ANALYSIS OF Vβ17a EXPRESSION IN NEW MOUSE STRAINS BEARING THE Vβa HAPLOTYP

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In the mouse the Vβ elements of the TCR-α/β are encoded in a complex of tightly linked genes on chromosome six (1-3). Two major Vβ haplotypes have been found. Most common strains carry the Vβb haplotype, which encodes ~20 Vβ elements (4-8). A few strains carry the Vβa haplotype, in which a large deletion has eliminated 10 Vβ gene elements (9). In addition, the Vβa haplotype carries a functional gene for Vβ17 (Vβ17a) (10). A Vβ17 gene is present in the Vβb haplotype (Vβ17b), but is inactive due to a mutation in the coding region that generates a termination codon (11).

We recently reported (10) that TCR-α/β that utilize Vβ17a react with very high frequency to the murine MHCI class II product, IE. This reactivity is demonstrated most dramatically in IEα mice, where during the establishment of self-tolerance, Vβ17a+ T cells are virtually eliminated (12). This phenomenon has provided a useful tool to study the process of self-tolerance; however, experiments have been limited because only a few strains carry the Vβa haplotype and these strains are of diverse genetic backgrounds, making it difficult to distinguish H-2 and non-H-2 effects on Vβ17a expression.

We report here the characteristics of Vβ17a expression in a set of new mouse strains constructed to have the Vβa haplotype associated with various H-2 haplotypes in related C57 mice. The results not only confirm the association of the deletion of Vβ17a+ T cells in IEα mice, but also establish a second H-2-encoded ligand for Vβ17a+ T cells mapping to the K end of the H-2a haplotype, and suggest that CD4+ Vβ17a+ T cells are differentially selected in mice of different H-2 types.

Materials and Methods

Mice. Mice were either bred in our own facility or purchased from the Jackson Laboratory, Bar Harbor, ME.

mAbs and Flow Cytometric Analyses. mAbs used to characterize purified peripheral T cells are listed in Table 1. For use in flow cytometric analyses, in some cases, purified antibodies were labeled with FITC or biotin-N-hydroxysuccinimide by standard procedures. Biotinylated

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antibodies were detected with phycoerythrin-streptavidin (PEAv; Tago Inc., Burlingame, CA) as a secondary reagent. Unconjugated murine antibodies were detected with an FITC-labeled goat anti-mouse Ig secondary reagent (Calbiochem-Behring Corp., San Diego, CA). All samples were analyzed with an Epics C flow cytometer.

**Vβ and H-2 Typing of Mice.** Approximately 10 drops of peripheral blood were obtained from the tail artery of mice to be typed. RBC were removed with Gey’s solution (20) and T cells enriched on nylon fiber columns (21, 22). Aliquots of cells were stained with anti-H-2 antibodies. For estimating T cell Vβ expression, cells were stained with FITC-anti-CD4 and FITC-anti-CD8 (separately or in combination) and simultaneously with Bio-anti-Vβ plus PEA  to estimate Vβ usage. The sum of CD4+ plus CD8+ T cells in these preparations varied from 75% to 90%. In some experiments mice were killed and T cells from lymph node cell suspensions were similarly prepared and stained. In this case the sum of CD4+ and CD8+ T cells varied from 95% to 100%.

**Table I**

| mAb   | Relevant specificity | Reference |
|-------|----------------------|-----------|
| MK-Q8 | Dβ1                  | 10*       |
| 11.4.1| Kβ                  | 13        |
| 34.2-12| Dβ                  | 14        |
| 28-13-3 | Kβ                  | 15        |
| GK-1.5 | CD4               | 16        |
| 53.6 | CD8                  | 17        |
| KJ23a | Vβ17a               | 10        |
| KJ16 | Vβ (8.1 + 8.2)      | 18, 19    |

* Produced from the same fusion that yielded KJ23a. Typed on H-2 congenic mice.

**Results**

**Production of New Vβ+ Strains.** We produced F1 mice by crossing either C57BR(H-2k) or C57L(H-2b) mice to any of a number of H-2 congenic mice on the C57BL/10 (B10) background. The F1 mice were crossed to produce F2 progeny. T cells from these mice were typed for H-2 haplotype. Mice homozygous at H-2 were distinguished from heterozygous mice both with antibodies specific for the parental H-2 haplotypes and by the fact that individual T cells from heterozygous mice expressed approximately one half the amount of each parental H-2 haplotype. The T cells were also typed for Vβ haplotype, using KJ23a to detect Vβ17a of the Vβb haplotype and KJ16 to detect Vβ8.1/8.2 carried only by the Vβb haplotype. Vβ homozygous mice were identified by the complete lack of Vβ17a+ T cells (10-12) and a high level of Vβb+ T cells, similar to that seen in the original Vβb homozygous parent of the cross. Vβb/Vβb heterozygous mice were identified by the presence of both Vβb+ and Vβ17a+ T cells. In the case of Vβ8, the level of expression was ~50% of that seen in the Vβb homozygous parent of the cross, reflecting allelic exclusion in the Vβ complex in individual T cells (23). Levels of Vβ17a in the heterozygous mice were similarly reduced by allelic exclusion and, in addition, in a number of cases by the presence of a tolerizing MHC product, such as IE. However, residual

1 Abbreviations used in this paper: Bio, biotinylated; PEA, phycoerythrin-streptavidin.
Vß17a+ T cells were detectable in every case. The important Vßa homozygous mice were identified in every case by the complete absence of Vß8+ T cells and the presence of at least some Vß17a+ T cells, again even in the presence of a tolerizing MHC molecule. In fact, with no exception, staining with these anti-Vß antibodies placed the several hundred mice examined in these studies unequivocally into one of these three categories.

Some of these points are illustrated in Fig. 1 for an experiment in which 29 (B10.A(5R) x C57L)F2 mice were typed. Using antibodies to Dd and Db to distinguish the two H-2 types, homozygous mice of both types were detected. H-2 heterozygous mice were also identified, reacting with both antibodies, but with cells expressing ~50% of the level of each compared with homozygous mice. For each of these three groups, mice homozygous for Vßb contained ~16-19% Vß8.1/8.2+ T cells, while this percentage dropped about half to 9-10% in Vßb/Vßa heterozygous mice and to 0% in homozygous Vßa mice. In H-2a mice a reciprocal pattern of expression was seen for Vß17a with ~5% in Vßa homozygous mice, 2.5% in a Vßb/Vßa heterozygous mouse, and 0% in Vßb homozygous mice. Due to the presence of the IEb molecule, ~80% of Vß17a+ T cells were deleted in H-2 heterozygous mice and in those homozygous for the H-2 type of B10.A(5R). In this experiment one mouse was identified that was both homozygous for Vßa and for the H-2 type of B10.A(5R), and two Vßb/H-2b homozygous mice were found. Similar results were obtained with mice from each of the other crosses.

Mice homozygous for Vßa and for H-2 were used as founder stock to establish a new Vßa strain. These mice occurred approximately with the predicted 1 in 16 frequency so that often a founder pair was identified after typing 30-50 F2 mice. In some cases a male and a female double-homozygous pair was not isolated, so that one double-homozygous mouse was bred with a mouse homozygous at only one of the loci. The double-homozygous mice identified among the F3 progeny with a frequency of ~50% were used to establish the strain. Once established, the strains were maintained by breeders taken whenever possible from a single litter. New breeders also were selected for black coat color in order to select for the eventual loss of the leaden and brown coat color genes of C57L and C57BR.

The resulting strains, while neither completely congenic nor inbred, differed at H-2 and only some of the genes that distinguish the closely related C57BL, C57BR, and C57L strains. To control partially for these differences, H-2b/Vßa and H-2b/Vßa...
mice produced by the same strategy were compared with C57BR and C57L, respectively, to see if mixing C57BL genes with those of C57BR or C57L mice altered expression of Vβ17a. In the case of H-2k, this also produced a strain that bred much better than C57BR.

An additional Vβ strain was produced by backcrossing the SJL-derived Vβa complex to C57BL/10 mice, again typing progeny with anti-H-2h and anti-Vβ antibodies. At the F6 generation males and females from the same litter were mated to produce an N6 H-2b/Vβa homozygous strain.

The origins of the nine new Vβa strains are summarized in Table II.

**Deletion of Vβ17a+ T cells in IE+ Vβa Mice.** The new Vβa strains were compared with existing Vβa strains for the expression of Vβ17a in CD4+ and CD8+ T cells. Purified lymph node T cells were stained simultaneously with either FITC-labeled anti-CD4 or anti-CD8 and Bio-anti-Vβ17a plus PEAv. Sample fluorescence histograms are shown in Fig. 2 for some of the strains. The summarized results of all

| Vβa strain Type | K | A | E | D | Derived from |
|-----------------|---|---|---|---|---------------|
| B10. Q3BR      | q | q | q | - | (B10.Q x C57BR)F2/F3 |
| B10. 4R3                  | t2 | s | s | - | (B10.4R x C57L)F2 |
| B10.81                  | b | b | b | - | B10.HTT x C57L |
| B10. 4S3                  | b | b | b | - | (C57BL/10 x [C57BL/10 x SJL]F1)F6 |
| B10. A4R3                  | h4 | k | k | - | (B10.A4R x C57L)F2 |
| B10. 4D3                  | d | d | d | d | (B10.4D x C57L)F2/F3 |
| B10. 4S3                  | i5 | b | b | b | (B10.A5R x C57L)F2/F3 |
| B10. HTT3                  | t3 | s | s | s | (B10.HTT x C57L)F2/F3 |
| B10. BR3BR              | k | k | k | k | (B10.BR x C57BR)F2 |

**Figure 2.** Deletion of Vβ17a+ CD4* and CD8+ T cells in IE+ mice. Nylon fiber-purified lymph node T cells from B10.Q3BR, B10.4R3, and C57BR mice were stained with either FITC-anti-CD4 or FITC-anti-CD8 and with Bio-anti-Vβ17a plus PEAv. Histograms show the red fluorescence of the green fluorescent cells. Percentages refer to the percentage of total green fluorescence cells staining red in the shaded area.
TABLE III

**Vβ17a Expression in Vβ0** Mice

| Vβ0 strain | Type | K | A | E | D | Percent of CD4⁺ T cells | Percent of CD8⁺ T cells | Percent of total T cells |
|------------|------|---|---|---|---|-------------------------|-------------------------|-------------------------|
| SWR        | q    | q | q | - | q | 18.5 ± 0.6               | 4.6 ± 0.4               | 15.1 ± 0.6               |
| B10.QBBR   | q    | q | q | - | q | 13.2 ± 0.5               | 3.7 ± 0.2               | 9.9 ± 0.4               |
| SJL        | s    | s | s | - | s | 10.9 ± 0.2               | 7.1 ± 0.3               | 9.8 ± 0.2               |
| B10.S(7R)BL| t2   | s | s | - | d | 5.0 ± 0.5                | 3.6 ± 0.1               | 4.3 ± 0.3               |
| C57L       | b    | b | b | - | b | 2.8 ± 0.2                | 6.3 ± 0.7               | 4.2 ± 0.4               |
| B10KI      | b    | b | b | - | b | 3.1 ± 0.2                | 6.3 ± 0.3               | 4.1 ± 0.4               |
| B10.8(N6)  | b    | b | b | - | b | 3.4 ± 0.3                | 7.3 ± 0.2               | 4.9 ± 0.2               |
| B10.A(4R)BL| h4   | k | k | - | b | 1.7 ± 0.1                | 0.9 ± 0.0               | 1.3 ± 0.0               |
| B10.D2hL   | d    | d | d | d | d | 1.3 ± 0.2                | 2.5 ± 0.3               | 1.8 ± 0.2               |
| B10.A(3R)BL| t5   | b | b | b | d | 0.5 ± 0.2                | 2.2 ± 0.5               | 1.1 ± 0.3               |
| B10.HTTBL  | t3   | s | s | s | d | 0.6 ± 0.1                | 1.1 ± 0.3               | 0.8 ± 0.2               |
| B10.BRBBR  | k    | k | k | k | k | 0.2 ± 0.1                | 0.3 ± 0.2               | 0.3 ± 0.1               |
| C57BR      | k    | k | k | k | k | 0.1 ± 0.1                | 0.1 ± 0.0               | 0.1 ± 0.0               |

* Average ± SEM of determinations made in three or more mice.
† Total T cells equals the sum of CD4⁺ plus CD8⁺ T cells.

The strains are listed in Table III and as the average percent of either CD4⁺, CD8⁺, or total T cells expressing Vβ17a.

As expected, Vβ17a⁺ T cells were depressed in all of the new Vβ0 strains bearing an IE molecule. That this low expression was due to tolerance to IE was confirmed by the dominant low expression in the Vβ0 homozygous/IE heterozygous F2 and F3 mice examined in the course of isolating these strains (Fig. 3). In each case the deletion of Vβ17a⁺ T cells was as striking in IE⁺ × IE⁻ heterozygous mice as in IE⁺ homozygous mice.

In our previous experiments it was difficult to compare the efficiencies of different IE molecules in this deletion because we had to use F1 mice in which either the IE or the Vβ17a structural gene or both were heterozygous (12). However, in these new
Vβa strains, where both the IE and Vβ17a genes are homozygous, we could clearly see that not all IE molecules delete Vβ17a+ T cells equally well (Table III, Fig. 4). Complementing our previous finding (10, 24) that Vβ17a+ T cell hybridomas responded with lowest frequency to IE4, the B10.D2BL mice bearing IE4 had the greatest number of Vβ17a+ T cells surviving tolerance. This was followed by B10.A(5R)BL(IEa), B10.HTTBL (IEa), and finally, B10.BRBR(IEa). In the last case the interpretation is clouded somewhat by a second deleting gene mapping to the K end of H-2k (see below). It is worth noting that combination of the B10 background genes with those from C57BR to construct B10.BRBR strain did not significantly alter the virtually complete deletion of Vβ17a+ T cells.

In each of the IE+ strains, deletion of Vβ17a+ T cells was evident among both the CD4+ and CD8+ T cells, although deletion among CD4+ T cells appeared more efficient (Table III, Fig. 2). For many CD4+/Vβ17a+ T cell hybridomas, loss of or blockage of the CD4+ molecule results in loss of IE reactivity (data not shown). Thus, the deletion of CD8+/Vβ17a+ T cells by IE suggests that deletion occurred at a stage of development when these cells bore CD4 and, therefore, reinforces the accumulating evidence for a CD4+/CD8+ intermediate in T cell development with at least some self-tolerance induction at this stage (25, 26). The skewing of Vβ17a+ T cell deletion toward CD4+ T cells may suggest that, in addition, some of the deletion occurs after the cells have become CD4 or CD8 single-positive T cells, or could simply indicate that the small proportion of Vβ17a+ T cells that are not IE reactive may be enriched in T cells selected by class I ligands in the thymus and are destined to become CD8+.

**Differences in Expression of Vβ17a in IE- Vβa Mice.** Some of the most noteworthy results on Vβ17a expression were obtained in the new IE- Vβa strains. For example, direct evidence that non-H-2 genes affect the levels of Vβ17a+ T cells came from comparing the two Vβa H-2a mice. There were considerably more Vβ17a+ T cells in SWR mice than in B10.QBR mice, especially among CD4+ T cells. Vβ17a expression was also considerably higher in SJL than in B10.S(7R), although the difference at H-2D as well as in the non-H-2 background genes may play a role here. On the other hand, Vβ17a expression did not differ significantly among the three H-2b mice carrying different mixes of the background genes from C57L and B10 mice, again pointing out the relatedness of the C57 mice.

One surprising result was the low expression of Vβ17a+ T cells (1.3%) in IE- B10.A(4R)BL mice (Table III). Comparison of these mice to any of the H-2b Vβa mice suggests that a second H-2 gene mapping to the K end of H-2k also causes significant deletion of Vβ17a+ T cells. Alternatively, it is possible that the B10.A(4R) H-2 products simply poorly select Vβ17a+ T cells during T cell development. Evi-
dence that this low expression is in fact due to deletion was seen during the analysis of the F2 mice used to construct this strain (Fig. 4). Low expression of Vβ17a was seen both among the mice homozygous and heterozygous for the H-2 type of B10.A(4R), reflecting the dominance expected of deletion due to self-tolerance. This non-IE-deleting elements perhaps explains why in H-2k mice, which carry both this element and the IEk molecule, the deletion of Vβ17a+ T cells is virtually complete.

While the deleting K end gene product could be either IAk or Kk, two observations favor Kk. First, although, as with IE, this deleting element deletes both CD4+ and CD8+ T cells in B10.A(4R)βl, mice, in this case the effect is more dramatic on CD8+ than CD4+ T cells. Second, in our studies of the reactivity of Vβ17a+ T cell hybridomas (10, 24), we have found only occasional responsiveness to IAk. At present, we are unable to study most cases of class I recognition in T cell hybridomas because the fusion partner, BW5147, supports CD4, but not CD8, expression in its hybrids (27). Therefore, it is likely in these hybridoma studies that a CD4-dependent reactivity to IAk would have been found were it to exist, but a CD8-dependent response against Kk would have been missed.

With the exception of the B10.A(4R)βl strain, expression of Vβ17a among CD8+ T cells varied only by a factor of about two (3.6-7.3%). On the other hand there was a much greater variation among CD4+ T cells, with a low of 2.8% in C57L mice and a high of 18.5% in SWR. The high expression in B10.Q6R (13.2%) vs. low expression in the H-2b mice (2.8-3.4%) established that much of this difference was due to H-2. This phenomenon could reflect another gene deleting Vβ17a+ T cells in the H-2b haplotype specific for CD4+ T cells; however, in this case, preliminary results have shown that high expression is dominant in (H-2a × H-2b)F1 mice (Blackman, M., P. Marrack, and J. Kappler, manuscript in preparation) favoring the view that IAa is a much better selector of Vβ17a+ T cells than is IAk. We are currently testing this idea in the appropriate chimeric mice.

Discussion

Mice carrying the Vβa haplotype should be useful in T cell repertoire studies for two reasons. First, they carry a functional structural gene for Vβ17 (10, 11). Since receptors utilizing Vβ17a have the unusual property of reacting with high frequency to B cell-presented IE molecules (10, 12, 28), Vβa mice can be used to study the phenomenon of self-tolerance to class II MHC ligands. A possible class I ligand for Vβ17a+ T cells in H-2k may make the B10.A(4R)βl strain useful in studying tolerance induction to class I MHC ligands. In addition, the dramatic difference in CD4+ Vβ17a+ T cells between H-2a and H-2b suggests that IAa selects Vβ17a+ better than IAk, perhaps offering a system in which the process of thymic positive selection can be studied.

A second feature of the Vβa haplotype is the remarkable deletion in the middle of the complex of about half of the Vβ elements (9). Although one might suppose that this loss of receptor elements would be detrimental because it would limit the diversity of the αβ receptor repertoire, the deletion has apparently been tolerated evolutionarily for some time (29). The immunological consequences of this deletion have not been extensively explored. We have suggested that this deletion and other genetic mechanisms that limit the expression of particular Vβ elements in different mice may play a role in the mouse population at large in balancing the advantage...
of a large T cell repertoire against the potential involvement of these Vß elements in autoimmune reactions (11, 12, 19, 30). The development of a set of Vßa mice of similar background and carrying different IE+ and IE− H-2 haplotypes should provide a useful tool to study this question.

Summary

A set of new mouse strains were produced that carry the Vßa haplotype of the TCR-α/β and any of a number of different H-2 haplotypes on backgrounds derived from related C57BL, C57L, and C57BR mice. Study of Vß17a expression in these mice confirms the association between the presence of IE and the deletion of Vß17a+ T cells. A second H-2 gene causing deletion of Vß17a+ T cells was mapped in these mice to the K end of H-2k, and H-2 influences on the level of selection of CD4+ Vß17a+ T cells were indicated.

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References

1. Chou, H., C. Nelson, S. Godambe, D. Chaplin, and D. Loh. 1987. Germline organization of the murine T cell receptor β-chain genes. Science (Wash. DC). 238:545.
2. Lai, E., R. Barth, and L. Hood. 1987. Genomic organization of the mouse T-cell receptor β-chain gene family. Proc. Natl. Acad. Sci. USA. 84:3846.
3. Lee, N., and M. Davis. 1988. T cell receptor β-chain genes in BW5147 and other AKR tumors. Deletion order of murine Vß gene segments and possible S' regulatory regions. J. Immunol. 140:1665.
4. Behlke, M., D. Spinella, H. Chou, W. Sha, D. Hartt, and D. Loh. 1985. T cell receptor β-chain expression: dependence on relatively few variable region genes. Science (Wash. DC). 229:566.
5. Barth, R., S. Kim, N. Lan, T. Hunkapillar, N. Sobieck, A. Winoto, H. Gershengeld, C. Okada, D. Hansburg, I. Weissman, and L. Hood. 1985. The murine T-cell receptor uses a limited repertoire of expressed Vß gene segments. Nature (Lond.). 316:517.
6. Patten, P., T. Yokota, J. Rothbard, Y. Chien, K. Arai, and M. Davis. 1984. Structure, expression and divergence of T-cell receptor β-chain variable regions. Nature (Lond.). 312:40.
7. Singer, P., R. McEvilly, D. Noonan, F. Dixon, and A. Theofilopoulos. 1986. Clonal diversity and T-cell receptor β-chain variable gene expression in enlarged lymph nodes of MRL-lpr/lpr lupus mice. Proc. Natl. Acad. Sci. USA. 83:7018.
8. Malissen, M., C. McCoy, D. Blanc, J. Trucy, C. Devaux, A. Schmitt-Verhulst, F. Fitch, L. Hood, and B. Malissen. 1986. Direct evidence for chromosomal inversion during T-cell receptor β-gene rearrangements. Nature (Lond.). 319:28.
9. Behlke, M., H. Chou, K. Huppi, and D. Loh. 1986. Murine T cell receptor mutants with deletions of β-chain variable region genes. Proc. Natl. Acad. Sci. USA. 83:767.
10. Kappler, J., T. Wade, J. White, E. Kushnir, M. Blackman, J. Bill, R. Roehm, and P. Marrack. 1987. A T cell receptor Vß segment that imparts reactivity to a class II major histocompatibility complex product. Cell. 49:263.
11. Wade, T., J. Bill, P. Marrack, P. C. Palmer, and J. Kappler. 1988. Molecular basis for the non-expression of Vß17 in some strains of mice. J. Immunol. 141:2165.
12. Kappler, J., N. Roehm, and P. Marrack. 1987. T cell tolerance by clonal elimination in the thymus. Cell. 49:273.
1. Oi, V., P. Jones, J. Goding, L. Herzenberg, and L. Herzenberg. 1978. Properties of monoclonal antibodies to mouse Ig allotypes, H-2 and Ia antigens. *Curr. Top. Microbiol. Immunol.* 81:115.

2. Ozato, K., N. Mayer, and D. Sachs. 1982. Monoclonal antibodies to MHC antigens. IV. A series of hybrid clones producing anti-H-2\(^d\) antibodies and an estimation of expression of H-2\(^d\) antigens on the surface of these cells. *Transplantation (Baltimore).* 34:113.

3. Ozato, K., and D. H. Sachs. 1981. Monoclonal antibodies to mouse MHC antigens. III. Hybridoma antibodies reacting to antigens of the H-2\(^b\) haplotype reveal genetic control of isotype expression. *J. Immunol.* 126:317.

4. Dialynas, D., Z. Quan, K. Wall, A. Pierres, J. Quintans, M. Loken, M. Pierres, and F. Fitch. 1983. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK-1.5: similarity of L3T4 to the human Leu 3/T4 molecule and the possible involvement of L3T4 in Class II MHC antigen reactivity. *J. Immunol.* 131:2445.

5. Gey, G., and M. Gey. 1936. The maintenance of human normal and tumor cells in continuous culture. I. Preliminary report: cultivation of mesoblastic tumors and normal tissue and notes on methods of cultivation. *Am. J. Cancer.* 27:45.

6. Julius, M., E. Simpson, and L. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived lymphocytes. *Eur. J. Immunol.* 3:645.

7. Kappler, J., and P. Marrack. 1977. The role of H-2-linked genes in helper T cell function. I. In vitro expression in B cells of immune response genes controlling helper T cell activity. *J. Exp. Med.* 146:1748.

8. Carbone, A. M., P. Marrack, and J. Kappler. 1988. Remethylation at sites 5' of the murine Lyt 2 gene in association with shut down of Lyt 2 expression. *J. Immunol.* 333:1569.

9. Marrack, P., and J. Kappler. 1988. T cells can distinguish between allogeneic major histocompatibility complex products or difference cell types. *Nature (Lond.)* 332:6167.

10. Pullen, A. M., P. Marrack, and J. W. Kappler. 1988. The T cell repertoire is heavily influenced by tolerance to polymorphic self antigens. *Nature (Lond.)* 335:796.