Brief Definitive Report

Murine Thymic CD4+ T Cell Subsets: A Subset (Thy0) That Secretes Diverse Cytokines and Overexpresses the Vβ8 T Cell Receptor Gene Family

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Summary

We demonstrate here the presence of a distinct mature $CD4^+8^-$ T cell subset in mouse thymus. This subset, termed "Thy0," is delineated by the absence of 3G11 expression from about half of the $6C10^-/HSA^{low/-}$ fraction of $CD4^+8^-$ thymic cells. Thy0 is detectable from the neonatal period and largely contributes the Th0-type diverse cytokine production previously reported for the $HSA^{low/-}CD4^+$ thymic population. Further, cells expressing the T cell receptor Vβ8 gene family are found at increasing frequency in Thy0 with age, comprising 40–60% of Thy0 in adult BALB/c mice. This alteration of $V\beta8^+$ cell frequency is unique to Thy0, since no other $CD4^+$ subset in thymus or spleen shows such Vβ8 overusage. All functional $CD4^+$ T cell subsets, including Thy0, show appropriate Vβ clonal deletion associated with endogenous superantigens. Thus, it appears that Thy0 is an intrathymically generated secondary cell subset produced after $CD4^+$ T cell selection.

The thymus is the primary site for T lineage differentiation, and is responsible for generating functionally competent T cells. After initial generation of $CD4^+$ and $CD8^+$ T cells, further cell differentiation takes place in the thymus, during which time the functional competence to respond to antigen is increasing. This progression of cells into an immunocompetent state can be recognized by phenotypic alterations, such as loss of the heat-stable antigen (HSA) (1–3). However, it has never been established whether all (or any) $HSA^{low/-}$ cells in thymus are comparable to peripheral naive T cells (3). Distinctive cytokine production similar to Th0 type cells by this mature thymic $CD4^+$ T fraction was recently demonstrated by Bendelac and Schwartz (4, 5), distinguishing them from the majority of peripheral T cells. Thus, the question has arisen whether this intrathymic Th0 cell stage is an authentic intermediate in the pathway for peripheral $CD4^+$ T cell generation, or instead, whether such cells are generated from naive cells in the thymus by secondary events (3, 4). Data we present here show that the $HSA^{low/-}$ cell fraction of thymic $CD4^+$ T cells can also be identified as a $6C10^-$ population and, further, that it is comprised of two subsets resolvable by expression of 3G11: one that resembles peripheral T cells and another that possesses a unique cell surface phenotype, cytokine potential, and TCR Vβ gene family representation.

Materials and Methods

Mice. BALB/cAnN, C57BL/6, and SJL mice were bred and maintained in our animal facility. 2–4-mo-old female BALB/c mice were used in most experiments, except where noted.

Immunofluorescence Staining and Flow Cytometry Analysis and Sorting. Cell preparation for immunofluorescence staining has been described previously (6). Antibodies used here for analysis and sorting are as follows: CD4 (GK1.5), CD8 (53-6), CD3 (500A-A2), HSA (J11d), 3G11 (SM3G11), 6C10 (SM6C10), Vβ8.1,2,3 (F23.1), Vβ8.1,2 (KJ16), Vβ3 (KJ25), Vβ11 (RR3-15), and Vβ6 (RR4-7). Most were purified in our laboratory and then fluorochrome labeled as described previously (7). FL-RR4-7 was purchased from PharMingen (San Diego, CA). Staining data analysis and cell sorting were carried out using a FACStar Plus® (Becton Dickinson Immunocytometry Systems, San Jose, CA) equipped with two lasers, the second laser being a tunable dye laser. Most data were obtained by four-color immunofluorescence analysis as described to the legends to the figures. To quantitate the frequencies of $V\beta^+$ cells in the rare $HSA^-3G11^-CD4^+8^-$ cell subset in the thymus, 2×10^7 cells were stained for each group, and data on 10^5 cells within the $CD4^+CD8^-(HSA + 3G11)^-$ cell gate were collected to the computer, so that 0.1% cells correspond to 100 positive cells (improving statistical validity).

Anti-CD3 Stimulation and Cytokine Assay. $1–2 \times 10^5$ sorted cells were deposited into an anti-CD3 precoated 96-well culture plate (500A-A2, 0.1 μg/0.1 ml/well; no. 3598; Costar, Cambridge, MA) together with 10^5 irradiated (2,500 rad) syngeneic spleen

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cells. Supernatant was harvested at 2 and 4 d after initiation of cell culture and then used for cytokine assay as described elsewhere (6, 8).

Results and Discussion

3G11 ganglioside expression increases as T cells mature in the thymus (9). As shown in Fig. 1 *a*, after selection into the $CD4^+8^-$ stage in the thymus (referred to here as $CD4^+$ T cells), a large fraction of $CD4^+$ T cells (>80%) express 3G11 regardless of HSA levels. However, in contrast to peripheral $CD4^+$ T cells, which are HSA^- and mostly $3G11^+$ (6), we found that between 30 and 50% of cells in the thymic HSA^-CD4^+ T cell fraction are $3G11^-$ (Fig. 1 *a*). Further, the level of several other cell surface molecules also differs within the HSA^- fraction depending on 3G11 expression. The HSA^-3G11^- subset exhibits a unique phenotype; that is, Mel-14^{low}, Thy-1^{low}, CD44 (Pgp-$1)^{high}$, and heterogeneous for CD3, often including cells with low CD3 expression. CD45RB levels are similar to (or slightly higher than) those seen on peripheral native T cells. In contrast, the HSA^- $3G11^+$ subset bears the closest resemblance to the peripheral native $CD4^+$T cell phenotype with comparable levels of expression for all these molecules (not shown).

During the neonatal period when the generation and emigration of the peripheral naive T cell pool is expected to be most active, HSA^- T cells are rare in the thymus (Fig. 1 *b*), while functional HSA^- T cells are already present in the periphery. In contrast with HSA, analysis for 6C10 expression clearly resolves a discrete functional cell population in the thymus at this time. We found that only $6C10^-$ cells, the majority of which are HSA^{low} with some HSA^- (Fig. 1 *c*, *boxed regions*), respond to anti-CD3 stimulation whereas $6C10^+$ cells do not respond, consistent with a previous report using HSA fractionation (3). As Fig. 1 *d* demonstrates, two subsets, $3G11^+$ and $3G11^-$, within this functionally mature $6C10^-$ ($HSA^{low/-}$) cell population can be resolved in the neonatal thymus, similar to adult. These two subsets were identifiable as early as day 1 after birth. Thus, our analysis reveals the continuing presence of two subsets within the functionally mature $CD4^+$ cell population in the thymus from the neonatal period to the adult.

We next investigated the cytokines produced by these mature thymic $6C10^-$ (and HSA^-) subsets (Table 1). As previously shown (5), stimulation of the $HSA^{low/-}$ fraction of thymic $CD4^+$ T cells resulted in secretion of high levels of IL-4, IL-5, and IFN-γ, unlike peripheral T cells. We found that this striking ability to secrete diverse cytokines is largely contributed by the $3G11^-$ fraction. Upon anti-CD3 stimulation, $6C10^-3G11^-$ cells rapidly secrete diverse cytokines, regardless of the animal's age. IFN-γ and IL-5 were also detected in 2-d culture supernatant. Based on this consistent Th0-like cytokine profile for the thymic $6C10^-(HSA^{low/-})$ $3G11^-CD4^+$ subset, we propose to name it "Thy0" (for thymic Th0). In comparison, predominant IL-2 secretion was obtained from the $6C10^-3G11^+$ cell subset in neonatal mice. However, IL-4 was also detected from this fraction in adult mice. Further, some IL-5 (and IFN-γ) was also detectable, although only at later times after stimulation. Sorting for HSA^- cells, which are present at increased numbers in adult thymus, yielded essentially similar results (Table 1), distinguishing them from peripheral naive T cells.

A question arises whether the Thy0 subset might represent an intermediate differentiation stage between HSA^- $3G11^+$ cells in the thymus and those in the periphery. One approach to determining the relationships of T lymphocyte subsets is to examine their respective TCR repertoires. If Thy0 is the direct precursor for the vast majority of peripheral $CD4^+$ T cells, then its TCR gene usage would be expected to be similar and stable with age. However, we found (Fig. 2 and Table 2) that Thy0 in adult mice shows an increasing frequency of TCR $V\beta8^+$ cells with age. In the thymus of adult BALB/c mice, 40–60% of Thy0 ($3G11^-$) are $V\beta8^+$,

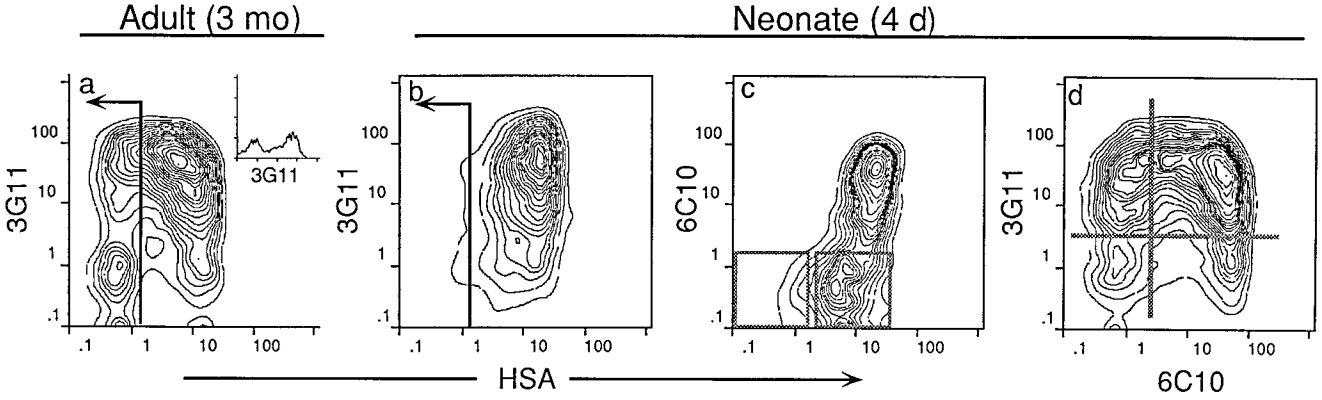

Figure 1. Presence of two subsets in the mature thymic $CD4^+$ T cell fraction resolved by 3G11. All figures display data of the $CD4^+8^-$ analytically gated fraction in thymus of either 3-mo-old (adult; *a*) or 4-d-old (neonatal; *b–d*) BALB/c. (*a*) Adult thymocytes were stained with APC-anti-CD4, PE-anti-CD8, FL-anti-HSA, and Bi-SM3G11 + Texas Red® (TR)-avidin. A histogram showing 3G11 expression within the HSA^- cell fraction (marked on the plot) of adult thymus is shown in the inset. The thymic $CD4^+HSA^-3G11^-$ subset comprises 1–2% of total thymocytes. (*b*) The same analysis with neonatal thymus is shown. (*c*) Cells were stained with APC-anti-CD4, Bi-anti-CD8 + TR-avidin, FL-HSA, and SM6C10 + PE-anti-IgM. HSA^{low} and HSA^- regions are boxed. (*d*) FL-SM6C10 was used in place of FL-HSA. Most $CD4^+8^-$ cell subsets express similar levels of CD3 except for the $6C10^+3G11^-$ cells which are $CD3^-$.

Table 1. *Diverse Cytokine Secretion from Thymic CD4⁺ Cells Predominantly Derives from 6C10⁻ (HSA$^{low/-}$)3G11⁻CD4⁺ Subset (Thy0)*

Age		CD4⁺ cell fractions	Day 2 IL-2	Day 2 IL-4	Day 4 IFN-γ	Day 4 IL-5
			U/ml	*U/ml*	*U/ml*	*pg/ml*
7 d		6C10⁻3G11⁺ thymus	35	<10	<20	<40
		6C10⁻3G11⁻ thymus	153	215	165	1200
3 mo	(Exp. 1)	6C10⁻3G11⁺ thymus	123	192	<20	440
		6C10⁻3G11⁻ thymus	113	229	707	8000
	(Exp. 2)	HSA⁻3G11⁺ thymus	490	280	210	890
		HSA⁻3G11⁻ thymus	130	440	1010	5700
		HSA⁻3G11⁺ spleen*	900	<10	<20	<40

3G11⁺ or 3G11⁻ cells in the 6C10⁻ (or HSA⁻) CD4⁺8⁻ fraction in thymus of BALB/c mice were sorted (see Fig. 1) and stimulated with anti-CD3. 2- and 4-d culture supernatants were tested for the presence of indicated cytokines. Representative data are shown. Exclusive IL-2 secretion from the 6C10⁻3G11⁺ fraction was consistently found in eight experiments with young (<2-wk-old) mice. More than 20 experiments were performed with adult (>3 mo) thymus, where the thymic HSA⁻3G11⁻ cell subset consistently showed the highest levels of IL-4, IFN-γ, and IL-5 secretion compared with cultures of HSA⁻3G11⁺ fraction.

* Data with HSA⁻ spleen cells were obtained by sorting for 6C10⁻3G11⁺ cells (6, 9).

whereas only 25–30% of either 3G11⁺ thymic or splenic CD4⁺ T cells are Vβ8⁺. Reciprocally, the relative frequency of Vβ6 is decreased in Thy0. Vβ3⁺ or Vβ11⁺ cells, with potential for binding to self-ligands on BALB/c cells (endogenously expressed retroviral genome products) (10–12), are not detectable in any cell fraction. No other CD4⁺ or CD8⁺ cell subset in the thymus or peripheral lymphoid organs showed such a bias, with the exception of the thymic CD4⁻8⁻TCR-α/β⁺ subset, as reported previously (Table 2, legend). The recently described Ly-6C⁺ CD4⁺ cell population with Vβ8⁺ cell overrepresentation (13) is contained within the Thy0 subset since we found that these cells are HSA⁻, 6C10⁻, and 3G11⁻, consistent with the Thy0 phenotype (our unpublished observations).

Table 2 also indicates that the frequency of Vβ8⁺ cells is dependent on strain background. In comparison with BALB/c, C57BL/6 mice show less increase of Vβ8⁺ cells in Thy0. Further, in SJL mice, which have a deletion of the Vβ8 gene family (14, 15), Thy0 frequency is reduced (10% of HSA⁻ cell fraction in adult mice) and Vβ8⁺ cells are undetectable (as expected). Furthermore, we found that Vβ8 overrepresentation by Thy0 is age dependent (Fig. 3). In neonatal (<1

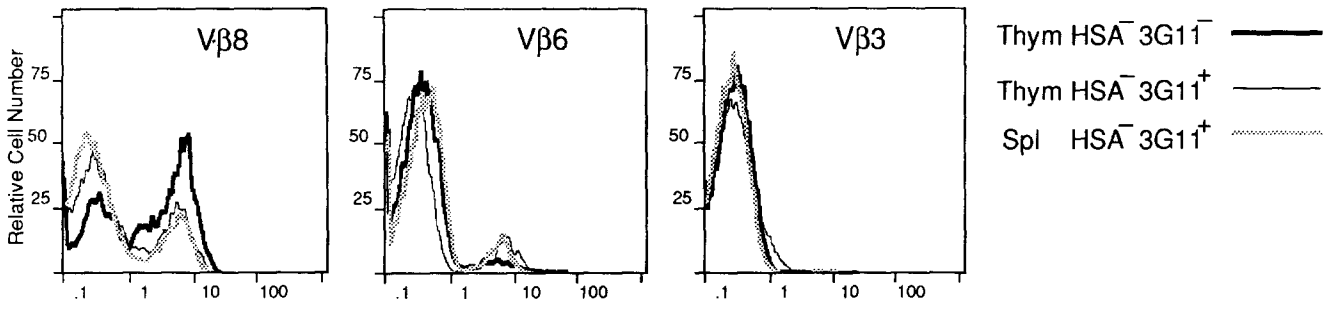

Figure 2. Elevated frequency of Vβ8⁺ cells in Thy0. Adult BALB/c thymocytes and spleen cells were stained with the PE-labeled anti-Vβ antibodies shown (except for RR4-7) together with APC-anti-CD4, FL-anti-CD8 plus FL-anti-HSA, and Bi-3G11+TR-avidin. PE histograms of the 3G11⁺ or 3G11⁻ cells in the CD4⁺CD8⁻HSA⁻ fraction are shown. For Vβ6⁺ cell analysis, FL-RR4-7 was used together with APC-anti-CD4, PE-anti-CD8, and either Bi-SM3G11 plus Bi-HSA (for HSA⁻3G11⁻ cell gate) or Bi-SM3G11 alone (for 3G11⁺ cell gate). The frequencies of Vβ⁺ cells in the 3G11⁺CD3⁺ thymic CD4⁺ cell fractions are the same regardless of HSA levels.

Table 2. *Frequency of Expression of Various Vβ TCR Gene Families in HSA⁻3G11⁺ and 3G11⁻ Thymic CD4⁺ T Cell Subsets*

Strain (*n*)	Thymic ($CD4^+HSA^-$)	Vβ8 (F23.1)	Vβ8.1,2 (KJ16)	Vβ6 (RR4-7)	Vβ3 (KJ25)	Vβ11 (RR3-15)
BALB/c (16)	$3G11^+$	27.4 ± 4.5	20.0 ± 2.5	10.3 ± 0.9	<1.0	<1.0
	$3G11^-$	51.9 ± 7.6	38.0 ± 6.7	5.0 ± 0.8	<1.0	<1.0
C57BL/6 (6)	$3G11^+$	21.9 ± 2.8	14.8 ± 0.9	10.3 ± 3.9	4.5 ± 0.6	5.0 ± 0.1
	$3G11^-$	31.4 ± 3.7	25.0 ± 2.6	6.3 ± 1.3	3.8 ± 0.5	4.0 ± 0.1
SJL (4)	$3G11^+$	<1.0	<1.0	9, 8	6.0 ± 0.0	<1.0
	$3G11^-$	<1.0	<1.0	23, 13	5.5 ± 0.6	<1.0

2–4-mo-old BALB/c, C57BL/6, and SJL mouse thymocytes (numbers of mice in parenthesis) were stained as described in Fig. 2. Vβ6 in SJL is based on two animals. Data shown indicate standard deviation. $V\beta8^+$ cell frequency of other cell subsets in BALB/c mice (data with seven adult mice) are: thymic $CD8^+$ cells (HSA^+, 32 ± 4%; HSA^-, 35 ± 4%), thymic $CD4^-8^-TCR\text{-}\alpha/\beta^+$ cells (67 ± 7%), and splenic $CD4^+$ subsets (Fr. I-V, 25–29%) (6).

wk) mice, the frequency of Thy0 itself is low, and the frequency of Vβ8 within Thy0 is similar to any other $CD4^+$ cell subset. At ~1 mo of age, the increase in $V\beta8^+$ cells becomes evident. The $V\beta8^+$ cell frequency peaks at 2–4 mo of age and declines thereafter. In contrast, the percentage of $V\beta8^+$ cells in the $6C10^-3G11^+$ cell subset in the thymus does not show any significant alteration with ontogeny (Fig. 3). In addition, the distinctive surface phenotype of Thy0 described above is not clear during the neonatal period, but becomes increasingly evident with age. Thy0 in aged mice shows the lowest levels of TCR. In all cases, Th0 type cytokine activity is associated with the Thy0 subset.

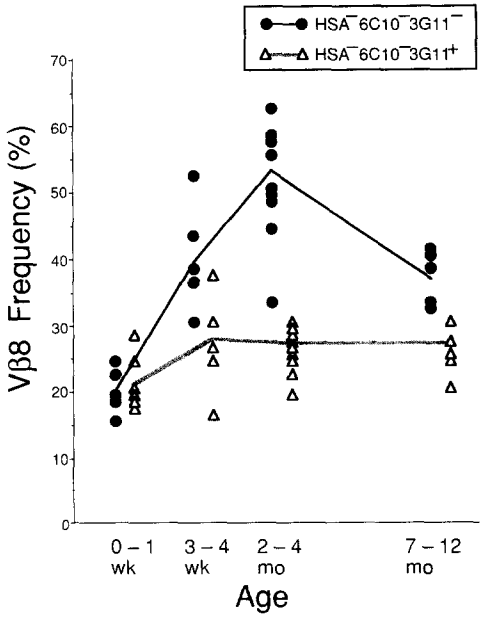

Figure 3. Age-dependent alteration of $V\beta8^+$ frequency in Thy0. BALB/c thymocytes of different ages were stained to obtain the $V\beta8^+$ cell frequency (using F23.1) in the HSA^-3G11^- (Thy0) and $3G11^+$ cell fractions as described in the legend to Fig. 2. For analysis of neonatal mice (<1 wk), FL-SM6C10 staining (in place of FL-anti-HSA) was also carried out. In both analyses (HSA and 6C10) the same results were obtained; i.e., no increase of $V\beta8^+$ cells in the neonatal Thy0 fraction.

Taken together, these data suggest that while $V\beta8^+$ overrepresentation is not an inherent character of Thy0, the mechanism for generating Thy0 is consistent regardless of age or mouse strains. It appears likely that Thy0 accumulates in the thymus due to secondary event(s) starting early in development; thereafter, further cell differentiation continues within this subset. Ontogenic and strain variation could be explained if this Thy0 fraction is comprised of cells at various stages after activation, responding to stimulation by different environmental antigens depending on genetic background. Thy0 is already present early in ontogeny but shows increased levels of $V\beta8^+$ cells only in adult mice. This could be accounted for either by preferential stimulation of $V\beta8^+$ cells in the adult thymus or else by generation of Thy0 cells earlier in development followed by preferential survival of $V\beta8^+$ cells.

Whichever alternative holds, it is interesting to note the phenotypic and functional resemblance of Thy0 to the $CD4^-8^-TCR\text{-}\alpha/\beta^+$ cell subset (double-negative [DN]α/β) in the thymus. DNα/β cells are HSA^- (16, 17) and $3G11^-$ (our unpublished observations), with characteristic levels of expression of cell surface molecules similar to Thy0 described above. Significantly, DNα/β exhibits age-dependent $V\beta8^+$ overrepresentation (17–19) with the appropriate deletion of self-reactive Vβs similar to Thy0 (17), and with a potential to secrete IL-4 and IL-5 cytokines (4). The CD8 gene is demethylated in DNα/β cells, suggesting its previous expression (17). If $DN\alpha/\beta^+$ cells have progressed through a $CD4^+8^+$ stage, then it is reasonable to speculate that Thy0 represents a transient stage in the maturation pathway of $CD4^+$ T lineage cells to $CD4^-8^-$ cells induced by intrathymic stimulation. $V\beta8^+$ overrepresentation is more stable, and often is more profound, in the DNα/β subset than in the $CD4^+$ subset. This could be explained by

progressive loss of CD4 expression and selection for $V\beta8^+$ cells with time, perhaps due to continual stimulation. In this regard, our finding may carry implications for the study of autoimmune *lpr* mice since T-lineage cells proliferating in mice with this defect (*lpr* cells) share features with $DN\alpha/\beta$ cells, as described elsewhere (18, 20).

$CD4^+$ T cells play a key role in the immune system. Recent findings of their functional heterogeneity have evoked considerable attention as to how such subsets are generated and how they relate to $CD4^+$ T cell differentiation and maturation (9). In these studies, defining the precursor population in the thymus that provides the naive $CD4^+$ T cell repertoire in the periphery is an important goal. As yet, our data do not completely exclude the possibility that cells differentiate through the Thy0 stage, wherein the bias of TCR $V\beta$ usage is contributed by only a fraction of cells within Thy0. However, it appears more likely that the $6C10^-$ $3G11^+$ subset represents the immediate precursor population for the peripheral naive cell pool. Although data from the thymic $6C10^-3G11^+$ subset in adult mice did not show absolute IL-2 predominance, this could be explained by contamination of naive cells with an increasing proportion of secondary cells that have yet to lose 3G11. Finding IL-5 or IFN-γ during the later stage of HSA^-3G11^+ cell culture may imply a rapid progression of such cells into the Thy0 fraction. These arguments will be clarified by analysis at the single cell level by work that is in progress. Finally, as recent data have demonstrated, Thy0 can also be exported into the periphery while retaining CD4 expression (21). The functional significance of Thy0 poses an interesting puzzle for further investigation.

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References

1. Scollay, R., P. Bartlett, and K. Shortman. 1984. T cell development in the adult murine thymus: changes in the expression of the surface antigens Ly1, L3T4 and B2A2 during development from early precursor cells to emigrants. *Immunol. Rev.* 82:79.
2. Crispe, N., and M.J. Bevan. 1987. Expression and functional significance of the J11d marker on mouse thymocytes. *J. Immunol.* 138:2013.
3. Wilson, A., L.M. Day, R. Scollay, and K. Shortman. 1988. Subpopulations of mature murine thymocytes: Properties of $CD4^-CD8^+$ and $CD4^+CD8^-$ thymocytes lacking the heat-stable antigen. *Cell. Immunol.* 117:312.
4. Bendelac, A., and R.H. Schwartz. 1991. Th0 cells in the thymus. The question of T helper lineages. *Immunol. Rev.* 123:169.
5. Bendelac, A., and R.H. Schwartz. 1991. $CD4^+$ and $CD8^+$ T cells acquire specific lymphokine secretion potentials during thymic maturation. *Nature (Lond.).* 353:68.
6. Hayakawa, K., and R.R. Hardy. 1988. Murine $CD4^+$ T cell subsets defined. *J. Exp. Med.* 168:1825.
7. Hardy, R.R. 1986. Purification and coupling of fluorescent proteins for use in flow cytometry. *In* Handbook of Experimental Immunology, 4th ed. D.M. Weir, L.A. Herzenberg, C. Blackwell, and L.A. Herzenberg, editors. Blackwell Scientific Publications, Edinburgh, Scotland. Chapter 31.
8. Mosmann, T.R., and T.A.T. Fong. 1989. Specific assays for cytokine production by T cells. *J. Immunol. Methods.* 116:151.
9. Hayakawa, K., and R.R. Hardy. 1991. Murine $CD4^+$ T cell subsets. *Immunol. Rev.* 123:145.
10. Bill, J., O. Kanagawa, D.L. Woodland, and E. Palmer. 1989. The MHC molecule I-E is necessary but not sufficient for the clonal deletion of $V\beta11$-bearing T cells. *J. Exp. Med.* 169:1405.
11. Dyson, P.J., A.M. Knight, S. Fairchild, E. Simpson, and K. Tomonari. 1991. Genes encoding ligands for deletion of $V\beta11$ T cells cosegregate with mammary tumour virus genomes. *Nature (Lond.).* 349:531.
12. Frankel, W.N., C. Rudy, J.M. Coffin, and B.T. Huber. 1991. Linkage of *Mls* genes to endogenous mammary tumour viruses of inbred mice. *Nature (Lond.).* 349:526.
13. Takahama, Y., S.O. Sharrow, and A. Singer. 1991. Expression of an unusual T cell receptor (TCR) $V\beta$ repertoire by Ly-6C subpopulations of $CD4^+$ and/or $CD8^+$ thymocytes. Evidence for a developmental relationship between CD4/CD8 positive $Ly\text{-}6C^+$ thymocytes and $CD4^-CD8^-$ $TCR\alpha\beta^+$ thymocytes. *J. Immunol.* 147:2883.
14. Behlke, M.A., H.S. Chou, K. Huppi, and D.Y. Loh. 1986. Murine T-cell receptor mutants with deletions of β-chain variable region genes. *Proc. Natl. Acad. Sci. USA.* 83:767.
15. Sim, G.K., and A.A. Augustin. 1985. $V\beta$ gene polymorphism and a major polyclonal T cell receptor idiotype. *Cell.* 42:89.

16. Pearse, M., P. Gallagher, A. Wilson, L. Wu, N. Fisicaro, J.F.A.P. Miller, R. Scollay, and K. Shortman. 1988. Molecular characterization of T-cell antigen receptor expression by subsets of $CD4^-CD8^-$ murine thymocytes. *Proc. Natl. Acad. Sci. USA.* 85:6082.
17. Takahama, Y., A. Kosugi, and A. Singer. 1991. Phenotype, ontogeny, and repertoire of $CD4^-CD8^-$ T cell receptor $\alpha\beta^+$ thymocytes. Variable influence of self-antigens on T cell receptor $V\beta$ usage. *J. Immunol.* 146:1134.
18. Fowlkes, B.J., A.M. Kruisbeek, H. Ton-That, M.A. Weston, J.E. Coligan, R.H. Schwartz, and D.M. Pardoll. 1987. A novel population of T-cell receptor $\alpha\beta$-bearing thymocytes which predominantly expresses a single $V\beta$ gene family. *Nature (Lond.).* 329:251.
19. Budd, R.C., G.C. Miescher, R.C. Howe, R.K. Lees, C. Bron, and H.R. MacDonald. 1987. Developmentally regulated expression of T cell receptor β chain variable domains in immature thymocytes. *J. Exp. Med.* 166:577.
20. Cohen, P.L., and R.A. Eisenberg. 1992. *Lpr* and *gld*: single gene models of systemic autoimmunity and lymphoproliferative disease. *Annu. Rev. Immunol.* 9:243.
21. Bendelac, A., P. Matzinger, R.A. Seder, W.E. Paul, and R.H. Schwartz. 1992. Activation events during thymic selection. *J. Exp. Med.* 175:731.