCR α and β chain nucleotide and amino acid sequences at the V–J junctions. In the absence of available germ-line sequence for Vα11.b and Vα11.bm, the Vα–N boundary is shown with the last two codons of the Vα segment based on comparison to Vα11.1 and Vα11.2 (33). The 3′ boundary of Vβ3 segment is based on germ-line sequence (34). The β chain cDNA sequence for BO3H.25 is identical to the published genomic sequence (34). The N–J boundaries were determined by comparison to the germ-line sequences of Jα33 (28), Jα18 and Jα40 (B. Koop, R. Wilson, and L.H., unpublished data), Jβ1.2 (35), and Jβ2.6 (36). The N regions for the β chains are indicated under the Dβ heading with the Dβ1 (37) or Dβ2 (38) germ-line sequences underlined.

may be quite different. For example, the same p74–88 may bind to the antigen binding groove of the MHC molecules in more than one conformation and thus give rise to distinct ligand structures as hypothesized for the p52–61–A^k complex (27). (*ii*) Even if a single stable conformation of p74–88–MHC ligand exists, the TCRs may bind to distinct surfaces of the same ligand. In this context, it is interesting that different nonapeptides bound to the antigen binding site of human HLA-B27 class I MHC molecules appear to occupy a fixed orientation (40). Whether the generally larger peptides such as the 15-residue p74–88 described here and those recently eluted from class II MHC molecules (41) will also occupy similar fixed positions within the antigen binding groove of class II MHC molecules is not known. (*iii*) A single ligand may be recognized by the TCRs in a similar orientation, and the combining sites formed by the distinct α and β chains may in fact be functionally equivalent. The first two possibilities predict that differences in $\alpha\beta$ TCR structure are due to their different ligand specificities, whereas the third possibility predicts that, despite different primary sequences, functionally equivalent TCR combining sites may be generated. These three possibilities cannot be distinguished by sequence comparisons alone but are amenable to experimental test (42).

Interestingly, four TCRs from B6 (BO3H.25 and BO4H9.1) and bm-12 (bm2T3.1 and bm4T6.2) mice expressed the same Vβ3 segment with two members of the Vα11 subfamily. Furthermore, both pairs of B6 TCRs used the same Jβ1.2 and Jα33 segments and the bm-12 TCRs expressed the Jβ2.6 and Jα18 segments. The repeated occurrence of these segments in the TCRs is unlikely to be fortuitous because these TCRs were independently isolated. Also, these particular combinations of gene segments were not found among the random set of 178 B6 TCRs alloreactive to the A^{bm-12} MHC molecules (43). The differences in ligand specificity of these TCRs must, therefore, be accounted for by amino acid differences within Vβ–Jβ junctions and α polypeptides. Extensive analysis of V segment usage among cytochrome-specific TCRs has shown that expression of Vα11.1 or Vα11.2 segments with alternative Vβ segments correlates strongly with recognition of the cytochrome peptide by different alleles of the E class II MHC molecule (6, 9). However, the fact that our TCRs clearly distinguish the A^b vs. A^{bm-12} molecules and yet use identical Vβ3 segments shows that Vβ3 segments do not by themselves play a major role in determining peptide or MHC β chain specificity of the TCRs. Instead our results point to the importance of other regions of α and β chains in determining ligand specificity.

Current models of TCR–ligand interactions suggest that contacts between the TCR and the MHC molecule primarily occur within residues of Vα and Vβ segments, whereas residues within Jα, Jβ, and junction regions contact the peptide antigen (5, 13). Because the antigenic peptides recognized by all our TCRs are similar, application of these models to our TCRs predicts that differences within the Vα11.b and Vα11.bm segments would be associated with recognition of the MHC-specific differences. The Vα11.b and Vα11.bm segments of the mature polypeptides differ in 24 out of a total of 87 amino acids. Fourteen of these residues occur within the first 30 amino acids, and a contiguous cluster of 5 amino acid substitutions occurs at residues 26–30. Significantly, residues 28–34 and 31–35 of the antibody light and heavy chains, respectively, contain the first CDR region. Thus, it is attractive to postulate that this cluster defines one of the CDR regions of the Vα segments. However, the importance of this cluster and of other regions of the TCR in determining the peptide–MHC specificity must await results of mutagenesis experiments.

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