Brief Definitive Report

CD4^+8^- Thymocytes Bearing Major Histocompatibility Complex Class I-restricted T Cell Receptors: Evidence for Homeostatic Control of Early Stages of CD4/CD8 Lineage Development

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Summary

During thymus development CD4^+ CD8^- precursor cells differentiate into mature CD4^+ and CD8^- T cells expressing T cell receptors (TCR) that recognize foreign antigens in association with major histocompatibility complex (MHC) class II or I molecules, respectively. Studies with TCR transgenic mice have shown that the accumulation of mature CD4^+ and CD8^- thymocytes is strongly skewed by the MHC restriction specificity of the TCR, thus suggesting that commitment of CD4^+ CD8^- precursors to the CD4 or CD8 lineage is a direct consequence of TCR/MHC interactions. However, we show here that CD4^+ cells expressing an inappropriate (MHC class I-specific) TCR appear transiently in the neonatal thymus of TCR transgenic mice and can also be found in the periphery of adult TCR transgenic recombination-deficient SCID mice. These data argue that the early stages of CD4 and CD8 lineage development in the thymus are (at least in part) controlled by homeostatic mechanisms independent of appropriate TCR/MHC interactions.

The α/β TCR on mature CD4^+ and CD8^- T cells recognizes foreign antigenic peptides in association with MHC class II or I molecules, respectively. During thymic development, CD4^+8^- and CD4^-8^+ single-positive (SP) cells arise from immature (TCR^-) CD4^-8^- precursors via a TCR^+ CD4^+8^+ double-positive (DP) intermediate stage (1). Experiments with TCR transgenic mice have shown that the development of mature CD4 SP or CD8 SP cells is strongly influenced by the MHC restriction specificity of the TCR (2). Thus, CD8 SP or CD4 SP cells accumulate preferentially in the thymus and periphery when the transgenic TCR is restricted by MHC class I or II molecules, respectively. However, the cellular and molecular basis of this phenomenon remains obscure.

Two models have been proposed to explain commitment of DP thymocytes to the CD4 or CD8 lineages. According to the instructional model (3), TCR engagement of MHC class I or II molecules on DP thymocytes leads to specific downregulation of CD4 or CD8, respectively. Alternatively, the stochastic model (4) predicts that CD4 or CD8 downregulation occurs randomly among DP thymocytes, but that only those SP cells expressing TCR of appropriate MHC specificity are selected to survive. Recent studies of T cell development in double-transgenic mice expressing both a MHC class I-specific TCR and a constitutive CD8 gene were interpreted to favor the instructional model (5, 6). However, we show here that CD4 SP cells expressing an inappropriate (MHC class I-specific) TCR appear transiently in the neonatal thymus of TCR transgenic mice and can also be found in the periphery of adult TCR transgenic recombination-deficient SCID mice. These findings have important implications for the control of T cell lineage commitment and homeostasis during development.

Materials and Methods

Mice. Mice bearing transgenic TCR α (Vα2-JαTA31) and TCR β (Vβ8.1 DJβ2.4) chains on a C57BL/6 (H-2^b) background were as described previously (7). In some experiments, neonatal TCR transgenic mice were injected daily with 50 μg protein A-purified mAbs directed against I-A^b (1D9; reference 8) according to published procedures (9). Injected and control mice were killed after 9 d. TCR transgenic SCID mice expressing H-2D^b were obtained by crossing C57BL/6 TCR transgenic mice with homozygous C.B-17 (H-2^a) SCID mice (IFFA-CREDO, L'Arbesle, France). TCR transgenic F1 mice were backcrossed to SCID and F2 progeny were typed for transgenic TCR, H-2D^b, IgM, and B220 in peripheral blood. H-2D^b-expressing TCR transgenic SCID mice with no detectable IgM^- or B220^- cells were then further analyzed at 6 wk of age.

mAbs and Flow Cytometry. Rat IgG mAbs directed against CD3 (17A2), CD4 (GK-1.5), CD8 (53-6.7), Vα2 (B20.1; reference 10), Vβ8 (KJ16), and heat-stable antigen (HSA; M1-69) were employed for staining as described (11). In some experiments rat IgM mAbs RL172.4 (anti-CD4) and 3.168.1 (anti-CD8) were used in the presence of rabbit complement to deplete thymus or lymph node sus-
pensions of CD4+ or CD8+ cells, respectively. Treated cells were routinely found to lack detectable CD4 (or CD8) expression.

Double staining of thymus or lymph node was performed with PE-conjugated anti-CD4 and biotinylated anti-CD8 (revealed by avidin-TANDEM; Southern Biotechnology, Birmingham, AL). For most triple-staining experiments, cells were first incubated with unconjugated mAbs against CD3, V\textsubscript{a}2, V\textsubscript{b}8, or HSA (revealed by FITC-conjugated goat anti-rat Ig). After a blocking step with normal rat Ig, the staining was completed for CD4 and CD8 as described above. In the case of CD4- or CD8-depleted cell suspensions, triple staining was performed with unconjugated anti-V\textsubscript{o}2 (revealed by FITC-conjugated goat anti-rat Ig), biotinylated anti-V\textsubscript{b}8 (revealed by avidin-TANDEM), and PE-conjugated anti-CD4 or anti-CD8.

Data shown are representative of at least three mice tested individually. For all histograms gated on CD4 SP or CD8 SP cells, background controls were obtained by omitting the primary Ab. In the case of cytograms, regions were established such that negative control staining fell within the lower left quadrant.

Results and Discussion

We examined the ontogeny of expression of a transgenic TCR \(\alpha\) chain (\(\alpha_T\)) and \(\beta\) chain (\(\beta_T\)) derived from a CD8 SP T cell clone specific for lymphocytic choriomeningitis virus presented in the context of H-2D\(^b\). Positive selection of T cells bearing this transgenic TCR and the concomitant skewing of adult thymocytes to the CD8 SP lineage had previously been shown to depend on the presence of the H-2D\(^b\) molecule (7, 12). As shown in Fig. 1, CD8 SP cells expressing the transgenic TCR were already present in increased numbers (20%) in the thymus of young (10-d) C57BL/6 (H-2\(^b\)) mice. Some CD4 SP thymocytes (~3%) were also present in these mice; however, these cells expressed predominantly endogenously rearranged TCR chains. It is important to note that, in contrast to other MHC class I-restricted TCR transgenic models (2), these CD4 SP thymocytes express endogenous TCR \(\beta\) (\(\beta_E\)) as well as TCR \(\alpha\) (\(\alpha_E\)) chains (Fig. 1).

Surprisingly, when we examined TCR expression in CD4 SP thymocytes of neonatal (1-d) C57BL/6 TCR transgenic mice, we found that >80% of these cells expressed both \(\alpha_T\) and \(\beta_T\) at the same high levels as the CD8 SP thymocytes (Fig. 1). The proportion of \(\alpha_T^-\beta_T^+\) CD4 SP cells declined rapidly so that by 10 d after birth \(\alpha_T^-\beta_T^+\) cells were virtually absent from the CD4 SP population, which contained approximately the same proportion of cells that stained singly with the V\textsubscript{o}2- and V\textsubscript{b}8-specific antibodies (used to detect \(\alpha_T\) or \(\beta_T\)) as nontransgenic C57BL/6 littermates (Fig. 2). As this \(\alpha_T\beta_T\) combination is restricted by MHC class I (H-2\(^b\)) in mature T cells, it seems likely that these neonatal CD4 SP thymocytes have made the transition from the DP to SP stage in the absence of an instructional signal given by appropriate TCR/MHC class II interaction. Since CD4 SP cells expressing the transgenic TCR were virtually undetectable in the periphery and thymus of older mice, we conclude that they were unable to survive and hence were rapidly replaced by CD4 SP cells expressing endogenously rearranged (presumably MHC class II-restricted) TCR.

Figure 1. Expression of transgenic (H-2D\(^b\)-restricted) \(\alpha/\beta\) TCR by CD4 SP and CD8 SP thymocytes from neonatal C57BL/6 mice. Neonatal (day 1) and day 10 total thymocytes were double stained with mAbs against CD4 and CD8 (top). In addition, CD8- and CD8-depleted thymocyte populations were prepared and triple stained with mAbs directed against \(\alpha_T\), \(\beta_T\), and either CD4 or CD8, respectively. (Middle) CD4 staining pattern of CD8-depleted thymocytes. (Bottom) Expression of \(\alpha_T\) and \(\beta_T\) by the CD4 SP subset (gated as shown in middle) or the CD8 SP subset (gated similarly on CD8; data not shown).
Figure 3. Detectable expression of transgenic TCR on CD4 SP thymocytes is prolonged in mice treated postnatally with anti-MHC class II mAbs. Thymocytes from control or anti-1-Ab-injected C57BL/6 TCR transgenic mice were prepared and analyzed for CD4, CD8, αT, and βT expression on day 9 as detailed in Fig. 1. CD8 SP thymocytes from both groups of mice homogeneously expressed αT and βT (not shown).

To confirm this latter point, neonatal TCR transgenic mice were injected daily with purified mAb directed against I-AL. As expected from previous studies with normal mice (9), this treatment selectively reduced the percentage of CD4 SP thymocytes by 10-fold after 9 d (Fig. 3). Significantly, a large proportion (~50%) of surviving CD4 SP thymocytes from anti-I-Ab-treated mice coexpressed αT and βT, whereas no αT+βT+ CD4 SP thymocytes were found in uninjected controls (Fig. 3). Taking into account the ~10-fold difference in cellularity between neonatal and day 9 thymus, these data indicate that CD4 SP thymocytes bearing MHC class I-restricted TCR continue to be produced at a relatively constant (low) rate during the early postnatal period but are diluted out by other CD4 SP cells (with endogenously rearranged α or β chains) that require TCR/MHC class II interactions for their survival and/or expansion.

In interpreting these data two important caveats must be considered. First, it could be argued (by analogy with other TCR transgenic models) that the αT+βT+ CD4 SP thymocytes in fact also express αE at low levels and thus have been selected to the CD4 lineage as a result of interaction of αE βT TCR with MHC class II. However, as noted above, our transgenic model is unique in that the surviving CD4 SP thymocytes in older H-2Kb mice express only αE and βE and hence even αE βT TCR (which are detected transiently in lymph node on day 7; see Fig. 2) cannot be stably selected to the CD4 lineage.

A second issue concerns the stage of development represented by the αT+βT+ CD4 SP thymocytes. It is formally possible that these cells are an immature (i.e., pre-DP) subset, analogous to the CD3+CD4 SP cells observed in low frequency in normal mice (13). We consider this highly unlikely, however, since kinetic studies of thymus regeneration after cortisone treatment in these TCR transgenic mice indicate that CD4 SP cells can only be detected after the appearance of DP thymocytes (11). Furthermore, light scatter analysis of early postnatal αT+βT+ CD4 SP thymocytes indicates that these cells are relatively small (Fig. 3; and data not shown), whereas CD3-CD4 SP thymocytes in normal mice are large cycling blasts (13).

Given the apparently nondirected transition from DP to CD4 SP thymocyte in the neonatal C57BL/6 TCR transgenic mice, we decided to examine TCR expression in recombination-deficient SCID TCR transgenic mice, where the likelihood of selection on endogenously rearranged α or β chains is further reduced. Surprisingly very few mature CD8 SP thymocytes were seen in young (<4 wk) H-2Db-expressing nonleaky SCID TCR transgenic mice, perhaps due to delayed functional maturity of the SCID thymic epithelium (14). However, in all 6-wk-old mice examined, we found the expected skewing to the CD8 SP lineage (15) in the thymus.

Figure 4. Expression of transgenic TCR and HSA by CD4 SP and CD8 SP cells from recombination-deficient SCID mice. Thymocytes or lymph node cells from TCR transgenic SCID mice were double stained for CD4 and CD8 (top). In addition, CD8-depleted thymocytes or lymph node cells were triple stained with mAbs against CD4, αT, and βT. (Middle) Expression of αT and βT by the gated CD4 SP subset. (Bottom) TCR transgenic SCID lymph node cells were triple stained with mAbs against CD4, CD8, and either CD3, βE, αE, or HSA. Histograms represent fluorescence of the latter markers gated on either CD4 SP or CD8 SP subsets. The dotted lines indicate control staining.
(Fig. 4), with only 1-2% CD4 SP cells. These few CD4 SP thymocytes expressed both αT and βT. In blood, lymph node (Fig. 4), and spleen, however, there were substantial numbers of CD4 SP cells, all of which expressed βT and at least 40% of which expressed αT. The remaining CD4 SP αT- cells, which have also been reported in other TCR transgenic SCID mice (15), presumably express αT, heavily selected for by introduction of the transgenic TCR, despite the nonleakiness of the SCID mice in terms of B cell development. Alternatively, it cannot be excluded that these cells express a βT dimer as described in other experimental situations (16, 17).

The CD3, αT, and βT expressions were lower on the CD4 SP SCID lymph node cells than on the CD8 SP cells, and the CD4 SP cells expressed higher levels of heat-stable antigen (HSA) (Fig. 4), consistent with a less mature phenotype (18). Thus, in a situation where endogenous TCR gene rearrangement is a very rare event, we found CD4 SP cells bearing a transgenic TCR α and β chain combination that is restricted by a MHC class I molecule. Interestingly, rare CD4 SP thymocytes and peripheral T cells with a similar phenotype (TCRlow HSAhigh) have been recently described in MHC class II-negative mice produced by gene disruption (19, 20).

Several models of thymic differentiation would be consistent with the transient existence of CD4 SP thymocytes expressing a TCR that cannot be positively selected by MHC class II. As discussed previously by Robey et al. (4, 5) and Borgulya et al. (6), such cells could arise stochastically if random downregulation of either CD4 or CD8 at the DP stage were followed by selective survival of SP cells expressing TCR of appropriate MHC specificity. One prediction of this model (at least in its simplest form) is that it should be possible to rescue CD4 SP thymocytes with inappropriate (MHC class I) TCR specificity by providing the cells with CD8. However, attempts to directly confirm this hypothesis by crossing TCR transgenic mice with other transgenic mice expressing CD8 constitutively have thus far not been successful (5, 6). Alternatively, it could be postulated that DP thymocytes are already precommitted to either the CD4 or CD8 SP lineages at an early stage (i.e., before TCR engagement). In this case, survival and further maturation of these committed DP precursor cells along the chosen SP lineage might normally depend (at least in part) upon positive signaling via appropriate TCR/MHC and/or CD4-CD8/MHC interactions. In situations where there are no (or few) DP thymocytes fulfilling these criteria, homeostatic control mechanisms may nevertheless guarantee the production of a limited number of SP thymocytes with inappropriate TCR specificity for MHC, as is the case in the SCID and neonatal TCR transgenic mice described here.

Finally, it should be emphasized that the data presented here pertain only to early stages of T cell development in the thymus and hence do not in any way contradict the concept that positive selection of functional T cells is ultimately dependent upon appropriate TCR/MHC interactions. Indeed, the rapid disappearance of CD4 SP thymocytes bearing MHC class I-restricted transgenic TCR in the neonatal thymus and their replacement by other CD4 SP thymocytes expressing endogenous MHC class II-restricted TCR is entirely consistent with positive selection models.

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