ANTIGENS EXPRESSED BY HUMAN B LYMPHOCYTES 
AND MYELOID STEM CELLS*

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It has previously been reported that rabbit antisera prepared with papain digests of human malignant spleen cell membranes recognize antigens expressed by normal and neoplastic human B lymphocytes (1-3). The antigens recognized by these antisera appear to be similar to, and may be identical with, the p23, 30 antigen identified by Humphreys and colleagues and by Schlossman and colleagues on normal B cells, 15-20% of null cells, and the majority of acute leukemia cells (4, 5). A surprising and unexplained observation was the frequent detection of similar antigens on acute and chronic myeloid (granulocytic) leukemia cells. It was not clear why neoplastic myeloid cells should express an antigen found on mature B cells but not mature granulocytes. To answer this question, we examined the effects of similar antisera on normal human myeloid stem cells. These cells, colony-forming unit-culture (CFU-C), were assayed for their ability to form granulocyte and macrophage colonies in agar, in vitro (6, 7) in the presence or absence of antiserum and complement (C). It is now clear that the antigens recognized by such antisera also occur on normal myeloid progenitors.

Materials and Methods

Antisera. As previously described, antisera were raised in rabbits immunized with papain digests of cell membranes from the spleen of patients with a variety of hematologic neoplasms (1).

Cells. Heparinized aspirates of bone marrow were obtained from appropriately informed normal adult volunteers, and single cell suspensions were prepared as previously described (8). Heparinized peripheral blood of patients with chronic lymphocytic leukemia (CLL) was used to prepare lymphocyte populations of >98% purity for absorption of antisera. Permanent tissue culture lines of B cells (pooled lines B44, 45, and 47) and T cells (MOLT-4) were also used for absorption (1).

Cytotoxicity Testing. Normal bone marrow cells, 2 × 10^6/ml, in 15% heat-inactivated fetal calf serum in McCoy's 5A medium were incubated with 1/10 volume of heated rabbit antiserum or heated normal rabbit serum at room temperature for 30 min. Normal rabbit serum, 1/10 volume, as a source of C was added for an additional 60 min. Cells were then plated in 0.3% agar above a feeder layer of 1 × 10^6 peripheral blood leukocytes for assay of CFU-C as previously described (8). After 10 days incubation the number of colonies with >40 cells were determined. Results were expressed as the percentage inhibition (mean ± SD) of colony formation in experimental assays relative to appropriate controls. The concentration of fresh rabbit serum used to provide C had no inhibitory effect on human marrow colony formation.

Results

Antisera to papain-digested spleen membranes inhibited normal marrow

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CFU-C in a concentration- and C-dependent fashion (Table I). Equivalent concentrations of fresh and heated serum from unimmunized rabbits had no inhibitory activity. The cytotoxic effect of antiserum and C appeared to be directed against myeloid stem cells rather than against mature end cells, since no abortive colony formation was observed during the culture period and the serum was not cytotoxic against mature granulocytes as tested by the method of Terasaki and McClelland (9).

The following experiments were performed to ascertain whether the antisera were detecting similar antigens on CFU-C and B lymphocytes. Antisera (300 µl) were absorbed with 50 × 10⁶ CLL cells, pooled lymphoblastoid B cells, or lymphoblastoid T cells (MOLT 4). Absorbed antisera were then tested for their inhibitory activity against normal marrow CFU-C. The results are shown in Table I and indicate identity of the antigens present on CFU-C and on neoplastic (CLL) B cells and permanently growing lymphoblastoid B cells. A permanent cell line of T cells did not absorb inhibitory activity. To exclude the possibility that the B-lymphoblastoid cells were abrogating the inhibitory activity by the production of colony-stimulating activity (CSA), separate studies were performed with B-cell-conditioned media. No CSA was detected.

**Discussion**

Prior studies led to the conclusion that antisera prepared against papain-digested spleen cell membranes recognized antigens present on normal B lymphocytes and also expressed by the majority (>70%) of acute and chronic lymphocytic and myelocytic leukemia cells and lymphoblastoid B-cell lines (1). The antisera have been shown to inhibit the mixed lymphocyte reaction. From these and F(ab')₂ blocking studies (10), it appears that the B antigen represents the common region of the HLA-D molecule. Detailed studies suggested that the antisera to papainized cell membranes reacted with a larger population of peripheral blood lymphocytes than those cells possessing surface-bound immunoglobulin. Additionally, bone marrow preparations from 61 leukemic patients in remission and from normal subjects had low numbers of antigen-positive cells (0–4%). The morphology of these positive cells was not determined. It is now clear that normal human myeloid stem cells also possess the antigen detected by these antisera. This observation probably explains the reaction of antisera with a larger population of cells than those expressing surface immunoglobulin and may explain the occurrence of antigen in association with neoplastic myeloid leukemia cells as well as neoplastic B cells (11).
Summary

Antisera prepared against papain-digested spleen cell membranes were known to be cytotoxic for normal and neoplastic human B lymphocytes and for a majority of acute and chronic myeloid leukemic cells. It is now shown that these antisera are also cytotoxic for normal myeloid stem cells (CFU-C), thus providing a probable explanation for their occurrence in myeloid neoplasia.

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