In a Small Multideterminant Peptide, Each Determinant Is Recognized by a Different Vβ Gene Segment

By Navreet K. Nanda, Karo K. Arzoo, and Eli E. Sercarz

From the Department of Microbiology and Molecular Genetics, University of California, Los Angeles, California 90024

Summary

Given the vast potential for diversification of the T cell receptor (TCR) repertoire and the fact that Vβ mice exist in the wild, it would have been predicted that in spite of the absence of 10 TCR Vβ gene segments, Vβ mice would still have been able to produce an antigen-specific T cell response to all determinants. We have recently shown that Vβ mice, with a wild-type TCR Vβ repertoire, respond to peptide 110-121 of sperm whale myoglobin, with a majority of T cells expressing TCR Vβ8.2 and restricted to a hybrid I-A4/I-E4 major histocompatibility complex molecule, and a smaller number of T cells expressing TCR Vβ8.1 and restricted to the I-A4d molecule. However, Vβ mice, lacking members of the TCR Vβ8 gene family, responded only with I-A4d-restricted T cells. Thus, it appeared that the I-A4d-restricted response was less constrained, or more plastic. We now show that the two separate panels of I-A4d-restricted T cell hybrids derived from Vβ or Vβ mice in fact recognize distinct determinants within the same peptide 110-121. The determinant recognized by Vβ T cells is NH2 terminal (core: 110-118) with an absolute requirement for the residue Ala-110 for a successful interaction with TCRs. On the other hand, Vβ T cells recognize the COOH-terminal region (core:112-118) on the same peptide with an absolute requirement for COOH-terminal residue 118. In the dominance hierarchy displayed by the three distinct determinants of peptide 110-121, Vβ mice cannot recognize the two most dominant: the hybrid I-Aα/I-Eα-restricted determinant and the COOH-terminal, I-Aα-restricted determinant. They instead respond with T cells specific for a third, distinctly NH2-terminal determinant. Our results show a strict association between recognition of a particular specificity and TCR Vβ usage. This evidence suggests that even when a small peptide induces a heterogenous group of TCR Vβs, this need not be considered evidence for plasticity. Rather, at the level of individual determinants within the peptide, the results can point in the opposite direction, towards serious constraints in recognition at the level of Vβ expression.

Mouse populations have recently been shown to have highly variable peripheral repertoires of TCR Vβ gene segments. Deletion mutations have caused the loss of half of the TCR Vβ gene segments from the germline repertoire of a number of wild and inbred strains of mice (1). These mice define a new genotype at the Vβ locus, the TCR Vβ-truncated (Vβ) genotype. Self-tolerance to self-super-antigens, recently defined to be mammary tumor virus (Mtv) integrants, has been shown to result in the loss of multiple Vβ gene segments from the peripheral repertoire of various strains of mice (2-4). Given the virtually unlimited potential available for repertoire diversification by recombination between V, D, and J gene segments, as well as the introduction of junctional residues, it would have been reasonable to predict that mice with different peripheral repertoires of TCR Vβ gene segments would be able to construct TCR structures reactive with all possible specificities (5, 6).

We have recently reported that this is not the case for certain antigen determinants, such as sperm whale myoglobin (SWM) 111-121, recognized in the context of a hybrid MHC molecule, I-A4d/I-E4, and myelin basic protein (MBP) 1-11 recognized in the context of I-Aa. These determinants can only be recognized by T cells expressing TCR Vβ8.2 in the former case and TCR Vβ8.2 or 13 in the latter (7). Thus, Vβ mice lacking both TCR Vβ8.2 and TCR Vβ13 cannot make a response to either of these determinants using alternative gene segments. We had expected that this would have been true only in those cases where T cell recognition demanded the usage of a particular specific TCR Vβ gene segment. In our study of the plasticity of the TCR repertoire, we described panels of T cell hybridomas specific for the peptide (p)110-121 of SWM from Vβ or Vβ (wild-type repertoire) haplotype mice (7). It was of interest that the Vβ mice responded to this peptide with: (a) hybrid I-A4d/I-E4 MHC-
Materials and Methods

Mice. Mice were obtained from The Jackson Laboratory (Bar Harbor, ME).

Antigens. SWM was obtained from Accurate Chemical & Scientific Corp. (Westbury, NY). SWM peptides were synthesized (M. McMillan and L. Williams; University of Southern California) using a peptide synthesizer (430 A; Applied Biosystems, Inc., Foster City, CA) and purified as reported previously (7). The series of truncated and substituted peptides were made by synthesis on pins followed by cleavage into 96-well plates (8).

Generation of T Cell Hybrids. T cell blasts from antigen-specific cell lines were fused with the α/β− variant of BW5147 (9) as a fusion partner, as described earlier (7), and cloned by limiting dilutions in the presence of SWM or its peptides: pl10-121 and p105-118. The antigen-reactive hybrids were analyzed for function and TCR Vβ expression (7).

T Cell Hybrids. T hybridoma cells (5 × 10⁴) were cultured with various concentrations of the peptide SWM 110-121 with 5 × 10⁵ irradiated BALB/c (I-A², I-E²) spleen cells as APC in 0.2 ml of supplemented DMEM (ICN Flow, Costa Mesa, CA) (7). The culture supernatants collected 24 h later were assayed for IL-2 activity on the IL-2/IL-4-dependent cell line, HT-2, as described earlier (7). p110-121 and p105-118 (synthesized by conventional methods) were used as positive control peptides in experiments using peptides synthesized with the pin method. In our previous extensive use of peptides synthesized on pins, we (13, and our unpublished observations) have not experienced cases of false negatives and positives, in comparison with conventionally synthesized peptides. Negative results with certain peptides and particular hybrids have been matched by positive results with other T cells.

restricted T cells expressing TCR Vβ8.2, as well as (b) a smaller number of I-A²-restricted T cells, expressing TCR Vβ8.1. The Vβ mice with their truncated repertoire, missing all members of the TCR Vβ gene family, responded with only I-A²-restricted T cells. The response to the I-A²-restricted specificity thus appeared to be plastic, as the Vβ mice lacking the Vβ8.1 gene actually succeeded in raising SWM 110-121-specific, I-A²-restricted T cells.

We have now compared the antigen-specificity of the two panels of pl10-121-specific and I-A²-restricted T hybridomas derived from either Vβ or Vβ mice using a panel of truncated or substituted peptides. To our surprise, we find that: (a) in fact, T cells from Vβ and Vβ mice recognize distinct I-A²-restricted determinants within p110-121. (b) The I-A²-restricted determinant recognized by T cells from the Vβ mouse is relatively NH₂-terminal with a core region of 110-118; the NH₂-terminal residue, Ala-110, is absolutely essential for recognition by these T cells. The Vβ-derived T cells recognize an overlapping, more COOH-terminal I-A²-determinant, with a core region of 112-118; the COOH-terminal Arg-118 is a crucial residue, required for triggering the TCR of this panel of T cells.

Our results show that T cell responses, when aligned with the distinct determinants that they recognize, are actually very narrowly constrained with respect to Vβ gene usage. Only a particular Vβ gene is compatible with TCR recognition of a given determinant.

Results

SWM 110-121 Can Induce T Cells Restricted to I-A² in Both Vβ and Vβ Haplotype Mice. T cells clones, derived from DBA/2 mice, and specific for SWM 110-121, had been described earlier (10), and all (13/13) were reported to be restricted to I-E², now known to be restricted to the hybrid I-A²/I-E² molecule (7, 11). We recently reported 22 T cell hybridomas, specific for SWM 110-121 from BALB/c mice (H-2², Vβ mice), and contrary to expectation, found six of them to be restricted to I-A² molecules (7), 16 hybrids, as expected, were restricted to the hybrid I-A²/I-E² molecule. An additional six cloned T cell hybrids obtained from recombinant inbred (C/Jx)3 mice, (H-2², Vβ mice, with a truncated TCR Vβ repertoire), also could respond in the context of I-A² molecules.

Table 1. Requirement of Residue Ala-110 by Vβ but Not by Vβ (SWM 110-121-specific, I-A²-restricted) T Cell Hybrids

| Peptide                        | Exp. 1 | Exp. 2 | Exp. 1 | Exp. 2 |
|-------------------------------|--------|--------|--------|--------|
|                               | cpm x 10⁻³ |        |        |        |
| No peptide                    | 6.3    | 1.3    | 15.6   | 6.0    |
| 109-121 (native: A110)         | 47.2   | 42.4   | 67.7   | 19.5   |
| 109-121, L110                  | 10.2   | 13.4   | 11.2   | 5.7    |
| 109-121, E110                  | 6.6    | 5.0    | 10.0   | 8.8    |

Vβ-derived hybrids

| CM8-16 | CM8-18 |
|--------|--------|
| Peptide | Exp. 1 | Exp. 2 | Exp. 1 | Exp. 2 |
| No peptide | 6.6 | 11.5 | 5.9 | 2.7 |
| 109-121 (native: A110) | 91.1 | 383.9 | 71.8 | 116.1 |
| 109-121, L110 | 84.0 | 309.9 | 72.5 | 162.4 |
| 109-121, E110 | 90.0 | 157.8 | 71.3 | 129.0 |

Data are from two representatives of four to five experiments, shown at the optimal dose of 7 µM of peptide. All experiments with the above variant peptides were done in single wells for each peptide, at a given dose. 3.5- and 14-µM doses of peptides gave results similar to 7 µM of peptide (data not shown). The stimulatory potential of p110-121 and p105-118 used as control peptides (synthesized by conventional methods) was the same as that of native p109-121 (synthesized on pins) (data not shown).
mice primed with SWM 110-121 did not show any recall response in vitro to p110-121, although they did respond to p110-121. Lymph node cells from Vβ mice primed with p110-121 in the same way, however, made an excellent response to p110-121 as well as p111-121. This differential response was the basis for the development of the idea that responses induced in the Vβ and Vγ mice were different, and resulted in our finding that Vβ mice could not be induced to make any hybrid I-A^d/I-Ed-restricted response.

Substitutions were made at residue Ala-110 by replacing it with conservative (Leu-110) or nonconservative (Glu-110) amino acids. p109-121 was chosen as it was highly stimulatory to all T cell hybridomas in both the panels. The results are shown in Table 1. Even a semi-conservative substitution at position 110 from Ala to Leu results in near abrogation of activity for Vβ I-A^d-restricted T hybrids. Nonconservative substitution at this residue to Glu leads to complete loss of stimulatory potential of this peptide for the Vβ hybrids. Both substituted peptides, however, are as stimulatory to the I-A^d-restricted, Vβ T cells as the native p109-121, indicating the importance of residue 110 in triggering the TCR of Vβ T cells, rather than in binding to the I-A^d MHC molecules.

**Differential Requirement for Residue Arg-118: Arg-118 Is Essential for Triggering Vβ-derived but Not Vβ-derived T Hybridomas.** Position Arg-118 of p109-121 was substituted either with conservative (Lys-118) or nonconservative (Ala-118 and Glu-118) amino acids. A fourth peptide with dual conservative substitutions, at positions 118 (Arg to Lys) and 112 (Ile to Leu), was also synthesized.

Surprisingly, even a conservative substitution of Arg to Lys at position 118 resulted in a complete loss of activity for all the Vβ hybridomas, although the altered peptide remains stimulatory for all Vβ hybrids (Table 2), and hence can be presented by I-A^d molecules. The small decrease in activity for some of the Vβ T cells with this substituted peptide appears to be nullified when using the double substituent (Lys-118 and Leu-112, both conservative replacements). The doubly substituted peptide, however, remains nonstimulatory for all Vβ T hybrids. A nonconservative substitution at position Arg-118 to Ala-118 has the same effect as the Lys-118 substitution, resulting in complete loss of activity for the Vβ hybrids, but with no effect on the Vγ hybrids. A reversal of charge, by using Glu at this position, however, is detrimental to both the Vβ and Vγ hybrids.

The above experiments show that nonresponsiveness of the I-A^d-restricted T cell hybrids derived from Vβ mice resulting from substitutions at Arg-118 (to Lys-118 or Ala-118) is exclusively due to the inability of these variant peptides to interact with the TCR, and not due to their binding to the I-A^d molecule, as the same substituents are able to stimulate all Vβ I-A^d-restricted T hybridomas.

Panels of SWM 110-121-specific, I-A^d-restricted T Cells, Derived from Vβ or Vγ Mice, Recognize Different Core Regions on p110-121. The “core” of a peptide determinant has been

### Table 2. Requirement of Residue Arg-118 by Vβ but Not by Vβ (SWM 110-121-specific, I-A^d-restricted) T Cell Hybrids

| Peptide            | CJM4-16 | CJM4-18 | CJM4-29-1 | CJM4-29-17 |
|--------------------|---------|---------|-----------|------------|
| No peptide         | 6.2     | 15.6    | 30.0      | 5.0        |
| 109-121 (native:R118) | 47.2    | 67.7    | 172.4     | 145.4      |
| 109-121, K118      | 36.6    | 32.5    | 84.7      | 93.0       |
| 109-121, A118      | 33.9    | 35.4    | –         | –          |
| 109-121, E118      | 13.1    | 17.6    | –         | –          |
| 109-121, K118, L112 | 56.2    | 58.7    | 120.3     | 173.2      |
| Vγ-derived hybrids |
| No peptide         | 8.7     | 5.9     | 9.6       | 1.5        |
| 109-121 (native:R118) | 91.1    | 71.8    | 241.0     | 352.7      |
| 109-121, K118      | 7.8     | 7.4     | 13.1      | 3.0        |
| 109-121, A118      | 6.3     | 7.0     | –         | 3.5        |
| 109-121, E118      | 6.7     | 6.8     | –         | 2.7        |
| 109-121, K118, L112 | 11.7    | 6.3     | 12.8      | 5.4        |

Data are from one representative of four experiments; see footnotes to Table 1.
defined as the minimal residues required for both: (a) binding to MHC molecules and (b) interacting with the TCR for a set of T cell clones using the same restricting molecule (12). The core regions recognized by the V\textsubscript{\gamma} and V\textsubscript{\delta} I-A\textsuperscript{\gamma}-restricted T cells were studied by using a series of 12-mer peptides, sequentially traversing the peptide from its NH\textsubscript{2} to its COOH terminus, and spanning residues 105-126 (data not shown for peptides spanning region 105-123). Table 3 shows the response to these peptides of four representative V\textsubscript{\gamma} and four representative V\textsubscript{\delta} T hybrids. The V\textsubscript{\gamma} hybrids responded optimally to peptides 106-117 to 110-121 (cores 110-117 to 110-118) (Table 3). All of the I-A\textsuperscript{\gamma}-restricted V\textsubscript{\delta} hybrids, however, were only well stimulated by the peptide series between 107-118 and 112-123 (data shown for four of six, and thus the core region recognized by T hybrids from V\textsubscript{\gamma} mice was 112-118 (Table 3). The peptides 113-124, 114-125, and 115-126 were tested in separate experiments and were non-stimulatory for all T cells in both panels (data not shown).

These experiments once again demonstrate that the region recognized by the two panels of T cells is overlapping but distinct. Residues Ala-110 and Ile-111 at the NH\textsubscript{2} terminus of the p110-121, although forming a part of the core region for V\textsubscript{\delta} T cells, are certainly not required in the core of V\textsubscript{\gamma} T hybrids. The core region recognized by the V\textsubscript{\gamma} T cells thus appeared to show a shift of two amino acids towards the NH\textsubscript{2} terminus. Conversely, the COOH-terminal residue, Arg-118, though an integral part of the core for V\textsubscript{\delta} T cells, was not required as part of the core for all V\textsubscript{\gamma} T hybrids.

Discussion

A number of recent reports have investigated a correlation between the antigen specificity of T cells and the structure of their TCR heterodimeric molecules: a striking restriction of TCR V\textsubscript{\gamma} gene usage by T cells recognizing a given antigen has been reported in several cases (10, 13-15). In a most recent example, murine T cells specific for a nonapeptide of Plasmodium berghei show expression of the TCR V\textsubscript{\gamma}13 gene segment in 60% of cells in spite of diversity in their V\textsubscript{\alpha} chains as well as the joining regions of both the \alpha and \beta chains (14). Even more surprising is the restricted usage of the TCR V\textsubscript{\beta} 2 gene segment reported in human T cells specific for a tetanus toxoid peptide, despite the utilization of a variety of MHC molecules as restriction elements (15). No correlation has yet been examined between the use of members of the TCR V\textsubscript{\gamma} gene repertoire and the expression of T cell function at the fine specificity level of unique determinants.

We now find that for each of the unique determinants that are recognized within the peptide SWM p110-121, an exclusive TCR V\textsubscript{\gamma} gene segment is required for its recognition. Thus, when p110-121, is used to immunize H-2\textsuperscript{d} mice, three distinct determinants are recognized in a hierarchical order: (a) the dominant, hybrid I-Ad/I-Ed-restricted determinant as described in a previous report, requiring TCR V\textsubscript{\gamma}8.2 and therefore only expressed in V\textsubscript{\gamma} mice; (b) a subdominant, I-A\textsuperscript{\gamma}-restricted COOH-terminal determinant (core: 112-118), requiring TCR V\textsubscript{\beta} 8.1 and only expressed in V\textsubscript{\delta} mice; and (c) an I-A\textsuperscript{\gamma}-restricted NH\textsubscript{2}-terminal determinant (core: 110-118) that is only expressed in V\textsubscript{\delta} mice.

These experiments once again demonstrate that the region recognized by the two panels of T cells is overlapping but distinct. Residues Ala-110 and Ile-111 at the NH\textsubscript{2} terminus of the p110-121, although forming a part of the core region for V\textsubscript{\delta} T cells, are certainly not required in the core of V\textsubscript{\gamma} T hybrids. The core region recognized by the V\textsubscript{\gamma} T cells thus appeared to show a shift of two amino acids towards the NH\textsubscript{2} terminus. Conversely, the COOH-terminal residue, Arg-118, though an integral part of the core for V\textsubscript{\delta} T cells, was not required as part of the core for all V\textsubscript{\gamma} T hybrids.

Table 3. Minimal Core Region Recognized by SWM 110-121-specific Hybrids

| NH\textsubscript{2}-terminal residue | Peptide sequence | V\textsubscript{\gamma} hybrids | V\textsubscript{\delta} hybrids |
|------------------------------------|-----------------|-----------------------------|-----------------------------|
|                                   |                 | CJM4-16* | CJM4-18* | CJM4-29-1* | CJM4-29-2* | CM8-16* | CM8-18* | CM8-30* | CM8-34* |
| 105                                | EFISEAIHVVLH    | 21.2    | 144.7   | 45.0    | 8.8    | 7.3    | 12.8   | 12.1   | 1.6    |
| 106                                | FISEAIHVHLHS    | 119.5   | 279.0   | 23.9    | 9.4    | 65.8   | 50.6   | 86.3   | ND     |
| 107                                | ISEAIHVHLHSR    | 156.5   | 212.0   | 339.6   | 168.4  | 187.0  | 178.0  | 287.0  | 290.7  |
| 108                                | SEAIHVHLHSRKH   | 247.5   | 222.5   | 350.0   | 225.4  | 157.5  | 186.5  | 257.5  | 426.6  |
| 109                                | EAIIHVHLHSRHP   | 181.0   | 206.0   | 245.7   | 131.2  | 174.0  | 150.0  | 204.0  | 511.8  |
| 110                                | IAIHVHLHSRPG    | 100.1   | 135.0   | 290.8   | 103.2  | 196.0  | 267.0  | 295.0  | 324.2  |
| 111                                | IIVLHSRHPGD     | 40.0    | 38.4    | 63.5    | 49.7   | 183.0  | 188.5  | 305.0  | 452.3  |
| 112                                | IIVLHSRHPGDF    | 12.0    | 44.5    | 79.1    | 16.3   | 185.0  | 195.0  | 213.0  | 70.9   |

Core regions: 110-116/117/118 112-118

Data are from one representative of four experiments; all experiments showed similar results (data not shown). The doses of the peptides used are as in Table 1. The p110-121 (synthesized by conventional methods) showed the same results as p110-121 shown in the Table (synthesized on pins) (data not shown).

* Data are expressed as mean of duplicate cultures.

† Data are from single-well cultures for each peptide.

Each Determinant on a Peptide Is Recognized by a Single V\textsubscript{\delta} Gene Segment
The two I-A^d-restricted determinants (b and c above) are further distinguishable from each other in that COOH-terminal Arg-118 is only essential for triggering the TCR molecules specific for the COOH-terminal determinant (V~), and NH2-terminal Ala-110 is only essential for TCR molecules in V~ hybrids. A conservative change in the COOH-terminal residue Arg-118 results in complete loss of activity in the V~ cells (Table 2), but has no effect on V~ T cells. Conversely, a conservative change in NH2-terminal Ala-110 abrogates its stimulation of V~ but not V~ T cells (Table 1).

Our results show that in the absence of TCR Va8.1, in V~ mice, alternative TCR V~ gene segments cannot be used to construct TCR molecules that can recognize the COOH-terminal, I-A^d-restricted specificity of p110-121. It is interesting that all SWM 110-121-specific T cell clones derived from V~ DBA/2 mice were reported to be restricted to the hybrid I-A^d/I-E^d MHC molecule (10), even though low I-A^d-restricted reactivity has been subsequently reported in primed lymph nodes of these mice (11). The DBA/2 strain does not express TCR Va8.1 in the periphery due to expression of the self-superantigen Mls-1^*, which induces deletion of Va8.1 cells in the thymus during T cell development. The absence of TCR Va8.1 apparently results in difficulty in raising I-A^d-restricted clones to the COOH-terminal determinant.

We believe that these results have more general implications for T cell recognition: (a) recognition of distinct determinants, even when they overlap on a single peptide, may require exclusive TCR Va gene usage for each of the individual specificities. Conversely, it can be predicted that certain peptides displaying a multiplicity of TCR V~ gene segments for recognition may in fact be comprised of distinct specificities within that peptide. (b) Without a particular V~ gene segment, the response to the precise specificity that it dictates should be absent, although the response to other specificities should remain intact and new specificities lower in the dominance hierarchy may appear. Such a strict association between recognition of a specificity and TCR Va usage is evidence for a very highly constrained T cell repertoire. (c) The nonplasticity of the TCR repertoire, as evidenced in the current and a previous (7) report, would have important implications for proposed models of TCR recognition of its ligand, the peptide-MHC complex. According to the Davis and Bjorkman model of TCR structure (6), the V regions of TCR x and b chains form the first and second hypervariable regions of the molecule, which only interact with non-polymorphic regions of the MHC molecule. The third hypervariable region, formed by the junctional region between V, D, and J gene segments of the two chains, is largely responsible for the nominal antigen specificity of the TCR molecules. This model, if strictly applied, would fail to explain our results that the presence or absence of particular V~ chains strongly influences the precise determinant recognized by TCR structures, as multiple (if not all) V chains should be able to interact with the MHC (I-A^d) molecules. The results presented here, along with those reported by Casanova et al. (14) and Boitel et al. (15), where T cells recognizing the same peptide show restricted V~ gene usage, even though the junctional CDR3 region is highly diverse, would argue for the idea that the V~ segment of the TCR molecule plays a significant role in establishing key contacts with the peptide-MHC complex as a whole. In fact, the results of Boitel et al. (15) describing restricted TCR V~ gene usage, even among T cells recognizing the same peptide and restricted to different class II molecules, as well as data presented in this report, suggest that the peptide acts as a selective force for choosing the V~ segment used by T cells.

Our results would have implications for responsiveness of an individual to an antigen as a whole. It is now well known that both MHC genes and non-MHC (self-superantigen) genes provide a potent influence (the former by both positive and negative selection, and the latter by negative selection) to regulate the expression of TCR V~ gene segments in the periphery (2-4, 17). If a response to a dominant peptide is restricted to one or two V~ gene segments, the lack of these segments might result in loss of the response to the whole antigen (7). However, if the determinant region were responded to in three ways, for example, and two genes were missing in the strain, a residual response might appear, which in fact would be a highly limited one. The crux of the argument presented here is that a varied response to a peptide comprised of several different TCR V~ and V~-containing TCR may in fact be quite restricted, when viewed from the perspective of particular determinants.

We thank Dr. Mario Geysen (Chiron Mimotopes, Clayton, Victoria, Australia), for providing pin peptides. This work has been supported in part by National Institutes of Health grants CA-24442 and AI-11183, and by grants from the National Multiple Sclerosis Society (PP0150 and RG-1755-B2).

Address correspondence to Eli E. Sercarz, Department of Microbiology and Molecular Genetics, 5304 Life Sciences Building, UCLA, Los Angeles, CA 90024.

Received for publication 6 March 1992 and in revised form 20 April 1992.
Each Determinant on a Peptide Is Recognized by a Single V\textsubscript{\beta} Gene Segment