Thymic Dendritic Cell Precursors: Relationship to the T Lymphocyte Lineage and Phenotype of the Dendritic Cell Progeny

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Summary

Successive T-precursors isolated from adult mouse thymus were examined for their developmental potential, by transfer to irradiated Ly 5–disparate recipients. The earliest, "low CD4" precursors formed T, B, and dendritic cells (DC), but not myeloid cells, in accordance with earlier studies. Surprisingly, the next downstream $CD4^-8^-3^-44^+25^+$ precursor population still formed DC as well as T cells although it no longer formed B or myeloid cells. Further downstream, the $CD4^-8^-3^-44^-25^+$ population formed only T cells. The thymic and splenic DC progeny of the early thymic precursors all expressed high levels of CD8α, in contrast with normal splenic DC and the splenic DC progeny of bone marrow stem cells, which consisted of both $CD8^-$ and $CD8^+$ DC. A common precursor of T cells and of a subclass of DC is proposed, with CD8α as a marker of the lymphoid-related DC lineage.

The earliest precursor of T lymphocytes so far identified in the adult mouse thymus resembles bone marrow hematopoietic stem cells (BMSC)[1] in surface phenotype, except for the expression of the antigen Sca-2 and of a low level of CD4: for this reason we have termed it the "low CD4 precursor" (1–3). This population has TCR β and γ genes in germline configuration. Despite the presence of some enzymes associated with recombination, it still lacks even D-J β gene rearrangements (1, 4). A striking feature of this population is its ability to generate dendritic cells (DC), B, and NK cells, as well as T cells, although it has lost the capacity to generate erythroid and most myeloid cells (2, 5–9). This suggested the same early intrathymic precursor cell gives rise to both thymic DC and to the thymic T-lineages (5, 6), in contrast to the established myeloid origin for most DC (10, 11). Strict clonal evidence for this hypothesis of a lymphoid origin for thymic DC is still not available. However, some support of the concept has come from the finding that thymic DC express several surface molecules normally considered characteristic of lymphoid cells, in particular the early B cell marker BP-1 (6) and the T cell marker CD8, in the form of an αα homodimer (6, 12).

Another early T-precursor cell was delineated by Godfrey and Zlotnik (13) and Godfrey et al. (14) within the $CD4^-8^-3^-$ (triple negative) population. This precursor is $CD44^+$ c-*kit*$^+$, like the low CD4 precursor, but as well as losing CD4 it has gained CD25 (IL-2Rα) and shows some upregulation of surface Thy-1. Like the low CD4 precursor, it has the Cβ (14) and the D-J β (4, 15) gene regions in germline state. For this reason it is termed a pro-T cell and it has been assumed to be downstream from the low CD4 precursor. This precursor population has been found to lack both B and NK cell developmental potential and accordingly has been assumed to be the point of T cell commitment (7, 9).

Still further downstream is the "triple negative pre-T cell" (3, 13), which has the surface phenotype $CD4^-8^-3^-$ $CD44^-25^+$ c-*kit*$^-$ Thy-1^+ (although both CD44 and c-*kit* should be more precisely defined as low rather than negative). This precursor population represents the stage where TCR β genes are rearranged (4, 13, 15), and on this basis would be considered a T-committed population. Exit from this stage and further development towards α/β T cells involves signals from the newly described pre-T α chain (16).

We now assess the ability of all three of these early T-lineage precursor populations to generate DC, and compare this to their capacity to produce other hemopoietic lineages. The results suggest a sequential rather than simultaneous loss of other developmental potentials en route to T cells, with the ability to form DC only being lost at the stage of TCR β gene rearrangement. Since the artificial transfer of the low CD4 thymic precursor population by intravenous injection produced progeny DC in the spleen as well as in the thymus, we have been able to assess whether the unique surface phenotype of thymic DC is dictated by the environment or by the nature of the precursor. Although some markers varied with the site of development, CD8α was always present and served as a marker of the DC lineage derived from the T cell precursors.

[1] *Abbreviations used in this paper:* BMSC, bone marrow stem cell; DC, dendritic cell; Lin^-BM, lineage marker negative bone marrow cells.

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Materials and Methods

Mice. The mice were bred at The Walter and Eliza Hall Institute under specific pathogen-free conditions. The donors in reconstitution experiments were 4–6-wk-old C57BL/Ka Thy−1.1 (Ly 5.2) or C57BL/6 (Thy-1.2, Ly 5.2) mice and the recipients were 7–8-wk-old C57BL/6 Ly 5.1-Pep^{3b} (Thy−1.2) mice.

mAbs and Fluorescent Reagents. The mAbs and hybridoma clones used for depletion and for immunofluorescent labeling, the fluorochromes employed, and the fluorescent reagents used, are all specified elsewhere (4, 6, 12).

Purification of Intrathymic Precursor Populations. The low CD4 precursors were isolated from C57BL/Ka Thy-1.1 or C57BL/6 donor thymuses as described previously (1, 2, 5). This involved complement-mediated cytotoxic depletion, adherence depletion, and then immunomagnetic bead depletion of mature thymocytes, of CD4$^+$8$^+$ thymocytes, of more developed precursors bearing CD2 or CD25, and of non T-lineage cells. The <1% remaining cells were then stained in three fluorescent colors and the low CD4 precursors (5–10% of the depleted population) sorted as Thy-1^{lo} heat-stable antigen (HSA)^{int} class II MHC$^-$ cells. Purity on reanalysis was 98–99%. In some experiments, an improved procedure (4) involving density separation, adherence, double immunomagnetic bead depletion, then sorting the precursors as Thy-1^{lo} HSA^{int} c-*kit*$^+$ cells, was also used with identical results. The triple negative, CD44$^+$25$^+$ and CD44$^-$25$^+$ populations were isolated as described previously (4). This involved cytotoxic and immunomagnetic bead depletion of cells bearing CD4, CD8, and CD3, together with depletion of non T-lineage cells. The depleted cells were then immunofluorescent stained and the precursors sorted on the basis of CD25 and c-*kit* expression. Purity on reanalysis for CD25, c-*kit*, and CD44 expression was 97–98%.

Purification of BMSC. The isolation of pure long-term reconstituting BMSC was as described elsewhere (17), with these stem cells being finally sorted as Lin$^-$ c-*kit*$^+$ Ly 6A/E$^+$ Rhodamine 123^{lo} cells. In some experiments, an enriched but not pure lineage marker negative bone marrow (Lin$^-$BM) stem cell preparation was obtained by incubating cells with an antibody mix and then using anti-Ig–coated magnetic beads to deplete cells bearing markers of hemopoietic lineages including T cells, B cells, erythrocytes, macrophages, and granulocytes.

Reconstitution of Irradiated Recipients with Precursor Cells. The procedures have been presented in detail elsewhere (2, 5, 6). For intrathymic transfer, the recipient mice were γ-irradiated (7.5 Gy); 10^4 low CD4 precursors, 10^4 CD44$^+$25$^+$ precursors, 10^5 CD44$^-$25$^+$ precursors, or 10^2 BMSC were injected into one thymus lobe. The injected lobes were then analyzed 7–21 d later. For intravenous transfer, the recipient mice received two doses of γ-irradiation (each 5.5 Gy, 3 h apart) and, to ensure survival, received 4×10^4 recipient-type bone marrow cells intravenously. The donor cells transferred intravenously were 3×10^4 low CD4 precursors, 3×10^4 CD44$^+$25$^+$ precursors, 3×10^5 CD44$^-$25$^+$ precursors, 10^2 purified BMSC, or 4×10^3 Lin$^-$BM. The spleens and thymuses were analyzed 1–4 wk later.

Enrichment of DC. Before analysis for reconstitution by donor-derived cells, it was necessary to enrich the DC by purification from pooled recipient thymuses or spleens. The methods have been described in detail elsewhere (5, 6, 12). The organs were chopped, digested with collagenase, and treated with EDTA. Light density cells were collected by a density centrifugation procedure. Finally, non DC-lineage cells were depleted from the light density cells by coating them with a mixture of mAbs and then depleting the coated cells with anti-Ig–coated magnetic beads.

Analysis of the Progeny of Transferred Precursors. The presence of donor-derived (Ly 5.2$^+$) cells bearing markers for T cells (Thy-1), B cells (B220), or granulocytes and macrophages (Gr-1, Mac-1) after intravenous transfer was determined by direct two- or three-color immunofluorescent staining on suspensions of the recipient spleens or thymuses using FITC-conjugated anti donor-type Ly 5.2, PE-conjugated anti-Thy-1, and either biotinylated anti-B220 or biotinylated anti-Mac-1 together with biotinylated anti-Gr-1, followed by Texas red–avidin as the second stage. The presence of donor-derived (Ly 5.2$^+$) cells bearing DC markers (class II MHC, CD11c, DEC-205, CD8α, BP-1) was assessed on DC-enriched preparations extracted from pooled recipient thymuses or spleens. The enriched DC were stained in three fluorescent colors with FITC-anti-Ly 5.2, allophycocyanin anti-class II MHC and biotinylated antibody against one other marker followed by PE-avidin as the second stage. During flow cytometric analysis, the cells were gated for donor origin (Ly 5.2$^+$) and DC characteristics (high class II MHC and characteristic high forward and side light scatter), then analyzed for expression of a third DC marker. Full details are given elsewhere (5, 6).

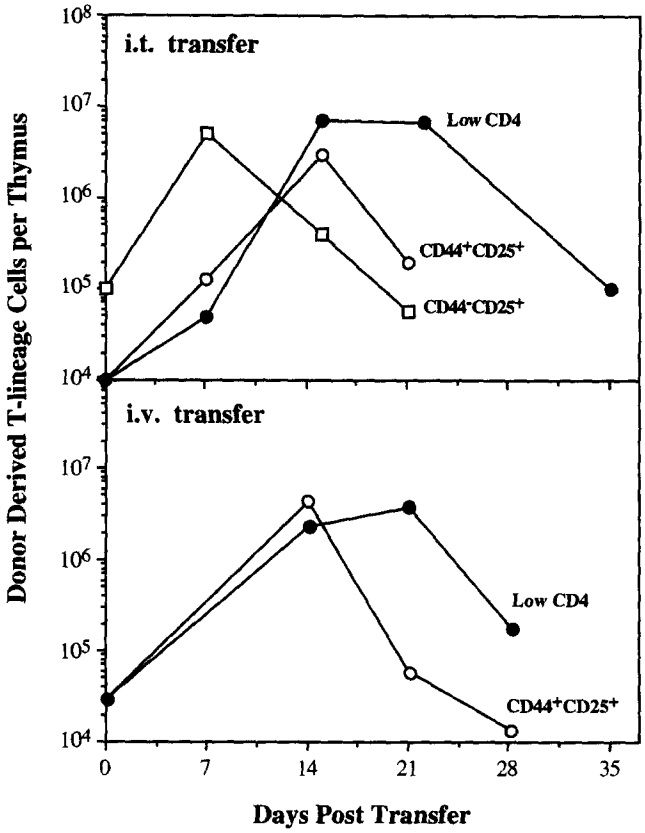

Figure 1. The kinetics of generation of T-lineage progeny in the thymus after intrathymic (*i.t.*) and intravenous (*i.v.*) transfer of different thymic precursor cells. The purified precursor populations were transferred into irradiated recipient mice differing in Thy-1 and Ly 5 allotype. Donor-derived T-lineage cells were revealed by two-color staining of cells with antidonor type Ly 5.2 and anti-Thy-1, then gating for Ly 5.2$^+$ Thy-1$^+$ cells. Full details are in Materials and Methods.

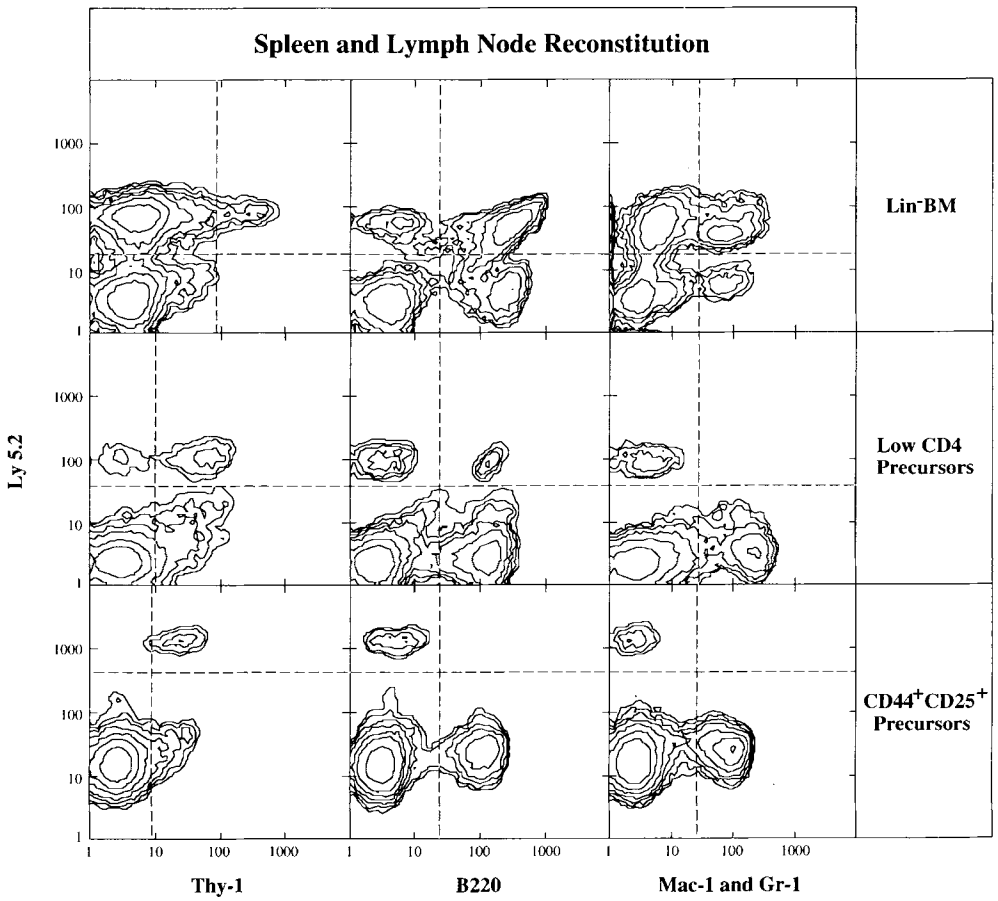

Figure 2. The generation of T and B lymphocytes in the pooled spleen and lymph nodes of recipient mice after intravenous transfer of different purified thymic precursor cells or stem cell–enriched bone marrow cells (*Lin⁻BM*). The purified thymic precursors (3×10^4) and Lin⁻BM (4×10^3) from Ly 5.2 mice were transferred into irradiated Ly 5.1 recipient mice. 3 wk after transfer, the recipient spleen and lymph nodes were pooled and the lineage of the progeny was determined by staining cell suspensions for donor type Ly 5.2, together with staining for Thy-1 (T cells), B220 (B cells), or for Mac-1 and Gr-1 together (myeloid cells). Three separate experiments, one for each precursor population, are shown; each result was typical of three experiments. The quadrants were set based on the background staining for each experiment. The differences in staining intensity between experiments was due to changes in the reagents used and variations in instrument settings. Note that there is no staining for recipient type Thy-1⁺ cell in the spleen and lymph nodes reconstituted by Lin⁻BM. This is because in these particular experiments, the Lin⁻BM cells were obtained from C57BL/6 Ka Thy-1.1 (*Ly 5.2*) mice, and in the analysis for donor-derived cells, only anti-Thy-1.1 antibody was used together with anti-Ly 5.2 to reveal the donor type Thy-1⁺ cells but not the recipient type Thy-1⁺ (Thy-1.2) cells. In the experiments with thymic precursors, both donor and recipient were Thy-1.2 mice, and the anti-Thy-1.2 antibody revealed both donor and recipient T cells.

Results

Reconstitution Kinetics. To check that the various thymic T-precursor populations behaved as sequential steps in T cell development, the kinetics of development of T-lineage progeny after intrathymic and intravenous transfer into irradiated recipients differing in Ly 5 allotype, was compared (Fig. 1), using criteria previously established in this laboratory (18, 19). Upon intrathymic transfer, the fastest reconstitution was obtained with the CD44⁻25⁺ triple negative pre-T precursor, the next fastest with the CD44⁺25⁺ triple negative pro-T precursor, and the slowest reconstitution from the low CD4 precursor (Fig. 1). This result is in line with the fetal thymus organ culture repopulation studies of Godfrey et al. (14), assuming that their CD44⁺25⁻ population corresponds to our low CD4 precursors. Previously we had established that the low CD4 precursor, in turn, gave faster reconstitution than BMSC (1). Similar reconstitution kinetics were obtained after intravenous transfer (Fig. 1), except that very few progeny were detected with the CD44⁻CD25⁺ precursor population. This population was deficient in its ability to seed the thymus, presumably because of its low expression of CD44, a thymus-homing molecule (20, 21). In addition to this kinetic evidence, we have previously demonstrated that after transfer, the progeny of the low CD4 precursors lose CD4 and gain CD25 (1, 2). Accordingly, all these results support the earlier models (3, 13) of a linear, sequential developmental process with the precursors in the sequence: low CD4 precursor, then CD44⁺25⁺ pro-T, then CD44⁻25⁺ pre-T.

Development of Lymphoid and Myeloid Cells from Intrathymic Precursors. To provide a side-by-side control for the DC precursor studies and to check previous conclusions, the nature of the predominant progeny formed in the spleen and lymph nodes of irradiated recipients after intravenous injection of the intrathymic precursors was determined. Suspensions of pooled recipient spleen and lymph nodes were stained for donor type Ly 5.2, together with anti-Thy-1 (for T cells), anti-B220 (for B cells), and anti-Mac-1 together with anti-Gr-1 (for myeloid cells). The results were compared with those obtained by transfer of bone marrow cells depleted of cells bearing markers of the hemopoietic lineages (Lin⁻BM), an enriched source of BMSC. Fig. 2 gives examples of the analysis. The low CD4 precursor formed not only T cell progeny but also B cell progeny, in

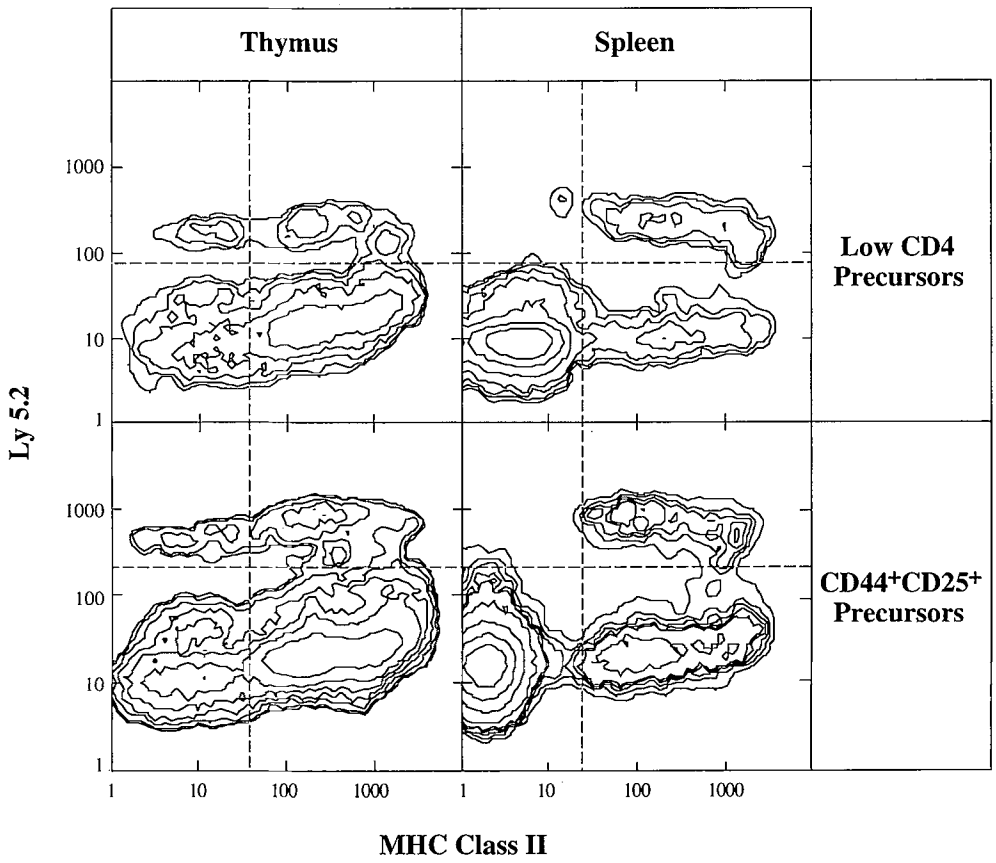

Figure 3. The generation of dendritic cells in the spleen and thymus of recipient mice after intravenous transfer of different thymic precursor cells. The purified precursors (3×10^4) were transferred intravenously into irradiated Ly 5 congenic recipient mice. 2 wk after transfer, the recipient thymuses and spleens were collected and DC enriched as described in Materials and Methods. Donor-derived DC were identified by staining cells with antidonor type Ly 5.2 and anti-MHC class II and then gating for Ly 5.2^+ MHC class II^+ cells. Note that the relative level of MHC class II fluorescence was high, the sensitivity being reduced to keep all cells on scale. Equivalent results were obtained when CD11c or DEC-205 was used to distinguish DC. The results are typical of five such experiments.

agreement with our previous findings (2). As reported previously, these $B220^+$ progeny were also surface Ig positive, and appeared to be typical small B cells (data not shown). No discrete population of myeloid (Mac-1^+ or Gr-1^+) cells was obtained (Fig. 2), in accordance with our previous results. In contrast, the $CD3^-4^-8^-44^+25^+$ pro-T precursors formed T cells but very few, if any, B cells, and the apparent yield of myeloid cells was within the range of the background levels. This lack of potential for B cell development by this triple negative precursor has already been reported by others, for both adult and fetal thymus (7, 9). As expected, the later $CD3^-4^-8^-$ 44^-25^+ pre-T population was also unable to form B or myeloid cells in the recipient spleens and lymph nodes (data not shown). As we have previously reported (2), at these time points Mac-1^+/Gr-1^+, $B220^+$, and Thy-1^+ progeny were all readily detected and resolved as discrete populations, when a preparation enriched for BMSC (Lin^-BM) was transferred, with $B220^+$ B cells being the dominant progeny (Fig. 2). If the numbers of cells found in the positive quadrants were compared, the ratio of donor-derived Thy-1^+ T cells to donor-derived Mac-1^+/Gr-1^+ myeloid cells 4 wk after intravenous transfer was: Lin^-BM, 0.6; low CD4 precursors, 66; and $CD44^+25^+$ precursors, 163. Thus, the thymic precursors were almost devoid of myeloid potential, an important issue when considering their DC developmental potential.

Table 1. *The Generation of DC Progeny from Intrathymic Precursor Populations*

Precursor population	No. of cells transferred	Route of transfer	Donor-derived DC per thymus	Donor-derived DC per spleen
Low CD4	3×10^4	intravenous	1.4×10^3	17×10^3
$CD44^+CD25^+$	3×10^4	intravenous	1.6×10^3	14×10^3
$CD44^-CD25^+$	30×10^4	intravenous	0.3×10^3	0.8×10^3
$CD44^-CD25^+$	10×10^4	intrathymic	0.1×10^3	ND

Results are the means of two experiments, each experiment involving a pool of four to eight recipient mice. In the case of the $CD44^-25^+$ precursor population, although some cells were counted in the DC progeny quadrants, no clear donor type DC population was delineated after either intravenous or intrathymic transfer.

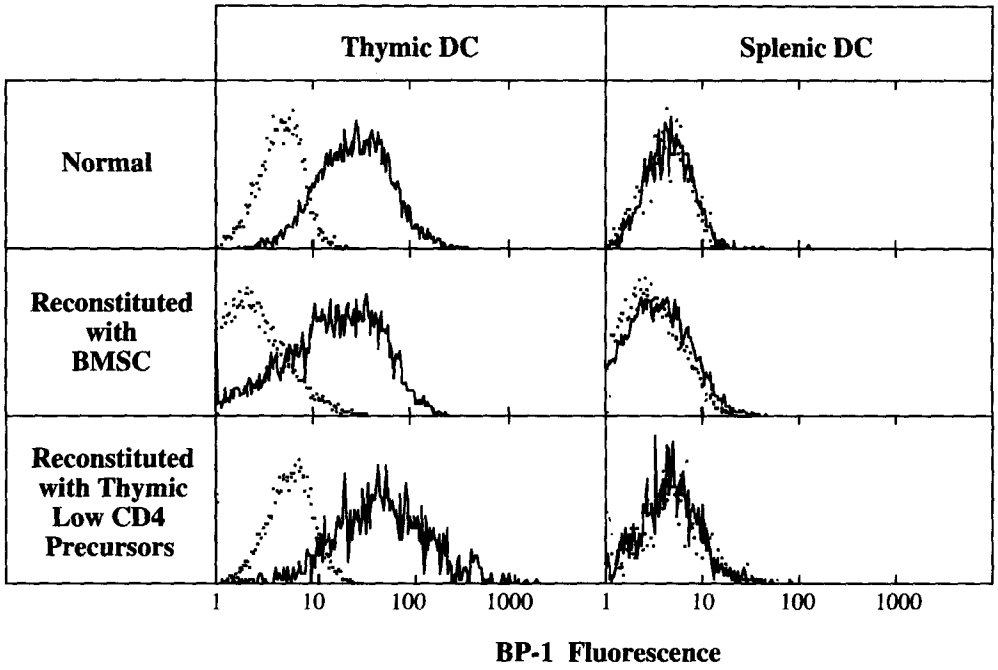

Figure 4. The expression of BP-1 on the surface of dendritic cell progeny in the spleen and thymus after intravenous transfer of thymic precursor cells or BMSC. Purified low CD4 precursors (3×10^4) or purified BMSC (10^2) were transfered into irradiated Ly 5 congenic recipients. 2 wk after transfer, thymic and splenic DC enriched from pooled recipient organs and stained in three colors with anti-donor type Ly 5.2, anti-MHC class II, and anti-BP-1. The progeny DC were detected and gated as Ly 5.2^+ MHC class II^+, and the BP-1 expression then determined using the third fluorescent color. (*Dotted lines*) Isotype control background staining. The results are compared with the DC present in normal, nonirradiated mice (*top*). The results are typical of three such experiments.

Development of DC from Intrathymic Precursors. Having confirmed the sequential loss of myeloid and then B lymphocyte potential by these early T-precursor populations, we used the same procedures to assess DC developmental potential. In particular, since the $CD44^+25^+$ triple negative pro-T cell was confirmed to have lost the capacity to form B cells despite still being in germline configuration for the TCR genes tested (4, 13, 15), its capacity to form DC in thymus and spleen was compared with that of the low CD4 precursor. Because DC are relatively rare components, it was necessary to pool recipient organs and enrich for DC before analysis. This enabled even low levels of DC to be detected above the background. Examples of the analysis of these enriched preparations for donor type (Ly 5.2^+) DC (class II MHC^{hi}) after intravenous transfer of precursors is given in Fig. 3; it is important to note that the results were similar when other markers of DC, including CD11c and DEC-205, were used instead or in combination with class II MHC. The total number of DC progeny obtained after transfer of different precursors is given in Table 1.

As we have reported previously (5, 6), the low CD4 precursor population formed DC in both the thymus and the spleen after intravenous transfer. In surprising contrast to the result for B cell progeny, the later $CD44^+25^+$ triple negative pro-T cells showed an undiminished capacity to form DC progeny in thymus and spleen, compared with the low CD4 precursors. However, although 10-fold more cells were transfered, the downstream $CD44^-25^+$ triple negative pre-T cells formed very few, if any, DC progeny regardless of the route of transfer (Table 1). Thus, during early thymic precursor cell development, the capacity to form B cells was lost before the capacity to form DC.

Nature of the DC Developing in the Thymus and in the Spleen from Thymic Precursors. To test whether the surface phenotype of thymic DC was determined by the thymic environment or by the nature of the DC precursor, the thymic precursors were transferred intravenously into irradiated recipients and the surface phenotype of the DC progeny in spleen and thymus determined. The DC progeny of the thymic precursors were compared with normal splenic and thymic DC. They were also compared with the DC generated by pure, long-term reconstituting BMSC, since these should generate all DC populations, and should indicate the type of DC likely to be produced if any multipotent stem cells contaminated the thymic precursor population.

In the first series of experiments, the level of Thy-1 on the surface of the DC progeny was analyzed, since freshly isolated thymic DC have some surface Thy-1, whereas splenic DC have very little (12). The progeny of thymic DC precursors showed some low surface Thy-1 staining in the thymus, but none in the spleen (data not shown). However, the reason for this proved to be trivial, as we have since demonstrated that most Thy-1 on the surface of thymic DC is picked up from thymocytes, rather than being synthesized by the DC themselves (22).

In the second series of experiments, the level of BP-1, a surface peptidase expressed on early B cells (23), was investigated. Many thymic DC are $BP\text{-}1^+$ and contain BP-1 mRNA, whereas splenic DC are surface $BP\text{-}1^-$ and lack BP-1 mRNA (6, 22). The progeny of thymic low CD4 DC precursors in the spleen proved to be clearly $BP\text{-}1^-$, like normal splenic DC, whereas the progeny in the thymus were $BP\text{-}1^+$, like normal thymic DC (Fig. 4). The same was found for the DC progeny of BMSC (Fig. 4). Accordingly, it appeared that BP-1 was induced on DC by the thymic environment, rather than being dictated by the precursor cell.

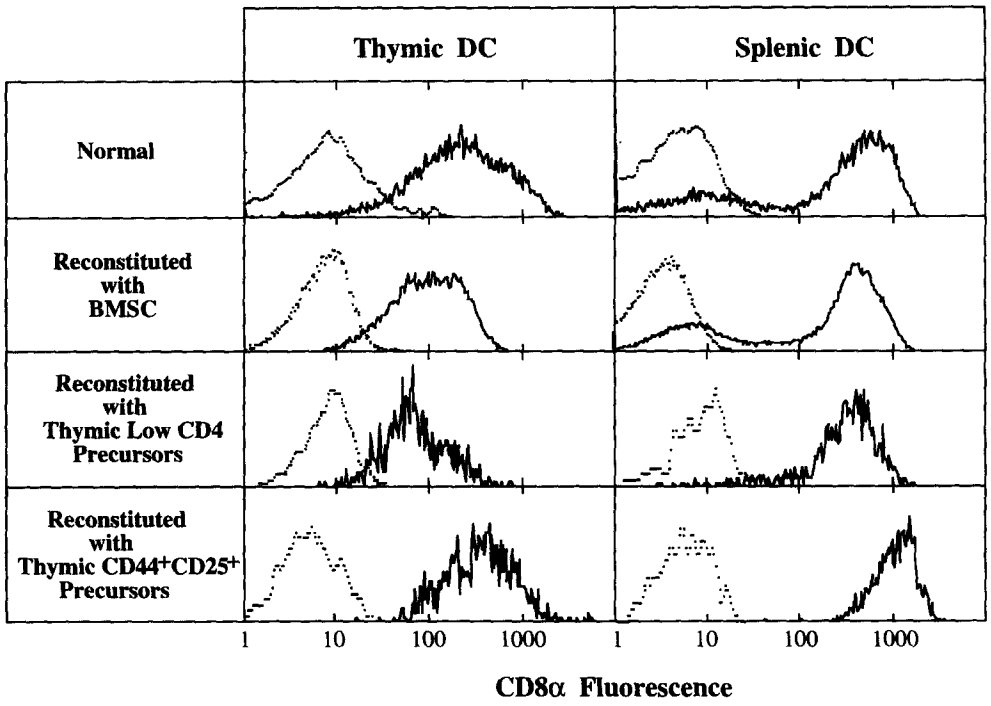

Figure 5. The expression of CD8α on the surface of dendritic cell progeny in the spleen or the thymus after intravenous transfer of thymic precursor cells or BMSC. The details are similar to Fig. 4, CD8α fluorescence being determined after gating for donor-derived DC (Ly 5.2^+ MHC class II^+). Three separate experiments with separate precursor populations are compared with the DC present in normal mouse thymus or spleen. The intensity of CD8α on DC was similar to that on T cells, but staining intensity varied between experiments with change of reagents and instrument used. The results are typical of five such experiments.

In the third series of experiments, the level of CD8α on the surface of the DC progeny was determined, since thymic DC are almost all $CD8\alpha^+$, whereas a definite population of splenic DC are $CD8\alpha^-$ (6, 12). It has also been established that $CD8\alpha^+$ DC contain CD8α mRNA (but not CD8β mRNA), so the CD8α is synthesized by the DC themselves (12). The pure BMSC reconstituted both the $CD8^-$ and $CD8^+$ DC populations of spleen, in a proportion almost identical to the normal splenic DC populations (Fig. 5). In contrast, the thymic low CD4 precursor and the thymic $CD44^+CD25^+$ precursor both produced only $CD8^+$ DC, in both the thymus and the spleen (Fig. 5). Since the animals injected with the Ly 5.2^+ thymic precursors were also injected with recipient type Ly 5.1^+ bone marrow cells to ensure survival after irradiation, these experiments also had an internal control. In contrast to the Ly 5.2^+ DC progeny of the thymic precursors, the Ly 5.1^+ recipient type DC had the normal proportion of $CD8^-$DC, exactly as for the BMSC reconstituted animals (data not shown).

A kinetic study of the DC progeny after intravenous transfer of different precursor populations was performed to ensure these results did not alter with time after reconstitution. It was found that 87% of splenic DC progeny of the low CD4 precursors were strongly $CD8^+$ at day 17, 93% were $CD8^+$ at day 21, and 96% were $CD8^+$ at day 28. At no time point was a discrete $CD8^-$ population obtained. Although a small proportion of DC expressing lower levels of CD8 was evident among the progeny, especially early after transfer, these were probably less mature cells in the process of acquiring CD8; we have already demonstrated that the level of CD8 on thymic DC increases with their maturation (6). In comparison, the BMSC gave rise to 76% $CD8^+$ splenic DC progeny at day 17, 78% at day 21, and 60% at day 28, with a discrete $CD8^-$ DC population being obtained at all time points.

Our procedure for DC isolation (12) normally includes a step of macrophage depletion, using graded levels of anti-Mac-1 and immunomagnetic beads, which removes cells expressing high levels of Mac-1 but leaves cells expressing

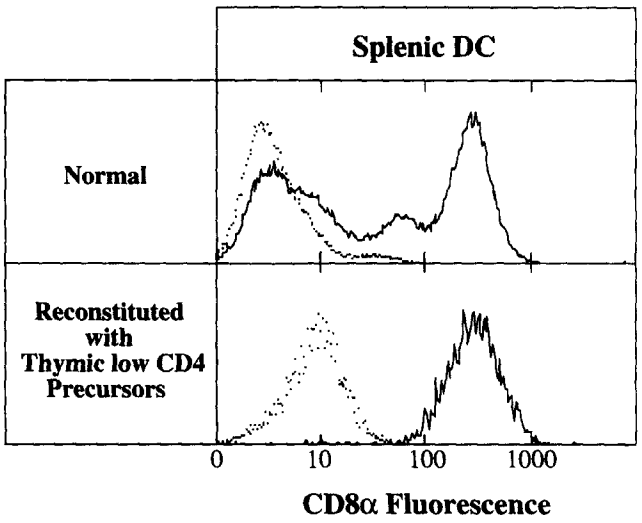

Figure 6. The expression of CD8α on the surface of DC progeny when the splenic DC are prepared without depleting Mac-1–bearing cells. The DC progeny in the spleen after intravenous transfer of thymic low CD4 precursor cells are compared with normal splenic DC for CD8α expression. In this experiment, anti-Mac-1 was not used in the antibody mix for depletion of other lineages during DC enrichment, however F4/80 was retained to aid macrophage depletion.

low levels of Mac-1. We have recently noted that a higher proportion of $CD8^-$ DC can be extracted from spleen if Mac-1 depletion is avoided entirely during DC enrichment. Accordingly, the DC progeny of the thymic precursors were examined using this less stringent enrichment procedure (Fig. 6). Even though this increased the relative level of $CD8^-$ DC detected in normal spleen, no $CD8^-$ DC progeny, only $CD8^+$ DC progeny, were obtained from the thymic precursors.

Discussion

Our kinetic studies on thymus reconstitution reinforce the view (1, 3, 13) that the low CD4 precursors represent the earliest T-precursor population so far defined and isolated from the adult mouse thymus. The rate of thymus reconstitution, as well as the surface phenotype, suggests these precursors are intermediate in development between BMSC and the pro-T $CD4^-8^-3^-44^+25^+$ thymic subset. However, the relatively broad peak of reconstitution from the low CD4 precursors suggests that more than a single wave of reconstitution is involved. This precursor population may therefore include both very early cells and cells almost as mature as the $CD44^+25^+$ population. This fits with our concept that the low CD4 precursor population includes cells from some 7 d of development after the initial seeding event (3, 24). This implies that the initial cells seeding from the bone marrow, and their immediate products, might still be minority components of this preparation. These still earlier precursors could even be multipotent, and responsible for the traces of myeloid precursor activity we sometimes see in the low CD4 precursor population. This trace of possible myeloid precursor activity has largely disappeared from the later, $CD4^-8^-3^-44^+25^+$ pro-T population.

Even allowing for some heterogeneity in developmental state, the low CD4 early precursor population of the adult mouse thymus displayed some surprising developmental capabilities, apparently differing from those of the early precursor cells in embryonic mouse thymus (25) or blood (26). The capacity to form both T and B cells, but very few erythrocytes, granulocytes, or macrophages (2), suggested the low CD4 precursor population might correspond to a "lymphoid-restricted precursor." Such a cell was not new in concept, even though evidence for its existence had been meagre. However, the capacity of the low CD4 precursor to form DC as well as lymphoid cells (5) was startling, since at least some types of DC have been found to be of myeloid origin (10, 11) and the low CD4 precursor showed little capacity to form other myeloid cells (2). One possible explanation was that the low CD4 precursor population consisted of a group of separate unipotent precursors with similar surface antigenic phenotype, although it was then not clear why the thymus would accumulate a B-restricted precursor cell it would never use (2).

The explanation that this early T precursor population only appeared to be oligopotent because it was "contaminated" with separate DC and B precursors, or with multipotent stem cells, now seems less likely, since the downstream c-*kit*$^+$ $CD44^+25^+$ pro-T cell also shows DC precursor activity. The procedure for the isolation of this $CD44^+25^+$ precursor population differs totally from that of the low CD4 precursor, and the level of possible myeloid precursor activity is still lower and now indistinguishable from the background. Yet this $CD44^+25^+$ precursor shows undiminished DC precursor activity per cell and shows a ratio of DC to T cell progeny similar to that of the low CD4 precursor population. Furthermore, the low CD4 precursor and the $CD44^+25^+$ precursor both produce only the CD8α type of DC, even when they seed the spleen, in contrast to BMSC; this is further evidence that they are not contaminated with multipotent stem cells or myeloid precursors. These results indicate there is a coincidence, or at least a very strong linkage, between the thymic DC precursors and the earliest T-lineage precursors. Of course the results with the $CD44^+25^+$ precursor do not strengthen the evidence for a common T and B cell precursor potential at the low CD4 precursor stage. However, in support of the view of a common DC/lymphoid precursor, a cell with T, B, and DC generative capacity, but apparently unable to form other lineages, has now been isolated from human bone marrow by Galy et al. (27). Linked pathways of T cell and DC development from human thymus $CD34^+$ precursors have also been described by Márquez et al. (28), although these precursors also had monocyte developmental potential. We favor the concept that the T, B, and DC progenitor activities are developmentally linked, having a common precursor downstream from the BMSC. Since the capacity to form B cells is lost before DC, thymic DC would then be a relatively late branch off the T lineage. This concept still requires direct confirmation, either by a clonal approach or by further evidence that DC bear markers of a lymphoid past.

It is of interest that the c-*kit*$^-$$CD44^-25^+$ pre-T cell, the cell undergoing TCR β gene rearrangement (3, 13, 15), lacks detectable B or DC precursor activity. By this stage this precursor seems fully committed to the T cell lineage, although the issue of α/β versus γ/δ lineage commitment is still under debate. It is not clear whether TCR gene rearrangement itself, or some linked control mechanism, determines this T-lineage commitment.

If thymic DC are a discrete DC lineage, either lymphoid derived or in some other way linked to T cell development, can this lineage be distinguished from other DC on the basis of surface markers? It is now clear that certain molecules on the surface of thymic DC are simply picked up from associated thymocytes and cannot serve as useful markers: Thy-1 is clearly in this category (6, 22) and it is likely that a small amount of CD4 and CD8α/β can also be absorbed onto DC (6). Nor do surface molecules made by the DC themselves in response to a particular inductive environment serve as useful permanent markers of a lineage. BP-1, although formed by thymic DC themselves (6), appears to be induced by the thymic environment, since in

the spleen the progeny of thymic DC precursors are BP-1$^-$. However, our evidence suggests that CD8α expression does mark a distinct thymic type of DC lineage, since the thymic DC precursors formed only CD8α$^+$ DC even when allowed to produce DC in the spleen. This was in contrast to BMSC that formed both CD8$^-$ and CD8$^+$ DC in the spleen. To date, CD8α is the only marker present on thymic DC that is consistently associated with the progeny of the thymic precursors regardless of whether they develop in the spleen or the thymus.

If CD8α marks a lymphoid-derived or lymphoid-related thymic DC population, we must now question the origin of the CD8α$^+$ DC found in the spleen and other lymphoid tissues. To date, there is no evidence that the CD8$^+$ and CD8$^-$ DC of spleen have a precursor–product relationship, although a few CD8$^-$ DC could be immature cells en route to CD8$^+$ (6). On overnight incubation these CD8$^+$ and CD8$^-$ DC retain their phenotype, the only change being some increase in the CD8α expression by the CD8$^+$ DC (6). The CD8$^+$ DC in spleen are unlikely to be thymus derived since they are present at normal levels in athymic "nude" mouse spleen (Vremec, D., and K. Shortman, unpublished data). We have been unable to detect the exit from the thymus of either the precursor cells or the DC progeny after intrathymic transfer (Wu, L., unpublished data). Our experiment of injecting the thymic precursors intravenously was therefore artificial, since there is no evidence that such precursors ever exit the thymus. Nevertheless this experiment suggests that an equivalent lymphoid-derived or lymphoid-related precursor may exist outside the thymus and give rise to a line of splenic CD8α$^+$ DC, separate from but related to thymic DC. An important issue is whether these proposed CD8$^+$ and CD8$^-$ splenic DC lineages differ in biological function. Our recent studies indicate that they do differ in function. Although both CD8$^+$ and CD8$^-$ DC are able to activate CD4 T cells, the Fas ligand-bearing CD8$^+$ DC then kill these activated T cells by Fas-mediated apoptosis (29). The CD8$^+$ DC may therefore have a regulatory function distinct from the better known stimulatory CD8$^-$ DC.

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