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Positive Selection of V\(\beta\)8+CD4−8− Thymocytes by Class I Molecules Expressed by Hematopoietic Cells  

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Summary

A small subset of T cells of mature phenotype express the \(\alpha/\beta\) T cell receptor, but not CD4 and CD8 coreceptors (\(\alpha/\beta\) double-negative [DN] cells). The repertoire of V\(\beta\) usage of \(\alpha/\beta\) DN cells is strongly biased towards V\(\beta\)8 expression, suggesting that the formation of the population is subject to selection. We now report that deficiency of class I expression leads to a strongly depressed frequency of V\(\beta\)8+ DN cells, but has little effect on V\(\beta\)8− DN cells. Studies of hematopoietic chimeras between class I+ and class I− mice demonstrated that expression of class I molecules by hematopoietic cells is necessary and sufficient for selection of most V\(\beta\)8 DN cells. The lack of a role for class I expression by thymic epithelial cells suggests that the mechanism of selection of these cells by class I differs significantly from the mechanism of selection of conventional T cells. Models to explain the selection of these cells as well as their possible function in vivo are discussed.

In most strains of mice, \(\sim\)15–30% of adult thymic CD4−8− (double-negative [DN]) cells express the TCR-\(\alpha/\beta\) (thymic \(\alpha/\beta\) DN cell) (1–3). These cells resemble mature conventional T cells in their pattern of phenotypic markers (CD2hi, CD5hi, Qa-2+, HSA−, and pgp-1hi). In addition, they can be stimulated by engagement of their TCRs to proliferate and secrete IL-4 (2, 4, 5). Also, like conventional TCR-\(\alpha/\beta\)+ T cells, \(\alpha/\beta\)+ DN thymocytes are susceptible to cyclosporin A−induced arrest during development, suggesting that a TCR engagement event is required for their maturation (6). Unlike conventional T cells, \(\alpha/\beta\)+ DN thymocytes appear rather late in ontogeny (at about the time of birth) (2, 6). It is not known whether \(\alpha/\beta\) DN cells carry out a specific immunological function or, alternatively, represent a byproduct of the developmental process that generates conventional CD4+ or CD8+, TCRhi T cells. A possibly related population of TCR-\(\alpha/\beta\)+ DN cells is enormously expanded in the peripheral lymphoid organs of mice with the inherited lymphoproliferative disorders conferred by the lpr and gld mutations.

Approximately 60% of thymic \(\alpha/\beta\) DN cells express a TCR-V\(\beta\)8 (V\(\beta\)8) chain (1–3, 6, and Fig. 1). In contrast, V\(\beta\)8 is expressed by only \(\sim\)20% of mature thymic and peripheral T cells. The V\(\beta\)8 family comprises three closely related genes: V\(\beta\)8.1, V\(\beta\)8.2, and V\(\beta\)8.3. The disproportionately high frequency of thymic V\(\beta\)8+ DN cells is due primarily to the overexpression of V\(\beta\)8.2 (1, 6, 7). Among conventional T cells, skewed V\(\beta\) repertoires often indicate biased selection dependent upon self-MHC and/or self-superantigen expression. Such selection can be either negative or positive (8–12). Surveys of different mouse strains have failed to establish a link between V\(\beta\)8 overexpression among thymic \(\alpha/\beta\) DN cells and expression of specific MHC haplotypes (1, 6). On the other hand, MHC and superantigen expression can in some instances result in depressed numbers of thymic \(\alpha/\beta\) DN cells expressing certain VBs, mirroring the effect of superantigens upon the development of conventional T cells. This includes V\(\beta\)8.2+ DN cells, which are reduced in frequency in mice that are administered the bacterial superantigen SEB at birth (6). Thus, while there is evidence that self-antigen expression can delete thymic \(\alpha/\beta\) DN cells, there is no evidence that interactions with MHC or MHC-like molecules are required to generate these cells.

A rigorous way to assess the role of MHC molecules in shaping the skewed V\(\beta\) repertoire of thymic \(\alpha/\beta\) DN cells is to examine mice deficient in expression of MHC molecules. Mice homozygous for a defective \(\beta\)2-microglobulin gene (\(\beta\)2m−) are grossly deficient in cell surface expression of class I molecules (13–15). Only very low levels of functional class I molecules have been detected on lymphoid cells of \(\beta\)2m− mice (15, 16). However, these levels are insufficient for positive selection of the vast majority of mature CD8+ T cells, as shown by the 20–50-fold reduction in their frequency in \(\beta\)2m− compared with \(\beta\)2m+ mice (13, 14, 17). Using such mice as well as class II−deficient mice, we have asked what role MHC expression might play in shaping the
Thymocytes were resuspended at 20–25 × 10⁶ cells/ml in 5% FCS, the optimal dilution of anti-CD4 antibody (RL172) and anti-CD8-PE (or anti-CD8-FL) were added to exclude residual CD4⁺ and CD8⁺ T cells, where thymic epithelial cells and not hematopoietic cells play the major role in directing positive selection. These results suggest that production of Vβ⁺ DN cells represents a novel thymus selection pathway.

Materials and Methods

Mice. C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). β₂m mutant mice (+/-) and wild-type littermates (+/+ and +/+ −) were bred in a pathogen-free environment at the University of California, Berkeley. In most experiments the β₂m⁻ mice used were the fifth generation backcross generation of the original 129 strain to C57BL/6. In some experiments (see legend to Fig. 1), third generation backcross of 129 to C57BL/6 and (129 × C57BL/6)F₂,4 animals were also used. β₂m genotype was assayed by Southern blot and PCR (18). All nonchimeric mice were used between 4 and 12 wk of age. Mice deficient for class II expression due to a targeted mutation in the Aβ² gene (19), and backcrossed five times to B6, were purchased from GenPharm International (Mountain View CA).

Antibodies. F23.1 (anti-Vβ8.1, 8.2, 8.3) (20), F23.2 (anti-Vβ8.2) (20, 21), and H57-597-2.1 (anti TCR β) (22) were purified from hybridoma culture supernatants and biotinylated according to standard procedures. Other antibodies staining reagents were obtained from commercial vendors: RM4.4-PE (anti-CD4: Pharmingen, San Diego, CA), 53.67.2-PE (anti-CD8α: Pharmingen), 53.67.2-FL (Becton Dickinson & Co., Sunnyvale, CA), tricolor-streptavidin (APC-SA) (Caltag Laboratories, South San Francisco, CA), and AF6-88.5-FL (anti-K⁺: Pharmingen).

Production of Irradiation Fetal Liver Chimeric Mice. 10⁷ fetal liver cells, obtained from embryonic day 16 fetuses, were injected intra-uterine into groups of mice that had received 980 rad from a 137Cs source ~2 h earlier. All β₂m⁻ recipients and β₂m⁺ fetal liver donors were fifth generation C57BL/6 backcross mice. All β₂m⁻ mice and β₂m⁺ fetal liver donors were inbred C57BL/6 mice. Chimeric mice were housed in a pathogen-free environment before being killed for analysis between 14 and 19 wk after reconstitution.

Antibody plus Complement Depletion of CD4⁺ and CD8⁺ Cells. Thymocytes were resuspended at 20–25 × 10⁶ cells/ml in 5% FCS, the optimal dilution of anti-CD4 antibody (RL172) and anti-CD8α antibody (AD4[15]), and a mixture of rabbit and guinea pig complement. After incubation at 37°C for 40 min, viable cells were recovered by passage over a Ficoll gradient and washed several times before further analysis.

Immunofluorescence Staining and FACS® Analysis. A two-step staining protocol was used to analyze enriched DN thymocytes. In the first step, biotinylated anti-TCR reagent, anti-CD4-PE, and anti-CD8-PE (or anti-CD8-FL) were added to exclude residual CD4⁺ and CD8⁺ cells; the cytotoxic antibodies used to enrich DN thymocytes do not significantly block staining by the anti-CD4 and anti-CD8 immunofluorescence reagents used (data not shown). The second step consisted of tricolor-streptavidin. To discriminate between donor and host-derived cells when chimeric mice were analyzed, anti-K⁺-FL was added to the cocktail of first-step reagents. Suspensions of 10⁵ to 10⁷ cells were stained in a final volume of 25 μl for 20 min on ice. Staining buffer consisted of PBS, 5% FCS, 0.02% NaN₃. Between staining steps, cells were washed two times in 200 μl of staining buffer. Before fixation in 200 μl of 1% paraformaldehyde, cells were washed two times in staining buffer and one time in PBS. Stained cells were stored, foil wrapped, at 4°C until analysis. Two- and three-color analysis was performed on a FACSscan® flow cytometer (Becton Dickinson & Co.). Typically, 1–5 × 10⁶ events were collected per sample. Dead cells and debris were electronically excluded from analysis by forward and side scatter characteristics.

Results

Reduced Frequency of Thymic α/β DN Cells in Class I-deficient Mice. To examine the frequencies of thymic α/β DN cells, DN thymocytes were prepared by cytotoxic elimination of CD4⁺ and CD8⁺ cells. The surviving cells were examined by two-color flow cytometric analysis, with TCR-specific antibodies vs. CD4⁺ and CD8⁺-specific antibodies, to exclude any CD4⁺ or CD8⁺ cells that escaped cytotoxic elimination (see Materials and Methods). The results revealed that the frequency of thymic Vβ⁺ DN cells in MHC class I-deficient (β₂m⁻) mice was reduced approximately fivefold in comparison with β₂m⁺ mice (Fig. 1). Most of the Vβ⁺ cells among thymic α/β DN cells in normal mice are Vβ8.2⁺, prompting us to examine Vβ8.2 expression as well. The frequency of thymic DN cells expressing Vβ8.2 was reduced >10-fold in class I-deficient (β₂m⁻) mice in comparison with β₂m⁺ mice (Fig. 1). The frequency of thymic DN cells expressing any TCR-α/β was reduced only about twofold in β₂m⁻ mice (Fig. 1). Because Vβ⁺ cells account for ~50% of α/β DN cells in normal mice, these results suggest the deficit in the β₂m⁻ mice is primarily in the Vβ8⁺ subset. The sizes of the thymi and the proportion of total DN cells were similar in class I-deficient and normal mice (data not shown). The results demonstrate that
normal cell surface expression of class I molecules is required for the appearance of most thymic Vβ8+ DN cells.

Role of MHC Expression by Hematopoietic Cells in the Appearance of Thymic α/β DN Cells. The processes of positive and negative selection involve intercellular interactions between developing thymocytes and thymic stromal cells, of which there are two broad types: bone marrow-derived hematopoietic cells and thymic epithelial cells. Thymic epithelial cells play the major role in directing positive selection which there are two broad types: bone marrow-derived hematopoietic cells and thymic epithelial cells. Thymic epithelial cells play the major role in directing positive selection (24, 25), and in some instances when positive selection by thymic epithelial cells is prevented (26, 27) (B. J. Fowlkes, personal communication). We asked whether the requirement for class I molecules exhibited by thymic Vβ8+ DN cells could be differentially met by expression on hematopoietic cells vs. thymic epithelial cells.

We used irradiation hematopoietic chimeras to target class I expression to specific tissues. B2m− or B2m+ fetal liver cells were transferred into groups of lethally irradiated B2m− or B2m+ recipients. The thymic epithelial cells in such chimeras are of host origin, whereas almost all the hematopoietic cells are derived from the fetal liver donor. 3–4 mo later the chimeras were killed and the frequencies of donor-derived thymic Vβ8+ DN cells, TCR-α/β+ DN cells, and CD8+ TCR-α/β+ T cells were determined.

Normal hosts reconstituted with class I+ fetal liver cells had a frequency of thymic Vβ8+ DN cells similar to control class I+ mice, demonstrating that these cells can develop normally in chimeras. However, normal hosts reconstituted with B2m− fetal liver cells displayed a low frequency of thymic Vβ8+ DN cells, similar to that of unmanipulated B2m− mice (Figs. 2 A and 3 A). These results indicate that class I expression by hematopoietic cells is necessary for the appearance of most thymic Vβ8+ DN cells. In the same type of chimera, the frequency of CD8+ TCR-α/β+ T cells was as high as in normal control animals (Fig. 3 B) (17), reflecting the lack of a requirement for class I expression by hematopoietic cells for the differentiation of CD8+ TCR-α/β+ T cells.

To ask whether class I expression by thymic epithelial cells is also important to direct the elevated frequency of thymic Vβ8+ DN cells, we analyzed chimeras in which the fetal liver recipients were B2m−. B2m− hosts reconstituted with B2m+ fetal liver displayed an elevated frequency of Vβ8+ DN cells similar to the frequency in control B2m+ mice, despite the lack of class I on host thymic epithelial cells (Figs. 2 A and 3 A, and data not shown). By contrast, B2m− hosts reconstituted with B2m− fetal liver cells displayed a low frequency of thymic Vβ8+ DN cells, similar to that found in control B2m− mice. Development of CD8+ TCR-α/β+ cells was impaired in B2m− recipients, regardless of whether the fetal liver cells expressed B2m, reflecting a requirement for class I expression by thymic epithelial cells for efficient differentiation of these cells (Fig. 3 B) (17). These results indicate that class I expression by hematopoietic cells is both necessary and sufficient for the appearance of elevated numbers of thymic Vβ8+ DN cells. Conversely, class I expression by hematopoietic cells is neither necessary nor sufficient for the development of normal numbers of CD8+ TCR-α/β+ cells.

The substantial drop in the frequency of Vβ8+ DN cells in recipients of B2m− fetal liver cells was paralleled in each case by a modest drop in the frequency of total α/β+ DN cells (Fig. 2 B). As was the case in comparing unmanipulated B2m+ and B2m− mice, the magnitude of the reduction in total α/β+ DN cells was approximately what would be expected if Vβ8+ cells were primarily affected in the chimeras.

In Mixed Chimeras of B2m+ and B2m− Fetal Liver, High Expression of Vβ8+ DN Cells Is Dominant. There are several possible reasons for the reduced frequency of thymic Vβ8+ DN cells in animals containing class I− hematopoietic cells. Class I molecules may be involved in positively selecting thymic Vβ8+ DN cells, resulting in an elevated frequency of these cells. Alternatively, considering that thymic Vβ8+ DN cells are hematopoietic cells, it is possible that the development
of such cells requires that they themselves express class I molecules. A less likely third possibility is that the absence of class I expression by hematopoietic cells "unmasks" an antigen that deletes or prevents the development of Vβ8⁺ DN cells.

These models can be distinguished by examination of mixed fetal liver chimeras in which both β2m⁺ and β2m⁻ hematopoietic cells ccodevelop within the same animal. Therefore, we transferred a mixture containing equal numbers of β2m⁺ and β2m⁻ fetal liver cells to groups of irradiated β2m⁺ recipients. 3–4 mo later, the chimeras were killed and the frequencies of thymic Vβ8⁺ and TCR-α/β⁺ DN cells were determined separately among class I⁺ and class I⁻ populations, with the use of three-color flow cytometry (see Materials and Methods, and Fig. 4 legend). Both class I⁺ and class I⁻ populations exhibited an elevated frequency of thymic Vβ8⁺ DN cells comparable to the levels found in control class I⁺ animals (Fig. 4 and data not shown). Once again, the overall frequency of thymic TCR-α/β⁺ DN cells reflected the lower frequency of Vβ8⁺ DN cells in these animals. Therefore, class I molecules on one cell can select positively for neighboring Vβ8 DN cells that do not themselves express class I. These results strongly support a model in which "positive selection" of Vβ8⁺ DN cells requires recognition of class I molecules expressed by hematopoietic cells.

Class II Mutation Has a Modest Effect on the Frequency of TCR-α/β⁺ DN cells. We examined the status of TCR-α/β⁺ DN cells in mice deficient for class II MHC expression by virtue of a disrupted Aβ gene. The frequencies of TCR-α/β⁺, Vβ8⁺, or Vβ8.2⁺ in the thymic DN population were each reduced by ~40–50% in the class II⁻ deficient mice compared with normal mice (Table 1).

Discussion

Positive Selection of Vβ8⁺DN Cells. The biased expression of Vβ8 by α/β DN cells initially suggested that the repertoire of these cells was formed by specific selection processes. Some recent evidence suggests, in fact, that negative selection of some superantigen-specific cells occurs in this population or a progenitor population, since Vβ11⁺ and Vβ17a⁺ DN cells are specifically reduced in the IIE⁺ strains where superantigen-mediated deletion of conventional T cells bearing these Vβs occurs. Not all superantigens mediated deletion in this population, however, since little or no Mls-1⁻ mediated deletion of Vβ6⁺ or Vβ8.1⁺ DN cells was observed (6, 28–30).

In contrast, no previous evidence has been reported that the overall repertoire of thymic DN cells, and in particular the predominance of Vβ8⁺ cells in the population, is determined by positive selection events. Analysis of α/β DN cells in mice expressing Vβ8⁺ TCR transgenes revealed no requirement for positive selection by the known MHC-restricting elements recognized by the receptors, and no negative selection mediated by expression of the nominal antigens recognized by the transgenic receptors (31, 32). Based on the latter results it has been suggested that α/β DN cells are

Figure 3. The frequencies of thymic Vβ8⁺ DN cells and CD8⁺4⁻ cells show a reciprocal pattern of dependence on class I expressed by thymic epithelial cells vs. thymic hematopoietic cells. (A) One-parameter histograms from three-color analysis showing TCR-β8 staining of CD4⁺ and CD8⁻ depleted thymocytes electronically gated to exclude residual CD4⁺ and CD8⁺ cells. Staining with KFL was used to exclude any residual host-derived cells in (+ → +) and (- → +) chimeras. (B) One-parameter histograms showing staining of total thymocytes, (electronically gated to exclude CD4⁺ cells) with anti-TCR-β antibody. Cells in A and B are from different animals. Chimeric animal designations are as described in the legend to Fig. 2.

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Table 1. Status of TCR-α/β+ DN Thymocytes in Class II-deficient mice

| Mouse | TCR-Vβ(8.1,8.2,8.3) | TCR-αβ |
|-------|---------------------|--------|
| +     | ![Graph A]           | ![Graph B] |
| mix−+ | ![Bar 0.8]          | ![Bar 0.5] |
| + from (mix−+) | ![Bar 0.2] | ![Bar 0.3] |
| - from (mix−+) | ![Bar 0.0] | ![Bar 0.1] |
| B6    | 0 2 4 6 8 10 12 14 16 18 20 | 0 2 4 6 8 10 12 14 16 18 20 |
| % of Thymic CD4+8+ | ![Bar Graph] | ![Bar Graph] |

CD8+ thymocytes, anti-Kb-FL was used to allow independent analysis of βm+ (+ from mix −+) or βm− (− from mix −+) cells. This experiment was repeated two more times with similar results in a separate study where the mice had received intraperitoneal injections of 200 μg of poly(I:C).

The frequency of Vβ8+ DN cells in the thymus is apparently not influenced by class I expression. Since these cells are only modestly reduced in class II-deficient mice, their appearance may be independent of selection by MHC molecules or they may be selected by either class I or class II molecules.

The failure to observe a requirement for positive selection of Vβ8 DN cells in TCR transgenic mice is still unexplained. One possibility is that cells of this phenotype in transgenic mice are not the counterparts of α/β DN cells in normal mice. In fact, based on phenotypic differences it has been suggested that the transgenic Vβ8 DN cells correspond to γ/δ lineage cells that are precluded from expressing γ/δ receptors due to expression of the TCR-α/β transgenes (B. J. Fowlkes, personal communication, and reference 33). The development of most γ/δ lineage cells is not dependent on class I expression (36). Another possibility is that the transgenic Vβ8 DN cells are selected by nonpolymorphic class I molecules distinct from the restricting class I molecule (see below).

The “positive selection” of Vβ8+ DN cells differs in at least one striking respect from positive selection events that control formation of the conventional T cell pool. Whereas thymic epithelial class I expression is both necessary and sufficient for efficient positive selection of CD8+ T cells, it is neither necessary nor sufficient to select high usage of Vβ8+ by DN cells. Instead, hematopoietic cell class I expression is important for selecting these cells. High usage of Vβ8 among class I+ α/β+ DN cells occurs in hematopoietic chimeras containing a mixture of class I+ and class I− cells, demonstrating that the effects of class I expression are mediated by selection rather than by a requirement for Vβ8 DN cells to express class I molecules.

The “positive selection” induced by class I+ hematopoietic cells may reflect differentiation of the cells from immature precursor cells. Alternatively, it is possible that Vβ8+ DN cells mature without selection, and “positive selection” in this system reflects class I-mediated activation and expansion of already mature cells. This would account for our finding that hematopoietic cells, which include APC, mediate selection of Vβ8 DN cells by class I molecules. It would also account for the observation that the frequency of Vβ8+ cells...
in the $\alpha/\beta$ DN population increases for several weeks post-
natally (6). Peptides of endogenous or environmental origin
bound to class I molecules might drive this cellular expansion.

*Are $\nu B8$DN Cells Positively Selected by Nonpolymorphic Class
I Molecules?* The striking difference in cell types mediating
positive selection may indicate that the development of $\nu B8$
DN cells is governed by a signaling mechanism distinct from
that governing the development of conventional T cells, per-
haps reflecting a unique biological role for these cells. Recent
reports describe human $\alpha/\beta$+ DN cell lines with specificity
for the class I-like CD1 molecules (37, 38). Some of these
CD1-reactive $\alpha/\beta$ DN T cell lines were autoreactive (37).
Mycobacterial antigen-specific, CD1β-restricted human $\alpha/\beta$
DN clones have also recently been reported (38).

These results invite speculation that murine $\nu B8$ DN cells
recognize and are selected by a specific set of nonclassical class
I molecules, such as the class Ib molecules, which map telo-
meric to H-2, or the class I-like CD1 molecules, which map
on chromosome 3 in the mouse (39). Several features of the
data concerning $\nu B8$ DN cells are consistent with the possi-
bility that they are selected by a specific set of nonclassical class I molecules: (a) most class Ib and CD1 genes display
limited or no polymorphism (39), which could explain why
the elevated $\nu B8$ usage at a similar extent in mouse
strains that differ at MHC. (b) Recent evidence suggests that
some class Ib molecules present a highly specific set of pep-
tides (40). Specific peptide/MHC complexes have been shown
to stimulate T cells with restricted $\nu C$ or $\nu B$ usage (41, 42).
It seems plausible, therefore, that stimulation or selection of
$\alpha/\beta$ DN cells by a specific complex of peptide and nonclas-
classical class I molecule could account for the predominance of
$\nu B8$+ cells in the population. (c) The class Ib and CD1 mol-
ecules do not have associated (39). $\beta_{a m}$ is generally required for
functional cell surface expression of class I molecules. This
would fit with the deficiency of $\nu B8$ DN cells in $\beta_{a m}$-
deficient mice.

Assuming that $\nu B8$ DN cells generally recognize class Ib
or CD1 molecules, and are positively selected by them, it
is still unclear what their biological role might be. One pos-
sibility, originally proposed for various subsets of $\gamma/\delta$ T cells
(43-45), is that $\alpha/\beta$ DN cells recognize stress-induced au-
tologous antigens bound to class I-like molecules. Alterna-
tively, perhaps one of the nonclassical class I molecules is
specialized to present specific antigens, corresponding to a
specific class of pathogen, to $\alpha/\beta$ DN cells. A precedent is
the recent evidence that the H-2M3 class I molecule is special-
ized to present N-formylated bacterially derived peptides to
T cells (40, 46).

*Precursor Cells of $\nu B8$ DN Thymocytes.* Our studies are
also relevant to the question of the identity of progenitor
cells of $\alpha/\beta$ DN cells. Studies of the methylation patterns of
the CD8 gene in thymic $\alpha/\beta$ DN cells suggest passage
of thymic $\alpha/\beta$ DN cells through an intermediate CD8+
stage (6, 47). In $[\beta_{a m}^{-} \rightarrow \beta_{a m}^{+}]$ chimeras, thymic $\nu B8$+
DN cells do not pass through a CD8- stage while the develop-
ment of CD4+8+ TCR- cells is strongly impaired. Converse-
ly, in $[\beta_{a m}^{-} \rightarrow \beta_{a m}^{+}]$ chimeras, most $\nu B8$+ DN cells fail to appear while CD4+8+ TCR- cells appear
at normal frequencies. The inverse correlation between
the appearance of CD4-8+ TCR- cells and $\nu B8$+ T cells in $\nu B8$+
DN cells argues against a precursor-product relationship be-
tween them, and suggests that if $\nu B8$+ cells do in fact pass through a CD8- intermediate, the transient CD8-4-
TCR stage and/or the CD4+8+ TCR stage would be
likely candidates. Alternatively, it remains possible that the
$\nu B8$ DN cells arise specifically from a subset of CD8+
CD4- TCRβ+ cells that are positively selected by hemato-
poietic cells (17).

Of interest is the relationship of class I-selected $\nu B8$ DN
cells to recently described Ly6C+ thymocytes, which are
found in all four thymic subsets defined by expression of CD4
and CD8, and which display a $\nu B$ repertoire skewed toward
$\nu B8$ (45). We find that in normal B6 mice ~66% of $\nu B8$+
DN thymocytes express Ly6C. In $\beta_{a m}$ mice the frequen-
cies of both the Ly6C+ $\nu B8$+ and Ly6C- $\nu B8$+ DN cells are
reduced, although the reduction is more pronounced in the
Ly6C+ $\nu B8$+ subset (data not shown). These results suggest
that both Ly6C+ and Ly6C- subsets of $\nu B8$ DN cells are
class I dependent.

Obviously, much remains to be learned regarding the origin,
selection, and function of $\alpha/\beta$ DN cells. Analysis of various
other mutant mouse strains, including mice deficient for CD4,
CD8, or both class I and class II should provide additional
clues about the selective events that act on the cells and their
lineage precursors. These results will be important in de-
veloping a complete understanding of the biological role of
these intriguing cells.

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