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Early Ontogeny of Thymocytes in Pigs: Sequential Colonization of the Thymus by T Cell Progenitors

Marek Šinkora,2,3* Jirí Šinkora,3* Zuzana Reháková,* and John E. Butler†

Successive colonization of the thymus by waves of thymocyte progenitors has been described in chicken-quail chimeras and suggested from studies in mice. In swine, we show that the first CD3ε-bearing thymocytes appear on day 40 of gestation (DG40). These early thymocytes were CD3εab+ and belonged to the γε T cell lineage. Mature CD3εabαβ thymocytes were observed 15 days later (DG55), and their occurrence was preceded by the appearance of CD3εεε thymocytes (DG45). Thereafter, we observed transient changes in thymocyte subset composition (DG56-DG74), which can be explained by a gap in pro-T cell delivery to the thymus. This delivery gap corresponds with the expression of the pan-leukocyte CD45 and pan-myelomonocytic SWC3a markers in fetal liver and bone marrow and is probably caused by shifting of primary lymphopoiesis between these organs. Therefore, we conclude that the embryonic thymus is colonized by at least two successive waves of hemopoietic progenitors during embryogenesis and that the influx of thymocyte progenitors is discontinuous. Surface immunophenotyping and cell cycle analysis of thymocyte subsets allowed us to compare thymocyte differentiation in pigs with that described for rodents and humans and to propose a model for T cell lymphopoiesis in swine. We also observed that the porcine IL-2Rα (CD25), a typical differentiation marker of pre-T cells in mice and humans, was not expressed on thymocyte precursors in pigs and could only be found on mature thymocytes. Finally, we observed a subset of TCRγδ+ thymocytes that were cycling late during their development in the thymus. The Journal of Immunology, 2000, 165: 1832–1839.

In comparison to other species, swine express a number of unpredicted differences in the T cell peripheral pool that may reflect differences in T lymphocyte ontogeny and differentiation. Swine, together with other ungulates and birds, have an abundance of γε T lymphocytes in their peripheral lymphoid pool (1–4). Furthermore, a substantial number of resting αβ T lymphocytes in porcine periphery is CD4+CD8− double positive (DP)4 (3–9). TCRγδ+CD2+ lymphocytes are numerous in pigs (3, 4), and there is a large number of CD8αα-bearing cells that includes NK cells, γε T cells, and at least two subsets of αβ T cells (10). Despite these unique features of peripheral T cells, thymocytes in young and adult pigs resemble those in other species, and their characteristics can be summarized in the following manner. The majority of thymocytes is DP, while double negative (DN, CD4−CD8−) and single positive (SP, CD4+CD8− or CD4+CD8+) subsets are less frequent (3, 8–12). The majority of DP thymocytes bears no or very little CD3, and although some DP cells can be found among large, blast-like cells, most of them belong to the well-known population of small cortical DP thymocytes (2, 3). CD3+ porcine thymocytes differ in their staining intensity such that small TCRγδ+ thymocytes express CD3 at low to medium density and belong to the αβ T cell lineage (3). In contrast, medium-sized thymocytes express CD3 at medium to high density and may belong to either the TCRαβ or the TCRγδ lineage (2, 3). Finally, the expression of other T cell markers is also consistent with findings in other mammalian species, i.e., all DP and SP thymocytes are positive for CD5 and CD6 (9, 12), and almost all are positive for CD1 and CD2 (7, 8). In contrast, the majority of DN thymocytes expresses CD1 at low density (7, 8), while a minor subset is negative for CD2, CD5, and CD6 (8, 9, 12). All DP and DN thymocytes are believed to represent less mature phenotypes. This is supported by the findings that they express lower amounts of both CD5 and CD6 than more differentiated SP thymocytes (9, 12). It has also been shown that almost all CD8+ positive cells in the porcine thymus express the CD8αβ heterodimers, while a substantial proportion of the CD8-bearing cells in the periphery expresses the CD8αα homodimers (10).

In this report, we describe the use of various leukocyte surface markers to analyze the development of thymocytes in pig fetuses. Our results suggest that the embryonic thymus is colonized by progenitors in at least two successive waves during embryogenesis. Moreover, we show that the expression of CD25 does not appear to be required for successful generation of normal T cells in pigs.

Materials and Methods

Animals

Animals used in the study were derived from Minnesota miniature pigs by repeated crossing with outbred black Vietnam-Asian and white Malaysian-derived pigs and selected for high fecundity and small body size. All sows were serum negative for common swine pathogens. Fetuses were obtained by hysterectomy under systemic halothane-oxygen anesthesia. Gestation age was calculated from the day of mating. All experiments were approved by the Ethical Committee of the Institute of Microbiology, Czech Academy of Sciences, according to guidelines in the Animal Protection Act.
Preparation of cells

Cell suspensions from thymus and fetal liver were prepared in RPMI 1640 medium supplemented with 2% FCS (2% FCS-RPMI; Sigma, St. Louis, MO) by careful teasing of tissues using two forceps. Bone marrow cells were flushed from tibiae and/or femur with cold PBS. Cells from early bone marrow were isolated by collagenase digestion. Briefly, the excised organs were transferred into a digestion medium (2% FCS-RPMI with 100 U/ml collagenase type V; Sigma), and incubated for 30–60 min on an orbital shaker at 37°C. All cell suspensions were filtered through a 40 µm nylon mesh and washed twice in cold PBS containing 0.1% sodium azide and 0.2% gelatin (PBS-GEL). Erythrocytes were removed from the pelleted cells using hypotonic lysis by treating them with 25 ml of distilled water for 30 s followed by osmotic reconstitution with 2 x PBS. Finally, the cells were washed twice in cold PBS-GEL, counted, and adjusted to a density of 5 x 10^6 to 1 x 10^7 cells per ml.

Immunoreagents

The following mouse anti-pig mAbs were used as primary immunoreagents: anti-CD3ε (PPT3, IgG1 or PPT6, IgG2b), anti-TCRγδ (PPT17, IgG1 or PPT16, IgG2b), anti-CD4 (10.2H2, IgG2b), anti-CD8 (76-2-11, IgG2b), anti-CD25 (K231.3B2, IgG1), anti-CD45 (K252.1E4, IgG1, pan-leukocyte Ag), anti-IgM (L14G1, IgG1), and anti-SWC3a (74-22-15; IgG2b; panel of monoclonal Ag) (13). Anti-CD4, anti-CD8, and anti-CD45 mAb were also labeled with biotin-N-hydroxysuccinimide ester (Vector Laboratories, Burlingham, CA) according to a protocol recommended by the manufacturer.

F(ab')2 of goat polyclonal Abs (pAb) specific for mouse Ig subclasses (Southern Biotechnologies Associates, Birmingham, AL) labeled with FITC or PE were used as secondary pAbs. Biotinylated primary mAb were visualized using a streptavidin-PE conjugate or using a streptavidin-Cy5-Chrome complex (St-Cy5); both streptavidin reagents were fluorochromes in two- and three-color staining experiments. Damaged and

dead cells were excluded from analysis using propidium iodide fluorescence. A FACSort doublet discrimination module was used in DNA content analysis that allowed single-cell events to be discriminated from doublets and higher multiplets.

Results

Thymocyte subsets in pigs

Thymic cell subsets in pigs were characterized by mAb specific for porcine CD45, SWC3a, CD4, CD8, CD3ε, TCRγδ, and IgM. Two-color staining for the pan-leukocyte CD45 and the pan-myelomonocytic SWC3a markers allowed discrimination of lymphoid cells (CD45+SWC3a−) from nonlymphoid ones, i.e., myelomonocytic (CD45+SWC3a+) cells and nonleukocytes (CD45−SWC3a−). Thymic B cells represented a minor population (<1%) during the fetal ages studied (data not shown). These were identified by surface IgM expression. All IgM+CD45−SWC3− leukocytes were hypothesized to belong to the T cell lineage, their number was normalized to 100%, and they are hereafter referred to as thymocytes.

Analysis of CD4, CD8, and CD3ε expression on porcine thymocytes identified at least: 1) three subsets of large-sized CD3ε1 (CD4+CD8+, CD4−CD8+, and CD4+CD8−; Fig. 1B), 2) two subsets of small-sized CD3ε2 (CD4−CD8+ and CD4+CD8−; Fig. 1C), 3) two subsets of small-sized CD3ε3 (CD4+CD8+ and CD4−CD8−; Fig. 1D), 4) two subsets of large-sized CD3ε4 (CD4+CD8+ and CD4−CD8−; Fig. 1F) thymocytes. We also observed that γδ thymocytes were medium-sized (Fig. 1G), and most of them did not express both CD4 and CD8 (Fig. 1H). However, a small number of γδ thymocytes with the CD4+CD8− or CD4−CD8+ surface phenotype was always present (Fig. 1H). It is also important to note that TCRγδ expression appeared with no observable CD3εlow or TCRγδlow transitional stage (Fig. 1G). Because the proportion of CD3εhighCD4+CD8− thymocytes (Fig. 1F, lower left quadrant) corresponded to the number of TCRγδhighCD4−CD8− cells (Fig. 1H, lower left quadrant), we have concluded that all thymic DN cells expressing high levels of the CD3/TCR complex are γδ thymocytes. Because of the current unavailability of a mAb specific for TCRαβ, putative αβ thymocytes in this work were detected as CD3εhighTCRγδ− cells. These are hereafter referred to as αβ thymocytes. Because TCRγδhigh DN cells represent γδ thymocytes, the majority of αβ thymocytes was SP cells, which is typical for mature αβ T cells (Fig. 1F).

As in other species, small thymocytes were a rather homogenous population, most of them being DP cells (Fig. 1, C–E). By contrast, a population of large cells differed markedly in surface phenotype (Fig. 1, B and F). Fig. 2 shows the analysis of large CD4+ and/or CD8− thymocytes in terms of their expression of CD3ε and TCRγδ. This analysis suggests that, among CD3ε− precursors, CD8+ SP cells (Fig. 2C, upper left quadrant) greatly outnumbered CD4+ SP cells (Fig. 2D, upper left quadrant). Moreover, only low CD4 expression was found on CD3ε−CD4−CD8− precursor cells (Fig. 2D, upper left quadrant). Once again, when the number of cells in the lower right quadrants of Fig. 2, C–F was compared, results suggested that CD3ε− DN thymocytes belong to the γδ T cell lineage.

Thymocyte subsets during prenatal ontogeny in pigs

The first leukocytes in the thymic rudiment appeared at the end of the first trimester of intrauterine life (day 38 of gestation, DG38). These were triple negative (TN), and the majority expressed the pan-myelomonocytic Ag SWC3a (Fig. 3). One fourth of all leukocytes, however, were SWC3a− cells; these may have represented early thymocyte progenitors (Fig. 3A).
We first found CD3ε expression on DG40 in the fetal thymus (Fig. 4A). At this time, TCRγδ+ thymocytes accounted for all CD3εhigh cells, and CD3εlow thymocytes were almost absent (Fig. 4B). Until DG50, TCRγδ+ thymocytes represented almost exclusively all CD3εhigh thymocytes (Fig. 4B). Thereafter, the proportion of TCRγδ+ thymocytes decreased as TCRαβ+ cells became the predominant CD3εhigh thymocyte subset (DG55; Fig. 4B). Figure 4, A and B shows gated CD4+ cells, while dot plots C and D shows gated CD8+ cells.

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After DG56–58, we observed major changes in the composition of both leukocyte and lymphoid subsets. First, the proportion of large CD3ε+ thymocytes increased, and CD3εhigh cells became the dominant thymocyte subset (Fig. 4A). This temporary dominance
persisted between DG56 and DG74 and included both the TCRαβ⁺ and the TCRγδ⁺ thymocytes (Fig. 4B). Consistent with this observation, the proportion of CD3⁺low and CD3⁺thymocytes decreased (Fig. 4, A and C, respectively). Second, the percentage of SP and DN thymocytes increased (Fig. 5, B–D), and the number of DP cells decreased (Fig. 5A). Finally, large thymocytes (Fig. 6A) and leukocytes with a typical myelomonocytic phenotype (CD45⁺ SWC3a⁻) became more apparent during this period (Fig. 6B). The change in the number of fetal thymic leukocytes was most pronounced between DG58 and DG67 (10 days in total). Thereafter, the frequency of individual subsets slowly returned to the levels observed on DG55 (Figs. 4–6).

After DG74, only minor changes in the composition of thymocyte subsets were observed. The majority of CD3⁺-positive thymocytes was small CD3⁺low cells while a minor proportion was CD3⁺high cells (Fig. 4A). The proportion of TCRαβ⁺ thymocytes was always slightly higher than that of TCRγδ⁺ thymocytes. As regards the expression of CD4 and CD8, the majority of thymocytes was DP cells while the minority were DN or SP cells (Fig. 5). The number of myelomonocytic cells and B cells was negligible (Fig. 6).

FIGURE 3. The phenotype of the first thymic leukocytes recovered. Cells isolated from the rudimentary thymus of 38-day-old fetuses were double-stained with combinations of anti-CD45 and anti-SWC3a (A), anti-CD3e and anti-TCRγδ (B), or anti-CD8 and anti-CD4 (C) mAb. The majority of leukocytes (CD45⁺ cells) in the early thymus expressed the pan-myelomonocytic marker SWC3a (A). While virtually no expression of CD4, CD8, and CD3e could be observed (B and C), some leukocytes had the lymphoid phenotype CD45⁺SWC3a⁻ (A).

FIGURE 4. Expression of CD3e and TCRγδ on fetal pig thymocytes. A shows the frequency of CD3e⁺ thymocytes as a percentage of all thymocytes (solid line). The contribution to this value of large CD3e⁺high (dashed line) and small CD3e⁺low (dotted line) thymocytes is also indicated. B shows the frequency of TCRγδ⁺high (solid line) and TCRαβ⁺high (dotted line) thymocytes. TCRγδ⁺CD3e⁺low cells are considered to be TCRαβ⁺high thymocytes. C shows the frequency of small (solid line) and large (dotted line) CD3e⁺ thymocytes. Data are mean values ± SEM from at least three animals for each stage of gestation.

FIGURE 5. Expression of CD4 and CD8 on fetal pig thymocytes. The proportion of CD4⁺CD8⁺ (A), CD4⁺CD8⁻ (B), CD4⁻CD8⁺ (C), and CD4⁻CD8⁻ (D) subsets expressed as a percentage of total thymocytes is shown. In each graph, the proportions of total (solid line), small (dashed line), and large (dotted line) thymocytes with selected phenotypes is presented. Data are mean values ± SEM from at least three animals for each stage of gestation.
Characterization of leukocyte subsets in early primary hemopoietic centers

Precursor and progenitor stages of lymphocyte differentiation in pigs have not been completely phenotyped. While mature lymphocytes and myelomonocytic cells can be characterized as CD45\(^{\text{high}}\) SWC3\(^{a2}\) and CD45\(^{\text{high}}\) SWC3\(^{a}\) high leukocytes, respectively (13, 14), precursor stages of myelomonocytic lineages have been characterized by their specific expression of CD45 and SWC3a on fetal pig thymocytes. Myelomonocytic CD45\(^{1}\) SWC3a\(^{1}\) cells are also shown (solid line). Data are mean values ± SEM from at least three animals for each stage of gestation.

FIGURE 6. Cell size analysis and CD45/SWC3a expression on fetal pig thymocytes. A shows the frequency of small (solid line) and medium plus large (dotted line) thymocytes as determined by forward scatter. B shows the frequency of CD45\(^{5}\)/SWC3a\(^{a}\) (dashed line) and CD45\(^{5}\)/SWC3a\(^{a}\) (dotted line) thymocytes. Myelomonocytic CD45\(^{1}\) SWC3a\(^{a}\) cells are also shown (solid line). Data are mean values ± SEM from at least three animals for each stage of gestation.

FIGURE 7. Expression of CD45 and SWC3a on mononuclear cells (defined by low side scatter values) isolated from fetal liver (A) and bone marrow (B). The proportions of CD45\(^{5}\)/SWC3a\(^{a}\) (solid line) and CD45\(^{5}\)/SWC3a\(^{a}\) (dotted line) lymphoid subsets are presented as a percentage of total CD45\(^{5}\) cells. The combined proportions of CD45\(^{5}\)/SWC3a\(^{a}\) and CD45\(^{5}\)/SWC3a\(^{a}\) subsets do not equal 100% because data on the myelomonocytic CD45\(^{1}\) SWC3a\(^{a}\) subset are not shown to reduce clutter. Data are mean values ± SEM for at least three animals at each stage of development.

FIGURE 8. Analysis of CD25 expression on pig thymocytes. Thymocytes isolated from 3-day-old piglets were double-stained with combinations of anti-CD25 and either anti-CD3\(\varepsilon\) (A) or anti-TCR\(\gamma\delta\) (B) mAb or were triple stained with combination of anti-CD25, anti-CD8, and either anti-CD4 or anti-TCR\(\gamma\delta\) mAb (D–F). The size of the thymocytes expressing IL-2R\(\alpha\) (CD25) is also demonstrated (C). When CD25/CD8/CD4 triple-stained thymocytes were gated for CD25\(^{+}\) cells (D, R1) and analyzed, the majority of gated cells was found within the CD4\(^{-}\)CD8\(^{-}\) or CD4\(^{-}\)CD8\(^{+}\) SP thymocyte subsets (E). The same type of analysis for CD25/CD8/TCR\(\gamma\delta\) triple-stained thymocytes showed that both CD8\(^{-}\) and CD8\(^{+}\) cells are present within the TCR\(\gamma\delta\)\(^{-}\)CD25\(^{+}\) subset (F). G shows the frequency of CD25\(^{+}\) thymocytes during prenatal and postnatal development. Data are mean values ± SEM from at least three animals for each stage of gestation.
suggested to represent the majority of the SWC3low population in bone marrow in young pigs (14). Moreover, late pre-B II cells in pigs were defined as CD45lowSWC3− lymphoid cells dominating among small mononuclear leukocytes in the fetal bone marrow (15). Finally, our unpublished results show that all pre-B II stages among small mononuclear leukocytes in the fetal bone marrow as mature CD3+ cells (Fig. 8A). Therefore represented more mature stages of thymocyte development (Fig. 8A). Thereby, CD25low cells showed that the majority of CD25low thymocytes for TCRγδ vs CD25 showed that the majority of CD25low thymocytes were TCRγδ− and thus presumed to belong to the αβ T cell lineage (Fig. 8B). Further support for the argument that CD25 is present predominantly on mature stages of thymocytes comes from the finding that the CD25+ cells displayed mostly a SP phenotype (Fig. 8E). A small proportion of TCRγδ+ thymocytes also expressed CD25, but the expression was lower than on αβ thymocytes (Fig. 8B). Both CD8+ and CD8− γδ T cells expressing CD25+ were observed (Fig. 8F). Based on forward scatter, CD25+ thymocytes represented a homogeneous population of medium-sized cells (Fig. 8C). The proportion of CD25+ thymocytes during ontogeny increased during ontogeny increased as mature CD3ehigh thymocytes became prominent between DG56 and DG74 (compare Fig. 8G with Fig. 5 and Fig. 4). While the number of CD25+ cells decreased after DG74, their proportion gradually increased after birth, reaching a relatively high frequency in the adult thymus (Fig. 8G).

Cell cycle analysis of thymocytes in pigs

Identification of cycling thymocyte subsets (i.e., cells in S+G2/M cell cycle phase) was performed by simultaneous surface immunophenotyping and staining of DNA with 7-AAD (Fig. 9). All cycling thymocytes were large (Fig. 9B), and the majority of them was found among DN and DP cells, while relatively few SP thymocytes synthesized DNA (Fig. 9F). We also observed that all cycling DP thymocytes were medium-sized, whereas cycling DN cells were generally larger (data not shown). CD3e− thymocytes prevailed among proliferating cells (Fig. 9D). While most dividing CD3ehigh thymocytes expressed TCRγδ, a small but significant subset of CD3ehigh cycling thymocytes was found to be TCRγδ− cells and presumed to be a mature stage of the αβ lineage (Fig. 9D). No cycling CD3elow thymocytes were detected (Fig. 9D).

CD25 expression on porcine thymocytes

Analysis of CD25 expression on thymocytes in pigs revealed that essentially all cells expressing CD25 were also CD3ehigh and therefore represented more mature stages of thymocyte development (Fig. 8A). Virtually no CD3e−CD25+ cells were found in fetal thymus since DG38. Staining of thymocytes for TCRγδ vs CD25 showed that the majority of CD25+ cells was TCRγδ− and thus presumed to belong to the αβ T cell lineage (Fig. 8B). Further support for the argument that CD25 is present predominantly on mature stages of thymocytes comes from the finding that the CD25+ cells displayed mostly a SP phenotype (Fig. 8E). A small proportion of TCRγδ+ thymocytes also expressed CD25, but the expression was lower than on αβ thymocytes (Fig. 8B). Both CD8+ and CD8− γδ T cells expressing CD25+ were observed (Fig. 8F). Based on forward scatter, CD25+ thymocytes represented a homogeneous population of medium-sized cells (Fig. 8C). The proportion of CD25+ thymocytes during ontogeny increased as mature CD3ehigh thymocytes became prominent between DG56 and DG74 (compare Fig. 8G with Fig. 5 and Fig. 4). While the number of CD25+ cells decreased after DG74, their proportion gradually increased after birth, reaching a relatively high frequency in the adult thymus (Fig. 8G).

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Discussion
Most detailed studies on early thymocyte development have been performed in rodents (16–21) and chickens (22–25). Data from other species are fragmentary. Early T cell progenitors, i.e., pro-T cells, are derived from stem cells in primary hemopoietic centers and migrate to the thymus where further differentiation takes place (19, 20, 22, 23). It is well established that there are different hemopoietic centers during prenatal and early postnatal ontogeny (19, 22, 23). Although the activity of these primary centers overlaps, the gradual shift of primary hemopoiesis from one anatomical site to another during ontogeny has been hypothesized to account for the waves of thymic colonization by pro-T cells that have been reported in chicken/quail chimeras and suggested from studies in mice (16–25). However, none of these studies provides a direct proof for a discontinuous influx of thymocyte progenitors in an intact system. In chicken, the microsurgical techniques used to introduce xenogeneic cells may have interfered with normal immune system development. The disadvantage of mice is their very short gestation so hypothetical waves of thymic colonization overlap and are difficult to observe. The fetal pigs are more convenient for studies on prenatal ontogeny because of their much longer gestation.

We have used CD3/CD4/CD8 immunophenotyping to correlate thymocyte maturation in pigs with the generally accepted model of intrathymic T cell differentiation developed from studies in mice (26). In pig embryos, the first TN lymphoid elements, probably pro-T cells, could be observed in the thymic rudiment on DG38. The precursor nature of these early nonmyelomonocytic leukocytes is supported by their low expression of CD45. CD3ε+ thymocytes appeared 2 days later, all of which remained brightly TCRγδ+. Differentiating thymocytes belonging to the αβ lineage followed a progression from less-differentiated, large TN precursors to small CD3ε−DP and CD3εlow DP cells and finally to SP thymocytes (Fig. 10). This scenario is also consistent with our cell cycle studies where the majority of cycling thymocytes was large TN and CD3ε−DP cells, while small thymocytes, either CD3ε− or CD3εlow, were not dividing. Moreover, CD3εlow thymocytes appeared ~10 days before the first mature αβ thymocytes. Our data suggest that porcine αβ thymocytes require about 15 days to fully differentiate, while γδ thymocytes do so in <3 days. Combined with our previous findings (4), the data presented here also suggest that αβ and γδ T cells migrate asynchronously from the thymus to the periphery with γδ T cells populating the periphery before αβ T cells. This is consistent with studies in other species demonstrating that γδ T cells require a shorter time period for maturation than αβ T cells, perhaps because the latter are subjected to more rigorous positive and negative selection (16–18, 24, 25, 27). Interestingly, a large numbers of proliferating TCRγδ+ cells were always observed. This is in a sharp contrast to mice, where only a few γδ thymocytes have the capacity to divide (28). This finding together with the higher frequency of γδ thymocytes in the porcine thymus compared with its murine counterpart may explain why γδ T cells are so prominent a T cell population in the peripheral blood of young piglets (3, 4).

Once the major differentiation stages of thymocyte development had been identified, we tested the hypothesis of discontinuous colonization of the rudimentary thymus with pro-T cells. In our previous work (4), we observed no dramatic changes in the relative proportion of thymocytes in different stages of prenatal development. In the current study, we tested additional time points and focused on the interval between DG36 and DG76, which represents the period between the first half and the first two-thirds of gestation. During this period, we identified noteworthy changes in thymocyte subset composition. In particular, the proportion of both CD4+ and CD8+ mature αβ thymocytes as well as γδ cells increased while the proportion of CD3ε− and CD3εlow DP thymocytes decreased. Cell recovery from the thymus during this time was unexpectedly low (data not shown), and the proportion of myelomonocytic cells (CD45+SWC3a+) was high. Altogether these findings suggest that the influx of pro-T cells is temporarily interrupted after midgestation because there is a decline in the absolute numbers of early TN precursors and the otherwise predominant CD3εlow DP thymocytes. In accordance with studies in mice and chickens, this can be explained by the shift of hemopoiesis from one primary center (fetal liver) to another (bone marrow). While CD3ε+ thymocytes were detected on DG40 and peripheral T cells on DG45 (4), almost no leukocytes could be recovered from the bone marrow before DG45. This indicates that

![FIGURE 10. Proposed model of αβ T cell development in the porcine thymus. This schematic representation is deduced from: 1) the appearance of individual thymocyte subsets during early prenatal ontogeny, 2) the finding that the level of surface CD3ε expression increased during thymocyte development, and 3) staining of DNA using 7-AAD. Large circles demonstrate differentiation stages of large, mitotically active thymocytes, while small circles represent small, nondividing populations. The phenotype of individual subsets is given. An analysis of subsets A–C, D, E–F, G, and H is presented in Fig. 1, B, C, D, E, and F, respectively.](http://www.jimmunol.org/)
the fetal pig bone marrow cannot function as a significant source of blood-forming cells before approximately DG45, and other organs must play the role of primary hematopoietic centers during the early period of prenatal development. The recovery of CD45<sup>hi</sup>SWC3a<sup>-</sup> mononuclear cells in different organs implies that the fetal liver is the primary site of lymphopoiesis before DG45. This conclusion is also supported by the finding that the fetal liver contains cells of the B cell lineage at DG30 (29). After DG45, the proportion of CD45<sup>hi</sup>SWC3a<sup>-</sup> cells progressively increased among cells recovered from the bone marrow, while this subset gradually disappeared from the fetal liver with increasing fetal age.

In attempt to distinguish between early and late pre-T cells (30–31) we stained thymocytes from fetal, neonatal, and young piglets ered from the bone marrow, while this subset gradually disappeared from the fetal liver with increasing fetal age. 

References


