RAPID COMMUNICATION

Localization of Hematopoietic Progenitor Cells in Tissue With the Anti-My-10 Monoclonal Antibody

W. E. BESCHORNER, C. I. CIVIN, and L. C. STRAUSS

From the Departments of Pathology and the Division of Pediatric Oncology of the Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland

Putative hematopoietic progenitor cells were localized in human lymphoid and nonlymphoid tissues with the use of the anti-My-10 antibody and avidin–biotin immunoperoxidase. Groups of My-10$^+$ round mononuclear cells were evident in the splenic marginal zone near the follicular mantle. My-10$^+$ cells were also evident in the hepatic portal triads and rare cells were seen near Peyer's patches. Except for My-10$^+$ endothelial cells, there was no staining of the thymus, lymph node, skin, or kidney specimens. (Am J Pathol 1985, 119:1–4)

PLURIPOTENT hematopoietic stem cells reside in large numbers in the fetal liver, spleen, and bone marrow, but are later found principally in the spleen and marrow.[1,2] The spleen is not only a rich source of stem cells but provides an environment ideal for the homing and maturation of stem cells, providing the basis for the colony-forming unit in spleen (CFUs) assay for enumerating the stem cells injected into lethally irradiated mice. It is not currently known, however, where in the normal spleen, the stem cells are located.

The anti-My-10 antibody, an IgG1k against a 115-kd membrane antigen, has been shown to specifically label human hematopoietic progenitor cells.[3] Although the My-10 antigen is detectable on only 2% of normal marrow cells, My-10$^+$ cells isolated from marrow account for over 90% of the immature hematopoietic cells as detected by *in vitro* colony-forming assays. My-10$^+$ marrow cells have an undifferentiated blast morphology. All bone marrow cells containing nuclear terminal deoxynucleotidyl transferase also express My-10. Approximately 30% of acute lymphocyte and nonlymphocytic leukemias are My-10$^+$, but chronic leukemias and lymphomas have not been found to express this antigen.

Using the anti-My-10 antibody and the avidin–biotin immunoperoxidase technique, we report here the localization of hematopoietic progenitor cells in lymphoid and select nonlymphoid human tissues.

Supported in part by grants from U.S. Department of Health and Human Services (CA15396, AI28703, and CA32318). Curt I. Civin is a scholar of the Leukemia Society of America. Lewis C. Strauss is a Fellow of the Leukemia Society of America.

Accepted for publication February 4, 1985.

Address reprint requests to William E. Beschorner, MD, Department of Pathology, The Johns Hopkins Hospital, Baltimore, MD 21205.

Materials and Methods

Tissue was obtained from postmortem (postmortem interval 2–5 hours), and surgical specimens. The specimens included 10 spleen specimens (6 normal, ages 2 months to 24 years, 2 with acquired immune deficiency syndrome, 1 with T-cell leukemia in remission, and 1 from a staging laparotomy for Stage 2 nodular sclerosis Hodgkin's lymphoma), 4 thymuses (ages 2 months to 3 years), 2 ileums with Peyer's patches, 16 lymph nodes with reactive hyperplasia, 2 liver specimens (ages 2 months, 10 months), 2 sections of skin, and 3 kidney specimens. The tissue was frozen in isopentane at −105 C and stored in liquid nitrogen.

The anti-My-10 monoclonal antibody was developed against the KG-1a human leukemic cell line, and its specificity for hematopoietic progenitor cells was established as described earlier.[3] Briefly, mouse spleen cells immunized against KG-1a blasts were fused with the non-Ig-secreting SP-2/0-AG14 mouse plasmacytoma cells. The resulting anti-My-10 antibody was selected for its binding to the KG-1a cells but not mature granulocytes, and is an IgG1k immunoglobulin which recognizes a 115-kd surface membrane protein. It has been extensively tested against normal marrow, peripheral blood, acute leukemias, and lymphomas. Anti-My-10 stains less than 2% of the peripheral blood mononuclear cells and marrow cells. Suspensions enriched for My-10$^+$ cells contain predominantly undifferentiated cells consisting of colony-forming cells for granulocytes-monocytes (CFC-GM) and burst-forming units-erythroid (BFU-E) as well as of TdT$^+$ cells. Suspensions depleted of My-10$^+$ cells are also depleted of CFC-GM, BFU-E, and TdT$^+$ cells.

Neat spent media from the My-10 hybridoma was used as the source of monoclonal antibody. The avidin–biotin immunoperoxidase technique used in this study has been described in detail elsewhere.[4] The hybridoma supernatant was diluted 1:10 with phosphate-buffered saline with 0.2% bovine serum albumin (PBS-BSA). Six-micron sections of the tissue were cut with a cryostat, air-dried and fixed in acetone. After washing, the slides were incubated 30 minutes with anti-My-10 mouse monoclonal antibody, or irrelevant IgG and washed. Endogenous peroxidase was inactivated with methanol and hydrogen peroxide. Endogenous avidin binding sites in the liver and kidney specimens were blocked with avidin D and biotin. The second antibody was biotinylated horse anti-mouse IgG (1:50, Vector Laboratories, Burlingame, Calif). The sections were exposed to the avidin–biotin complex, developed with diaminobenzidine and copper sulfate, and counterstained with Terry's methylene blue. Serial sections of spleen were also stained with mouse anti-Factor VIII (1:20, Cappel, Cochranville, Pa).

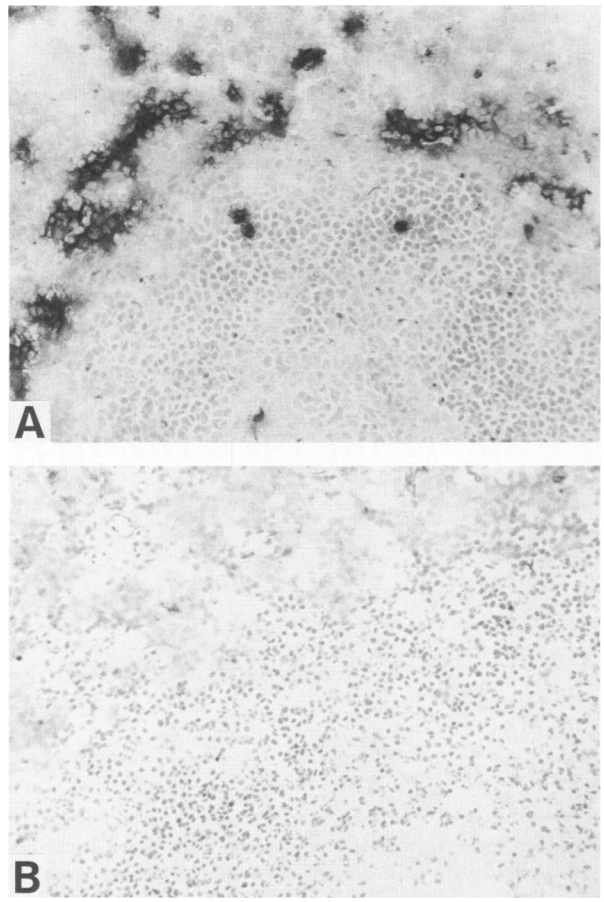

Figure 1—Spleen of a 24-month-old boy, stained with anti-My-10 antibody and avidin–biotin immunoperoxidase. **A**—Anti-My-10. Note the groups of My-10$^+$ cells in the marginal zone near the mantle zone. **B**—Negative control. (Terry polychrome methylene blue counterstain, ×250).

Results

Membrane expression of the My-10 antigen was apparent in groups of small mononuclear cells in 9 of 10 spleen sections, including all of the pathologically normal specimens. No My-10$^+$ lymphocytes were seen in the splenectomy of a staging laparotomy for Hodgkin's lymphoma. These collections of cells were present in the marginal zone near the interface with the follicular mantle zone (Figure 1). Elsewhere in the spleen sections and in other tissues there was also staining of the endothelial cells of arteries and veins. To exclude the possibility that the groups of My-10$^+$ cells were groups of capillaries, the stains were repeated, and sequential frozen sections were stained with anti-Factor VIII, an endothelial cell marker. Although scattered small vessels

were evident between the marginal and mantle zones, they were only scattered individual capillaries, considerably more sparse than the My-10$^+$ cells in these regions.

In the thymus sections, the My-10 antigen was detected only on the endothelium of vessels of the septa and occasional elongated endothelial cells in the medulla. The thymocytes of the cortex and medulla were negative.

None of the reactive lymph nodes contained My-10$^+$ lymphocytes.

Both of the pediatric liver specimens showed scatterd My-10$^+$ round mononculear cells in the portal triads, often near the hepatic artery in the larger triads. The bile duct epithelium, hepatocytes, and Kupffer cells did not express the antigen.

Scattered individual My-10$^+$ round mononuclear cells were observed adjacent to the Peyer's patches, next to the muscularis in two ileal specimens. We cannot totally exclude the possibility that these are capillaries.

Except for the staining of endothelium, the My-10 antigen was detected neither in 3 kidney specimens nor in 2 skin sections.

Discussion

With the use of the anti-My-10 antibody and immunoperoxidase procedures, the present study suggests that among the analyzed tissues, hematopoietic progenitor cells reside in greatest numbers in the spleen between the T- and B-cell zones of the follicles, in small groups in the hepatic portal triads, and possible rare cells near the Peyer's patches. Aside from the vascular staining, no My-10$^+$ cells were detected in the thymus, lymph node, skin, or kidney.

The significance of the splenic location of putative progenitor cells is not known. Located at the interface of mature T cells in the marginal zone and the B cells in the mantle zone,[5,6] they could represent committed lymphoid progenitor cells which provide a source of pre-B-lymphocytes and pre-T-lymphocytes. In this region, they would also be near mature helper T-lymphocytes and suppressor T-lymphocytes, allowing for regulation of hematopoiesis or lymphopoiesis.[7-10]

The My-10$^+$ putative hematopoietic stem cells observed in the liver were much less prevalent than those seen in the spleen. Do these cells in the portal triads permanently reside there, or are they simply in temporary residence downstream from the spleen in the portal circulation? It would be most interesting to evaluate specimens from splenectomized patients. Because the specimens examined were from infants, it is also uncertain whether these cells are remnants of the source of fetal hematopoiesis or represent a more mature "home" for progenitor cells.[2]

The relative numbers of My-10$^+$ cells in the spleen was quite variable, from rare cells to numerous small groups of cells. Many factors could contribute to this variability, including age, disease, environmental factors, etc. Interestingly, the greatest number of My-10$^+$ cells were observed in a 22-year-old man with T-cell leukemia in remission. The important factors, therefore, may not be so easy to discern. The relationship of circulating or marrow progenitor cells and splenic My-10$^+$ cells would also be enlightening.

Apparent endothelial staining with the anti-MY-10 antibody was evident in all tissues. Immunogold electron microscopy could readily determine whether the anti-My-10 antibody is binding to endothelial cells by the presence of Weibel–Palade bodies within the My-10$^+$ cells.[11] These My-10$^+$ cells could reflect margination of circulating stem cells, retention of early antigens in endothelial cells evolved from hematopoietic stem cells, or simple unrelated antigens. In addition, unrelated cells may share the same cell-surface protein. There is certainly precedent for the last possibility. Anti-OKT9 reacts with early thymocytes and plasma cells.[12] Anti-OKT6 reacts with cortical thymocytes and epidermal Langerhans cells.[12,13] Anti-cALLa reacts with the common acute lymphocytic leukemias and with glomerular basement membrane.[14]

An alternate possibility is that the endothelial cells developed from hematopoietic cells. The endothelium of skin xenografts have been shown to repopulate with host cells,[15] which raises the possibility that progenitor cells of the endothelial cells do, in fact, circulate.

We believe that this report represents the first microanatomic localization of lymphohematopoietic progenitor nonmyeloid human tissues.

References

1. Jacobson LO, Simmons EL, Marks EK, Robson MJ, Bethard WF, Gaston EO: The role of the spleen in radiation injury and recovery. J Lab Clin Med 1950, 35:746–770
2. Hann IM, Bodger MP, Hoffbrand AV: Development of pluripotent hematopoietic progenitor cells in the human fetus. Blood 1983, 62:118–123
3. Civin CI, Strauss LC, Brovall C, Fackler MF, Schwartz JF, Shaper JH: Antigenic analysis of hematopoiesis: III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J Immunol 1984, 133:157–165
4. Parfrey NA, Mann RB, Selonick SE, Beschorner WE: Malignant large cell lymphoma of B cell type with multilobated nuclei: Report of a case and review of the literature. Cancer (In press)
5. Brubacker DB, Whiteside TL: Localization of T cells in

sections by rosetting technique. Am J Pathol 1977, 88:323–332
6. Poppema S, Bhan AK, Reinherz EL, McCluskey RT, Schlossman SF: Distribution of T cell subsets in human lymph nodes. J Exp Med 1981, 153:30–41
7. Sieber F, Sharkis SJ: Modulation of murine erythropoiesis by syngeneic thymocytes. Interactions of enhancing and suppressing subpopulations with fluorescent anti-Theta antibody and polyamino acids. Blood 1982, 60:845–850
8. Pandolfi F, Strong DM, Slease RB, Smith ML, Ortado JR, Herberman RB: Characterization of a suppressor T-cell chronic lymphocytic leukemia with ADCC but not NK activity. Blood 1980, 56:653–660
9. Tsoi M-D, Warren RP, Storb R, Witherspoon RP, Michelson E, Giblett ER, Schanfield MS, Weiden P, Thomas ED: Autologous marrow recovery and sensitization to non-HLA antigens after HLA-identical marrow transplantation for aplastic anemia. Exp Hematol 1983, 11:73–81
10. Speck B, Gratwohl A, Nissen C, Osterwalder B, Signer E, Jeannet M: Bone marrow graft vs. anti-lymphocyte globulin in patients with aplastic anemia. Biomed Pharmacother 1983, 37:139–143
11. Hoffman-Fezer G, Lohmeyer J, Doxiadis I, Stuenkel K, Rieber EP, Kummer U, Eulitz M, Thierfelder S: Monoclonal antibodies against T cell antigens studied by immunohistochemistry. Blut 1982, 44:275–288
12. Murphy GF, Bhan AK, Sato S, Harrist TJ, Mihm MC: Characterization of Langerhans cells by the use of monoclonal antibodies. Lab Invest 1981, 45:465–470
13. Beschorner WE: Unpublished observations
14. Weibel ER, Palade, GE: New cytoplasmic components in arterial endothelium. J Cell Biol 1964, 23:101–112
15. Jooste SV, Colvin RB, Winn HJ: The vascular bed as the primary target in the destruction of skin grafts by antiserum: II. Loss of sensitivity to antiserum in long-term xenografts of the skin. J Exp Med 1981, 154:1332–1341

Acknowledgments

We gratefully acknowledge the technical assistance of Donna Suresch, Sharyn Hedrick, and Rosalind Richardson, the secretarial assistance of Nancy Folker, and the photographic assistance of Raymond Lund.