CONCISE REPORT

Altered Expression of Histocompatibility Antigens on “B” Large Cell Lymphomas

By Bruce A. Woda, Barbara Racklin, and Henry Rappaport

We describe three malignant lymphomas of the diffuse “histiocytic” (large noncleaved) type in which most of the large lymphoid cells express mononuclear surface immuno-

HISTOCOMPATIBILITY antigens play an essential role in transplantation rejection, cellular cooperation in immune responses, and recognition of foreign antigens on the cell surface. With few exceptions, mature lymphoid cells express surface histocompatibility antigens. In the course of our studies on the expression of HLA-A,B,C (HLA), HLA-DR (DR), and beta-2-microglobulin (β2m) by normal and neoplastic lymphoid cells, we found three diffuse “histiocytic” (large noncleaved) lymphomas in which the neoplastic cells did not express cell surface HLA or β2m as assessed by indirect immunofluorescence.

MATERIALS AND METHODS

Patient Specimens

Fresh lymph node and extranodal tissue was obtained from the Los Angeles Regional Lymphoma Cooperative, comprised of 24 hospitals that submit specimens of suspected lymphoid neoplasms to the City of Hope National Medical Center for morphological and immunologic studies. Lymph node were processed for light microscopy as previously described. Lymphomas were classified according to both the Rappaport and Lukes and Collins classifications. Cell suspensions were prepared as previously reported.

Antibodies

Fluorescein (Fl) labeled F(ab')2, fragments of goat anti-human mu, delta, gamma, and burro anti-kappa and -lambda chains were obtained from Kallestadt Laboratories, Chaska, Minn. Specificity of the antisera was ascertained by double diffusion in gel. Fl-IgG goat anti-human β2m was obtained from Atlantic Antibodies, Scarborough, Me. Monoclonal antibody specific for HLA antigens was obtained from Sera Laboratories, Sussex, England. Monoclonal antibody specific for DR antigens, SG157, kindly donated by J. Silver and S. M. Goyert, Research Institute of Scripps Clinic, was produced from a fusion of SP2/0-Ag myeloma cells with splenocytes from a BALT/c mouse immunized with human lymphoblastoid cells expressing HLA-DR antigens. This antibody precipitates a complex from cell lysates consisting of two polypeptide chains whose molecular weights and amino terminal sequences correspond to HLA-DR antigens.

Bound monoclonal antibodies were detected with Fl-F(ab')2, goat antimouse IgG (Cappel Laboratories, Downington, Pa.).

Surface Marker Studies

E-rosette-forming cells were determined as previously described. Surface immunoglobulin and β2m were detected by direct immunofluorescence. A quantity of 2 x 106 cells were suspended in 50 μl of the appropriate dilution of Fl-labeled anti-isotype, anti-light chain or anti-β2m, incubated on ice for 30 min, washed 3 times with Hanks' balanced salt solution–1% BSA–10 mM NaN3, fixed with paraformaldehyde (final concentration 1.4%), washed, and resuspended in 50 μl of media. Ten microliters were placed on a glass slide, covered slipped, and sealed with nail polish. In cases 1 and 3, the remainder of the cells were analyzed for fluorescence intensity by flow microfluorimetry (see below). HLA and DR were detected by indirect immunofluorescence. The 2 x 106 cells were suspended in 50 μl of the appropriate dilution of monoclonal antibody for 30 min on ice, washed 2 times, and stained with 1:20 dilution of Fl-F(ab')2, goat anti-mouse IgG, washed 3 times, and then processed as described above. Fluorescent cells were not detected when normal mouse serum was substituted for the monoclonal antibody.

Cells were examined with a Leitz microscope, equipped with an epifluorescence illuminator, rhodamine (Rh), and Fl filters and phase contrast optics. Two-hundred cells were counted to determine the percentage of stained cells. In cases in which small lymphoid cells were admixed with large lymphoid cells, each population was counted separately.

Experiments were done to ascertain the presence of HLA-negative cells in normal peripheral blood and tonsil. Cells stained with Fl-F(ab'), goat anti-human immunoglobulin (polyvalent) were incubated with anti-HLA and stained with Rh goat anti-mouse IgG. Two-thousand cells were examined to determine the presence of HLA. When HLA-negative cells were encountered, the presence or absence of surface immunoglobulin was noted.

Flow Microfluorimetry

Stained cells were analyzed for fluorescence intensity and light scatter in a fluorescence-activated cell sorter (FACS) (FACS IV, Becton Dickinson, FACS Systems, Mountain View, Calif.) as outlined previously. The percent of positively stained cells was determined as follows: cells above minimum fluorescence channel/total scatter.

Enzyme Digestion

In case 3, cells were incubated with media alone, 5 U/ml neuraminidase (Sigma Chemical Company, St. Louis, Mo.), or 0.1% trypsin (GIBCO, Long Island, N.Y.) for 30 min at 37°C and then stained to detect HLA antigens.

From the Department of Anatomic Pathology, City of Hope National Medical Center, Duarte, Calif. Submitted November 7, 1980; accepted December 4, 1980. Address reprint requests to Bruce A. Woda, M.D., Department of Anatomic Pathology, City of Hope National Medical Center, Duarte, Calif. 91010.

© 1981 by Grune & Stratton, Inc.

0006-4971/81/5704-0027$01.00/0
RESULTS

The three cases that did not have detectable HLA or $\beta_{3m}$ were diffuse histiocytic (large noncleaved) lymphomas of B-cell origin. As shown in Fig. 1, the cell suspension from case 1 was composed predominantly of large cells, which did not stain for HLA antigens. Analysis of this population in the FACS showed that 16% of the cells carried HLA (Fig. 2) and 11% $\beta_{3m}$. Eighty-five percent expressed gamma heavy chains, 90% kappa light chains, and 81% DR antigens.

The cell suspension from case 2 consisted of a mixture of small and large cells. Over 90% of the large, presumably neoplastic cells, carried mu heavy chains, kappa light chains, and DR antigens. Seven percent of the large cells expressed HLA and $\beta_{3m}$. Of the small, morphologically non-neoplastic cells, 7% carried mu heavy chains and 94% HLA antigens. FACS analysis of case 3 showed that 46% of the cells carried gamma heavy chains, 49% lambda light chains, and 60% DR antigens. HLA and $\beta_{3m}$ were detectable on 6% and 9% of the large cells, respectively. Neuraminidase or trypsin treatment did not increase the number of cells with detectable HLA.

During the course of this investigation, five additional large cell lymphomas (diffuse histiocytic) (two T-cell and three B-cell) that expressed HLA and $\beta_{3m}$ were studied (Fig. 2).

We tried to ascertain whether B-lymphoid cells without detectable HLA were present in normal peripheral blood (two specimens) or tonsil (one specimen) by performing dual immunofluorescence studies of cells stained with anti-immunoglobulin and anti-HLA. These studies showed that 1% of the cells did not express HLA; however, none of these cells had surface immunoglobulin.

DISCUSSION

In this study we have shown that HLA and $\beta_{3m}$ were not detectable on the plasma membrane of some large cell lymphomas of B-cell origin. We will not know the incidence and clinical significance of this subtype of large cell lymphoma until more cases are studied. These lymphomas may have arisen in a B-cell subset that expresses surface immunoglobulin and DR antigens, but lacks HLA and $\beta_{3m}$, however, we could not find such cells in normal peripheral blood or tonsillar lymphocytes. It appears that this cell does not...
have a normal counterpart, as has also been reported in T-cell acute lymphoblastic leukemia. It is intriguing to hypothesize that these lymphomas originally expressed HLA and β₂m, but that in order to escape immune surveillance by cytotoxic T lymphocytes, which recognize foreign antigens in the context of self-histocompatibility antigens, lost these antigens from the cell surface. The simplest explanation for this loss would be that these cells have mutations that cause deletion of HLA and β₂m from the cell surface.

Two Burkitt's lymphoma lines, Daudi and Chevalier, and K562, a line derived from the blood of a patient with chronic myelogenous leukemia in blast crisis, also do not express HLA. In addition, Daudi also lacks β₂m. The loss of HLA expression by Daudi is secondary to a loss of β₂m expression. A similar event may have occurred in the cases we reported; alternatively, there may be abnormalities in both HLA and β₂m or HLA alone. Alterations in HLA expression have also been reported in immunodeficient children and in a patient with Hodgkin's disease undergoing radiotherapy.

Factors other than the absence of HLA and β₂m may have contributed to our inability to detect these cell surface proteins. (1) They may have been present at a low level, below the threshold of detection of our system, which can detect proteins with as little as 10,000–15,000 molecules per cell. (2) These proteins may not have been available for antibody binding due to their being masked by autoantibodies directed against HLA, β₂m, or tumor-associated antigens. This is not likely, as such masking would have been reflected in the neoplasms staining with both anti-kappa and anti-lambda antibody. (3) The proteins could have been masked by additional sialic acid residues or trypsin-sensitive glycoproteins. This possibility was diminished as we could not detect HLA or β₂m on case 3 after trypsin or neuraminidase treatment. (4) Altered membrane fluidity may have accounted for the alterations we detected, but this is improbable because it should have been reflected in difficulty in detecting other membrane proteins.

It has been shown that the anti-HLA antibody we used binds better to the HLA-β₂m complex than to HLA alone, however, the loss of β₂m should not have abolished the anti-HLA binding. We cannot exclude the possibility that antigenic variation of both HLA and β₂m prevented their detection. Whether these proteins were not synthesized or, if synthesized, were not inserted into the plasma membrane awaits further study.

REFERENCES

Altered expression of histocompatibility antigens on "B" large cell lymphomas

BA Woda, B Racklin and H Rappaport