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Citation for published version:
PEAULT, B, WEISSMAN, IL, BAUM, C, MCCUNE, JM & TSUKAMOTO, A 1991, 'Lymphoid reconstitution of the human fetal thymus in SCID mice with CD34+ precursor cells', Journal of Experimental Medicine, vol. 174, no. 5, pp. 1283-1286. https://doi.org/10.1084/jem.174.5.1283

Digital Object Identifier (DOI):
10.1084/jem.174.5.1283

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published in:
Journal of Experimental Medicine

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Lymphoid Reconstitution of the Human Fetal Thymus in SCID Mice with CD34+ Precursor Cells

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Summary

The search for human hematopoietic stem cells has been hampered by the lack of appropriate assay systems. Demonstration of the ability of precursor cell candidates to give rise to T cells is of significant difficulty since dissociated in vitro cultured thymus stroma cells lose their ability to sustain thymocyte maturation. To define further the differentiative capacities of the rare human fetal liver and bone marrow cells that express the CD34 surface antigen and exhibit in vitro myeloid and pre-B cell activities, we have microinjected them into HLA-mismatched fetal thymus fragments, partially depleted of hematopoietic cells by low temperature culture. In vitro colonized thymuses have then been allowed to develop upon engraftment into immunodeficient SCID mice. Using this modification of the SCID-hu system, we show that low numbers of fetal CD34+ progenitor cells can repopulate the lymphoid compartment in the human thymus.

Materials and Methods

Hematopoietic Depletion and Reconstitution of the Human Fetal Thymus. Individual lobules from 19-22-wk fetal thymuses obtained from elective abortions were placed on sterile nitrocellulose filter squares (1 cm × 1 cm) (Millipore Corp., Bedford, MA) supported by absorbent gelatin sponges (Gelfoam; UpJohn, Kalamazoo, MI), in 3.5-cm petri dishes containing 2 ml of RPMI, 10% heat-inactivated FCS, and penicillin/streptomycin, for 7 d at 25°C in a 5% CO₂ atmosphere. An oil-filled syringe (Hamilton Co., Reno, NV) with a screw-operated plunger was used to inject, through glass micropipettes, suspensions (0.5-1 µl) of HLA-mismatched CD34+ or CD34- cells into low temperature-cultured thymus fragments. In vitro colonized thymuses were inserted under the kidney capsule of Nembutal-anesthetized CB17 scid/scid mice bred in our own facility.

Sorting of Hematopoietic Precursor Cells from Human Fetal Blood-forming Organs. Human fetal marrow cell suspensions were prepared from long bones of 15-20-wk-fetuses. The bones were split lengthwise, then placed in a 1-mg/ml solution of collagenase/dis-
cells were lysed by low osmolarity ammonium chloride treatment. Cell suspensions were mechanically prepared from livers of 14–20-wk fetuses, then placed on a Ficoll-Hypaque gradient (Sigma Chemical Co., St. Louis, MO) to remove hepatocytes, red blood cells, and debris.

CD34+ and CD34- cells were isolated on a FACStar® (Becton Dickinson & Co., Mountain View, CA) from fresh fetal liver or bone marrow mononucleated cell suspensions, after labeling with a monoclonal anti-CD34 IgM (kindly provided by Dr. Irving Bernstein, Fred Hutchinson Cancer Center, Seattle, WA), then with a goat antibody anti-mouse IgM, non-cross-reactive to human IgG, conjugated to PE or to FITC (Caltag Labs., South San Francisco, CA).

Analysis of T Cell Repopulation in Human Fetal Thymus Grafts. For analysis of chimerism, mice were killed by cervical dislocation and human thymus grafts dissected free of kidney tissue. Thymocytes were then mechanically dispersed and stained by two-color immunofluorescence, resuspended in the presence of propidium iodide, and analyzed on a FACScan® (Becton Dickinson & Co.). PE-labeled anti-CD3, anti-CD4, and anti-CD8 mAbs were purchased from Becton Dickinson & Co. Hybridomas secreting the following anti-MHC class I antibodies: BB7.2 (anti-A2), MA2.1 (anti-A2 and B7), GAPA3 (anti-A3), W632 (anti-A, B, C), BB7.1 (anti-B7), and MB40.2 (anti-B7 and B40) were obtained from the American Type Culture Collection (Rockville, MD). mAbs were purified from ascitic fluids and directly labeled with FITC.

Immunohistochemical Analysis of CD34+ Cell-reconstituted Human Fetal Thymus Grafts. 6-μm cryostat sections of frozen grafts were air-dried and fixed with acetone. Rehydrated sections were incubated successively with mouse monoclonal anti-human class I antibody, biotinylated-horse anti-mouse Ig antibody (Vector Labs., Inc., Burlingame, CA), and avidin-alkaline phosphatase at room temperature. For development, a mixture of Fast Blue BB salt (2.5 g in 50 μl dimethylformamide) and naphthol AS phosphate (2.5 mg in 50 μl dimethylformamide) was used, in 2.5 ml of 0.05 M propanediol buffer, pH 9.75, containing 60 μl of 10 mM levamisole to block endogenous enzyme activity. Sections were then postfixed with 20% formalin for 5 min, washed in distilled water, counterstained with hematoxylin, and mounted in gelatin-glycerol.

Results and Discussion

Reducing the size of the thymus hematopoietic cell compartment is a prerequisite to experimental reconstitution and can be achieved by low temperature in vitro organ culture (11). Human thymus tissue cultured at 25°C appeared as a loose epithelial network, showing large lymphocyte-free areas (not shown), as previously described in the mouse model (11). HLA-mismatched CD34+ or CD34- cell suspensions sorted from fresh human fetal liver or bone marrow were delivered by microinjection to the depleted thymus pieces. After engraftment under the kidney capsule of SCID mice, low temperature–cultured, in vitro colonized thymus fragments were vascularized and grew. At intervals, grafts were harvested, reduced into cell suspensions, and analyzed for chimerism in the T cell compartment. Using a two-color immunofluorescence assay class I HLA antigens served as markers for donor vs. host-derived lymphocytes. The initial phase of thymus lymphoid repopulation from injected donor cells was difficult to trace given the fact that nonmature and immature cortical thymocytes express very low quantities of class I antigens. Chimerism could be evidenced when the progeny of transferred cells was differentiated far enough to express detectable amounts of class I molecules, which occurred 3–4 wk after the engraftment of thymus colonized with 10⁶ CD34+ cells (Fig. 1 A). From this time onwards, the donor-derived population was observed to expand and mature, with larger numbers of thymocytes in the graft expressing higher amounts of the donor class I antigens. After 6 wk, the donor-derived thymocyte population in the graft was similar to that of a fresh normal thymus, with respect to its range of CL I antigen expression (Fig. 1 C and 2, A–C). These cells were analyzed by three-color immunofluorescence and found to include, in the expected proportions, donor-derived elements that were either CD4+ or CD8+ (not shown). In contrast, no T cell repopulation was ever observed after transfer of fetal liver or bone marrow CD34+ counterpart cells, which were analyzed in the same conditions in all experiments. The successful...
engraftment and differentiation of $10^4$ CD34+ cells prompted an attempt to repopulate the thymus with lower numbers of these precursors. As shown in Fig. 2, complete T cell repopulation was observed after supplying the thymus with $10^3$ and even $10^2$ CD34+ cells, the lowest number assayed so far.

To visualize the tissue distribution of the cells generated by the microinjected precursor cell population, sections from thymus grafts fully reconstituted with $10^3$ CD34+ cells were stained with mAbs specific for either host or donor class I HLA antigens. Host class I molecules were restricted to epithelial cells in the cortex and at the corticomedullary junction, and of cells with a dendritic morphology in the medulla where, by contrast, no stained lymphocytes were present (Fig. 3). The donor-specific anti-class I reagent stained almost exclusively packed lymphocytes in the medullary region. Whether cortical thymocytes were all donor derived or included resurgent host cells could not be determined in the present experimental system.

These results show that at least a fraction of the CD34+ cell population present in the fetal liver and bone marrow has the ability to differentiate into thymic lymphocytes when experimentally introduced into the thymic microenvironment.

Human CD34+ cells are phenotypically heterogenous and can be subdivided into several subsets coexpressing, among others, the myeloid-specific CD33, B cell–specific CD19 and CD10, and T cell–restricted CD7 lineage markers. Interest-
ingly, human in vitro colony-forming cells can be distinguished from their precursors since only the former express CD33, whereas all of them are CD34⁺ (12). CD7 is believed to be expressed by extrathymic pre-T cells (13, 14). The above-described thymic reconstitution assay should allow the definition of the subsets of human bone marrow cells that can give rise to thymic colonization and T cell differentiation, using techniques that defined at least two subsets of mouse bone marrow cells with this potential (15).

Due to the unavoidable variability of human material, differences are noted in the kinetics and extent of the T cell repopulation observed after in vitro intrathymic transfer of precursor cells. Also, in some cases, precursor cells sorted from one given donor were unable to reconstitute a particular thymus, at any number of transferred cells, suggesting that allogeneic reconstitution may be dependent upon yet unknown compatibility requirements. These constraints may be similar to those genetically defined factors enforcing host resistance to donor hematolymphoid transplants, even in irradiated hosts (16). However, despite these limitations, >40 successful T cell repopulations have been observed after CD34⁺ cell transfer.

In animal models, experimental chimeras have been instrumental at elucidating the complex cell interactions involved in the ontogeny of the thymus (17). Defining the ability of potential progenitors to differentiate into T lymphocytes is an important step towards the characterization and purification of human hematopoietic stem cells, as well as in the study of positive selection (MHC restriction) and negative selection (self-tolerance) of developing human thymocytes from genotypically defined precursors in contact with genotypically defined thymic stroma. Also, this system may allow studies towards the elucidation of potential human bone marrow targets of pathogenic human lymphotropic viruses such as HIV, HTLV, and EBV.

We thank the following people from SyStemix for their contribution to this work: Nancy Mori and Carrie Garneau for expert technical assistance; Shelly Heimfeld, Yukoh Aihara, and Hideko Kaneshima for helpful discussions; Dennis Sasaki for assistance with the FACS®; the SyStemix animal facility for providing SCID, yet healthy, mice; and Nita Chapman for preparation of the manuscript.

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Received for publication 1 March 1991 and in revised form 5 August 1991.

References

1. Whitlock, C., K. Denis, D. Robertson, and O. Witte. 1985. In vitro analysis of murine B-cell development. *Annu. Rev. Immunol.* 3:213.

2. Toogood, I.R.G., T.M. Dexter, T.D. Allen, T. Suda, and L.G. Latsha. 1980. The development of a liquid culture system for the growth of human bone marrow cultures. *Leuk. Res.* 4:449.

3. Ezine, S., I. Weissman, and R. Rouse. 1984. Bone marrow cells give rise to distinct clones within the thymus. *Nature (Lond.)* 309:629.

4. Goldschneider, I., K.L. Komschlies, and D.L. Greiner. 1986. Studies of thymocytopoiesis in rats and mice. I. Kinetics of appearance of thymocytes using a direct intrathymic adoptive transfer assay for thymocyte precursors. *J. Exp Med.* 163:1.

5. Guidos, C.J., I.L. Weissman, and B.J. Adkins. 1989. Developmental potential of CD4⁻8⁻ thymocytes. Peripheral progeny include mature CD4⁻8⁻ T cells bearing αβ T cell receptor. *J. Immunol.* 142:3773.

6. McCune, J.M., R. Namikawa, R. Kaneshima, L.D. Schultz, M. Lieberman, and I.L. Weissman. 1988. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science (Wash. DC)* 241:1652.

7. Civin, C.I., L.C. Strauss, C. Brovall, M.J. Fackler, J.F. Schwatz, and J.H. Shaper. 1984. Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. *J. Immunol.* 133:157.

8. Andrews, R.G., J.W. Singer, and I.D. Bernstein. 1986. Monoclonal antibody 12-8 recognizes a 115 kD molecule present on both unipotent and multipotent colony-forming cells and their precursors. *Blood.* 67:842.

9. Lu, L., D. Walker, H.E. Broxmeyer, R. Hoffman, W. Hu, and E.J. Walker. 1987. Characterization of adult human marrow hematopoietic progenitors highly enriched by two-color cell sorting with MY-10 and major histocompatibility class II monoclonal antibodies. *J. Immunol.* 139:1823.

10. Brandt, J., N. Baird, L. Lu, E. Srou, and R.J. Hoffman. 1988. Characterization of a human hematopoietic progenitor cell capable of forming blast cell containing colonies in vitro. *Clin. Invest.* 82:1017.

11. Robinson, J.H., and R.K. Jordan. 1983. Thymus in vitro. *Immunol. Today.* 4:41.

12. Andrews, R.G., J.W. Singer, and I.D. Bernstein. 1989. Precursors of colony-forming cells in humans can be distinguished from colony-forming cells by expression of the CD33 and CD34 antigens and light scatter properties. *J. Exp Med.* 169:1721.

13. Haynes, B.F., M.E. Martin, H.H. Kay, and J.J. Kurtzberg. 1988. Early events in human T cell ontogeny. Phenotypic characterization and immunohistologic localization of T cell precursors in early human fetal tissues. *J. Exp Med.* 168:1061.

14. Bertho, J.M., M.D. Mossalayi, A.H. Dalloul, G. Mouterde, and P. Debré. 1990. Isolation of an early Tcell precursor (CFU-TL) from human bone marrow. *Blood.* 75:1064.

15. Spangrude, G.J., J. Klein, S. Heimfeld, Y. Aihara, and I.L. Weissman. 1989. Two monoclonal antibodies identify thymic-repopulating cells in mouse bone marrow. *J. Immunol.* 142:425.

16. Cudkowicz, G., and J.H. Stimpfling. 1965. Hybrid resistance on control of data. *Science (Wash. DC)* 147:1056.

17. Le Douarin, N.M. 1978. Ontogeny of hematopoietic organs studied in avian embryo interspecific chimeras. *In Differentiation of Normal and Neoplastic Hematopoietic Cells.* Cold Spring Harbor Laboratory. Cold Spring Harbor, NY. 5–31.