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# Structure and Specificity of a Class II MHC Alloreactive γδ T Cell Receptor Heterodimer

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**Two distinct CD3-associated T cell receptors (TCRαβ and TCRγδ) are expressed in a mutually exclusive fashion on separate subsets of T lymphocytes. While the specificity of the TCRαβ repertoire for major histocompatibility complex (MHC) antigens is well established, the diversity of expressed γδ receptors and the ligands they recognize are less well understood. An alloreactive $CD3^+CD4^-CD8^-$ T cell line specific for murine class II MHC (Ia) antigens encoded in the I-E subregion of the H-2 gene complex was identified, and the primary structure of its γδ receptor heterodimer was characterized. In contrast to a TCRαβ-expressing alloreactive T cell line selected for similar specificity, the TCRγδ line displayed broad cross-reactivity for multiple distinct I-E–encoded allogeneic Ia molecules.**

TCRγδ T LYMPHOCYTES CONSTITUTE a distinct T cell lineage (1% to 10% of $CD3^+$ T cells) with cytolytic activity and the capacity to produce lymphokines (*1*). The major unresolved question concerning TCRγδ T cells is the nature of the ligands they recognize. The TCRαβ repertoire displays specificity for both class I and class II MHC (Ia) antigens. Several developmental parallels between TCRγδ and TCRαβ suggest that some TCRγδ might also recognize MHC antigens. For example, the TCRδ locus is situated with the TCRα locus and $V_\alpha$ gene elements can rearrange productively to DJδ (*2–4*).

In this light, several murine and human alloreactive TCRγδ T cell lines and clones specific for class I MHC antigens have been identified (*3, 5*). Evidence for different antigen-processing pathways for presentation of peptides by class I versus class II MHC molecules (*6*) suggests that class I and class II MHC–restricted T cells may recognize distinct sets of foreign protein antigens. Therefore, in order to better characterize the potential diversity of the MHC-specific TCRγδ repertoire, it was important to establish whether TCRγδ T cells have the capacity for class II MHC molecule recognition.

Alloreactive T cell lines were derived from the peripheral lymph nodes of athymic B10 ($H\text{-}2^b$) *nu/nu* mice by in vitro stimulation of T cells with H-2–congenic B10.BR ($H\text{-}2^k$) splenic antigen-presenting cells (APCs) (*7*). The structure of the receptor proteins expressed by these lines was examined by performing immunoprecipitations with a monoclonal antibody specific for the CD3ε chain of the TCR complex (*8*). One $CD3^+CD4^+CD8^-$ line, LBK4, expressed a CD3-associated heterodimer with a molecular mass of 41 to 43 kD, which appeared to be an αβ receptor (Fig. 1A and Fig. 1B, lane 1). That LBK4 was an αβ T cell line was confirmed by showing that an antiserum to $C_\beta 1$ (*9*) precipitated an identical 41- to 43-kD heterodimer (Fig. 1B, lane 2).

A second independently derived line, LBK5, with a $CD3^+CD4^-CD8^-$ surface phenotype, expressed a markedly different CD3-associated heterodimer consisting of proteins of 31 and 45 kD (Fig. 1C). A clone derived from this line, G11, expressed an identical heterodimer (Fig. 1D, lane 1). After reduction and alkylation of the immunoprecipitate of the G11 clone obtained with an antibody to CD3, the 31-kD and 45-kD proteins were immunoprecipitated by an antiserum to the $C_\gamma 1/C_\gamma 2$ peptide (*9*) (Fig. 1D, lane 3) and an antiserum to TCRδ (*10*) (Fig. 1D, lane 2), respectively.

The specificities of the TCRαβ-bearing LBK4 line and the TCRγδ-expressing LBK5 line were tested in cell-mediated cytotoxicity assays with target cells from various H-2 recombinant mouse strains (Fig. 2A). Because TCRγδ T cells cultured in the presence of exogenous growth factors may develop nonspecific cytolytic activity (*5*), the assays of cytolytic T lymphocytes (CTLs) were performed with cells that had been cultured in the absence of interleukin-2 (IL-2) for 24 to 48 hours. First, the MHC-linked specificity of both lines was shown by the fact that they lysed H-2 congenic B10.BR ($H\text{-}2^k$) (not shown) and B10.A ($H\text{-}2^a$), but not syngeneic B10 ($H\text{-}2^b$) or allogeneic B10.D2 ($H\text{-}2^d$) target cells (Fig. 2A, Exp. 1). The LBK5 but not the LBK4 T cells also killed B10.A(5R) ($H\text{-}2^{i5}$), B10.S(9R) ($H\text{-}2^{t4}$), and B10.RIII ($H\text{-}2^r$) targets (Fig. 2A, Exp. 2).

More detailed analysis of the specificity of the γδ receptor expressed by LBK5 was

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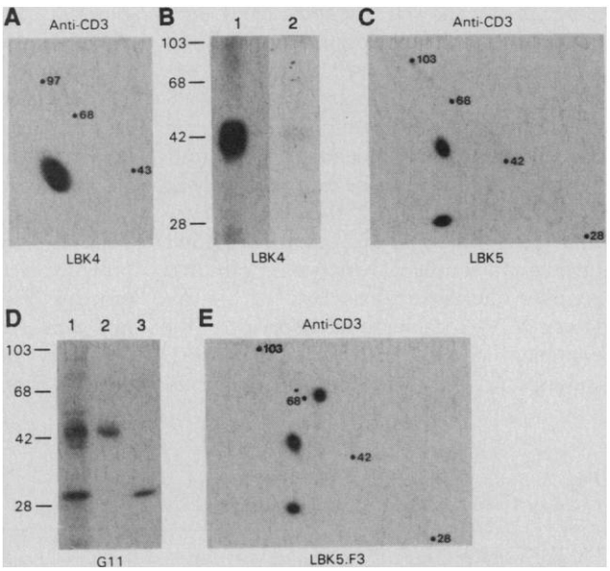


**Fig. 1.** CD3-associated TCR heterodimers expressed by alloreactive T cell lines derived from athymic B10 *nu/nu* mice. Specific immunoprecipitations with 145-2C11, a monoclonal antibody directed against CD3ε (*8*), and the antisera to $C_\beta$ (*9*), TCRγ (*25*), and TCRδ (*10*), were performed as described (*9*) on lysates of surface radiolabeled cells. Two-dimensional SDS-polyacrylamide gels were run under nonreducing conditions along the horizontal axis followed by reducing conditions along the vertical axis. The molecular masses (kilodaltons) of protein standard markers are indicated. (**A**) Two-dimensional gel analysis of an anti-CD3 immunoprecipitate of the $CD3^+CD4^+CD8^-$ LBK4 cell line. (**B**) One-dimensional SDS-polyacrylamide gel analysis of surface radioiodinated LBK4 cells run under reducing conditions. The same labeled cell lysate was immunoprecipitated with the anti-CD3ε monoclonal antibody 145-2C11 and with the antiserum to $C_\beta$. (**C**) Two-dimensional gel analysis of an anti-CD3ε immunoprecipitate of the $CD3^+CD4^-CD8^-$ cell line LBK5. (**D**) One-dimensional gel analysis of surface-labeled cloned G11 cells, run under reducing conditions. The clone was derived from the LBK5 line by limiting dilution. The anti-CD3ε immunoprecipitate of surface-labeled G11 cells is shown in the first lane. After reduction/alkylation of the anti-CD3ε immunoprecipitate, the sample was reprecipitated with the TCRδ-specific antiserum (second lane) or the TCR $C_\gamma1/C_\gamma2$–specific antiserum (third lane). (**E**) Two-dimensional nonreducing/reducing SDS-polyacrylamide gel analysis of an anti-CD3ε immunoprecipitate of cloned LBK5.F3 hybridoma cells derived by fusion of LBK5 T cells to a TCRβ-negative variant of the BW5147 thymoma.

performed with T cell hybridomas generated by fusing LBK5 cells to a TCRβ-negative variant of the BW5147 thymoma. One cloned hybrid cell line, LBK5.F3, expressed the same CD3-associated γδ heterodimer as the LBK5 line and the G11 clone (Fig. 1E). The specificity of this hybrid was examined by assaying for lymphokine production after stimulation with APCs from various mouse strains. Supernatants from stimulated LBK5.F3 cells were tested for their ability to support the proliferation of DA.1, an IL-3–dependent and granulocyte-macrophage colony-stimulating factor (GM-CSF)–dependent cell line (*11*). LBK5.F3 was activated by B10.BR, B10.A, B10.A(5R), and B10.S(9R) APCs, but not by APCs from B10.D2, B10.A(4R), B10, or B10.S mice (Fig. 2B). APCs from the latter three strains do not express any I-E–encoded surface Ia molecules. In addition, the activation was inhibited only by I-E–specific and not I-A–specific monoclonal antibodies (*12*). Thus, the LBK5.F3 hybridoma recognizes the $I\text{-}E^{k,b,s}$ Ia molecules expressed by B10.A, B10.A(5R), and B10.S(9R) APCs, respectively (Fig. 2B).

The specificity pattern of this TCRγδ T cell clone for $I\text{-}E^{b,k,s,r}$ but not $I\text{-}E^d$ is of interest in that it is very similar to that of

**A**

| Effector cells | Target cells: B10 | B10.A | B10.A(5R) | B10.S(9R) | B10.RIII | B10.D2 |
|---|---|---|---|---|---|---|
| EXP 1 | | | % Specific lysis | | | |
| LBK5 (TCRγδ) | 2.4 | 17 | | | | 2.3 |
| LBK4 (TCRαβ) | -1.0 | 29 | | | | 3.1 |
| EXP 2 | | | | | | |
| LBK5 | 5.7 | 36 | 57 | 23 | 26.6 | |
| LBK4 | -1.5 | 23 | -2.2 | 4.1 | -0.6 | |

**B**

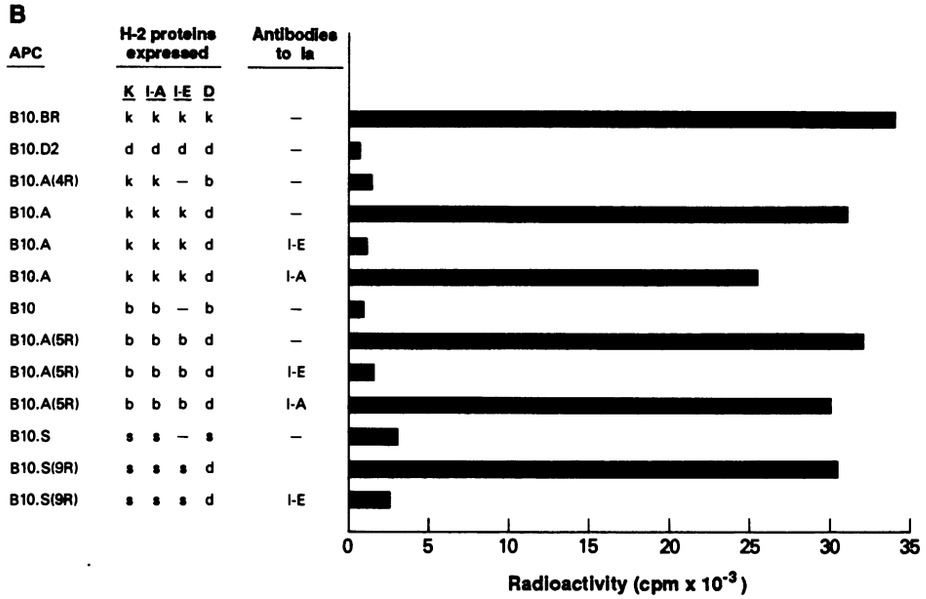


**Fig. 2.** (**A**) MHC-specific cytolytic activity of the TCRγδ LBK5 and the TCRαβ LBK4 T cell lines. $^{51}Cr$ release assay: $2 \times 10^6$ to $4 \times 10^6$ target cells (lipopolysaccharide-induced splenic blasts) were radiolabeled with 300 μCi of $^{51}NaCrO_3$ for 1 hour at 37°C. The preparation of the effector T cells, performance of the assays, and the calculations of percent specific lysis were exactly as described (*3*). Values represent the means of triplicate cultures. Standard errors were <10% of the mean. The specificity of CTL activity was examined with various H-2 recombinant target cells. In addition to the MHC proteins listed in (B), the MHC proteins for B10.RIII are ($K^rA^r$-$E^rD^r$). The effector/target ratio in these experiments was 10:1. (**B**) Class II MHC I-E subregion specificity of the γδ-receptor–expressing T cell hybridoma LBK5.F3. Hybridoma cells ($1.5 \times 10^6$) were cultured in individual wells of 24-well tissue culture plates in the presence of $4 \times 10^6$ low-density splenic APCs prepared as previously described (*3*). Supernatants were collected at 24 hours and frozen. Aliquots of each supernatant were added at a final concentration of 50% (v/v) to individual microtiter wells containing $3 \times 10^4$ IL-3/GM-CSF–dependent DA.1 cells. Proliferation of DA.1 cells was measured after 24 hours by an 8-hour pulse with 1 μCi per well of tritiated thymidine. The allelic variants of the proteins expressed by each H-2 locus of the various strains are shown. A dash represents the fact that B10.A(4R), B10, and B10.S mice express no I-E subregion–encoded Ia molecules. Where indicated, antibodies to Ia were added to the culture of hybridoma cells and APCs. The I-E–specific antibodies used were culture supernatants of the 14.4.4 and 17.3.3 hybridomas (*12*) added at final concentrations of 1:10 and 1:4, respectively. The $I\text{-}A^k$–specific antibody 10-2.16 (*12*) was added at a 1:10 dilution, and the $I\text{-}A^b$–specific antibody Y-3P (*12*) represented purified antibody added at 20 μg/ml. Values for radioactivity in counts per minute represent the means of triplicate cultures. Standard errors were uniformly <10% of the mean.

two previously described I-E–specific monoclonal antibodies (*13*). There are a number of shared amino acid residues in the β1 domain of the $E_\beta^{k,b,s}$ molecules that differ from the $E_\beta^d$ sequence (*14*) and therefore represent potential recognition sites for the LBK5 TCRγδ receptor.

To begin to examine the structural basis of MHC antigen recognition by TCRγδ, the primary structure of the receptor expressed by LBK5 and the G11 clone was characterized (Fig. 3). LBK5 expressed full-length TCRβ mRNA, but, unlike LBK4, no TCRα mRNA (Fig. 3B), confirming that it could not express any TCRαβ protein. DNA hybridization analyses of LBK5 and G11 demonstrated rearrangements of both the $V_\gamma1$ and $V_\gamma2$ genes (Fig. 3C) (*15*). RNA hybridization analysis (Fig. 3D) showed preferential expression of $V_\gamma1$ relative to $V_\gamma2$ mRNA. In addition, a $V_\gamma1$-specific antiserum precipitated the 31-kD protein (*16*). Although both the LBK5 line and the G11 clone expressed full-length $C_\gamma4$ mRNA (Fig. 3D), $V_\gamma1.1$-$J_\gamma4C_\gamma4$–encoded proteins are 40 kD (*9*). Thus, the combined biochemical and molecular data indicated that LBK5 and G11 express a $V_\gamma1.2$-$J_\gamma2C_\gamma2$ protein.

Examination of the TCRδ locus in the LBK5 line, G11 clone, and LBK5.F3 hybridoma with genomic $J_\delta$ probes revealed two $J_\delta1$ rearrangements of 9.6 and 7 kb (Fig. 3E) and no $J_\delta2$ rearrangements. Further analysis revealed that a probe specific for $V_\delta5$, the $V_\delta$ segment most commonly expressed in adult thymocyte populations (*4*), hybridized to the 9.6-kb Eco RI $J_\delta1$ rearrangement (Fig. 3E). The 7-kb $J_\delta1$ rearranged band was demonstrated to be an incomplete $D_\delta2$-$J_\delta1$ rearrangement, because it also hybridized to an upstream $D_\delta2$ probe (*2*).

To determine the primary structure of the receptor heterodimer expressed by these class II MHC alloreactive T cells, we cloned and sequenced the productively rearranged TCR γ and δ genes (Fig. 3F) (*17*). Significantly, the V(D)J junctional sequences of both the γ and δ chains (Fig. 3F) are unique with respect to all previously published TCR γ and δ sequences encoded by functionally rearranged $V_\gamma1.2$ and $V_\delta5$ genes (*4, 18, 19*). This analysis encompasses 13 sequenced $V_\gamma1.2$ and 10 full-length $V_\delta5$ cDNAs. As these junctionally encoded sequences correspond to the presumed third complementarity-determining region (CDR 3) of the γδ receptor heterodimer, they are likely to contribute significantly to the observed MHC specificity (*20*).

We have characterized (*3, 5*) several distinct clonal populations of TCRγδ T cells specific for both class I and class II MHC antigens. We showed that these MHC-specific γδ T cells may express both $V_\gamma1$ and $V_\gamma2$ family genes, as well as two $V_\delta$ elements, one highly homologous to a well-characterized $V_\alpha$ gene family, $V_\alpha11$ (*3*), and the other the single member $V_\delta5$ family gene thus far found to be expressed only in γδ heterodimers. Thus, there is evidence from examination of γδ receptors in adult thymus and peripheral lymphoid organs that germline diversity, particularly of $V_\delta$ elements, $V_\gamma V_\delta$ combinatorial association [for example, $V_\delta5$ also pairs with $V_\gamma2$ (*18, 19*)], and especially extensive junctional diversity generate a TCRγδ repertoire of considerable overall sequence diversity with the potential to recognize a large number of antigens. This contrasts with γδ receptor–bearing T cells populating the skin and intestinal epithelium, which express a more restricted TCRγδ repertoire (*21*).

It has also been shown recently that some TCRγδ T cells recognize highly conserved proteins such as mycobacterial heat-shock proteins (hsp's) (*22, 23*). A strong correlation was observed between mycobacterial specificity and the expression of proteins of one $V_\delta$ family, $V_\delta6$ (*23*). In contrast, we

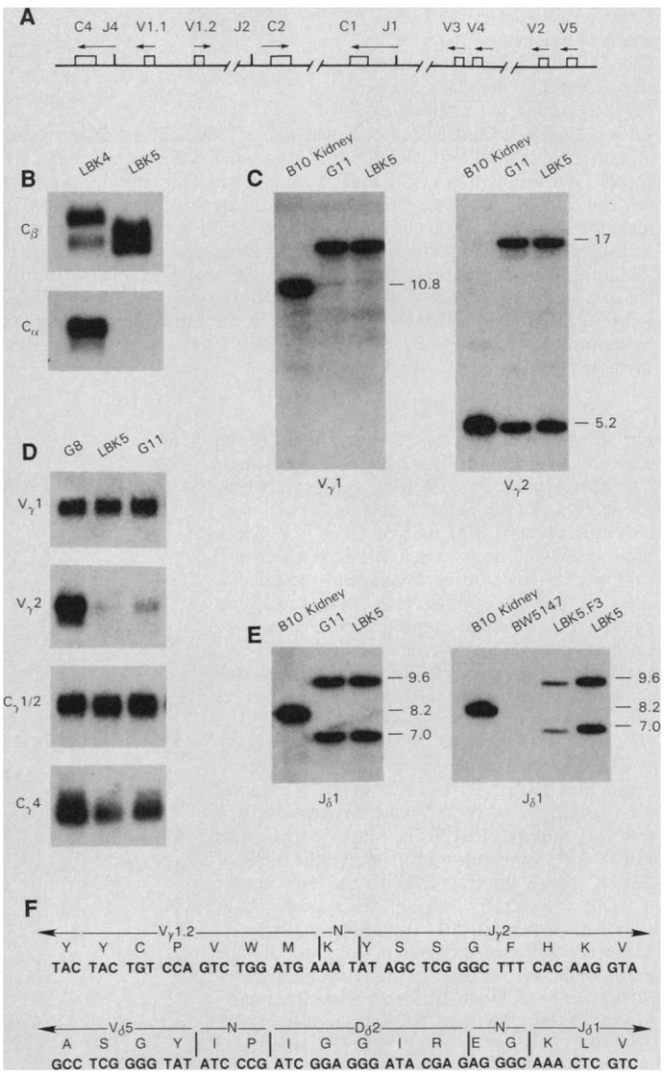


**Fig. 3.** Primary structure of a class II MHC alloreactive γδ receptor. (**A**) Germline organization of the TCR γ genes, adapted from Korman *et al.* (*18*). The arrows indicate the relative transcriptional orientations of the genes. (**B**) TCRβ and TCRα mRNA expression in the TCRαβ-expressing LBK4 T cell line and the TCRγδ-expressing LBK5 line. Preparation of total cellular RNA and RNA blot analysis were performed as described previously (*3*). (**C**) DNA blot analysis of $V_\gamma1$ and $V_\gamma2$ rearrangements in the TCRγδ LBK5 line and the LBK5-derived clone G11. DNA blot analysis was performed as previously described (*3*) on Eco RI–digested DNA derived from the T cell lines and from B10 kidney as a germline control. The $V_\gamma1$ and $V_\gamma2$ probes have been described (*15*). The sizes of the hybridizing bands are shown and were calculated from a Hind III digest of phage λ DNA. The 14-kb $V_\gamma1$ hybridizing Eco RI band represents both $V_\gamma1.1$-$J_\gamma4C_\gamma4$ and $V_\gamma1.2$-$J_\gamma2C_\gamma2$ rearrangements on the same chromosome (see Fig. 3A), and the 17-kb $V_\gamma2$ rearrangement results from a $V_\gamma2$-$J_\gamma1C_\gamma1$ joining (*15*). (**D**) TCR γ mRNA expression in the $V_\gamma2$ protein–expressing class I MHC–specific TCRγδ clone G8 (*3*), the LBK5 line, and the G11 clone. RNA extracted from each cell line was examined for the expression of $V_\gamma1$, $V_\gamma2$, $C_\gamma1/C_\gamma2$, and $C_\gamma4$. The $V_\gamma1$ probe hybridizes to both $V_\gamma1.1$- and $V_\gamma1.2$-encoded mRNAs. The $C_\gamma1/C_\gamma2$ (*15*) and the $C_\gamma4$ (*26*) probes have been described. (**E**) DNA blot analysis of TCR δ rearrangements in LBK5, G11, and the LBK5.F3 hybridoma. Eco RI–digested DNA was hybridized to a 3′ $J_\delta1$ probe (*2*). The TCRδ locus has been deleted from BW5147 because of $V_\alpha$-$J_\alpha$ rearrangements on both chromosomes. (**F**) DNA sequences including the junctional sequences of the productively rearranged TCRγ and TCRδ genes of the class II MHC–specific T cell clone G11. The V, D, and J elements are shown as are the nucleotides representing N-region additions. Full-length cDNA clones were isolated from a phage λ ZAP library prepared by Stratagene (La Jolla, California). The TCRγ and TCRδ cDNAs were subcloned into M13mp18 and sequenced by the dideoxy chain termination method. The protein sequences are represented by the single-letter code (*27*).

found that both our class II and class I MHC alloreactive γδ T cell lines failed to respond to heat-shocked syngeneic APCs, to crude mycobacterial sonicates rich in hsp's, or to partially purified extracts of 65-, 70-, and 12-kD hsp's derived from mycobacterium tuberculosis (*24*). Therefore, mycobacterial hsp-specific TCRγδ T cells and MHC-specific TCRγδ T cells may constitute distinct functional cell subsets.

Both the TCRγδ and TCRαβ T cell lines we used were derived from athymic mice and thus they represent examples of functional extrathymic T cell maturation for class II MHC molecule recognition. One striking feature of the specificity pattern of the class II MHC alloreactive TCRγδ-expressing T cells is their cross-reactivity for the products of multiple distinct I-E alleles. This is in contrast to the unique specificity of the TCRαβ LBK4 cells for the $E^k$ molecule expressed on the immunizing B10.BR APC. The class I MHC–specific TCRγδ cell line we described earlier also had a broad pattern of cross-reactivity for multiple allogeneic class I MHC antigens (*3, 5*). It is possible that TCRγδ in general may be specific for distinct determinants on MHC molecules less polymorphic than those recognized by TCRαβ.


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27. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.


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# Neonatal Thymectomy Results in a Repertoire Enriched in T Cells Deleted in Adult Thymus


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**In B6AF1 mice, T lymphocytes that use the $V_\beta11$-positive (and not $V_\beta6$-positive or $V_\beta8$-positive) segment in their receptor for antigen are greatly reduced in the thymus and peripheral lymphoid tissues, most likely as a result of clonal deletion. The relative number of $V_\beta11$-positive cells in adult lymph nodes was ten times as high in B6AF1 mice thymectomized 1 to 4 days after birth as in normal mice. Moreover, for the first 10 days of life of B6AF1 mice, mature $V_\beta11$-positive T cells were readily detected in the thymus and spleen. Thus neonatal thymectomy results in the maintenance of the receptor repertoire of early postnatal life, and this correlates with the subsequent development of organ-specific autoimmune diseases.**


ONE MECHANISM OF SELF TOLERance involves the deletion of T cell clones in the thymus. Thus T cells with antigen receptors of specific $V_\beta$ families do not mature intrathymically in mice expressing the major histocompatibility complex (MHC) molecule I-E (*1–3*), or the minor lymphocyte stimulatory (Mls) antigens (*4–6*). The elimination of such T cell clones appears to involve intrathymic interaction of T cell receptors (TCR) with MHC antigen on thymic stromal cells (*7–9*). This seminal concept implies that autoreactive T cells that encounter self antigens bound to MHC intrathymically would be similarly deleted. We now show that mice thymectomized soon after birth (days 1 to 4) contain in their peripheral lymphoid tissue T cells with TCR that would normally be deleted in the thymus. This finding indicates that clonal deletion is imperfect or leaky so that when thymopoiesis is ablated, autoreactive clones that emigrate from the neonatal thymus can expand in the periphery and become prominent. Such thymectomized mice exhibit autoimmune traits. Thus some autoimmune reactions may result from imperfect clonal deletion.


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