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Delineation of Intrathymic T, NK, and Dendritic Cell (DC) Progenitors in Fetal and Adult Rats: Demonstration of a Bipotent T/DC Intermediate Precursor

Luis M. Alonso-C.,* Juan J. Muñoz,* and Agustín G. Zapata†

We previously published study results stating that the early rat fetal liver contains a high frequency of T/dendritic cells (DCs), but rarely T/NK bipotent common progenitors. Now, by using xenogenic rat/SCID mouse fetal thymic organ cultures, we extend these observations to the thymus, in which conflicting data have been published in human and mouse. On the one hand, enriched adult intrathymic CD45×CD2– triple negative for CD8, CD4, and CD3 Ag cell progenitors, which contained both rearranged TCRβ chain and pre-TcR chain transcripts, completely lacked NKR-P1A expressing cells, and upon limiting dilution conditions, generated T- and T/DC-containing lobes, but no T/NK or NK ones were found. On the other hand, the CD45×CD2– triple negative for CD8, CD4, and CD3 Ags cell population obtained from 15- and 16-day-old fetal rat thymus can be divided into NKR-P1A– and NKR-P1Alow cell subpopulations that differ in several aspects. Both cell subsets expressed pre-TcRα chain transcripts, but only the former contained fully rearranged TCRβ chain transcripts. Upon limiting dilution, T cell-committed progenitors were only found in the NKR-P1A– cell population, whereas NK-committed progenitors were present in the NKR-P1Alow population. More importantly, bipotential T/NK progenitors were very rare and were found only in the NKR-P1Alow cell population, whereas bipotential T/DC progenitors, only previously suggested in the adult mouse thymus, were observed frequently in the NKR-P1A–CD2– cell subpopulation. Our results demonstrate, therefore, that a common intrathymic T/DC intermediate represents the main T cell developmental pathway in rat thymus. The Journal of Immunology, 2001, 167: 3635–3641.

The occurrence of a common cell progenitor for T, B, and NK cells and certain subsets of dendritic cells (DCs), called lymphoid DCs, is currently assumed (1). However, the intrathymic developmental pathway followed by DC, T, and NK cells is still a matter of discussion, and the identification of bipotential common progenitors for these cell lineages at the clonal level remains to be clarified. On the one hand, a large body of data has suggested that a common T/NK intrathymic progenitor could represent the main developmental intermediate (2–5), leading to the current view that DC first split off from the common T/NK/DC progenitor. However, most of these works have left the DC developmental potential unexplored, further precluding the T/NK intermediate hypothesis. In fact, studies focused on analyzing DC development have suggested that T and DC could share a common T/DC bipotent intermediate in the thymus. Thus, Wu et al. (6) and Lucas et al. (7) showed that the T and DC potentials of adult mouse intrathymic triple negative for CD8, CD4, and CD3 Ags (TN) progenitors are retained up to the CD44+CD25– stage, when the NK cell potential is nearly absent (5, 8). However, neither a formal demonstration for the existence of clonal T/DC bipotent progenitors has been reported yet nor has this issue been studied in the fetal thymus.

Therefore, it has not been possible to definitively conclude, from these studies, how the intrathymic development of T, NK, and DC takes place. In this report, we study the T, DC, and NK potencies of adult and fetal rat thymic progenitors, and we conclude that clonal bipotent T/DC progenitors represent the main, if not the only, developmental intermediate in the rat thymus.

Materials and Methods

Animals

Wistar Hanover rats and C.B-17 SCID mice were used in this study. They were maintained in the animal facilities of the Facultad de Biología (Universidad Complutense de Madrid, Madrid, Spain) or Centro de Biología Molecular (Universidad Autónoma de Madrid, Madrid, Spain), respectively.

Isolation of thymic cell populations

The adult CD45+CD2−Lin− cell population was isolated from cell suspensions of adult thymuses prepared in PBS containing 0.1% FCS and 5 mM EDTA, and they were centrifuged onto a 28% BSA cushion (30 min, 400 × g, 20°C). The cell fraction at the PBS/BSA interface contained nearly 40% CD4–CD8– cells and was further depleted of T cells, DCs, B cells, and myeloid lineages by negative depletion with autoMACS (Miltenyi Biotec, Bergisch Gladbach, Germany) by using mouse mAb against rat CD4 (OX-38), CD8α (OX-8), CD3 (G4.18), MHC class II (OX-6), CD1 (OX-12), and CD11b/c (OX-42) (all hybridoma supernatants were from the European Collection of Animal Cell Cultures), followed by rabbit anti-mouse MACS microbeads (Miltenyi Biotec). The negative cell fraction thus obtained, hereafter referred to as the Lineage− (Lin−) fraction, was further enriched in CD45+CD2– cells by three-color FACS sorting (FACStarPlus cell sorter; BD Biosciences, Mountain View, CA) after staining with mouse mAb against rat CD45 (rCD45) clone OX-1, FITC-conjugated; BD Pharmingen, San Diego, CA), CD2 (clone OX-34, PE-conjugated; BD Pharmingen), and propidium iodide, the latter to exclude nonviable cells. Cells for further RNA/DNA extraction were sorted in serum-free medium.
Fetal thymus (FT) cell suspensions were first depleted of CD2⁺ and MHC class II⁺ cells by negative selection in an autoMACS magnetic cell separator (Miltenyi Biotec). The CD2⁺ MHC class II⁺ cells thus obtained were further FACs sorted to obtain highly purified CD45⁺ NKR-P1A⁺ and CD45⁺ NKR-P1A⁺ cell subpopulations based on CD45 (clone OX-1, FITC-conjugated; BD Pharmingen) and NKR-P1A (clone 10/78, PE-conjugated; BD Pharmingen) staining combined with propidium iodide.

**Flow cytometric analysis**

For flow cytometric immunophenotyping, cell suspensions were incubated with saturating concentrations of mouse mAbs specific for rat Ags. Abs used for immunofluorescence staining were either prepared in the laboratory (purified rat MHC class II (clone OX-6), anti-CD53 (clone OX-44), anti-MHC class I (OX-18), anti-CD71 (clone OX-26), biotin-labeled anti-CD45 (clone OX-1), and anti-CD2 (clone OX-55); all from European Collection of Animal Cell Cultures) or purchased from BD Pharmingen (FITC-labeled anti-NKR-P1A (clone 10/78), anti-CD44 (clone OX-49), anti-CD4 (clone OX-35), anti-CD45R (His24), and anti-rat granulocyte Ag (His48); PE-labeled anti-TCRαβ (clone R73), anti-TCRγδ (clone V65), anti-CD25 (clone 390), anti-CD2 (clone OX-7), biotin-conjugated anti-CD54 (clone 1A29); and PerCP anti-CD8a (clone OX-8). Either streptavidin-CyChrome (SA-CY, FL-3H; BD Pharmingen) or SA-Cy5 (FL-4H; Jackson ImmunoResearch Laboratories, West Grove, PA) was used as a second-step reagent when a biotinylated mAb was present. Indirect stainings were performed first and were followed by incubation with rabbit anti-mouse IgGs conjugated to FITC, PE, or Cy5 (Jackson ImmunoResearch Laboratories). In all cases, nonspecific binding was blocked by the addition of saturating concentrations of purified mouse Ig (Sigma-Aldrich, Madrid, Spain) before incubation with biotin- or fluorochrome-conjugated mAb and following indirect stainings. Data acquisition, 20–50 × 10⁶ events, was performed in a FACSCalibur flow cytometer (BD Biosciences) of the Servicio Común de Investigación (Facultad de Biology, Complutense University of Madrid). Data were analyzed with CellQuest software (BD Biosciences).

**RNA isolation and RT-PCR analysis**

RNA was extracted from cell suspensions by using TRI-Reagent solution (Molecular Research Center, Cincinnati, OH) following the manufacturer’s instructions. RT (1–1.5 µg total RNA) and PCR were performed with a RT-PCR core kit from PerkinElmer (Roche Diagnostic Systems, Mannheim, Germany).

Detection of pre-TCRα chain (p7α) mRNA species was conducted by using a combination of primers that recognize highly homologous sequences between mouse and human p7α cDNA of the Ig-like (5’-CG GCACCCCCCTTTGGGCTTC-3’) and transmembrane (5’-GGCCAGAGGAGACATC-3’) domains. cDNA samples were denatured (94°C, 1 min), annealed (60°C, 1 min), and extended (72°C, 1 min) for 35 cycles, followed by Southern blot hybridization with a rat p7α-specific DNA probe cloned by us (9). For RT-PCR amplification of TCR Vβ8-Cβ mRNA species, a combination of 5’-Vβ8 primer (5’-GGGCTGGTGAGTTTGGGAAGC-3’) with 3’-Cβ (5’-AGGTTCTCTCACCTTCCTGAC) primers was used for 35 cycles (1 min at 94°C, 1 min at 55°C, and 1 min at 72°C). Specific PCR amplifications were detected by Southern blot hybridization with a digoxigenin (Dig)-labeled Cβ probe more internal than the 3’-Cβ primer used in the PCR.

Dig-dUTPs were obtained from Roche Diagnostics System. The hybridization was visualized using an alkaline-phosphatase-conjugated anti-Dig mAb (Roche Diagnostic Systems).

Primers for RT-PCR amplification of rat actin were derived from GenBank accession no v01217: forward, 5’-CATTTGGGCTATGCGCAG-3’; reverse, 5’-GCTTTAGCCGATAGTGATG-3’.

**Results**

**Studies on adult rat thymic progenitors**

A number of studies in mice and humans have revealed lineage relationships between T, NK, and B cells and a subtype of DC that presumably emerge from a common lymphoid progenitor, although later lymphoid cell lineage developmental intermediates are still a matter of controversy (1, 10, 11). Thus, the possible existence of a common bipotent T/DC intrathymic progenitor has only been referred at a population level in the adult mouse thymus (6), whereas an immature intermediate with T/NK bipotentiality has been claimed to occur in the mouse FT (4, 5). Interestingly, we previously found clonal bipotent T/DC progenitors in the early rat fetal liver (9), prompting us to investigate this subject in the rat thymus. After whole elimination of mature intrathymic T, B, DC, and myeloid cells (see Materials and Methods), adult thymus cell suspensions were enriched in the CD45⁺ CD2⁻ cell fraction by FACS sorting (further referred to as CD2⁻ cells). These cells were 100% positive for CD44, Thy-1, MHC class I, CD53, and the transferrin receptor, CD71 (Fig. 1), but in sharp contrast to mouse thymic TN cells, rat immature thymocytes did not express CD25, as previously found by Law et al. (12), precluding a further fractionation of the CD2⁻ TN rat subset based on CD44 and CD25 staining. Other tested Abs previously reported in rat immature progenitors like the OX22 determinant of CD45 and the CD5 Ag did not allow for a further phenotypic fractionation of the CD2⁻ cell population (not shown). In contrast, because no NK cell-specific marker was included in our lineage depletion protocol, we explored for the possible expression of NKR-P1A by some of these cells, as reported for mouse NK1.1 in fetal thymic progenitors (13). However, no NKR-P1A⁺ cells were found in the CD2⁻ enriched cell fraction obtained (Fig. 1).

![FIGURE 1. Cell surface phenotype of enriched rat CD45⁺ CD2⁻ Lin⁻ adult thymic cells. A, Cell sorting of CD45⁺ CD2⁻ cells from pre-enriched adult Lin⁻ TN intrathymic cells (see Materials and Methods). B, Cells from A, expressed CD53, Thy1, CD44, MHC class I, and CD71 and were negative for the NK-associated Ag (NKR-P1A). Control stainings (open histograms) were developed with fluorochrome-conjugated irrelevant mouse mAbs.](http://www.jimmunol.org/.../fig1.png)
Next, we developed a rSCID-FTOC assay, as previously described by us (14), to examine for the presence of T, NK, and DC progenitors in the CD45\(^{+}\)CD2\(^{-}\) cell fraction. First, we developed a limiting dilution analysis to calculate the number of thymic re- 

progenitors in the CD45\(^{+}\)CD2\(^{-}\) cell population obtained from adult rat thymus, which was estimated to be 1 in 21 cells of this population (Fig. 3A). Then, 12-day singly cultured rSCID-FTOC lobes, which were reconstituted with a limiting cell number of the CD2\(^{-}\) cell population per lobe, were individually analyzed by flow cytometry for the expression of rCD45, TCR\(\beta\) plus TCR\(\alpha\gamma\), NK-R-P1A, and MHC class II rat Ags in four colors (Fig. 3B). In these conditions, no rCD45\(^{+}\)Lin\(^{-}\) lobes were observed, but all the rCD45\(^{+}\) ones contained at least one of the lymphoid cell lineages studied. T cell-containing lobes were present, confirming the above-described results and suggesting the existence of T cell-committed cells already in the CD2\(^{-}\) adult rat thymic cell fraction. In addition, they represented a majority of rCD45\(^{+}\) lobes (Fig. 3C). Along with them, some rCD45\(^{+}\) lobes containing both TCR\(^{+}\)NK-R-P1A\(^{-}\)MHC class II\(^{+}\) and TCR\(^{+}\)NK-R-P1A\(^{-}\)MHC class II\(^{high}\) cell populations, possibly T/DC-containing lobes, were nicely observed, but no rCD45\(^{+}\)NK-R-P1A\(^{-}\)-containing lobes developed. We further confirmed that MHC class II\(^{high}\)-expressing 

cells in the lobes were true DC by visual inspection of cytoplasmic preparations prepared from every cultured lobe and stained for MHC class II Ag (data not shown). Finally, NK cell development occurred in rSCID-FTOC when a higher input number of progenitor cells was used (by using 100 or more of these cells), i.e., nonlimiting cell numbers (not shown), demonstrating NK cell potentiality by these cells, but at a very low frequency to be detected at limiting dilution. Thus, in accordance with previous reports in mice (11) and humans (15), immature adult rat thymic progenitors exhibit T, DC, and NK potentialities. In addition, we demonstrate for the first time the occurrence of clonal bipotent T/DC intrathy- 

mic progenitors in an in vitro system that allows T, NK, and DC development.

Studies on fetal rat thymic progenitors

As mentioned above, bipotential T/NK intrathymic progenitors have been claimed as an intermediate stage in both mouse (16) and human (15) fetal thymic lymphoid development. In mice, they could occur in either CD16/CD32\(^{+}\) (5, 17) or NK1.1\(^{+}\) (13) CD44\(^{+}\)CD25\(^{-}\) TN fetal thymic cell populations, although their existence in adult thymus remains to be demonstrated. More importantly, a parallel examination of the possible DC potentiality of presumptive bipotent T/NK fetal thymic progenitors is still lacking in mice, whereas in humans, a high frequency of DC progenitors was reported in a fetal intrathymic T/NK bipotent population (2), although there was no clonal demonstration of those results. All

![Figure 2.](https://example.com/figure2.png)

**FIGURE 2.** Expression of pT\(\alpha\) and TCR\(\beta\) chain transcripts by adult intrathymic CD45\(^{+}\)CD2\(^{-}\)Lin\(^{-}\) cells. Detection of pT\(\alpha\) and rearranged TCR\(\beta\) V-C transcripts was performed by RT-PCR analysis followed by Southern blot and hybridization with rat-specific probes for pT\(\alpha\) and TCR \(\beta\) (see Materials and Methods). Lane 1, CD2\(^{-}\) subset; lane 2, CD2\(^{-}\) subset; lane 3, negative control (isolated rat CD45\(^{+}\) 13-day-old fetal liver cells (see Ref. 9).**
these data suggest that bipotent T/NK progenitors could represent an intermediate stage in fetal thymic development that we did not detect in the adult rat thymus. Accordingly, we examined the developmental capabilities of rat fetal thymic cell populations from very early developmental days to later stages.

The first day to undoubtedly dissect the rat FT from the surrounding connective tissue was day 15 of gestation (15 gd), which corresponds to 1–1.5 days after the first thymus seeding by CD45+ progenitors in rats (18). At this time point, the CD45+ cell content per thymic lobe was ~2–5 × 10^3 cells, 15–20% of which have begun to express the CD2 Ag (Fig. 4). One day later (FT 16 gd), a sharp increase in the thymus cell content took place, with a CD45+ cell recovery of 60–80 × 10^3 cells per thymic lobe, which was accompanied by an increase of both the proportion of CD2+ cells, which reached nearly 70% of the CD45+ progenitors in rats (18). At this time point, the CD45+ corresponds to 1–1.5 days after the rounding connective tissue was day 15 of gestation (15 gd), which very early developmental days to later stages.

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We performed a phenotypic analysis by flow cytometry of the thymic cells from 15-, 16-, or 17-day-old FT by combining CD45 and CD2 stainings with other T and non-T cell-related Ags. As illustrated in Fig. 4, both FT 15 gd and FT 16 gd cells contained a small proportion of CD4+expressing cells that lacked CD2 or CD8 expression. In contrast, CD8-expressing cells were undetectable until 16 gd, and they were found to be the CD2+CD4–. From 16 gd to 17 gd, there was a sharp increase in both the proportion and absolute numbers of CD8+CD4– cells, and the first CD4+CD8+ cells were detected (Fig. 4). Upon staining for TCRb, the first surface reactive cells were detected at 17 gd (not shown).

To determine the presence of thymic DC during these early fetal stages of rat thymus development, we first looked for MHC class II expression on the CD45+ cells as a marker for rat thymic DCs. Low levels of class II expression were already observed at FT 15 gd and were stronger at FT 16 gd (Fig. 5). In both cases, the CD45+MHC class II+ cells were CD2– (Fig. 5). The CD8 and CD4 expression on these cells was also examined, and although no CD8 expression was correlated with that for MHC class II, the early CD4 staining detected at fetal days 15 and 16 was nicely associated with the CD45+ class II+ cell population, especially in the MHC class II^high population of the 16-day-old FT (Fig. 5). In the adult rat, we (19) and others (20) have reported the occurrence of a subpopulation of CD4+expressing DC in the thymus. A further phenotypic analysis of the class II^-CD2^-CD4^+ from 16-day-old FT demonstrated that they expressed higher ICAM-1, CD53, and CD44 and lower Thy1 levels of Ag expression than those found in the CD45+ class II+ thymic cells (Fig. 5). This phenotypic analysis suggested that these cells corresponded to thymic DC. In addition, rat fetal thymic MHC class II^high cells did not show reactivity for the mouse mAbs His24, which recognizes the CD45R isoform in developing rat B cells, and His48, specific for rat granulocytes, and weakly to negative for the myeloid determinant recognized by mAb OX42, recognizing a common epitope shared by CD11b and CD11c (not shown). In contrast, isolated MHC class II^high cells from FT 16 gd ended up, after some days of culture in suspension, to a major population of cells

![FIGURE 4. Phenotypic analysis of rat fetal thymic cell populations. Cell suspensions from 15-, 16-, or 17-day-old FTs were quadruple stained for rat CD45, CD2, CD4, and CD8α. CD2, CD4, and CD8 Ag expression was analyzed in gated CD45+ rat thymocytes. Days 15 and 16 of development represent the most immature stages, whereas day 17 already contains CD4+CD8+ immature thymocytes and most thymocytes have become CD2high cells.](image)

![FIGURE 5. Phenotypic analysis of MHC class II expressing rat fetal thymocytes by flow cytometry. A. Gated rat CD45+ cells were analyzed for the expression of MHC class II and CD2, CD4, or CD8α Ags. B. Rat CD45+ 16-day-old fetal thymocytes contain MHC class II+ and MHC class II+ cells that exhibit a differential expression of Thy-1, CD53, CD44, and ICAM-1 Ags.](image)
with long cytoplasmic processes, with an irregularly shaped nucleus and strong MHC class II expression, characteristic features of thymic DC (not shown).

In contrast to the adult condition, CD45\(^+\)CD2\(^-\) cells from 15- and 16-day-old FTs weakly expressed the NKR-P1A Ag (Fig. 6). In addition, NKR-P1A\(^{high}\) cells appeared for the first time in the FT 16 gd, associated with low levels of CD2 and Thy-1 Ags, suggesting their presumptive relationship to rat NK cells. In contrast, as NKR-P1A expression has been also reported in adult rat DCs (21), we confronted NKR-P1A and MHC class II Ag expression defined two mutually exclusive cell populations. Accordingly, three CD45\(^+\)CD2\(^-\) cell subpopulations can be defined in the early rat FT: NKR-P1A\(^{high}\) class II\(^+\) cells, CD45\(^{low}\) NKR-P1A\(^{high}\) class II\(^-\), and NKR-P1A\(^{low}\) class II\(^-\).

Finally, we enriched FT 16 gd thymocytes in the CD45\(^+\)CD2\(^-\) class II\(^-\) TN population, as above mentioned for adult studies. From this cell population, we FACS sorted NKR-P1A\(^-\) and NKR-P1A\(^{low}\) cell subpopulations to examine the possible presence of TCR\(\beta\) and pT\(\alpha\) transcripts. As shown in Fig. 7, fully rearranged TCR\(\beta\) V-C transcripts were only detected in the NKR-P1A\(^-\) cell population, but to the contrary, pT\(\alpha\) transcripts were detected in both the NKR-P1A\(^{low}\) and the NKR-P1A\(^-\)CD2\(^-\) cells. Accordingly, NKR-P1A\(^-\) cells could contain T-committed cells, whereas the NKR-P1A\(^{low}\) could contain the above-mentioned reported T/NK bipotent progenitors.

As in the adult studies, we performed rSCID-FTOC assays established with either limiting or nonlimiting cell numbers of the distinct fetal thymic cell populations. Based on limiting dilution analysis (not shown), there were important differences in the estimated frequency of SCID-FTOC repopulating cells between the two assayed CD2\(^-\) cell subsets, with higher frequency in the NKR-P1A\(^{low}\) subpopulation (one-fifth) than in the NKR-P1A\(^-\) one (<1/100). The differences between these two CD2\(^-\) cell subpopulations also extended to their potentialities. Thus, upon reconstitution with limiting cell numbers of NKR-P1A\(^-\)CD2\(^-\) rat thymic cells, most reconstituted lobes contained T cells, both TCR\(\alpha\)\(\beta\) and TCR\(\gamma\)\(\delta\) thymocytes (not shown), as the only cell lineage growing in the lobes (Fig. 8). Under the same limiting cell number conditions, we also found a lower but reproducible number of lobes containing both T and DC cell lineages, whereas the lowest frequency of rCD45\(^+\) corresponded to those that contained all of the three cell lineages studied (Fig. 8). When limiting numbers of the NKR-P1A\(^{low}\) cell subpopulation were used in the reconstitution of SCID-FTOC, we exclusively found NK-containing lobes, whereas no T- or DC-containing lobes grew (Fig. 8). However, under nonlimiting conditions, 100 or more cells of the NKR-P1A\(^{low}\) cell population gave rise to 100% reconstituted lobes, all of which contained NK cells. Some of these also developed T cells, but

![FIGURE 6. Analysis of NKR-P1A expression by rat CD45\(^+\) 15- and 16-day-old fetal thymocytes. Day 15 thymocytes only contained an NKR-P1A\(^{low}\) population, whereas day 16 thymocytes contained, in addition, an NKR-P1A\(^{high}\) population. At day 16, both NKR-P1A-expressing populations differed in Thy-1 and CD2 expression, but neither of them contained MHC class II-expressing cells.](Image 101x637 to 486x734)

![FIGURE 7. Expression of pT\(\alpha\) and rearranged TCR\(\beta\) V-C chain transcripts by 16-day-old fetal thymic populations. Detection of pT\(\alpha\) and TCR\(\beta\) V-C transcripts was performed by RT-PCR analysis followed by Southern blot and hybridization with rat-specific probes for pT\(\alpha\) and TCR C\(\beta\) (see Materials and Methods). Lane 1, CD45\(^+\)CD2\(^-\) NKR-P1A\(^{high}\) cells; lane 2, CD45\(^+\)CD2\(^-\) NKR-P1A\(^{low}\) subset; lane 3, CD45\(^+\)CD2\(^-\) cell population.](Image 358x137 to 487x395)

![FIGURE 8. Evaluation of T, NK, and DC potentialities of rCD45\(^+\)CD2\(^-\) fetal thymic progenitors. Limiting numbers of either NKR-P1A\(^-\) or NKR-P1A\(^{low}\) subpopulations were used to reconstitute singly cultured SCID fetal thymic lobes. After 12 days of culture, development of rat T, NK, and DC was studied in each individual lobe as described in Fig. 3. In these conditions, the NKR-P1A\(^-\) cell population mainly developed T-containing lobes, but T/DC and T/NK/DC were also detected, although no other types of precursors for these lineages were observed. On the contrary, the NKR-P1A\(^{low}\) cell subpopulation of CD45\(^+\)CD2\(^-\) fetal thymocytes only contained progenitors for NK cells.](Image 101x637 to 486x734)
DC-containing lobes remained rare (not shown), indicating that T/NK bipotent progenitors could exist in this fetal thymic population, but at a very low frequency to be detected in limiting dilution conditions.

Discussion

The current results indicate that the main bipotential cell intermediate for rat intrathymic development of T, DC, and NK cells is a common T/DC progenitor, rather than the T/NK progenitor previously proposed by others in mice and humans (2, 4, 5, 13). However, a similar proposal has been indirectly suggested in mice, although not firmly demonstrated at a clonal level (6).

We had already observed bipotent T/DC progenitors in the early rat fetal liver (9) where, in contrast, T/NK cells were rarely detected. It is still not known whether these results have implications for the commitment status of cell progenitors colonizing the thymus. In this regard, we have demonstrated DC- and, more importantly, T cell-committed progenitors in the early rat fetal liver, which could represent prethymic progenitors for these lymphoid cell lineages or an extrathymic pathway of their development (9).

In contrast, the existence of a common T/DC progenitor has remained largely unexplored because previous published articles have mainly focused on the common T/NK progenitor (2, 4, 5, 13). It is important, however, to remark that these studies were conducted with fetal but not adult thymic cells and did not examine the situation of DC. In contrast, most of those studies were based on cytokine-supplemented cultures, which could give rise to unpredictable results. Thus, Ikawa et al. (5) improved the proportion of NK cells to the detriment of T cell production in in vitro assays supplemented with IL-2. In these experiments the authors, however, did not show the effect of IL-2 addition on the reported frequency of clonal progenitors, although, as previously shown by others (3), it is presumable that thymic progenitors are selectively influenced toward NK differentiation upon IL-2 addition. More importantly, Márquez et al. (22) have shown that the addition of IL-2 to suspension cultures of early intrathymic human progenitors results in the appearance of NK cells with a reduction of DC cell recovery. Recently, the unpredictable influence of cytokines in cell lineage decisions of uncommitted progenitors has also been pointed out by Kondo et al. (23), who redirected common lymphoid progenitor toward myeloid differentiation. Finally, in agreement with our results, adult intrathymic CD44+CD25+ TN cells rarely form NK cells (5, 8), but still retain a robust capacity to develop T cells and DC upon both in vivo i.v. transfer (6) or in in vitro assays (7).

As stated above, the existence of a lymphoid DC lineage is widely supported by a large body of data in human and mouse (1, 11, 15, 24), and although there are no available specific markers for rat lymphoid DC, our current results make this situation now applicable to this species. With respect to this, experiments are in progress for further phenotypic characterization of rat DC derived from T/DC single colonized lobes.

As in mice (25), the intrathymic development of rat NK cells takes place very early in rat ontogeny, preceding the appearance of mature T cells. In agreement, the first NKR-P1AhighCD2− cells detected in the rat thymus during ontogeny were found at 16 gd when TCRαβ-expressing cells are still lacking. Interestingly, mature NK cells at 16 gd are preceded by a population of NKR-P1AlowCD2− cells at 15 gd, which is mainly committed to the NK cell lineage. This situation is similar to that reported in mouse fetal ontogeny for the NK1.1 molecule and NK-committed clonal progenitors (25). Despite these results, the significance of intrathymic NK cell development still remains intriguing, as nude animals contain extrathympically derived NK cells (26). This intrathymic NK cell development could reflect a special situation during ontogeny, as in the adult thymus, this developmental pathway should be extremely rare. Recently, Id-2 and Id-3 transcriptional inhibitors of bHLH factors have been shown to block the development of both human T and DC from a common progenitor, while promoting the NK cell fate (27). Thus, it could be that unknown microenvironmental signals, which promote NK cell fate through the regulation of Id proteins, block the T and DC development from a common T/NK/DC progenitor to a similar extent, further precluding a common T/NK intermediate as an obligatory T cell developmental pathway over the T/DC demonstrated in this study. In contrast, a parallel development of thymic T and DC, as demonstrated in mice (11), could be significantly relevant for eliminating potentially auto-reactive T cell clones during T cell development, while the significance of an intrathymic NK cell development still remains to be understood.

Expression of NKR-P1 by a subset of mature T cells has been correlated with a special T cell subset (28) and, in some cases, with an activated state of normal T cells (29). Remarkably, the rarely developed T cells in rSCID-FTOC colonized by the NKR-P1AlowCD2− 16 FT population did not show a preferential expression of the NKR-P1 Ag. However, some of these NK-T cells developed in rSCID-FTOC from fetal liver progenitors (14) and from adult and fetal thymic progenitors (results not shown), further precluding these fetal thymic NKR-P1Alow progenitors as an alternative route for the development of NK-T cells.

In summary, the present results conclusively demonstrate the occurrence of a common T/DC rather than a T/NK bipotential intermediate as the main, if not the only, pathway of rat T cell development in both adult and FT. Its existence in human and mouse thymus remains to be further demonstrated.

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References


