Regulated Expression and Function of CD122 (Interleukin-2/Interleukin-15R-β) During Lymphoid Development

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To determine whether signaling via CD122 (interleukin-2 [IL-2]/IL-15 receptor β-chain) plays a role in regulating the expansion and differentiation of lymphocyte precursors, we have characterized its expression and evaluated its ability to influence the activity of developing lymphoid cells. A significant fraction of Sca1+Lin- hematopoietic stem cells in day 12 fetal liver were found to be CD122+. CD122-mRNA+ and IL-2-mRNA+ cells were also localized in embryonic sections within pharyngeal blood vessels adjacent to and surrounding the thymus. This distribution is consistent with the migration of CD122+ progenitor cells from the liver to the developing thymus where a majority of Sca1+ intra-thymic T-cell progenitors were CD122+. Analysis of CD122 expression in the day 12 fetal liver revealed that the majority of B220+ cells were CD122+. Furthermore, CD122 expression was restricted to the earliest B220+ cells (CD43+CD24-; pre-pro B cells; fraction A) that proliferate vigorously to IL-2 in the absence of any stromal cells, but not to IL-15. Consistent with a role for the IL-2/IL-2R pathway in lymphocyte development is the progressive loss of B cells seen in IL-2-deficient mice. Together, these observations suggest that CD122 plays a role in regulating normal lymphocyte development in vivo.

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Materials and Methods

Animals. Adult (6 to 8 weeks of age) C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME) were bred and maintained by the University of Pennsylvania animal facilities. Fetal tissues were obtained from time-mated mice with the day of detection of a vaginal plug designated day 0. Adult C13-SCID mice (6 to 8 weeks of age) were provided by Dr John Cebra (Department of Biology, University of Pennsylvania).

Cell preparation. Liver and thymus obtained from fetuses of the appropriate age (day 12 through day 16 of gestation) were disaggregated in phosphate-buffered saline, pH 7.3 (PBS) using fine forceps and a single cell suspension obtained by repeated aspiration through a 1-mL syringe barrel. BM mononuclear cells were obtained from the femurs and tibias of adult SCID mice by flushing the bones with PBS and repeated aspiration through a 1-mL syringe barrel. Erythrocytes were removed by sedimentation centrifugation using a Ficoll Hypaque solution (Ficoll/Lite-LM, density 1.086 g/mL; Atlanta Biologicals, Norcross, GA). Viable mononuclear cells were obtained from the interface, washed, and resuspended in PBS containing 2% fetal calf serum (FCS; Atlanta Biologicals). Human peripheral blood mononuclear cells (PBMC) were obtained from whole blood donated by normal healthy adults after sedimentation centrifugation through Ficoll Hypaque solution (LSM, density 1.080 g/mL; Organon Teknika, Durham, NC).
Monoclonal antibodies (MoAbs). The following mouse and rat MoAbs were used to stain and analyze populations of cells isolated from the fetal liver, thymus, and adult BM by flow cytometry: anti-mouse CD32/CD16/FcRγII/III (2.4G2, ATCC); CD122/IL-2Rβ (TM-β1; gift from Dr Masayuki Miyasaka, Tokyo Metropolitan Institute of Medicine, Japan); CD45R/B220 (RA3-6B2; Pharmingen, San Diego, CA); Sca-1/Ly-6A/E (E13-161.7; Pharmingen); CD90/Thy 1.2 (30H.12; GIBCO-BRL, Gaithersburg, MD); CD24/HSAs (M1/69; Pharmingen); Mac-1 (M1/70.15; Caltag Laboratories, San Francisco, CA); Gr-1 (RB6-8C5; Pharmingen); CD43/Ly-48 (S7; Pharmingen); IgM (Fab2 fragments goat-antimouse; Jackson ImmunoResearch, Westgrove, PA). Streptavidin-phycocyanin and streptavidin-red 670 (SA-PE; SA-RED670; GIBCO-BRL) were also used to detect reactivity of biotinylated primary antibodies.

Flow cytometric analysis and cell sorting. All steps of the two- and three-color staining procedure were performed using 96-well, "V"-bottomed microtiter plates. Approximately 1 x 10^5 cells in 50 μL of staining buffer (PBS, 2% FCS) were incubated in 5 μg of antihuman FcRγ antibody for 1 hour at 4°C to block nonspecific binding of mouse and rat antibodies. The cells were then incubated with the appropriate biotin-conjugated antibodies for 45 minutes at 4°C, washed twice, incubated for 30 minutes with SA-PE or SA-RED670, and fluorochrome (FITC, Red 613, or PE)-conjugated antibodies and washed twice before flow cytometric analysis using a FACScan (Becton Dickinson, San Jose, CA). Antibody-positive populations of cells were distinguished according to the level of staining obtained with fluorochrome-conjugated, mouse and/or rat Ig isotype-matched, control antibodies of irrelevant specificity. Ten thousand events were collected and analyzed using Lysis II software (Becton Dickinson). Because preliminary characterization of CD122+ cells in the day 14 to 15 fetal liver indicated that a majority of CD122+ cells coexpressed FcRγII/III, they were in some instances first enriched for by positive immunomagnetic selection using the 2.4G2 antibody before flow cytometric analysis and cell sorting.

For cell sorting, 15 to 30 x 10^6 fetal liver or SCID BM cells (BMC) were stained with anti-B220 and anti-CD24 antibodies as described above except the staining buffer contained antibiotics (Penicillin/Streptomycin and Gentamycin; GIBCO-BRL). Stained cells were run on a FACStar Plus cell sorter (Becton Dickinson) and the B220+ and B220- fractions from fetal liver or B220+CD24- and B220-CD24+ fractions from adult BM were collected in 2 to 3 mL of DMEM (with 10% FCS).

Cellular proliferation assays. One to 5 x 10^5 nonfractionated or sorted populations of fetal liver or adult SCID mouse BMC were cultured in 100 μL of DMEM/10% FCS alone or in media containing 100 ng/mL of recombinant murine IL-2 (Boehringer Mannheim, Indianapolis, IN) or recombinant human IL-15 (Pepro Tech Inc., Rocky Hill, NJ) at 37°C for 48 hours. At this time, cultures were pulsed with 0.5 μC of [3H]-thymidine and the cells were procured 12 hours later. As a positive control for the effects of IL-2 on cellular proliferation, 10^6 freshly isolated human PBMC were cultured in RPMI 1640/5% FCS with 4 μg/mL PHA (Wellcome, Research Triangle Park, NC) for 48 hours, after which IL-2 or IL-15 was added for a further 48 hours before pulsing with [3H]-thymidine as described for murine BMC cultures.

Hybridization probes. All probes used for in situ hybridization were cDNA fragments consisting of sequences complementary to coding sequence only inserted into the polylinker site of the pGEM-3 or -4 plasmids (Promega Corp, Madison, WI) using standard procedures. The IL-2 and IL-2Ra (CD25) genes have been described previously.16,17 Two nonoverlapping IL-2Rβ probes corresponding to either the 176-729 or 985-1,744-bp region of the gene were used. The former cDNA clone was obtained from a day 15 mouse fetal thymus mRNA sample using a reverse transcriptase-polymerase chain reaction (RT-PCR) assay incorporating primers derived from the published sequence of the murine IL-2Rβ gene sequence. The latter cDNA probe was kindly provided by Dr Glen Gaulton (University of Pennsylvania). The identity of the cloned IL-2Rβ cDNAs were confirmed by DNA sequencing. pGEM/cytokine/cytokine receptor cDNA constructs were linearized and sense and antisense 35S-labeled RNA transcripts were synthesized using SP6 or T7 RNA polymerase according to the supplier's recommendations (Promega Corp).

In situ hybridization. Cyto centrifuge preparations of fetal liver mononuclear cells were processed for hybridization in situ as described previously.15 Coded slides were hybridized with sense and antisense probes in triplicate and the number of silver grains overlaying samples hybridized with sense probes was used to determine the level of nonspecific hybridization (routinely between 1 and 5 grains per cell) and to identify mRNA+ cells in samples hybridized with antisense probes. From each coded slide, between 500 and 1,000 positive cells from at least 10 different fields were examined to provide the frequency of positive cells per slide. For in situ hybridization of whole embryo sections, day 12 and day 13 mouse embryos were fixed in 4% (wt/vol) paraformaldehyde in PBS for 16 to 20 hours at 20°C and stored in 70% ethanol until processed for paraffin-embedding and sectioning (American Histolabs, Gaithersburg, MD). Six-micron sections were dewaxed in xylene, rehydrated in decreasing concentrations of ethanol, and then processed for hybridization in situ as described for cytocentrifuge samples. After hybridization (18 hours at 50°C) and posthybridization washes all of the samples were dipped in autoradiographic emulsion (NTB-2; Kodak, Rochester, NY) and developed after 1 to 4 days autoradiography at 4°C. Samples were counterstained with hematoxylin/ eosin before mounting and photomicroscopy.

Immunohistochernistry. Immunohistochemical staining of frozen sections of fetal liver and omentum was performed as described previously.19 The rat-antimouse IL-2 MoAb, S4B6, (Pharmingen), 1 mg/mL, was used to detect IL-2 protein. Normal rat IgG (Miles Inc, Kankakee, IL) was used as a control. As an additional specificity control the S4B6 antibody was preincubated with recombinant IL2 (1,000 U/mL) for 60 minutes at 20°C before staining.

RESULTS

Distribution of CD122 expression among murine fetal liver hematopoietic cells. Blood cell lineage-restricted stem cell populations are derived from a multipotential hematopoietic stem cell (HSC) in the fetal liver and can be distinguished by the expression of lineage-specific surface antigens. HSC activity has been shown to be contained within a population of cells that expresses low levels of Thy-1, high levels of the stem cell-associated antigen, (Sca-1) and undetectable levels of blood cell lineage-specific differentiation markers (Lin).20 To determine the stage in hematopoiesis at which CD122 is first expressed, we analyzed cells from the day 12 fetal liver by three color flow cytometry using antibodies able to distinguish HSC and lineage-specific progenitor populations. The results shown in Fig 1 are representative of four independent experiments. The Sca-1+Lin-HSC population, represented approximately 2% of the mononuclear cell fraction of day 12 C57BL/6 fetal liver cells (Fig 1C). Although CD122+ cells represented only 6% of the mononuclear fraction of the day 12 fetal liver (Fig 1B), the Sca-1+Lin-HSCs were enriched for cells expressing this cytokine receptor chain; approximately 20% of the Sca-1+Lin-HSCs were CD122+ (Fig 1D). Interestingly, in subsequent analyses of CD122-enriched fetal liver cell prep-
Expression of CD122 in the day 12 fetal liver was verified using in situ hybridization. Cytocentrifuged fetal liver mononuclear cells were probed with antisense cDNA probes specific for CD122, developed, and the number of positive cells on each slide scored as described in Materials and Methods. As shown in Table 1, the proportion of CD122° cells detected by in situ hybridization was similar to that observed by flow cytometry (4% to 6%). In addition, cells expressing mRNA encoding the α-chain (CD25) of the IL-2-receptor were present at a similar frequency. Although we have been unable to detect surface expression of CD25 on day 12 fetal liver cells, approximately 25% of CD122° cells in day 14 fetal liver coexpress IL2R α (data not shown). These cytocentrifuge preparations were also analyzed for IL-2-mRNA expression. As shown in Table 1, a significant number of IL-2-mRNA° cells were detected in the liver at this time in gestation.

Localization of IL-2 and IL-2Rβ-chain mRNA° cells in the day 12 embryo. To determine if CD122° fetal liver cells were destined to colonize the thymus, we examined frozen sections of 5, day 12, C57BL/6 mouse embryos for expression of CD122 mRNA within areas of the developing neck and pharyngeal region adjacent to the developing thymus (Fig 2, upper right panel). The ability to abolish reactivity of the anti-IL-2 antibody by preincubating with an excess of recombinant IL-2 (upper left panel) shows the specificity of the staining. In addition, we also found abundant expression of IL-2 in the day 15 fetal omentum (lower right panel, control shown on lower left), which is an active site of hematopoiesis during fetal development.25 In both of these tissues it is interesting to note that IL-2 is not localized to specific cells or compartmentalized to defined areas of the tissue but has instead a rather diffuse and even distribution.

Table 1. % mRNA° Cells in the Fetal Liver at Gestational Age

<table>
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<td>8.2±13</td>
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Cytocentrifuge preparations of cells probed with 35S-labeled RNA probes synthesized in either the antisense (a.s) or sense (s) orientation.

* Average grain count overlaying positive (>5 grains/cell) cells.
† Frequency of positive cells less than 10 in 1,000.
Fig 2. Immunohistochemical localization of IL-2 production in fetal hematopoietic tissues. Frozen sections of day 15 C57BL/6 fetal liver (upper panels) and omentum (lower panels) were stained with the rat-antimouse IL-2 antibody, S4B6 (upper and lower right panels) or with antibody previously incubated with 1,000 U/mL of recombinant IL-2 (upper and lower left panels). Bound antibody was visualized with a secondary biotinylated-antirat Ig antibody and an avidin-biotin-peroxidase conjugate (ABC reagent; Vector Labs). (Original magnification (OM) ×40.)

mus. Longitudinal sections from one of these embryos probed with 35S-labeled RNA probes complementary to either the coding or noncoding sequences of the β-chain of the IL-2R and IL-2 showed an interesting pattern of mRNA+ cells (Fig 3). Individual cells expressing high levels of CD122-mRNA+ were detected in close proximity to the vascular endothelium of a branchial blood vessel directly above the developing pharynx (Fig 3A and upper panel A') and within the connective tissue immediately below it (Fig 3, lower panel A'). On adjacent sections of the same embryo, distinct clusters of cells expressing CD122-mRNA were also identified in association with the connective tissue below the pharynx (Fig 3B) and adjacent to the developing pharyngeal pouches (Fig 3, upper and lower panels of B'). In addition, CD122-mRNA+ cells were also localized to the base of the stem of the thymic anlagen in the region of the third pharyngeal pouch (data not shown). A similar distribution of CD122-mRNA+ cells was seen using additional embryo sections processed in parallel or separately (Fig 3C). In contrast, no hybridization signal could be detected in any areas of adjacent embryo sections probed with either a control, sense, IL-2Rβ (Fig 3D) or IL-2 (Fig 4B and B') probe. Interestingly, on sections of day 12 embryos probed with an antisense IL-2–specific probe, IL-2–mRNA+ cells could be detected in close proximity to the developing thymus and within connective tissue surrounding the branchial arch (Fig 4A and A'). Consistent with findings from in situ hybridization analysis of cytocentrifuge preparations (Table 1), cells expressing the genes encoding the β-chain of the IL-2R and IL-2 were identified within the liver of day 12 fetal embryo sections.

The pattern of distribution of IL-2Rβ– and IL-2–positive cells within the pharyngeal region of day 12 embryos is consistent with their migration from the pharyngeal blood vessels after which they traverse the surrounding mesenchyme before entering the thymus.3,24 This suggested that at least some of the CD122+ progenitor cells in the pharyngeal region of the embryo sections may be destined to enter the thymus and ultimately give rise to T cells. To directly address this possibility we analyzed thymocyte populations present within the day 13 fetal thymus for expression of CD122 using multicolor flow cytometry.

CD122 is expressed by pre-T cell populations and is regulated during T-cell development. Although lymphoid cells are first detected in the mouse thymic rudiment at day 11 to 12 of gestation, it is not until day 13 of mouse gestation that sufficient numbers of thymocytes can be isolated for flow cytometric analysis. In the day 13 thymus a small population of Sca-1+ cells (15%) can be detected (Fig 5A). Analysis of CD122 expression revealed that more than 95% of these expressed CD122 (Fig 5B). In addition, this population of cells also expressed low levels of HSA (M169 antibody staining) and high levels of CD44 (data not shown). As thymocyte development progressed, the frequency of Sca1+ cells (Fig 5C) and in particular Sca1+ CD122+ cells (Fig 5D) declined, consistent with these cells being one of, if not the earliest thymic immigrant and T-cell precursor population.1
Expression of CD122 is regulated during B-cell development. In view of our finding that a subset of Lin− progenitor cells in the day 12 fetal liver express CD122 (Fig 1) we investigated the possibility that B-cell progenitor populations (pro-B cells) may express this cytokine receptor chain. Using an antibody to B220, which is thought to represent the earliest cell surface marker expressed by committed B cell progenitors,2,22 to analyze day 12 fetal liver cells it was determined that (Fig 6C) the majority (>60%) of B220+ cells were CD122+. The proportion of B220+ cells that were CD122− decreased during fetal life; whereas the proportion of B220− cells expressing CD122 was greater than 60% at day 12 of gestation, only 10% of B220+ cells were CD122− in the day 15 fetal liver. Compared to B220+ cells, only a...
Fig 4. Localization of IL-2–mRNA expressing cells in the neck region of a day 12 embryo. Six-micron paraffin-embedded sections of day 12 C57BL/6 mouse embryos were probed with either an 35S-labeled RNA probe complementary to mRNA encoding IL-2 (A and A') or as a control, an IL-2 probe transcribed in the sense orientation (B and B') as described in Materials and Methods. IL-2–mRNA+ cells present in (A) are shown at higher magnification in (A'). B, branchial arch; PP, pharyngeal pouches; T, thymus. Magnification: (A and B') ×320; (B) ×250; (A') ×500.

Fig 5. Expression of CD122 in the fetal thymus. Mononuclear cells isolated from the day 13 (A and B) and day 14 (C and D) fetal thymus were stained with anti-CD122, -Sca-1, and -CD45 antibodies and analyzed by three-color flow cytometry. CD45+, Sca-1+ T-cell progenitor cells (A and C) were analyzed for expression of CD122 (B and D). The percentage of Sca-1+ or CD122+ cells are indicated on each histogram. The results are representative of three independent experiments.
Fig 6. Expression of CD122 during B-cell development in the fetal liver. CD122 expression by developing B cells in the day 12 (A through C) and day 16 (D through F) fetal liver was analyzed by three-color flow cytometry using antibodies specific for CD122, B220, and CD24/HSA. The results are representative of those obtained from five independent experiments. In A through C, day 12 fetal liver mononuclear cells were stained with either an isotype control antibody (B) or the anti-CD122 antibody, TMpl (C). The boxed region in (A) identifies the B220<sup>+</sup> CD122<sup>+</sup> (2.4% of total cells analyzed) population shown as a histogram plot of CD122<sup>+</sup> cells in (C). Day 16 fetal liver cells (D through F) were used to analyze CD122 expression by pro-B cell populations, as defined by the B220 and HSA antibody staining profile (D). The boxed regions in (D) identify the HSA<sup>-</sup> B220<sup>+</sup> (upper box, representing 7% of cells analyzed) and the HSA<sup>-</sup> B220<sup>-</sup> (lower box, representing 1% of cells analyzed) populations analyzed for expression of CD122 (E and F, respectively). The frequency of CD122<sup>+</sup> cells among B220<sup>+</sup> HSA<sup>-</sup> (fraction A; E) and B220<sup>+</sup> HSA<sup>-</sup> (fraction B; F) pro-B cell populations is shown on each histogram.

A small proportion (between 10% and 20%) of developing erythroid (TER 119<sup>+</sup>) myeloid (Mac-1<sup>+</sup>) and granulocytic (Gr-1<sup>+</sup>) cells at this stage expressed CD122 (data not shown). To determine whether the expression of CD122 on B220<sup>+</sup> day 12 fetal liver cells was restricted to a particular subset of B cells, we analyzed its expression on populations of day 16 fetal liver cells using antibodies that have previously been used to resolve pro-B cells in the BM and fetal liver into three sequential stages (A through C) of development. Based on the differential expression of the surface markers B220, CD24 (HSA), and BP-1, pro-B cells can be resolved into pre-pro-B (fraction A; B220<sup>-</sup> S7<sup>-</sup> HSA<sup>-</sup>), early pro-B (fraction B; B220<sup>+</sup> S7<sup>+</sup> HSA<sup>-</sup>) and late pro-B (fraction C; B220<sup>+</sup> S7<sup>+</sup> HSA<sup>+</sup>) populations. Since fraction A can be distinguished from fractions B and C by the differential expression of HSA, a three-color flow cytometric analysis using HSA<sup>-</sup> B220<sup>-</sup> and CD 122-specific antibodies was performed. As can be seen in Fig 6E, by day 16 of gestation the proportion of B220<sup>+</sup> pre-pro B cells (HSA<sup>-</sup>) that express CD122 decreased to about 30% and of the newly emerging pro/late pro-B cells (HSA<sup>+</sup>) only 2% were CD122<sup>+</sup> (Fig 6F), consistent with its expression being restricted to an early B-cell precursor. These results were confirmed using BMC from SCID mice that serve as an enriched source of pro-B cells (Fig 7A). As in the fetal liver, the B220<sup>+</sup> cells in SCID BM were fractionated into pre-pro B cells (B220<sup>+</sup> HSA<sup>-</sup>) and late pro B cells (B220<sup>+</sup> HSA<sup>-</sup>) (Fig 7B). The pattern of expression of CD122 on these subpopulations was strikingly similar to that in fetal liver; approximately 80% of the B220<sup>+</sup> HSA<sup>-</sup> cells were CD122<sup>+</sup> (Fig 7C) whereas only 10% of B220<sup>+</sup> HSA<sup>-</sup> cells were CD122<sup>+</sup> (Fig 7D).

Lymphoid progenitor cells proliferate preferentially to IL-2. To determine the functional significance of CD122 expression by pro-B cell populations, cell fractions enriched for CD122 and/or B220 expression were isolated from either day 15 fetal liver or BM of adult SCID mice using the fluorescence activated cell sorter and cultured in the presence of either IL-2 or IL-15. As can be seen in Fig 8 (which is representative of three different experiments), the B220<sup>+</sup> pro-B cell populations from both sources proliferated vigorously to IL-2. The analysis of SCID BMC response to IL-2 shows that the responding cells within the HSA<sup>-</sup> fraction was further enriched for in the B220<sup>+</sup> fraction. This suggests that the ability to respond to IL-2 is primarily contained within this population of cells in the BM and fetal liver. Interestingly, although a small subset of B220<sup>+</sup> HSA<sup>-</sup> pro-B cells in SCID BM are CD122<sup>+</sup>, virtually no response to IL-2 could be detected. The difference in the response of the B220<sup>+</sup> HSA<sup>-</sup> cells obtained from the adult BM (stimulation index 100 to 125) and the fetal liver B220<sup>+</sup> (S.I. 3 to 4) cells probably reflects the difference in frequency of CD122<sup>-</sup> cells in each sample. Among the B220<sup>+</sup> cells isolated from day 15 fetal liver the CD122<sup>-</sup> population represented less than 10% whereas in the adult BM samples 80% of the B220<sup>+</sup> HSA<sup>-</sup> expressed CD122<sup>-</sup>. This increase in the size of the B220<sup>+</sup> CD122<sup>-</sup> cell pool in the SCID BM may simply reflect the expansion of these cells in the absence of more mature B-cell populations. It is also possible that it may be a conse-
expression of differences in the hematopoietic microenvironments present within the fetal liver and BM. These microenvironments may also change over time in such a way that they are only able to support the expansion and development of different progenitor populations during distinct periods of an animal’s development. The decline in the proportion of B220+ fetal liver cells that are CD122+ between day 12 and 15 of gestation may be a consequence of such a phenomenon. The specificity of the B220+CD122+ cells response to cytokines was shown by the absence of any proliferative response to IL-15. The optimal concentration of IL-2 (100 ng/mL) and IL-15 (100 ng/mL) for proliferation was determined by incubating PHA-stimulated human peripheral blood lymphocytes with varying concentrations of IL-2 or IL-15 (data not shown). In addition, we have also consistently failed to show any proliferative response to IL-15 over a wide range of concentrations in total or fractionated day 12 to 16 fetal liver cell populations (data not shown).

**A loss of B220+ cells in the bone marrow of IL-2−/− deficient mice.** The observation that large amounts of IL-2 protein are present in the fetal liver (Fig 2) and that B220+CD122+ cells in the fetal liver and adult BM proliferate in response to IL-2, suggests that they may utilize this cytokine in some way during their development in vivo. Consequently, we examined IL-2−/− mice for evidence of any disruption of B-cell generation. As previously reported, up to 2 weeks of age the B-cell composition of the IL-2−/− mice was very similar to that of wild type (IL-2+/+) mice. However, beyond 2 weeks of age a progressive loss B220+ cells was seen in IL-2−/− until by approximately 6 weeks of age the B220+ cells represented only 6% of the mononuclear cell fraction in the BM (Fig 9). Populations of immature (pro-, pre-) and mature (IgM+) B cells (B220+) were equally affected (T.R. and S.R.C., manuscript submitted). Considering the number of cells recovered from the femurs of IL-2−/− (6 × 10⁶) and IL-2+/+ (11 × 10⁶) shown in Fig 9 this reduction in frequency of B220+ cells represents a more than sixfold reduction in the absolute number of B cells in the IL-2−/− mice. Similar results have been obtained from the analysis of an additional 20 IL-2−/− mice.

**DISCUSSION**

The expression of CD122 (the IL-2/IL-15R β-chain) by developing hematopoietic cell populations during the fetal and adult phases of hematopoiesis in C57BL/6 mice has been characterized. We have shown that CD122 is expressed by multiple hematopoietic cell populations and in particular by T- and B-cell progenitors. In embryo sections, cells expressing this receptor were identified in and around the thymus during progenitor cell colonization and the majority of cells of the earliest pro-T cell population (expressing Sca1) also were found to express CD122. During B-cell development, expression of this cytokine receptor is also tightly regulated, being almost entirely restricted to the earliest known B-cell progenitor population, the pre-pro or fraction A² B cells. Moreover, we have shown that during lymphoid cell development in the fetal liver, large amounts of IL-2 are produced in situ at the time CD122+ progenitor populations are present. The significance of this observation was reinforced by our finding that highly purified adult BM and fetal liver CD122+ fraction A pro-B cells proliferate in response to IL-2 but not to IL-15. Interestingly, the ability of these cells to
respond to IL-2 was independent of any stromal cell contact. Finally, we have shown that in IL-2−deficient mice there is a progressive loss of B220+ cells in the BM, consistent with a requirement for IL-2 for the continued generation and/or viability of developing B cells.

The frequency of CD122+ cells (6% to 7%) in the mouse fetal liver contrasts with the much higher frequency of CD122 mRNA+ cells (approximately 75%) identified in the human fetal liver. Although the authors of this study inferred the presence of β-chain-containing IL-2R on the basis of showing the expression of intermediate-affinity IL-2R, using 125I-labeled IL-2, it was not possible to enumerate the cells expressing surface IL-2R-β chains. Therefore, it is possible that CD122-mRNA detected might not be translated and expressed on the surface of the mRNA+ cells. Alternatively, the difference in frequency of CD122+ cells reported in these studies could reflect differences in the regulation and/or cellular distribution of this cytokine receptor chain during hematopoiesis in humans and mice.

From our detailed analysis of the distribution of CD122 on various hematopoietic subpopulations in the day 12 fetal liver we have identified CD122 expression on a subset of...
Sca-1^Lin^- hematopoietic stem cells. However, because this fetal liver population does not express c-kit (data not shown), an antigen that has previously been shown to be expressed at high levels by pluripotent stem cells, it is possible that CD122 expression is restricted to lineage-committed progenitor populations. The expression of CD122 by cells committed to either the myeloid (Mac-1^), granulocytic (Gr-1^), or erythroid (Ter 119^; data not shown) progenitors is consistent with this interpretation. The lack of c-kit expression by B220^CD122^ fetal liver cells would appear, on the basis of previous phenotypic characterization of lymphocyte progenitor populations which have shown them to be c-kit^, to exclude it from being a pro-B population. However, there are several possible explanations for this inconsistency. A trivial explanation is that since we have used the anti-FcRyII/III antibody, 2.4G2, to enrich for CD122^ cells from the fetal liver it is possible that a B220^FcRy II/III-c-kit^-CD122^- progenitor population may also be present but was excluded from our analyses. Alternatively, it is possible that CD122 expression during B-cell development in the fetal liver is primarily restricted to a pre pro-B cell population that precedes the c-kit^ pro-B cell stage; the population we have described may acquire c-kit expression as they develop. Although we have not characterized c-kit expression on CD122^-mRNA^ cells that are found in close association with the day 12 thymus, if they are (Fig 3 and below) c-kit^, it may indicate that this antigen is acquired during their development upon entry into the thymus. Furthermore, during colonization, the thymus may be seeded by both c-kit^- and c-kit^+ progenitor cell populations. Finally, in view of the demonstration that fetal liver- and adult BM-derived T-cell progenitor populations can be distinguished by their maturational potential, it is possible that the phenotype of progenitor cells that colonize the thymus during fetal and adult life may also be different in that adult progenitors may express c-kit whereas fetal progenitors may not.

Previous studies have shown that during colonization, blood-borne pro-T cells leave adjacent vessels and traverse the surrounding mesenchyme before seeding the thymus. The presence of CD122 mRNA^ cells within the pharyngeal blood vessels and in close proximity to the developing thymus is consistent with them being in the process of migrating to the thymus and suggests that T-cell precursors migrating from the liver to the thymus express CD122. In agreement with this idea is our observation that the majority of Sca1^- intrathymic T-cell precursors within the day 13 thymus are CD122^ and the demonstration by Falk et al. that this population of cells isolated from day 14 fetal thymus can give rise to functionally mature T cells. Although the nature of the mechanism(s) involved in promoting or regulating stem cell migration and thymic colonization are not known, some studies have implied the involvement of specific homing molecules and thymic-chemotactic factors. The co-localization of IL2-mRNA^ cells in close proximity to the developing thymus in adjacent day 12 embryo sections suggests that IL-2 (or another cytokine able to interact with CD122) may also facilitate stem cell migration perhaps by maintaining their viability during transit.

From our analysis of B-cell development in the fetal liver and adult BM insights into the role CD122 may play in lymphopoiesis have been obtained. In both the day 12 fetal liver and adult SCID BM CD122 expression on B-lineage cells was developmentally regulated, being restricted to the earliest defined B-cell progenitor population, the pre-pro B cells that are B220^HSA^- (fraction A). Our finding that pre-pro B cells can be divided into CD122^ and CD122^- subsets implies, as originally proposed by Hardy et al, that this population is phenotypically heterogeneous. The observation that large numbers (10^5) of fraction A cells are required to reconstitute B cells in irradiated SCID recipients also implies that these cells are functionally heterogeneous. However, this result together with the finding that highly purified preparations of fraction A cells can give rise to pro-B cells (fraction B and C) after short term culture in vitro with stromal cells shows that this population of cells contains B-cell progenitors. At this time we do not know if this progenitor activity is contained within the CD122^- or CD122^ subset. Because several studies have shown that the B220-isoform of CD45 can also be expressed by mitogen- and cytokine-activated T cells, activated-natural killer (NK) cells and other lymphokine-activated major histocompatibility complex-nonrestricted killer (LAK) cells it is possible that the CD122^B220^-HSA^- cells we have detected in the fetal liver may contain cells not only of the B lineage but of other lineages as well. In view of the observation that CD122 is constitutively expressed by developing murine splenic NK cells it is possible that the B220^CD122^ fetal liver and BM population may be multipotential. However, because our studies have been performed at a time in gestation before the generation of functionally mature T cells and because CD122^-NK1.1^+ cells can only be detected after birth it is likely that the CD122^-B220^- cells we have identified in the day 12 fetal liver are primarily B-cell progenitors.

As cells progressed through subsequent stages of B-cell development CD122 expression was downmodulated with pre-B cells (fraction C) being essentially devoid of any CD122 expression. This developmentally regulated expression of CD122 is particularly interesting in light of recent results showing that among developing B-cell populations in the adult BM the IL-2Ra-chain (CD25) is restricted to pre-B cells. Although we have identified a population of CD122^- cells that express CD25 in the day 14 fetal liver (data not shown) it is possible that these cells are B220^-. Although the ability of CD25^- pre-B cells to respond to IL-2 was not directly tested, the results from previous studies showing that in the absence of the beta- and gamma-chains the alpha-chain is incapable of mediating signals in response to IL-2 suggest that CD122^- pre-pro B cells can be distinguished from CD25^- pre-B cells by their ability to respond to IL-2 in vitro.

Perhaps the most interesting feature of the proliferative response of the B220^HSA^-CD122^- cells to IL-2 is that it occurred in the absence of any stromal cell contact. Although this contrasts with the inability of other known B-cell growth factors (eg, IL-7 and steel locus factor) to promote the expansion of pre-B cells in the absence of stromal cells, it is similar to the IL-7-induced, stromal cell independent, proliferation of BM-derived pre-B cells. Together these
results suggest that different cytokines function in conjunction with, or independently of, stromal cells in a stage-specific manner to regulate the growth and differentiation of developing B cells.

The presence of IL-2 protein in the fetal liver at the time when CD122⁺ pro-B cells can be detected suggests that these cells may normally use IL-2 in vivo. A possible source of this IL-2 are γδ T cells that we have previously identified³¹ in the day 12 fetal liver of C57BL/6 mice. However, the production of IL-2 from a non T-cell source is also possible. Indeed, morphologic analysis of the IL-2-mRNA⁺ cells suggest that some may be of the myelo-granulocytic lineage. Further experimentation is required to definitively identify the cellular source of this IL-2-mRNA. Our finding that IL-2 was produced by cells in the fetal omentum is interesting because besides being an embryonic source of T and B lymphocytes in the mouse it is also the principal tissue from which CD5⁺ B cells arise.⁵⁰ These B cells represent a separate lineage that can be distinguished from conventional B cells with regard to several characteristics.⁴⁰⁻⁴⁴ Fetal omentum has also been shown to give rise to IgA producing cells in the gut.⁴⁷ Therefore, we cannot exclude the possibility that some B-cell populations may express and/or use IL-2 during their development.

Although it was originally reported that lymphocyte development was normal in IL-2-deficient mice⁶⁶ our finding that these animals have dramatically reduced numbers of B cells by 6 weeks of age clearly shows that B-cell generation, expansion and/or viability is severely compromised. Although we do not yet have an explanation for why this defect is not apparent earlier in these mice, it may reflect differences in the requirement for IL-2 by lymphocyte progenitor populations that arise at different times during the lifespan of the animal (eg, fetal, neonatal, and adult stages). Alternatively, IL-2 through its mitotic activity may serve to maintain the viability and the size of CD122⁺ B220⁺ cells in vivo as suggested by our observation that this population proliferates in response to this cytokine (Fig 7). Therefore, in the absence of an endogenous or exogenous supply of IL-2 this progenitor pool may eventually become exhausted as a consequence of the successive waves of B-cell development that occur before and after birth, eventually producing the phenotype we observe in the IL-2-deficient mice after 6 weeks of age. Interestingly, we have also identified a similar late-onset defect in other hematopoietic cell populations in these mice (T.R. and S.R.C., manuscript submitted). Very recently a similar late-onset defect in B-cell generation and/or viability has been observed in CD122-deficient mice.⁴⁸ Although the authors of this study suggest that this may be caused by the presence of large numbers of activated CD4⁺ T cells, the possibility that B-cell progenitor activity was also affected was not addressed. However, the observation that B-cell colony formation was significantly reduced in these mice even at 1 week of age, at which time the spleen contains very few T cells, implies that there may be an intrinsic B-cell defect in these mice. These mice also displayed abnormal levels of granulocyte and erythrocyte production, which in view of our finding that CD122 is expressed by immature granulocytic and erythrocyte cells in the fetal liver may reflect a requirement for IL-2 during their development in vivo.

In summary, all our results are consistent with a role for the IL-2–IL-2R signaling pathway in lymphocyte development perhaps by regulating the size of the progenitor pool or by promoting their progression through different developmental stages.

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REFERENCES

16. Carding SR, Jenkinson EI, Kingston R: Developmental con-
CD122 and Lymphoid Development

43. Waldschmidt TJ, Kroese FGM, Tygnett LT, Conrad DH, Lynch RG: The expression of B cell surface receptors. III. The murine low-affinity IgE FcεR is not expressed on Ly1 or ‘Ly1-like’ B cells. Int Immunol 3:305, 1991

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Regulated expression and function of CD122 (interleukin-2/interleukin-15R-beta) during lymphoid development

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