THE MHC MOLECULE I-E IS NECESSARY BUT NOT SUFFICIENT FOR THE CLONAL DELETION OF Vβ11-BEARING T CELLS

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The MHC-restricted TCR consists of a 90-kD heterodimeric glycoprotein composed of an α and a β chain that are encoded by somatically rearranging gene segments (for review, see references 1–3). Receptor diversity and antigen/MHC specificity are generated by rearrangement of the known variable elements (variable [V], diversity [D], and joining [J] segments), as well as by the addition of N-region nucleotides. While the α/β receptor confers both antigen recognition and MHC restriction (4, 5), no general rules for the relative contribution of specific variable elements to antigen or MHC specificity have emerged. Early work demonstrated that the same Va and Vβ gene segments can encode a receptor with specificity for antigen plus a class II MHC gene product or with specificity for a class I alloantigen (6). The same Vβ domain has been shown to participate in a TCR specific for cytochrome c/I-E$k$ as well as in a TCR reactive to hen egg white lysozyme/I-A$k$ (7). On the other hand, surveys of sperm whale myoglobin-reactive T cells (8) and of cytochrome c-reactive T cells (9, 10) have been consistent with the idea that the Va gene segment encodes a TCR's antigen specificity while the Vβ domain confers its MHC specificity. However, studies of additional cytochrome c-specific T cells have shown that in some cases, the α chain influences the MHC specificity of a particular TCR (11). Thus, no simple correlation has emerged.

Even though it has been difficult to establish a precise relationship between a V domain and a pattern of MHC restriction, TCR V region domains have been shown to confer an affinity for MHC-encoded gene products. Blackman et al. (12) found that the random pairing of a BW5147-derived α chain and a β chain derived from a normal T cell generates an MHC-reactive (anti-D$k$) TCR. Kappler et al. (13) have shown that the participation of the Vβ17a domain in a TCR predisposes to I-E reactivity and that T cells bearing Vβ17a+ TCRs are eliminated in I-E$k$ strains of mice (14). Two other Vβ elements (Vβ8.1 and Vβ6) have been shown to confer broad specificity to the Mls-1a gene product (15–17). We report here the production of an
mAb, RR3-15, which recognizes TCRs encoded by the Vβ11 gene segment. Using this antibody, we find that, as with Vβ17a⁺ T cells, T cells that express Vβ11-encoded TCRs are eliminated in the thymus of I-E-expressing strains of mice. Surprisingly, it has been difficult to demonstrate reactivity of T cells expressing Vβ11-encoded antigen receptors for I-E molecules. Finally, experiments with B6 × DBA/2 recombinant inbred mice suggest that I-E expression is necessary but not sufficient for the clonal elimination of Vβ11⁺ T cells.

Materials and Methods

**Mice.** All mice were purchased from The Jackson Laboratories, Bar Harbor, ME, or bred in the facilities of the National Jewish Center for Immunology and Respiratory Medicine, Denver, CO. Fisher rats were purchased from The Jackson Laboratory.

**Cell Lines and Antibodies.** To generate the RR3-15 mAb, a Fisher rat received four bi-weekly immunizations, each consisting of 2 × 10⁷ OH6 cells, injected intraperitoneally. The cytotoxic T cell clone OH6 has been described previously (18). It was derived from C57BL/6 and is specific for H-Y in the context of H-2D⁺. 1 mo after the fourth immunization, 2 × 10⁷ OH6 cells were injected intravenously, and 3 d later, the rat was killed and its spleen cells were fused to P3X63.Ag8.653 cells using established procedures. The hybridomas were cultured in HAT-containing medium and supernatants from growing hybrids were assayed as described (18). Briefly, culture supernatants were tested for their ability to facilitate lysis of labeled P815 cells by the OH6 T cell clone. Lysis depends on the presence in the culture supernatant of an antibody that can bridge target and killer cells by binding to the Fc receptor on P815 target cells and to the TCR on cytotoxic OH6 cells. In this manner, the mAb RR3-15 was identified.

The OH2 cytotoxic T cell clone and the mAb MR5-2 have been described (18). OH2 is also specific for H-D⁺ plus the H-Y antigen. MR5-2 recognizes TCRs containing Vβ8.1 or 8.2, including the TCR of OH2, which contains Vβ8.1. The T cell hybrid KL was obtained by fusing OH6 cells to the C58 rat thymoma as described (19). T cell hybrids K12 and K13 were generated by fusing Con A-stimulated T cell blasts from B10.A(4R) to C58 as above. The resultant hybrids were panned using RR3-15-coated plates, and two hybrids positive for surface staining with RR3-15 were obtained and cloned by limiting dilution.

**Cytotoxicity Assays.** Cytolytic activity was measured by the ⁵¹Cr release assay (20). Briefly, 10⁴ ⁵¹Cr-labeled P815 cells (100 µl) were added to dilutions of CTL cells in V-bottomed microtiter plates (100 µl). After incubation at 37°C for 3.5 h, the plates were centrifuged and 100 µl of supernatant was collected for measurement of ⁵¹Cr release.

**Radioiodination and Immunoprecipitation.** Cell surface proteins were labeled by lactoperoxidase-catalyzed radioiodination, immunoprecipitated using RR3-15 or RR3-18 and Staphylococcus protein A, and analyzed on one-dimensional SDS polyacrylamide gels under reducing and nonreducing conditions as described (18).

**Hybridization Analyses.** Total cellular RNA was isolated by the method of Cheley and Anderson (21) or by cell lysis in guanidine isothiocyanate and ultracentrifugation over a cesium chloride cushion (22). 3 × 10⁶ cell equivalents or 0.5 µg of total cellular RNA were blotted onto nitrocellulose filters that were hybridized with ³²P-labeled probes for TCR variable region genes as described (23).

**Immunofluorescent Staining.** T cell hybrids, thymocytes, and nylon wool-nonadherent lymph node cells were analyzed for expression of RR3-15 using indirect immunofluorescent staining. The primary reagent was either culture supernatant or biotinylated RR3-15, and the secondary reagent was fluorescein-conjugated goat anti-rat Fc (Rockland, Inc., Gilbertsville, PA) or phycoerythrin conjugated with streptavidin (Becton Dickinson & Co., Mountain View, CA), respectively. Thymocytes were precultured for 8 h in complete tumor medium (CTM) at 5 × 10⁶/ml to increase the level of TCR expression on the immature, cortical cells (14). Thymocytes were preincubated with 5% normal mouse serum (BALB/c) to reduce background. The level of I-E⁺ expression was measured on unfractionated lymph node cells using a biotinylated mAb 14.4.4., followed by phycoerythrin-conjugated streptavidin as above. The
presence of CD4 on T cell hybridomas was determined using the mAb GKL5, followed by
 goat anti-rat Fc as above. Samples were analyzed on an Epics C flow cytometer (Coulter
 Electronics Inc., Hialeah, FL).

**IL-2 Assays.** T cell hybridomas were stimulated with cells or antibodies and the amount
 of IL-2 released was measured using HT-2 indicator cells, as previously described (24).

**Results**

*Generation of the RR-3-15 Antibody.* We were interested in generating an mAb to
 the antigen receptor on the cytolytic T cell clone OH-6. This clone is specific for
 the male antigen, H-Y, and is D<sup>b</sup> restricted. Fisher rats were immunized with OH6
 cells as described in Materials and Methods and spleen cells were fused with the
 B cell hybridoma fusion parent, P3X63Ag8.653. The supernatants from HAT-resistant
 B cell hybridomas were tested for their ability to mediate lysis of labeled P815 cells
 by cytolytic OH6 T cells, as described previously (18, 25, 26). The principle of this
 assay is that an anti-TCR mAb can bridge TCR-bearing OH6 cells with Fc
 receptor-bearing P815 cells and mediate lysis of the P815 cells, even though P815
 cells do not bear the nominal antigen recognized by the OH6 cell line. The sensitivity
 of the assay resides in the fact that anti-TCR and anti-CD3 mAbs are highly
 efficient at activating the cytolytic T cells to kill P815. Similar activation with anti-
 Thy-1 mAbs, for example, requires much higher antibody concentrations (data not
 shown).

Using this assay, we identified a B cell hybridoma producing the mAb RR3-15.
 This mAb reproducibly mediates the killing of P815 cells by the immunizing clone,
 OH6, and the specificity of this antibody is demonstrated by the data in Table 1.
 RR3-15 mediates the killing of P815 by OH6 but not by unrelated OH2 cells. On
 the other hand, the anti-V<sub>G</sub>8 mAb, MR5-2, mediates the killing of P815 by OH2
 but not by OH6 cells. Thus, the RR3-15 antibody is specific for a determinant present
 on OH6 but absent from OH2 cells. That this antibody recognizes a determinant
 on the OH6 TCR is shown in Fig. 1. Lysates of radiolabeled surface proteins from
 OH6 cells were subjected to precipitation with the RR3-15 mAb and Staphylococcus
 protein A. Precipitated proteins were analyzed by SDS-PAGE and autoradiography.
 The major precipitated protein under nonreducing conditions (without 2-ME) is

| Antibody  | E/T ratio |
|-----------|-----------|
| OH6       | 20 10 3 1 |
| MR5-2     | 2 2 3 2  |
| RR3-15    | 56 52 58 40 |
| OH2       | 4 4 2 1 |
| (V<sub>G</sub>8.1) | 47 37 36 28 |
| MR5-2     | 7 6 4 3 |
| RR3-15    |           |

**Table I**

*Cytolytic Activity of OH2 and OH6 on P815 Cells*

OH6 and OH2 are cytotoxic T cell clones specific for H-Y/H-2D<sup>b</sup>. MR5-2 is
an mAb specific for TCR variable regions V<sub>G</sub>8.1 and V<sub>G</sub>8.2. In the absence
of nominal antigen, lysis of P815 cells results from bridging by anti-TCR mAbs
of P815 cells via Fc receptors and T cell clones via their TCR.
a 90-kD species that appears as a 43-kD species under reducing conditions (with 2-ME). This is characteristic of the disulfide-linked heterodimeric, (α/β) antigen receptor on T cells. The data in Fig. 1 also show that a similar protein can be precipitated from OH-6 cells using an antyclonotypic antibody, RR3-18.

The Determinant Recognized by the RR3-15 mAb Is Encoded by the Vβ11 Gene Segment. Cytofluorographic analysis demonstrated that the determinant detected by the RR3-15 mAb is present on ~5% of C57BL/10 peripheral T cells but on an undetectable fraction of SWR and C57L peripheral T cell (Table II). Since SWR and C57L mice carry an allele of the β chain gene complex that has deleted the Vβ5, Vβ8, Vβ9, Vβ11, Vβ12, and Vβ13 structural genes (27, 28), we considered the possibility that the RR3-15 mAb recognizes a determinant encoded by one of these gene segments. We had available to us a large collection of random T cell hybridomas in which we had determined the expression of particular Vα and Vβ gene segments by hybridization analysis of hybridoma RNA (23); the data in the lower portion of Table III demonstrates that none of the 10 hybridomas expressing the Vβ5, Vβ8, Vβ9, Vβ12, or Vβ13 gene segments expressed the epitope recognized by the RR3-15 mAb. In contrast, 31 of 39 hybrids that were shown to express Vβ11 RNA express the RR3-15 determinant. The hybridomas that express the RR3-15 epitope coexpress a variety of different Vα gene families. Therefore, it seems likely that the RR3-15 determinant is encoded by the Vβ11 gene segment, and its expression is not dependent on the presence of particular Vα, Jα, Dβ, or Jβ gene segments. This is similar to other mAbs with reactivity for other vβ gene products (13, 16, 17, 26, 29, 30). The eight hybrids expressing Vβ11 RNA but not the RR3-15 determinant probably

Figure 1. RR3-15 precipitates TCR from the T cell clone OH6. SDS-PAGE analysis of surface-labeled proteins immunoprecipitated from the OH6 T cell clone. Lane 1, immunoprecipitation with RR3-15; lane 2, immunoprecipitation with RR3-18, an antyclonotypic mAb specific for the TCR of OH6. Gels were run under nonreducing conditions (-2-ME) or under reducing conditions (+2-ME).
TABLE II

**Expression of Vβ11+ T Cells in Various Mouse Strains**

| Strain                              | H-2 | Percent of peripheral T cells expressing Vβ11 |
|-------------------------------------|-----|-----------------------------------------------|
|                                     | A   |                  |                        |                          |
| B10                                 | b   | b                | b                       | 4.6 ± 0.1                |
| B10.M                               | f   | f                | f                       | 4.5 ± 0.1                |
| B10.Q                               | q   | q                | q                       | 6.3 ± 0.5                |
| B10.S (7R)                          | s   | s                | d                       | 3.5 ± 0.7                |
| B10.D2                              | d   | d                | d                       | 1.7 ± 0.8                |
| B10.BR                              | k   | k                | k                       | 0.7 ± 0.1                |
| B10.PL                              | u   | u                | u                       | 0.2 ± 0.1                |
| C57L                                | b   | b                | b                       | 0.04 ± 0.03              |
| SWR                                 | q   | q                | q                       | 0.1                      |
| DBA/2                               | d   | d                | d                       | 1.6 ± 0.1                |
| CBA/Ca                              | k   | k                | k                       | 0.5 ± 0.1                |
| CBA/J                               | k   | k                | k                       | 1.2 ± 0.1                |
| C3H/HeJ                             | k   | k                | k                       | 0.9 ± 0.1                |
| (B10 x B10.BR)F1                    | b/k | b/k              | b/k                     | 0.8 ± 0.1                |
| BDF1                                | b/d | b/d              | b/d                     | 1.3 ± 0.1                |
| B10.A (4R)                          | k   | k                | b                       | 5.0 ± 0.6                |
| B10.A (2R)                          | k   | k                | k                       | 0.5 ± 0.3                |
| B10.A (3R)                          | b   | b                | b/k                     | 1.57                     |
| B10.MBR                             | b   | k                | k                       | 0.78 ± 0.39              |
| B10.TL                              | s   | s/k              | k                       | 0.56                     |
| B10.HTT                             | s   | s                | s/k                     | 0.45                     |

Vβ11 expression is determined by immunofluorescent staining with RR3-15 as described in Materials and Methods. H-2 genotype for recombinant loci is indicated according to chromosomal position relative to the centromere of chromosome 17 (K, I-Aβ, I-Aα, I-Eβ, I-Eα, D). For most strains, at least three mice were examined and the mean value ± SEM is given. For strains where only one or two mice were examined, the individual values are given.

Transcribe rearranged Vβ11 genes that are out-of-frame. Alternatively, some Vβ11-encoded β chains may not pair with particular α chains to form surface heterodimers.

The expression of Vβ-gene segments was also determined in several T cell hybrids known to express the RR3-15 epitope. RNA was prepared from a T cell hybridoma (KL) obtained by fusing the immunogen, OH6, with the rat thymoma, C58, and from two other RR3-15+ T cell hybridomas (K12 and K13). It can be seen in Fig. 2 that all three RR3-15+ T cell hybrids transcribe the Vβ11 gene. These data, along with those from Table III, demonstrate that all 34 T cell hybridomas expressing a surface molecule detected by the RR3-15 mAb transcribe the Vβ11 gene. An example of an RR3-15+ T cell line that does not express Vβ11 RNA has not yet been found. Since we have not exhaustively examined Vβ11+, RR3-15- T cells, the possibility cannot be excluded that the Vβ11 domain does not express the RR3-15 determinant in the context of certain combinations of Vα, Jα, Dβ, or Jβ segments. Nevertheless, the simplest interpretation of these data is that the RR3-15 determinant is present on the Vβ11 gene product and that all Vβ11-encoded TCRs express this epitope.
| Hybridoma | VB | Va | RR 3-15 |
|-----------|----|----|---------|
| 1 HB 27   | 11 | 3  | -       |
| 1 HB 36   | 11 | 3 and 11 | +   |
| 1 HB 88   | 11 | 3  | +       |
| 1 HB 133  | 11 | 3  | -       |
| 1 HB 188  | 11 | -  | +       |
| 1 HB 184  | 11 | 5  | -       |
| 2 HB 49   | 11 | 3 and 8 | +   |
| 3 HB 68   | 11 | -  | +       |
| 3 HB 120  | 11 | 3  | +       |
| 3 HB 123  | 11 | 4 and 13 | +   |
| 4 HB 32   | 11 | 13 | +       |
| 4 HB 40   | 11 | 3  | +       |
| 4 HB 42   | 11 | 8  | -       |
| 4 HB 56   | 11 | 4  | +       |
| 4 HB 81   | 11 | -  | -       |
| 4 HB 99   | 11 | 10 and 11 | +   |
| 1 Q 11    | 11 | -  | +       |
| 1 Q 53    | 11 | 3  | +       |
| 1 Q 59    | 11 | 8  | -       |
| 1 Q 62    | 11 | -  | +       |
| 2 Q 34    | 11 | 2 and 4 | -   |
| 2 Q 36    | 11 | -  | +       |
| 3 Q 69    | 11 | -  | +       |
| 3 Q 96    | 11 | -  | +       |
| 4 Q 41    | 11 | -  | +       |
| 5 Q 4     | 11 | 2 and 3 | +   |
| 5 Q 5     | 11 | 2  | +       |
| 5 Q 12    | 11 | 13 | +       |
| 5 Q 65    | 11 | 4  | +       |
| 5 Q 68    | 11 | 4  | +       |
| 5 Q 78    | 11 | 3 and 8 | -   |
| 5 Q 79    | 11 | -  | +       |
| 5 Q 125   | 11 | -  | +       |
| 1 BR 92   | 11 | 8  | +       |
| 1 BR 168  | 11 | 5 and 13 | -   |
| 2 BR 118  | 11 | 3 and 5 | +   |
| 4 BR 10   | 11 | 3  | +       |
| 4 BR 25   | 11 | 3 and 13 | +   |
| 4 BR 37   | 11 | 10 | +       |
| 2HB51.8   | 5  | 10 | -       |
| 4HB48.8   | 5  | 3 and 11 | -   |
| 4Q53.5    | 5  | 10 | -       |
| 1Q67.7    | 5  | 8  | -       |
| 18BBM4    | 8  | -  | -       |
| 17BBM32   | 8  | 3 and 8 | -   |
| 1HB111    | 9  | 3  | -       |
| 16BBM33   | 12 | 8  | -       |
| 5Q26      | 13 | 3  | -       |
| 2BR51     | 13 | 2  | -       |

TCR V region RNA expression was determined by hybridization with \(^{32}P\)-labeled V-specific probes to VB2-17 and Va2-8 and Va10,11, and 13 as described (23). HB, Q, and BR hybrids were derived from Con A-stimulated peripheral T cells from B10, B10.Q, and B10.BR mice, respectively. BBM hybrids were derived from B10 mice and are I-Abm12 reactive. Hybrids were stained with labeled RR3-15 antibody and analyzed in an Epics C flow cytometer as described in Materials and Methods. For Va expression, (-) indicates that the hybridoma expresses RNA from the Va1 family or from a Va family for which we have no probe.
The Frequency of \( V\beta 11^+ \) T Cells in Mice Is Dependent on the MHC Haplotype. To examine the effect of particular MHC haplotypes on the frequency of \( V\beta 11^+ \) T cells, we subjected lymph node T cells from a panel of mouse strains to cytofluorographic analysis using the RR3-15 mAb. The data in Table II show that B6 (H-2\(^b\)), B10 (H-2\(^k\)), B10.M (H-2\(^a\)), B10.Q (H-2\(^q\)), and B10.S(7R) (H-2\(^{cd\})\) mice express the largest fraction of \( V\beta 11^+ \) T cells (3.5-6.3\%). On the other hand, the fraction of \( V\beta 11^+ \) T cells in B10.D2 (H-2\(^d\)), B10.BR (H-2\(^o\)), and B10.PL (H-2\(^o\)) is significantly lower (0.2-1.7\%). Other H-2\(^d\) and H-2\(^k\) strains (DBA/2, CBA/Ca, CBA/J, and C3H/HeJ) have a relatively low frequency of \( V\beta 11^+ \) bearing T cells (0.5-1.6\%). The number of \( V\beta 11^+ \) T cells in C57L (H-2\(^b\)) and SWR (H-2\(^q\)) mice is negligible (0.04-0.1\%). Given that the \( V\beta 11 \) gene is deleted from the genome of these two strains, these values probably represent the true background in these cytofluorographic experiments.

Crossing a strain that carries a high frequency of \( V\beta 11^+ \) bearing T cells with a strain that carries a low frequency of \( V\beta 11^+ \) bearing T cells produces F\(_1\) mice that express a low frequency of \( V\beta 11^+ \) T cells (see Table II). The frequency of T cells with \( V\beta 11^+ \)-encoded TCRs in [B10 \(\times\) B10.BR]\(_F_1\) mice (0.8\%) is similar to that seen in B10.BR mice (0.7\%). Similarly, the frequency of \( V\beta 11^+ \) T cells in [B6 \(\times\) DBA/2]\(_F_1\) mice (1.3\%) is similar to that seen in DBA/2 mice (1.6\%). Thus, the phenotype of infrequent expression of \( V\beta 11^+ \) T cells is dominant, relative to the phenotype of frequent expression of \( V\beta 11^+ \) T cells in F\(_1\) mice. Considering that the frequency of T cells with \( V\beta 11^+ \)-encoded antigen receptors is low in [B10 \(\times\) B10.BR]\(_F_1\) mice, it is likely that a protein encoded by the MHC of B10.BR (H-2\(^o\)) mice is depressing the level of \( V\beta 11^+ \) T cells. To examine this point further, we compared the expression of \( V\beta 11^+ \) bearing T cells in MHC recombinant mice, and the most relevant MHC-recombinant strains are B10.A(4R) and B10.A(2R). The frequency of \( V\beta 11^+ \) T cells is high (5.0\%) in B10.A(4R) but low (0.5\%) in B10.A(2R) mice, yet the only genetic difference between these two strains is that B10.A(2R) mice express an I-E protein while B10.A(4R) mice do not. Thus, it seems likely that the majority of T cells expressing the \( V\beta 11 \) domain as part of their surface antigen receptor are clonally deleted in I-E-bearing mice. Consistent with this idea is the fact that the frequency of \( V\beta 11^+ \) T cells is generally high in mice that carry I-E\(^-\) haplotypes (H-2\(b\), H-2\(s\), H-2\(f\), H-2\(7R\)), and that the frequency of T cells with I-E\(^-\) haplotypes is generally low in mice that carry I-E\(^+\) haplotypes (H-2\(2d\), H-2\(k\), H-2\(u\)).

**Mature \( V\beta 11^+ \) Thymocytes Are Absent from I-E-bearing Mice.** We examined the \( V\beta 11^+ \) bearing thymocytes in young adult B10.A(4R) (I-E\(^-\)), B10.A(2R) (I-E\(^+\)), and C57L (\( V\beta 11 \) gene-deleted) mice. As shown in Fig. 3, \( V\beta 11 \) thymocytes expressing low levels of TCR (RR3-15 dull cells) are present in B10.A(4R) (I-E\(^-\)) and in B10.A(2R) (I-E\(^+\)).
mice; however, Vβ11+ thymocytes expressing high levels of TCR (RR3-15 bright cells) are present only in B10.A(4R) (I-E\(^-\)) mice. As expected, both RR3-15 dull and RR3-15 bright cells are absent from C57L (Vβ11 gene-deleted) mice. As a control, thymocytes from C57L mice were stained with an mAb, RR4-7 (17), specific for the V\(\beta 6\) gene product. Clearly, V\(\beta 6^+\) thymocytes (TCR dull and bright) are present in this strain. Thymocytes with low levels of surface TCRs are characteristically double positive (CD4\(^+\) and CD8\(^+\)), cortical, and functionally immature, while thymocytes with high levels of surface TCRs are characteristically single positive (CD4\(^+\) or CD8\(^+\)), medullary, and functionally mature (31). Our findings suggest that the presence of I-E prevents the appearance of the majority of Vβ11+ mature thymocytes and Vβ11+ peripheral T cells.

**Most Vβ11+ Hybridomas Are not Reactive with I-E-bearing Splenocytes.** Since I-E mediates the clonal deletion of Vβ11+ T cells, we have studied the reactivities of 25 T cell hybridomas that express Vβ11-encoded surface receptors. These hybrids were stimulated with I-E\(^a\)-bearing splenocytes or CH12 (I-E\(^a\)) lymphoma cells; surprisingly, we found only three (12%) that could be stimulated to produce IL-2. That all 25 hybrids were capable of IL-2 production was demonstrated by their response to stimulation with the anti-Vβ11 antibody, RR3-15 (data not shown). Since it is possible that the I-E reactivity of Vβ11+ hybridomas is dependent on the presence of surface CD4 molecules, we have studied a set of six Vβ11+ hybrids that express CD4 surface molecules (see Table IV). All six hybridomas secrete IL-2 when stimulated with a crosslinked form of the anti-TCR antibody, RR3-15, yet only one hybrid (SQ5) can be strongly stimulated with I-E-bearing splenocytes from B10.BR (I-E\(^a\))

**Figure 3.** Absence of Vβ11 expression among mature but not immature thymocytes in I-E+ mice. Fluorescence histograms of thymocytes from B10.A(2R) and B10.A(4R) or, as a negative control, C57L adult mice. RR3-15 and RR4-7 were biotinylated and phycoerythrin-conjugated streptavidin was used as a secondary staining reagent. Indicated percentages of Vβ11+ T cells were obtained by curve subtraction. Dashed lines indicate staining by secondary reagent alone. Thymocytes were cultured for 8 h before staining to increase the level of TCR on immature cells as described (14).
or B10.D2 (I-E\(^d\)) mice. Given the clonal deletion of \(\sim90\%\) of V\(\beta\)I\(^1\) T cells from I-E\(^d\)-bearing mice (see Table II and Fig. 3), the rarity of V\(\beta\)I\(^1\) hybridomas reactive with I-E\(^d\)-bearing splenocytes seems paradoxical.

**The MHC Molecule I-E Is Necessary but not Sufficient to Mediate the Clonal Deletion of V\(\beta\)I\(^1\)-bearing T Cells.** To confirm that the regulation of the number of V\(\beta\)I\(^1\) T cells is mediated by MHC-encoded proteins, the frequency of V\(\beta\)I\(^1\) cells was determined in 23 C57BL/6 \(\times\) DBA/2 (BXD) recombinant inbred (RI)\(^1\) strains. All loci in these strains are either B6 or DBA/2 in origin; consequently, the MHC in each of these strains is either H-2\(^b\) (B6-derived) or H-2\(^d\) (DBA/2 derived). The data in Fig. 4 show the frequency of V\(\beta\)I\(^1\) T cells in these RI strains. The level of V\(\beta\)I\(^1\)-bearing T cells in each strain is quite reproducible in that the SEM of these measurements is small relative to the total fraction of V\(\beta\)I\(^1\) cells. Thus, the frequency of V\(\beta\)I\(^1\) T cells represents a reproducible phenotype in each of these strains. Considering the H-2\(^b\) (I-\(E^d\)) strains, the frequency of V\(\beta\)I\(^1\)-bearing cells is high (\(\sim5.5\%\)), consistent with the absence of clonal deletion in I-\(E^d\) mice. On the other

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**Table IV**

| Hybridoma | IL-2 released when stimulated with: | Surface expression |
|-----------|-----------------------------------|------------------|
|           | RR3-15 (anti-V\(\beta\)I) | B10.BR(H-2\(^b\)) | B10.D2(H-2\(^d\)) | V\(\beta\)I | CD4 |
| 1HB36     | 640 <10 <10 + + |
| 1HB184    | 160 <10 <10 + + |
| 3HB123    | 320 <10 10 + + |
| 4HB56     | 320 <10 10 + + |
| 5Q4       | 80 10 10 + + |
| 5Q5       | 640 160 20 - + |

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\(^1\) Abbreviations used in this paper: RI, recombinant inbred.

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**Figure 4.** Frequency of V\(\beta\)I\(^1\)-bearing T cells in 23 strains of C57BL/6 \(\times\) DBA/2 RI mice. The frequency of V\(\beta\)I\(^1\) T cells was determined as described in the legend to Fig. 3. For most of the strains, the values in the figure represent the average of at least three animals. The SEM is indicated by the error bars. For strains 27, 28, and 29, only two animals were analyzed, and consequently, the SEM could not be calculated. The frequency of V\(\beta\)I\(^1\) T cells was 3.2\%, 3.7\% for strain 27; 3.6\%, 3.8\% for strain 28; and 5.4\%, 5.9\% for strain 29. C57BL/6, DBA/2, and (C57BL/6 \(\times\) DBA/2)\(F_1\) mice carry 5.6 \(\pm\) 0.2\%, 1.6 \(\pm\) 0.1\%, and 1.3 \(\pm\) 0.1\% V\(\beta\)I\(^1\) T cells, respectively.
hand, the frequency of Vβ11+ T cells varies considerably among the H-2d (I-E+) strains. Some I-E+ strains (Nos. 6, 9, and 16) carry a small fraction (~0.8%) of Vβ11+ T cells, while other I-E+ strains (Nos. 25, 27, and 28) carry a much larger fraction (~3.5%) of Vβ11+ T cells. To rule out the possibility that these differences are due to quantitative differences in the degree of I-E expression between these strains, the amount of the I-E gene product was measured on lymph node B cells from several RI strains (Nos. 6, 9, 11, 18, 21, 24, and 25) that carry different levels of Vβ11+ T cells (data not shown). The amount of I-E expressed on the surface of B cells in several of these RI strains is virtually identical. Thus, the variation in the frequency of Vβ11+ T cells among I-E+, BXD RI strains cannot be explained by quantitative differences in the expression of the I-E gene product.

**Discussion**

We have identified an mAb that recognizes a determinant on all or nearly all T cells expressing the Vβ11 domain as part of the TCR. Based on the following criteria, the antibody, RR3-15, recognizes an epitope encoded by the Vβ11 gene segment. RR3-15 precipitates a 90-kD heterodimer, characteristic of the TCR, from the immunizing T cell clone OH6. RR3-15 mediates the lysis of P815 cells by OH6 (Vβ11+) but not by another (Vβ8.1+) T cell clone, OH2. Of 34 T cell hybridomas that express the RR3-15 determinant all transcribe the Vβ11 gene segment (Table III and Fig. 2). RR3-15+ T cells are undetectable in SWR and C57L mice (see Table II); these strains carry an allele of the β chain gene complex that has deleted the Vβ11 structural gene (27, 28). Of 10 T cell hybrids expressing Vβ5, Vβ8, Vβ9, Vβ12, or Vβ13 RNA, none express a surface molecule recognized by the RR3-15 mAb. This is notable since SWR and C57L mice are deleted for these Vβ genes as well. Taken together, these data strongly suggest that the TCR determinant recognized by the mAb, RR3-15, is part of the Vβ11 domain. It is expected that an epitope encoded by a single Vβ gene would be present on ~5% of T cells as there are 21 functional Vβ gene segments in mice. Since ~5% of T cells from B10, B10.Q, and B10.M mice are RR3-15+, it seems likely that expression of Vβ11 gene is both necessary and sufficient for the expression this epitope. However, we cannot rule out the possibility that some combinations of Vα, Jα, Dβ, and Jβ gene segments do not permit the expression of the RR3-15 determinant on a few Vβ11-encoded TCRs.

The most striking aspect of our findings is that a majority of T cells with Vβ11-encoded antigen-receptors are clonally deleted from I-E-bearing mice. In general, the frequency of Vβ11+ T cells is much lower in I-E-bearing mice than it is in strains that do not express a functional I-E protein (see Table II). The most dramatic example of this effect can be seen when comparing the frequency of Vβ11+ T cells in B10.A(4R) and B10.A(2R) mice. These two strains share the same genetic background (B10) and the same H-2K, H-21-A, and H-2D genes; however, they differ in their expression of the I-E gene product (32). B10.A(4R) mice are genetically defective in their expression of an I-E gene product and 5% of their T cells express a Vβ11-encoded TCR. On the other hand, B10.A(2R) mice express the I-E gene product but only 0.5% of their T cells express a Vβ11-encoded TCR (see Table II). Thus, the frequency of Vβ11+ T cells is inversely correlated with the presence of an I-E molecule. The effect of I-E is genetically dominant in that F1 mice derived from I-E-expressing and I-E-nonexpressing parents express I-E and clonally delete the
majority of their Vβ11+ T cells (see Table II). A comparison of the frequency of Vβ11+ T cells in B10.D2(I-Ek), B10.BR(I-Eb), B10.PL(I-E0), B10.A(3R)(I-Eb), and B10.HTT(I-E') mice indicates that most if not all alleles of the I-E locus are capable (to varying degrees) of deleting Vβ11+ T cells from the repertoire. The elimination of Vβ11+ T cells encompasses both the CD4+ and CD8+ subsets, though the elimination of CD4+ cells is more dramatic (data not shown). Two closely related but I-E-disparate strains, B10.A(2R) (I-E+) and B10.A(4R) (I-E-), were examined for the expression of Vβ11 on both mature and immature thymocytes (Fig. 3). Both strains contained immature thymocytes expressing the Vβ11 domain; however, in B10.A(4R) (I-E-) but not in B10.A(2R) (I-E+) mice, there were thymocytes expressing high density Vβ11+ TCRs. Thus, the clonal deletion of Vβ11+ T cells extends to mature thymocytes in I-E+ mice.

These findings are remarkably similar to those reported to Kappler et al. (13, 14). They have determined that T cells bearing antigen receptors encoded by the Vβ17a gene segment are clonally deleted from I-E-bearing mice. They have further demonstrated that mature (high density TCR) Vβ17a+ thymocytes are absent from strains expressing I-E and that all I-E alleles examined mediate the clonal elimination of Vβ17a+ T cells (14). These properties apply to Vβ11+ T cells as well. Given that all allelic forms of the I-E protein mediate the clonal deletion of both Vβ11+ and Vβ17a+ T cells, it seems likely that these cells recognize an epitope that is common (public determinant) to all polymorphic forms of I-E. However, allelic differences between I-E molecules may determine the extent of clonal deletion. For example, I-Ek deletes a larger fraction of Vβ11+ and Vβ17a+ T cells than does I-Ed (Table II and reference 33).

One striking difference between Vβ11 and Vβ17a-encoded TCRs is that a large fraction (~90%) of Vβ17a+ hybridomas are strongly reactive to I-E when stimulated with I-E-bearing splenocytes or lymphomas (13). We have examined 25 Vβ11+ hybrids for their reactivity to I-E when stimulated with B10.BR (H-2b) spleen cells or CH12(H-2b) lymphoma cells and found only three potentially I-E-responsive hybridomas (data not shown). Since all 25 hybrids respond when their antigen receptors are crosslinked with the anti-Vβ11 reagent, RR3-15, these hybrids have Vβ11+ surface TCRs and are capable of secreting IL-2. Furthermore, we have examined six of these hybrids that express CD4 surface molecules and found only one to be potentially I-E reactive (see Table IV). Thus, it is difficult to argue that the absence of CD4 accessory molecules accounts for the lack of pronounced I-E recognition in most of these Vβ11+ hybrids. The explanation for the lack of I-E recognition by hybrids bearing Vβ11-encoded TCRs may be trivial. It is conceivable that the affinity of a Vβ11+ TCR for the I-E ligand is low (accounting for the lack of I-E reactivity among Vβ11+ hybrids) but that the in vivo clonal deletion of Vβ11+ T cells requires only a weak interaction between the TCR and I-E. A second, perhaps more intriguing, hypothesis to explain this discrepancy is that splenocytes, although I-E bearing, do not present the actual ligand for Vβ11-encoded TCRs and are not the tolerogenic cells in the mouse. This could be the case, if the actual ligand for Vβ11+ T cells is an I-E molecule plus a self-peptide. This protein fragment may not be produced by splenocytes but is expressed by other I-E-bearing cells that mediate the clonal deletion. This idea has been proposed recently (34) and experiments are underway to resolve this issue.
The idea that the tolerizing ligand(s) for Vβ11+ cells is an I-E/self-peptide complex is further supported by experiments examining the frequency of Vβ11+ cells in C57BL/6 × DBA/2 (B × D) RI mice (see Fig. 4). All RI strains that carry the H-2b (I-E+)' haplotype have a full complement of Vβ11+ T cells, demonstrating the lack of clonal deletion in the absence of I-E expression. On the other hand, the degree of clonal deletion of Vβ11-bearing T cells is not equivalent in all H-2d (I-E+), B × D RI strains even though the magnitude of I-E expression among these H-2d strains is similar. Some RI strains (Nos. 6, 9, and 16) show a strong deletion of Vβ11+ T cells, while other RI strains (Nos. 18, 25, 27, and 28) display a much weaker elimination of Vβ11-bearing T cells (see Fig. 4). Other RI strains display intermediate levels of clonal deletion. Taken together, these results demonstrate that expression of the class II molecule, I-E, is necessary but not sufficient to mediate the clonal deletion of Vβ11+ T cells. It is likely that some genes in the B6 genetic background and other genes in the DBA/2 genetic background complement the I-E genes to mediate the clonal deletion of Vβ11+ T cells. Furthermore, it is the segregation of these genes that most likely accounts for the varied extent of clonal deletion among these B × D RI strains. These background genes possibly encode self-proteins that generate peptides with I-E-binding properties. The implication of these findings is that the products of genes other than I-E control the extent of clonal deletion.

Given the plethora of Vβ domains that impart reactivity to I-E, Mls-1 or Mls-2 gene products (13, 15, 16, 17, 30), we have wondered whether Vβ or Vα domains exist that confer a generic reactivity to H-2 K, I-A, or D glycoproteins. In retrospect, it has been relatively easy to identify TCRs that are clonally deleted in the presence of I-E proteins because of the existence of I-E-expressing and nonexpressing strains of mice. Since no strains have been described that are nonexpressors of the H-2 K, I-A, or D gene products, it is experimentally difficult to identify these kinds of TCR domains. Nevertheless, it seems unlikely that V region domains exist that are pan-reactive with H-2 K, I-A, or D gene products because of enormous pressure against maintaining such V genes in the germline. A V domain that imparts reactivity for a gene product expressed in every member of a species and somatically eliminated from the repertoire would offer no protective value and be of no advantage to the organism nor to the species. In this context, it is conceivable that the Vβ11, Vβ17, Vβ8.1, Vβ6, and Vβ3 TCR domains have evolved to have a high affinity for their ligands (I-E, Mls-1, Mls-2), because these ligands are not ubiquitously expressed in all mice.

The selective pressure to maintain V genes encoding self-reactive domains should be weak or nonexistent and the sequence of these genes might drift resulting in the appearance of pseudogenes. The Vβ17b gene provides an illustration of this point. This Vβ17 allele carried by most strains of inbred mice is nonfunctional due to the presence of a termination signal at codon 88 (35). Since Vβ17a-encoded receptors are presumably eliminated from a large proportion of outbred mice, the selective pressure to maintain this Vβ gene is weak. Thus, it is not surprising that one of the Vβ17 alleles has drifted and become a pseudogene.

We have previously observed a decrease in the frequency of Vβ11+ T cells from B10.BR (I-E+) mice. By analyzing the RNA from large panels of random T cell hybridomas for the expression of 16 Vβ and 10 Vα gene families (23), we determined the frequency with which these V gene families are expressed among T cells from
three MHC congenic strains. From this analysis, we noticed a statistically significant decrease in the frequency of \( V_{\beta 11} \)-expressing hybrids in B10.BR (I-E\(^{+}\)) compared with B10 (I-E\(^{-}\)) and B10.Q(I-E\(^{-}\)) mice. The cytofouographic experiments described here with the anti-\( V_{\beta 11} \) reagent, RR3-15, have independently confirmed our previous observations.

Our analysis of V gene expression among MHC congenic strains (23) also reveals a low frequency of T cells expressing the \( V_{\beta 5.1}, V_{\beta 5.2}, \) and \( V_{\beta 12} \) gene segments in B10.BR (I-E\(^{+}\)) mice relative to B10 (I-E\(^{-}\)) and B10.Q(I-E\(^{-}\)) mice. It is at least conceivable that T cells bearing these \( V_{\beta} \) domains are clonally deleted from I-E\(^{+}\) mice as well. Thus, an I-E-bearing mouse may lose up to 20\% (4 of 20 \( V_{\beta} \) domains) of its TCR repertoire due to the somatic deletion of I-E-reactive T cells. While this is costly, in terms of the size of the repertoire, a mouse that expresses I-E has another MHC protein that can present antigens and can positively select (I-E-restricted) T cells. On balance, I-E\(^{+}\) and I-E\(^{-}\) haplotypes may generate equally protective cell-mediated immunity.

Summary

We have generated an mAb, RR3-15, that recognizes murine TCRs containing the \( V_{\beta 11} \) domain. Using this antibody to stain peripheral T cells, we have demonstrated that \( V_{\beta 11} \)-bearing T cells are largely absent from strains of mice that express the class II MHC molecule, I-E. Studies with F\(_1\) mice demonstrate that this effect is dominant, consistent with tolerance. The clonal deletion of \( V_{\beta 11} \)-bearing T cells appears to occur intrathymically, as immature but not mature \( V_{\beta 11}^{+} \) T cells are present in the thymus of I-E-bearing mice. Examination of B6 × DBA/2 recombinant inbred strains demonstrates that the expression of I-E molecules is necessary for the clonal deletion of \( V_{\beta 11} \)-bearing T cells, but that other non-MHC genes control the clonal deletion process, as well. Paradoxically, only a small fraction of \( V_{\beta 11}^{+} \) T cell hybridomas are I-E reactive.

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