A Hodgkin Cell-Specific Antigen is Expressed on a Subset of Auto-
and Alloactivated T (Helper) Lymphoblasts

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A Hodgkin cell-specific antigen detected by the monoclonal antibody Ki-1 was found on T helper lymphocytes after activation by autologous and allogeneic stimulator cells. About 50% of lymphoblasts generated by auto- and alloactivation reacted with the antibody. In contrast, only less than 6% of lymphoblasts stimulated with Con-A, phytohemagglutinin (PHA), or protein A, and none of lymphoblasts activated by oxidative mitogenesis, expressed this antigen. Among several permanent cell lines tested, the K562, MOLT-4, HL-60, and EBV transformed B lymphoblastoid cells reacted with the Ki-1 antibody. The results may indicate possible relationships between the autoreactive subset of T lymphocytes and the pathogenesis of Hodgkin's disease.

THE ORIGIN AND IDENTITY of the neoplastic cell in Hodgkin's lymphoma are still under debate. Recently, a monoclonal antibody (Ki-1) has been described that reacted specifically with Hodgkin (H) and Sternberg-Reed (SR) cells, but not with any other cells in the biopsy material. A small subset of cells in normal hyperplastic lymphoid tissue was also found to bind the Ki-1 antibody, but to lack markers for T cells, B cells, and monocytes. This novel cell type has been suggested to be the normal counterpart of H and SR cells. However, we report the transient expression of the Ki-1 antigen on T helper lymphocytes after activation by autologous and allogeneic stimulator cells. The results may help to further understand the immunopathologic abnormalities in patients with Hodgkin's disease and indicate a possible relationship between H (SR) cells and autoreactive T cells.

MATERIALS AND METHODS

Cells

Human mononuclear cells were separated from peripheral blood by the Ficoll-Hypaque method, washed 3 times in phosphate-buffered saline (PBS), resuspended and plated in RPMI 1640 supplemented with antibiotics, l-glutamine, 5 x 10^{-5} M 2-mercaptoethanol, and 10% fetal calf serum (FCS) at 5 x 10^6/ml. After 60 min, nonadherent cells were collected. Phenotyping with monoclonal antibodies revealed mainly B and T lymphocytes with less than 1% monocytes. For stimulation, irradiated (3,000 rad) allogeneic cells were mixed with responders at 1:1 to a final concentration of 10^6 cells/ml suppl. RPMI 1640; concanavalin A, (Con-A) phytohemagglutinin (PHA), and protein A were added at indicated concentrations. For oxidative mitogenesis, cells were incubated for 30 min with 2 mM sodium periodate (Merck Chemicals, Darmstadt, West Germany), washed 3 times with PBS, and plated at 10^6/ml.

Human permanent cell lines were kept in suppl. RPMI 1640 + 10% FCS and passed twice weekly.

Surface Marker Analysis

Cells were harvested, washed 3 times in phosphate-buffered saline (PBS), and transferred onto polylysine-coated glass slides for staining of surface antigens by the peroxidase-antiperoxidase method (PAP) using a technique described earlier with minor modifications. Cells attached to the positively charged glass surface are fixed with 0.05% glutaraldehyde for 15 min at 4°C to block Fc receptors and preserve cell morphology. A gelatin-containing medium is used for preincubation and dilution of antisera to avoid binding of immunoglobulins to the glass surface. The staining includes: (1) incubation with monoclonal antibodies, (2) rabbit anti-mouse immunoglobulin (Dakopatts, Copenhagen, Denmark), (3) swine antirabbit immunoglobulin (Dakopatts), (4) peroxidase-antiperoxidase complex from rabbit (Dakopatts), and (5) dianamobenzidine-H_2O_2, followed by postfixation with OsO_4. The slides are covered with glycerin and coverglass. Controls were routinely performed with the different sandwich antisera. A panel of murine monoclonal antibodies of different Ig classes that did not react with the cells to be tested were usually included in our phenotyping procedure and thus served as negative controls, indicating no unspecific Fc receptor-mediated binding. Ki-1 antibody (donated by Dr. H. Stein) was used at a 1:10 dilution, OKIa1, OKT4, OKT6, OKT8, OKT9, OKM1 (Ortho Pharmaceutical Corp., Raritan, NJ), anti-Leu-7, anti-lambda, anti-kappa (Becton Dickinson, D-6074 Rödermark), anti-human monocyte (Bethesda Research Lab. Inc., Gaithersburg, MD), Coulter clone B1 (Coulter Immunology, Hialeah, FL), and VIM-D5 (donated by Dr. Knapp, Vienna) were used at a 1:100 dilution. Cells were counted as positive when dark brown cell membranes were seen, as indicated in Fig. 2. Data are expressed as percentage of Ki-1-positive blast cells compared to total cells per culture; in Fig. 1, the results of 1 typical experiment are shown (1 of 3 experiments)

RESULTS

Functional and phenotypic analysis of H and SR cells revealed a striking similarity to the dendritic cell first described in the mouse and rat. In an attempt to use the Ki-1 antibody as a marker for distinct differentiation stages of the human dendritic cell and thereby prove the hypothesis that this is the normal equivalent cell, we phenotyped adherent mononuclear cells from the peripheral blood during long-term culture on hydrophobic Teflon. In about 1 of 15 individual cultures, we observed the appearance of a small Ki-1-positive nonadherent blast-like cell popul-
Fig. 1. Expression of the Ki-1 antigen during an autologous mixed lymphocyte culture. Nonadherent mononuclear cells were cultured in supl. RPMI 1640 + 10% FCS at 10⁶/ml. On indicated days, surface marker analysis was performed using the PAP method. Data are expressed as percentage of Ki-1-positive blast cells compared to total cells per culture; the results of 1 typical experiment (of 3) are shown.

Fig. 2. Ki-1-positive blast cell in a 6-day-old culture of nonadherent peripheral blood cells (magnification x640). Note the unstained small and large lymphocytes.

against B lymphocyte or monocyte lineage-specific antigens was observed.

The highest percentage of autoactivated Ki-1-positive blasts was yielded by simply cultivating unseparated nonadherent mononuclear cells from peripheral blood, but we obtained similar results in setting up cultures using purified T cells as responders and irradiated non-T cells as stimulator cells. Polyclonal activation of T lymphocytes with Con-A, PHA, and protein A generated only 2%-6% of Ki-1-positive blasts, whereas none were seen after oxidative mitogenesis (Table 1). Screening of different permanent cell lines revealed binding of the Ki-1 antibody to a T cell leukemia. EPS Epstein-Barr virus (EBV) transformed B lymphoblastoid cells (author's own laboratory), a chronic myelogenous leukemia cell line, and to a minority of leukemic promyelocytes.

However, none of freshly isolated cells of normal blood, various leukemias, and lymphoma types reacted with the Ki-1 antibody (not shown).

DISCUSSION

The normal counterpart of the malignant cell in Hodgkin's disease and its relation to the many immunologic defects seen in these patients has not yet been defined. A recently described hybridoma antibody (Ki-1), which reacted specifically with the H and SR cells in biopsy tissues, also stained a small cell population that lacked conventional markers for B, T, or monocytic cell origin. It was speculated that this cell might be the normal equivalent of the H and SR cells.

Our data clearly show that the surface antigen detectable by the Ki-1 antibody is found on auto- and alloactivated T helper cells, whereas only less than 6% of lymphoblasts generated after polyclonal stimulation reacted with the antibody (Table 1). From Fig. 1, it is obvious that at least the majority of autoactivated Ki-1-positive lymphoblasts are of helper (T4⁺) phenotype. This is in accordance with the reports of others that the responding cell in the autologous MLC belongs to the T4 subset. Thus, the Ki-1 monoclonal antibody might specifically bind to autoreactive T cells. The generation of T4⁺HLA-DR⁺Ki-1⁺ cells during alloactivation, however, could represent the involvement of autoreactive cells in the allogeneic MLC. Others have suggested that autoactivated T and B cells may assemble an inducer circuit to trigger the allospecific immune response. As nearly 50% of all alloactivated blasts react with the Ki-1 antibody, this inducer circuit may comprise the major part of the overall reaction in the MLC, as measured by increase of thymidine uptake, total blast cell count, and production of interleukines. The biologic relevance of the
rapid and effective lymphocyte response to an infinite number of possible alloantigens (within the species) is still not understood. It could reflect cross-reactivity of T cell receptors for autologous products of the major histocompatibility complex (MHC)\(^{20}\) or it might result from continuous in vivo stimulation via the autoreactive inducer circuit.

The expression of the Ki-1 antigen on EBV-transformed B cells cannot be explained yet. However, it is of interest that lymphoblastoid cell lines are potent stimulators in the autologous MLC\(^{21,22}\) and have been introduced as effective feeder cells for the growth of autologous cells of various types.\(^{23,24}\) As it has also been shown that autoreactive T cells are required for the synthesis of Ig by autologous B cells,\(^{25}\) it is tempting to speculate that the Ki-1 antibody might react with a common autospecific T and B cell receptor. The Ki-1 reactivity with tumor cell lines of otherwise different phenotype further indicates that the Ki-1 antigen is not cell lineage restricted. It may resemble a gene product, which is expressed upon activation of cells by various stimuli or upon permanent in vitro culture of malignant cell lines. Immunoprecipitation should clarify whether the surface structures stained with the Ki-1 antibody are identical or cross-reacting molecules. However, as autoreactive cells seem to be the only normal cells that can express the Ki-1 antigen, and as no other malignant cell types besides H and SR cells react with the Ki-1 antibody in situ, a close correlation between these autoreactive T cells and Hodgkin’s disease is suggestive. However, as other pertinent data from the literature are missing, it would be still too speculative to describe Hodgkin’s disease as the result of a malignant transformation of an autoactivated T cell clone.\(^{26}\) It should be noted, however, that T4 antigens indeed have been found in rare cases on H and SR cells in situ.\(^{3}\)

The data presented here may be at least helpful in explaining the anergy of lymphocytes in patients with Hodgkin’s disease to allo- and autoactivation.\(^{1,27,28}\) In addition, the Ki-1 antibody may be useful to define the activated autoreactive subset within the T cell lineage. The antibody could thus be of experimental value to further investigate the role of autoactivation in the immune response.

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