Intrathymic Differentiation of Vγ3 T Cells

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Summary

Whereas there is considerable information on the phenotypic and functional maturation of T cell receptor (TCR) α/β thymocytes, comparatively little is known of the maturational processes that affect development of TCR-γ/δ thymocytes. One class of γ/δ T cells, those bearing the Vγ3 gene product, are generated only during the early fetal stages of thymic development, and then migrate to the skin. Here we examine the intrathymic differentiation of these $V\gamma3^+$ cells. The earliest Vγ3 cells to appear in the thymus expressed low levels of TCR ($V\gamma3^{low}$) and high levels of heat stable antigen (HSA). Over the next few days, $V\gamma3^+$ thymocytes appeared which expressed high levels of TCR ($V\gamma3^{high}$) and very low levels of HSA. The antigens CD5, CD45RB, and MEL14 were also differentially expressed on $V\gamma3^{low}$ versus $V\gamma3^{high}$ thymocytes, but the shift in expression was the opposite as compared with immature and mature TCR-α/β thymocytes. Transfer experiments of sorted $V\gamma3^{low}/HSA^{high}$ thymocytes to SCID thymic lobes showed that these cells were indeed the precursors of $V\gamma3^{high}/HSA^{low}$ thymocytes. The phenotype of the $V\gamma3^{high}$ thymocytes was similar to that of the postthymic $V\gamma3^+$ cells found in the skin of adult mice. The differentiation of $V\gamma3^{low}$ in $V\gamma3^{high}$ thymocytes was also observed in fetal thymic organ culture. Addition of cyclosporin A (CsA) to these cultures had little effect on the appearance of $V\gamma3^{low}/HSA^{high}$ cells, but blocked the appearance of $V\gamma3^{high}/HSA^{low}$ cells. These results show that, like α/β T cells, $V\gamma3^+$ thymocytes differentiate from TCR^{low} precursors to cells with a mature phenotype and that CsA inhibits this transition.

The phenotypic changes that are associated with intrathymic differentiation of T cells that express TCR-α/β are well known (for a review see reference 1). The least mature cells are TCR^-, and express low levels of CD5 and high levels of heat stable antigen (HSA). They progress into $CD4^+CD8^+$ intermediate cells which express low levels of TCR, and finally into mature $CD4^+$ or $CD8^+$ cells that have elevated TCR levels, express high levels of CD5, and have lost expression of HSA. During this differentiation, and particularly at the $CD4^+CD8^+$ TCR^{low} stage, the functional repertoire of α/β T cells is shaped by both positive and negative selection.

Comparatively little is known of the developmental stages of TCR-γ/δ cells, or of the phenotypic changes that accompany their maturation. Several studies have demonstrated that the immunosuppressive drug cyclosporin A (CsA) has little or no effect in vivo or in vitro on the generation of γ/δ thymocytes (2–4). Since CsA profoundly blocks the maturation of α/β thymocytes (2–6), the reported lack of effect on generation of the bulk of γ/δ cells suggests either that these cells appear as fully mature cells, or at the very least, that the signals required for their maturation are very different from those of α/β cells. It has also been reported that the level of TCR expression on developing γ/δ thymocytes, unlike α/β thymocytes, does not increase during development (7). However, more recently, it has been shown that the majority of γ/δ cells in the thymus of adult mice express high levels of HSA, whereas those in peripheral lymphoid organs fail to express this antigen (8). This suggests that immature HSA^+ cells might mature into HSA^- cells.

A factor that complicates interpretation of the existing data with regard to thymic maturation of γ/δ cells is that these T cells can be divided into at least two distinct classes based on V gene usage, junctional diversity, ontogenic origin, and tissue localization (9). Cells that express invariant TCR composed of Vγ3 or Vγ4 paired with Vδ1-Dδ1-Jδ2 chains are the first T cells to appear during thymic development, and they migrate to the epidermis or the female reproductive epithelium. Later in thymic development, cells appear that express a TCR composed of Vγ2 or Vγ1 paired with a variety of Vδ products and containing extensive junctional diversity. They comprise the vast majority of γ/δ T cells in the peripheral lymphoid tissues. These two classes of γ/δ T cells, those with invariant and those with variable TCR, may well

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be subject to different developmental pathways and selective pressures.

In this report, we examine the phenotypic changes that accompany thymic development of T cells that express the invariant Vγ3/Vδ1 pair. We demonstrate that the earliest Vγ3 cells to appear in the fetal thymus express low levels of TCR and high levels of HSA. These cells are gradually replaced by cells that express high levels of TCR and are HSA^{low}. We further show that the same shift in phenotype occurs in fetal thymic organ culture (FTOC), and that addition of CsA blocks the appearance of the $V\gamma3^{high}/HSA^{low}$ population. The phenotype of the descendants of the thymic Vγ3 cells, the dendritic epidermal cells, is essentially the same as that of $V\gamma3^{high}$ thymocytes. Together, these results suggest that Vγ3 cells undergo a maturational process in the thymus that results in elevation of TCR expression and loss of HSA expression, and that this maturation involves signals that are susceptible to inhibition by CsA.

Materials and Methods

Animals. BALB/c and SCID mice, originally purchased from Proefdierencentrum (Catholic University Leuven, Leuven, Belgium) and Iffa Credo (L'Arbresle, France), respectively, were bred in our laboratory. Alternatively, BALB/c mice were purchased from Simonsen (Gilroy, CA). To obtain fetal mice, mice were mated for 15 h and the fetuses were removed at the indicated days of fetal development (plug date, day 0).

Media and Reagents. IMDM and RPMI-1640 medium supplemented with L-glutamine and 25 mM Hepes (Gibco Laboratories, Paisley, Scotland) were obtained commercially. Penicillin (100 IU/ml), streptomycin (100 μg/ml), 0.03% L-glutamine, and 10% heat-inactivated (30 min, 56°C) FCS (all products from Gibco Laboratories) were added. This medium will be referred to as complete medium.

CsA used was the commercially available Sandimmun (Sandoz, Basel, Switzerland). This contains 50 mg/ml CsA in 33% ethanol/67% cremophor. It was diluted in complete medium to obtain the indicated concentrations of CsA. An equivalent amount of the solvent (33% ethanol/67% cremophor) was added to the control cultures.

FTOC. Thymic lobes were removed from fetuses at day 14 of gestation. A FTOC was set up in IMDM complete medium as described previously (10) with addition to the culture medium of CsA or an equal amount of the solvent (control). After 7 or 12 d, a cell suspension was prepared from the thymic lobes by placing the lobes in culture medium without FCS and disrupting the tissue with a small Potter homogenizer. Cell viability was determined by trypan exclusion.

Transfer of Vγ3 Thymocytes to SCID Thymic Lobes. Thymocytes were prepared from fetal day (FD) 17 BALB/c embryos and labeled with FITC-conjugated anti-Vγ3 mAb and with biotin-conjugated anti-HSA mAb (second step streptavidin–PE). The cells were sorted on a FACS Star Plus® (Becton Dickinson Immunocytometry Systems, Mountain View, CA) in a $V\gamma3^{low}/HSA^{high}$ and a $V\gamma3^{high}/HSA^{low}$ population. A single FD14 SCID thymic lobe was placed into each well of a microwell plate (Terasaki, 60 × 10 μl; Nunc, Roskilde, Denmark) containing 25 μl of IMDM complete medium with 6,500 unsorted FD17 thymocytes, with 6,500 sorted $V\gamma3^{low}/HSA^{high}$ cells or with 3,250 sorted $V\gamma3^{high}/HSA^{low}$ cells. From each SCID embryo, one thymic lobe was incubated with Vγ3 cells as described above, the other lobe was incubated in medium alone. The plate was then inverted and cultured for 24 h in a humidified petri dish. The lobes were removed after this period, placed onto a filter, and set up as organ cultures and cultured for another 3 d. Afterwards, a cell suspension was prepared of each thymic lobe separately, cells were labeled with FITC-conjugated anti-Vγ3 mAb and with biotin-conjugated anti-HSA mAb (second step streptavidin–PE), and analyzed.

Dendritic Epidermal Cells. Skin was removed from killed adult BALB/c mice, digested with trypsin, and epidermal cells were isolated by Ficoll-Hypaque density gradient centrifugation (11). Cells were cultured overnight in RPMI-1640 complete medium with 100 U/ml rIL-2 (kindly provided by Dr. M. Gately, Hoffmann-La Roche, Nutley, NJ) to allow reexpression of the TCR that is cleaved off by the trypsin treatment.

Antibodies. mAb used for staining were PE and FITC conjugates of anti-CD4 and -CD8 purchased from Caltag Laboratories (San Francisco, CA) and Becton Dickinson & Co. FITC-conjugated anti-Vγ3 (hamster hybridoma F536) (12) and anti-α/β (hamster hybridoma H57-597) (13); biotin-conjugated anti-CD5 (Becton Dickinson & Co.) and anti-CD45RB (hybridoma 16A, rat IgG2a) (14); and unlabeled anti-HSA (J11d, rat IgM) (15) and MEL14 (rat IgG2a) (16).

Flow Cytometric (FCM) Analysis. Cells were incubated with saturating amounts of antibodies for 1 h at 4°C. After washing, biotin-conjugated antibodies were revealed with second step streptavidin–PE (Becton Dickinson & Co.). Unlabeled antibodies were revealed with anti-rat IgG PE (Tago, Inc., Burlingame, CA). 10% normal rat serum was added to the samples stained with anti-rat Ig PE for all subsequent incubations of cells with antibodies. Cells were analyzed for fluorescence using a FACScan® flow cytometer (Becton Dickinson & Co.) with the Lysis program for data analysis as described (Hewlett-Packard Co., Palo Alto, CA) (10). Propidium iodide (Sigma, Deisenhofen, Germany) was added to the cells (2 μg/ml) just before FCM analysis. Gating was done on propidium iodide negative cells to exclude the dead cells.

Results

Two Populations of Vγ3 Thymocytes: One with a Low, One with a High TCR Density. $V\gamma3^+$ cells appear in the thymus during early fetal development (12) and migrate to the skin before birth (17, 18). As a consequence, the differentiation from immature to mature Vγ3 thymocytes, if present at all, has to occur in a limited time period of 3–5 d in the fetal thymus. In the TCR-α/β lineage, differentiating cells gradually upregulate their TCR density. Therefore, we first analyzed freshly prepared thymocytes from FD15 to FD18 embryos for the density of the TCR on the Vγ3 cells. From the results (Fig. 1), it is clear that the major part of TCR-Vγ3 thymocytes at FD15 had a low TCR density. At FD16, some Vγ3 cells expressed the TCR at a higher level. At FD17 and FD18, two populations of Vγ3 thymocytes could be recognized: one with a low, and one with a high density of the TCR (these two populations will be further referred to as $V\gamma3^{low}$ and $V\gamma3^{high}$, respectively). These results suggest that $V\gamma3^{low}$ thymocytes differentiate to $V\gamma3^{high}$ cells.

Phenotypic Analysis of $V\gamma3^{low}$ and $V\gamma3^{high}$ Thymocytes. As in the TCR-α/β lineage (19), also in the TCR-γ/δ lineage HSA has been suggested to be a marker of immature, func-

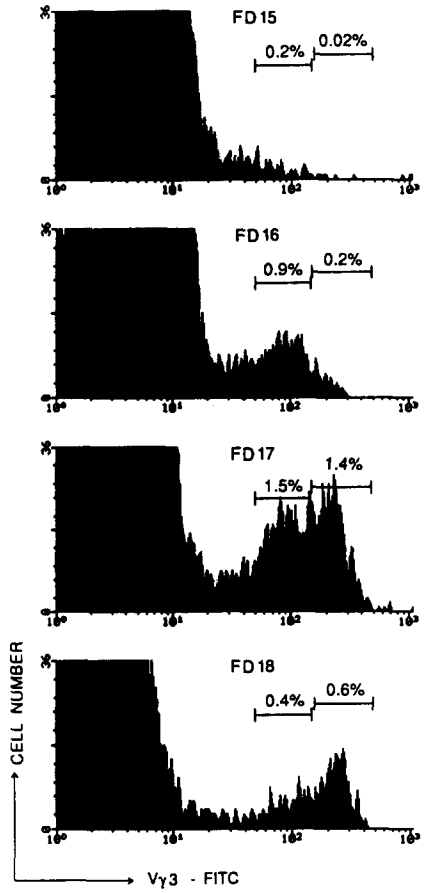

Figure 1. TCR density on developing Vγ3 thymocytes. Freshly prepared thymocytes from FD15 to FD18 embryos were labeled with FITC-labeled anti-Vγ3 mAb. Propidium iodide was added just before FCM analysis. Fluorescence histograms are shown for propidium iodide negative cells.

tionally incompetent cells, whereas HSA^- cells are mature and functionally competent (8, 20). The results of double color fluorescence analysis show that the major part of the $V\gamma3^{low}$ cells were bright for HSA staining, whereas $V\gamma3^{high}$ cells were mainly HSA low (Fig. 2). In addition, we analyzed expression of other surface antigens that have been described to be differentially expressed on immature and mature TCR-α/β thymocytes. For comparison, adult thymocytes were stained with anti-TCR-α/β mAb in combination with the same antibodies. With regard to Vγ3 thymocytes, expression of the antigens CD5 and MEL14 was downregulated, whereas CD45RB was upregulated. The shift in expression of these antigens on differentiating TCR-α/β thymocytes was the opposite. Other markers that are differentially expressed on immature and mature TCR-α/β thymocytes, namely CD2, CD28, and CD69, showed no difference in expression between the two populations of Vγ3 thymocytes (data not shown). At no time during fetal development did more than 15% of $V\gamma3^+$ thymocytes express CD4 or CD8, (data not shown). This low level expression of CD4 and CD8 was observed on both the $V\gamma3^+$ populations. Thus, it appears that $V\gamma3^+$ thymocytes do not pass through a $CD4^+CD8^+$ double position stage. From these data, we conclude that (*a*) two populations of TCR-Vγ3 cells are present in the thymus: one with an immature and one with a mature phenotype; and that (*b*) some phenotypic characteristics of this maturation are different from those in TCR-α/β thymocytes.

$V\gamma3^{low}/HSA^{high}$ Thymocytes Are the Precursors of $V\gamma3^{high}/HSA^{low}$ Thymocytes. To determine whether $V\gamma3^{low}/HSA^{high}$ thymocytes are the precursors of $V\gamma3^{high}/HSA^{low}$ thymocytes, we sorted these two populations from FD17 thymocytes and transferred them to FD14 SCID thymic lobes. The

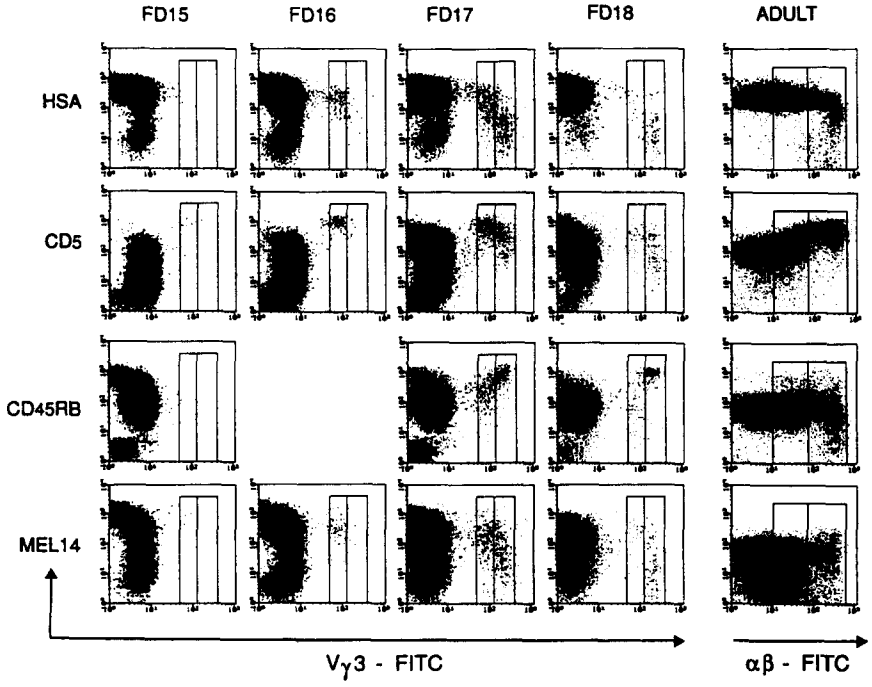

Figure 2. Phenotypic differences between the two populations of Vγ3 thymocytes. Freshly prepared thymocytes from FD15 to FD18 embryos were colabeled with anti-Vγ3 mAb and with several other antibodies as indicated. In parallel, adult thymocytes were labeled with anti-α/β mAb plus the same antibodies. Propidium iodide was added just before FCM analysis. Fluorescence dot plots are shown for propidium iodide negative cells. The HSA expression was obtained by labeling the cells with J11d anti-HSA mAb, but the same results were obtained with B2A2 and M1/69 anti-HSA mAb (data not shown).

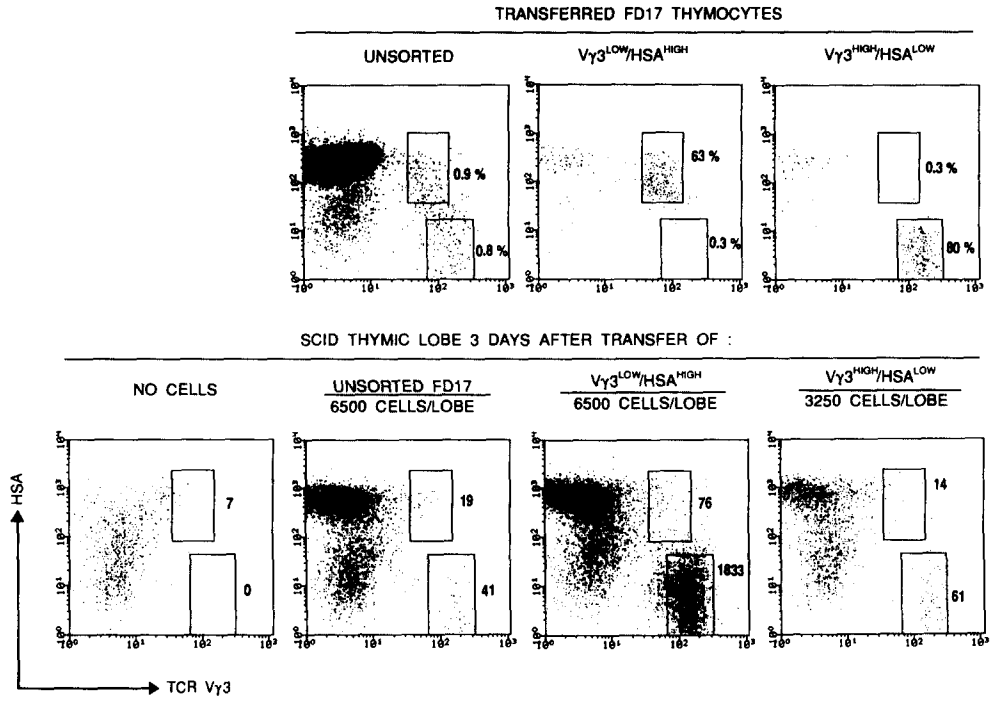

Figure 3. Transfer of sorted Vγ3 thymocytes to SCID thymic lobes. FD17 BALB/c thymocytes were colabeled with FITC-labeled anti-Vγ3 mAb and with biotinylated anti-HSA mAb (second step streptavidin-PE). $V\gamma3^{low}/HSA^{high}$ and $V\gamma3^{high}/HSA^{low}$ cells were sorted. FD14SCID thymic lobes were incubated in a hanging drop with medium alone, with 6,500 unsorted FD17 thymocytes, with 6,500 sorted $V\gamma3^{low}/HSA^{high}$ cells, or with 3,250 sorted $V\gamma3^{high}/HSA^{low}$ cells for 1 d. Lobes were cultured in FTOC for an additional 3 d. Cell suspensions were prepared from each lobe separately and they were labeled with FITC-labeled anti-Vγ3 mAb and with biotinylated anti-HSA mAb (second step streptavidin-PE). (*Top*) Dot plots of unsorted and sorted populations of FD17 BALB/c thymocytes. The numbers are the percentages of $V\gamma3^{low}/HSA^{high}$ and $V\gamma3^{high}/HSA^{low}$ cells. (*Bottom*) Dot plots of SCID lobes after FTOC. Numbers are the cell numbers per lobe of $V\gamma3^{low}/HSA^{high}$ and $V\gamma3^{high}/HSA^{low}$ cells. Propidium iodide was added just before FCM analysis. Dot plots are shown for propidium iodide negative cells. The results shown are representative for two experiments.

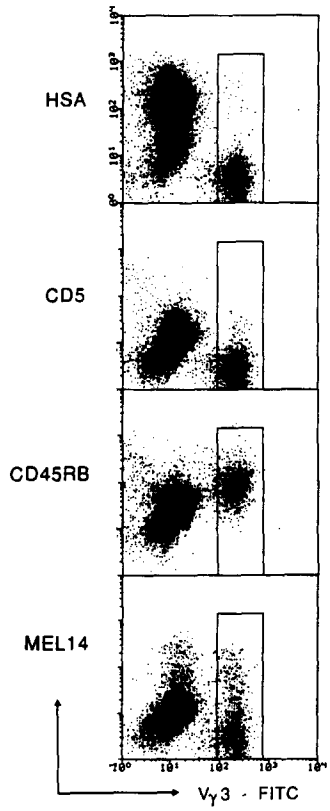

Figure 4. Phenotypic analysis of skin-located $V\gamma3^+$ T cells from adult mice. Epidermal cells were prepared from the skin of adult mice and were colabeled with anti-Vγ3 and with several other antibodies as indicated. Propidium iodide was added just before FCM analysis. Dot plots are shown for propidium iodide negative cells.

upper panel of Fig. 3 shows the FACS® analysis of unsorted FD17 thymocytes and of the two sorted populations. The lower panel of Fig. 3 shows the FACS® analysis of the SCID thymic lobes 3 d after transfer of these thymocyte populations. No Vγ3 cells could be detected in SCID thymic lobes cultured in medium alone. A low number of both $V\gamma3^{high}/HSA^{low}$ and $V\gamma3^{low}/HSA^{high}$ cells was clearly present in lobes that were incubated in a hanging drop with 6,500 unsorted FD17 thymocytes. These unsorted thymocytes were also labeled with anti-Vγ3 mAb and with anti-HSA mAb before the transfer. 3 d after transfer of sorted $V\gamma3^{low}/HSA^{high}$ cells, a high number of $V\gamma3^{high}/HSA^{low}$ cells could be recovered from the lobes. The main contaminating cells in the sorted population were $V\gamma3^-$ cells. These cells can not account for the generation of $V\gamma3^{high}/HSA^{low}$ cells as only a low number of these cells is detected after transfer of unsorted thymocytes (98% of these cells are $V\gamma3^-$). The appearance of $V\gamma3^{high}/HSA^{low}$ cells in SCID thymic lobes after transfer of sorted $V\gamma3^{low}/HSA^{high}$ cells could also not be due to contamination of these sorted cells with $V\gamma3^{high}/HSA^{low}$ cells. First of all, only few, if any, $V\gamma3^{high}/HSA^{low}$ cells were present in the sorted $V\gamma3^{low}/HSA^{high}$ population. In addition, these few contaminating cells cannot explain the high number of $V\gamma3^{high}/HSA^{low}$ cells after culture. Transfer of a high number of sorted $V\gamma3^{high}/HSA^{low}$ cells only yields a low number of these cells after culture. From other experi-

ments, we know that $V\gamma3^{high}/HSA^{low}$ thymocytes, contrary to $V\gamma3^{low}/HSA^{high}$ cells, die within 12 h when cultured in cell suspension (data not shown). Thus, the $V\gamma3^{high}/HSA^{low}$ cells may perish in the hanging drop culture or fail to enter the thymic lobe. This transfer experiment directly demonstrates that $V\gamma3^{low}$/ HSA^{high} thymocytes are indeed the precursors of $V\gamma3^{high}/HSA^{low}$ thymocytes.

The Phenotype of Skin-derived Vγ3 T Cells Is Similar to that of $V\gamma3^{high}$ Thymocytes. Since we have previously demonstrated that $V\gamma3^+$ fetal thymocytes are the precursors of $V\gamma3^+$ T cells in the epidermis (17), we also performed a phenotypic analysis of these cells. As shown in Fig. 4, these cells are negative for HSA and CD5, positive for CD45RB, and mainly negative for MEL14. Thus, the phenotype of the epidermal $V\gamma3^+$ T cells is essentially the same as that of $V\gamma3^{high}$ thymocytes.

CsA Blocks Differentiation of Vγ3 Thymocytes. As CsA has been described to block the development of α/β thymocytes, we examined its effect on differentiation of Vγ3 thymocytes. Pregnant mice were injected with CsA to study its effect on embryonic thymic development. However, as was frequently observed, this treatment caused growth retardation of the embryos, and sometimes even resulted in their death. For these reasons, experiments were done in FTOC. The results are shown in Table 1. It is shown, as described earlier, that 1 μg/ml CsA blocked development of single CD4 thymocytes. Concerning the effect on Vγ3 thymocytes, CsA treatment had little effect on the cell number of immature Vγ3 cells. However, generation of mature Vγ3 cells was largely inhibited. These results show that maturation from $V\gamma3^{low}/HSA^{high}$ to $V\gamma3^{high}/HSA^{low}$ thymocytes involves signals that can be inhibited by CsA.

Table 1. *Effect of CsA on Vγ3 Maturation*

	CsA concentration				
Cells/lobe	Control	0.16	0.4	1	2.5
			μg/ml		
Total	225,000	127,000	100,000	70,000	45,000
$CD4^+$	22,500	5,080	2,810	3,460	2,100
$CD4^+CD8^+$	112,500	36,410	33,010	24,400	10,520
$CD4^-CD8^-$	49,500	56,410	39,150	27,470	24,190
$CD8^+$	40,500	29,100	25,030	14,670	8,190
$V\gamma3^{low}/HSA^{high}$	3,820	4,190	4,200	4,410	2,200
$V\gamma3^{high}/HSA^{low}$	4,950	3,420	1,700	560	360

FD14 thymic lobes were cultured in a FTOC with the indicated concentrations of CsA. After 12 d, cell suspensions were prepared and the percentages of the different subpopulations were determined by FCM analysis. Data are expressed as the total cell number per lobe or the cell number per lobe for each subpopulation.

Discussion

We have shown that the first TCR-Vγ3 cells to appear in the fetal thymus are $V\gamma3^{low}$ cells and that they are gradually replaced by $V\gamma3^{high}$ cells. This upregulation of the TCR on γ/δ cells during thymic differentiation has not been previously discussed. We demonstrate that $V\gamma3^{low}$ thymocytes express high levels of HSA, whereas $V\gamma3^{high}$ cells are HSA^{low}, suggesting that $V\gamma3^{low}$ cells are immature cells, $V\gamma3^{high}$ cells are mature. Although this phenotypic change of differentiating Vγ3 thymocytes mimics the change associated with maturation of TCR-α/β thymocytes, there are clearly differences for other phenotypic characteristics of the maturation of TCR-Vγ3 versus TCR-α/β thymocytes. We directly demonstrate that $V\gamma3^{low}/HSA^{high}$ thymocytes are the precursors of $V\gamma3^{high}/HSA^{low}$ thymocytes and that the phenotype of these latter cells is essentially the same as that of their descendants in the skin, showing that indeed a maturational process is occurring. This is supported by the fact that CsA blocks the shift from $V\gamma3^{low}/HSA^{high}$ to $V\gamma3^{high}/HSA^{low}$ cells in FTOC.

Our CsA data are in contrast to previous reports that concluded that CsA did not affect the generation of γ/δ T cells (2–4). However, these studies dealt only with the effect of CsA on the yield of the total γ/δ population, and did not examine the effects on maturation of particular γ/δ subsets. Our conclusion is that at least one class of γ/δ T cells, those found in the adult skin and expressing an invariant Vγ3/Vδ1 TCR, undergoes maturation in the early fetal thymus, and that this maturation is susceptible to inhibition by CsA.

The maturation of Vγ3 thymocytes is phenotypically similar to the maturation of α/β thymocytes in that it is accompanied by an increase in levels of expression of the TCR and loss of expression of HSA. Antibodies against CD5, CD45RB, and MEL14 differentially stained the $V\gamma3^{low}$ and the $V\gamma3^{high}$ population, but the shift in expression was the opposite as compared with α/β thymocytes. We can only speculate on the reason for this, but it may reflect differences in function of mature α/β and γ/δ T cells, rather than differences in maturational mechanisms. MEL14 is a homing receptor for lymphocytes migrating to peripheral lymph node high endothelial venules (16). Vγ3 thymocytes migrate to the skin (17, 18), and a homing receptor has not been described. The ligand for CD5 has recently been described to be the B cell surface protein CD72 (21). It was suggested that the CD5–CD72 interaction is important in activation and proliferation of T cells by antigen-presenting B lymphocytes. Since skin-located Vγ3 T cells recognize antigens expressed on keratinocytes but not B cells (22), CD5 interactions may not be important for the function of mature Vγ3 cells, but rather may play a role in their early thymic differentiation. Another phenotypical difference between development of α/β thymocytes and Vγ3 thymocytes is their CD4-CD8 expression. Whereas the majority of α/β thymocytes are CD4-CD8 double positive at an intermediate stage in their intrathymic differentiation, we could never detect more than 15% $CD4^+CD8^+$ double positive Vγ3 thymocytes, in either the $V\gamma3^{low}/HSA^{high}$ population, or in the $V\gamma3^{high}/HSA^{low}$

population. These findings are in agreement with those recently described by Fisher et al. (23).

It is well accepted that maturation of α/β thymocytes involves rescue by positive selection of clones expressing a TCR with the potential for recognition of foreign peptides in the context of self-MHC gene products. Thus, for α/β cells, engagement of the TCR during positive selection has two effects: shaping of the repertoire by selection of particular TCRs, and stimulation of maturation of immature cells by TCR-mediated signal transduction. Whether both these processes occur during maturation of γ/δ thymocytes is not clear. Positive selection on class I MHC is not required for all γ/δ cells, since the distribution and function of most γ/δ cells, including the Vγ3 cells in the epidermis, is normal in β_2-microglobulin–deficient mice (8, 24, 25). It has been demonstrated that class I MHC expression is necessary for development of $\gamma\delta$ cells in mice transgenic for a class I MHC–specific TCR of the variable type, utilizing Vγ2 (20, 26). However, in no instance has an allele-specific requirement for MHC products been demonstrated. Concerning the invariant Vγ3 and Vγ4 T cells of the skin and reproductive epithelium, as well as their precursors in the fetal thymus, identical junctional sequences are found in MHC-disparate mice (27, 28). Although data have been reported that seem to support a role for positive selection in shaping the TCR repertoire of invariant γ/δ T cells (29), other data fail to support this conclusion (9). Two recent reports provide strong support for the notion that the invariant repertoire is largely shaped intracellularly by the recombinase machinery rather than by cellular selection (30, 31). In mice unable to express cell surface γ/δ TCR because of disruption of the Cδ gene, the frequency of canonical Vγ3 and Vγ4 rearrangements was no different than in control mice (30). Similarly, canonical Vγ3 and Vδ4 rearrangements in transgenic mice bearing recombination substrates containing termination codons were found at frequencies similar to those of the endogenous genes (31). Since in neither case was the product expressed at the cell surface, cellular selection is effectively excluded as a major force in shaping the repertoire.

Our results clearly demonstrate that V$\gamma3^+$ thymocytes undergo a phenotypic maturation, and that there is a requirement for CsA-sensitive signals, most likely generated by the TCR, in this maturation. This process is highly reminiscent of positive selection in α/β T cell development. However, since the TCR repertoire of the V$\gamma3^+$ cells appears to be determined by intracellular mechanisms, TCR-mediated signaling in these cells may serve merely to test the developing thymocytes for successful surface expression of a TCR whose composition and junctional sequence have been predetermined, and ensure the survival and maturation of these cells. This dual requirement for a stem cell–directed rearrangement process checked by a receptor-mediated signal could explain the observation that both fetal stem cells and the fetal thymic microenvironment are required for the generation of V$\gamma3^+$ T cells (32, 33).

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