Characterization and Classification of Rat Leukemias and Lymphomas by Membrane Markers

Tetsuya Moriuchi, Masaharu Kasai, Hideo Yamaguchi, and Hiroshi Kobayashi

Laboratory of Pathology, Cancer Institute, Hokkaido University School of Medicine, Kita-15-jo, Nishi-7-chome, Kita-ku, Sapporo 060, Japan

ABSTRACT

An immunological characterization of leukemias and lymphomas was made in the rat by using a panel of membrane markers in combination with morphological analysis. In the present study, five antigen markers and three surface markers were used for the characterization of 20 rat leukemias and lymphomas, and it was indicated that they could be divided into at least six groups. Of the lymphomas studied, six thymic lymphomas (Group 1) had the Thy-1.1 antigen, T-cell antigen, and receptors for guinea pig red blood cells; five extrathymic lymphomas (Group 2) lacked T- and B-cell antigens, receptors for guinea pig red blood cells, and surface immunoglobulin, but three of them had complement receptors. An absorption test revealed that Group 2 lymphomas possess a very low amount of the Thy-1.1 antigen compared to Group 1 lymphomas. None of the leukemias studied had detectable T- and B-cell antigen. Four leukemias had undifferentiated blast cell morphology and bore the Thy-1.1 antigen; three leukemias (Group 3) reacted with anti-lymphocyte serum, but one leukemia (Group 4) did not. Two leukemias (Group 5) had only the complement receptor and morphologically showed granulocytic appearance. Three leukemias (Group 6) had none of the membrane markers used and morphologically resembled erythroblasts. Based on these results, an attempt was made to classify these leukemias and lymphomas into T-cell lineage, B-cell lineage, stem cell, myeloid, and erythroid groups, respectively. Furthermore, the stage of differentiation in the lymphocyte maturation pathway of the leukemias and lymphomas belonging to Groups 1 to 4 is discussed.

INTRODUCTION

Different types of leukemias and lymphomas have been induced in rats by various chemicals and MuLV's (3, 6, 11, 12). For many years, the classification of rat leukemias and lymphomas has been essentially based on morphological and cytochemical criteria. Membrane markers are not yet routinely used for their characterization in the rat, although several murine leukemias and lymphomas have been well characterized by membrane markers (15). Recently, several membrane markers of lymphocytes and other hematopoietic cells have been reported in the rat (1, 5, 17). The identification of immunological membrane markers on leukemias and lymphomas in the rat made it possible to reclassify them on a more objective basis. In the rat, in contrast to the mouse, Thy-1 is not a T-cell marker (18). Furthermore, it has been reported that the rat pluripotent stem cell in hematopoietic tissues possesses the Thy-1 antigen (2). These recent reports indicate that Thy-1 antigen is a useful marker for the study of hemopoiesis in the rat. The present study indicated that the Thy-1 antigen might serve to differentiate “stem cell” leukemia from lymphoma of B-cell origin, i.e., if the antigen were present on the former and lacking or much less in the latter. In the present study, 20 rat leukemias and lymphomas were divided into 6 groups by using 5 antigenic and 3 surface markers. It was demonstrated that thymic lymphomas (Group 1) are of T-cell lineage, and it was suggested that extrathymic lymphomas (Group 2) are of B-cell lineage. The immunological characterization of morphologically unclassified leukemias, i.e., Groups 3 and 4, revealed that their antigenic profiles resemble those of lymphoid and pluripotent stem cells, respectively. Each position of the Thy-1-positive groups of leukemias and lymphomas in the lymphocyte maturation pathway is discussed.

MATERIALS AND METHODS

Animals. WKA/Hok rats were obtained from the Experimental Animal Center, Faculty of Science, Hokkaido University, Sapporo, Japan. Donryu rats were supplied from Nippon Rat Co., Ltd., Urawa, Japan. C3H/He and AKR/J mice were obtained from the First Department of Pathology, Hokkaido University, School of Medicine, Sapporo, Japan.

Tumors. Twenty ascites tumors were used in the present experiment. They were: (a) G-MuLV-induced lymphoma, WGT-13, -15, -20, and -25 (WKA/Hok); (b) LLVF-induced lymphoma, WFT-6 (WKA/Hok); (c) F-MuLV-induced lymphoma, WFT-2N, -22, -24, and -25 (WKA/Hok); (d) R-MuLV-induced lymphoma, WFT-5 and -6 (WKA/Hok); (e) BNU-induced leukemia, DBLA-1, -6, -9, and -10 (Donryu) (11), KNL-13 (WKA/Hok), and L1005 (W/Fu) (13); (f) ENU-induced leukemia, AL-604 (ACI/N); and (g) F-MuLV-induced leukemia, WFT-28E and -29E (WKA/Hok). WGT, WFT, WRT, and WLFT lines were derived from the enlarged thymus, spleen, or lymph nodes of WKA/Hok rats after neonatal infection with G-, F-, R-MuLV, or LLVF, respectively. These ascites tumor lines were maintained in the corresponding virus-infected tolerant rats (7). Chemically induced leukemia lines were maintained in ascites form in syngeneic rats.

Cell Suspensions. Normal lymphoid organs, thymus, spleen, and lymph nodes, were removed from donor animals, finely minced with scissors, and gently blended with a loose-fitting glass homogenizer (Bellco Glass, Inc., Vineland, N. J.) in MEM. Plugs of marrow were expressed from the femurs and tibias of
exsanguinated rats by perfusing the exposed marrow cavities with MEM. Clumps of cells were dispersed by gentle aspiration using a fire-polished Pasteur pipet. The crude cell suspensions were filtered through a cotton gauze sponge, washed twice by centrifugation at 1000 rpm for 5 min, and resuspended to a concentration of 5 x 10^6 cells/ml in MEM. Ascites tumor cells were obtained from peritoneal lavage. The tumor cells were washed twice in MEM. Viability as judged by trypan blue exclusion was 85 to 95% for normal, leukemia, and lymphoma cells.

**Antisera.** Two anti-Thy-1.1 alloantisera (Batch 1 and Batch 2) were used. Batch 1 was the gift of H. Sato, Department of Pathology, Asahikawa Medical College, Asahikawa, Japan. Batch 2 was produced in our laboratory by immunizing C3H/He mice with AKR/J thymocytes. The heteroantisera used were prepared in rabbits according to published methods (5, 9, 10). Briefly, rabbit ALS was produced by giving rabbits i.v. injections of WKA/Hok lymph node cells (4 x 10^6 cells) 3 times at 2-week intervals followed by bleeding 1 week after the last injection. The antisera were absorbed twice with a 1:1 packed volume of syngeneic KMT-17 fibrosarcoma cells and once with a 1:1 packed volume of WKA/Hok brain homogenate to absorb contaminating anti-Thy-1 antibodies. Rabbit anti-rat T-lymphocyte serum was prepared by the method of Ishii et al. (5). Rabbit anti-B-lymphocyte serum was produced by absorbing ALS with WKA/Hok thymocytes. Antisera against DBLA-6 cells were obtained by i.v. immunization of rabbits with 4 x 10^8 DBLA-6 cells 3 times at 2-week intervals and by bleeding them 1 week after the last injection. The antisera were absorbed 3 times with a 1:1 packed volume of syngeneic AH-66 hepatoma cells. The reactivity of anti-DBLA-6 serum on rat lymphomas and leukemias was reported previously (10). All antisera were heat inactivated at 56° for 30 min and stored at -70° until used.

**Rosette Assay for the Binding of YC and GpRBC.** The YC rosette method described by Rivero et al. (14) was used to detect complement receptors on cell membranes, based on the use of baker's yeast particles treated previously with human immunoglobulin, 5 x 10^6 normal lymphoid or tumor cells were incubated with 0.1 ml of 1:5 diluted goat anti-rat immunoglobulin serum conjugated with fluorescein isothiocyanate (Hyland Division, Travenol Laboratories, Inc., Costa Mesa, Calif.) for 60 min at 0°. The cells were then washed 3 times with MEM. The pellet was resuspended in 50% glycerol in MEM, and 1 drop of the suspension was examined with a Leitz fluorescent microscope.

**Cytotoxicity Test.** An equal volume (0.05 ml) of antiserum at doubling dilutions, a cell suspension (5 x 10^6/ml) in MEM, and guinea pig complement were incubated at 37° for 45 min and washed in cold MEM. The proportion of dead cells was determined by microscopy with trypan blue solution. More than 200 cells were counted in each specimen. The cytotoxicity index was calculated as follows: (c — t) x 100/c, where c is the percentage of unstained cells in the control sample and t is the percentage of unstained cells in the test sample. The mean control toxicity (target cells plus complement) for all tests was less than 10%. A cytotoxicity index higher than 50 was regarded as a positive reaction.

**Quantitative Absorption Test.** The capacity of different lymphoid cells to absorb the cytotoxic activity of antisera was determined by incubating 0.1 ml of adequately diluted antiserum with a decreasing number of cells from various sources. The test tubes were left at 4° for 60 min with occasional mixing. After centrifugation, the supernatants were tested for residual cytotoxic activity against WKA/Hok thymocytes. The number of cells required to reduce the cytotoxicity to 50% (B) were expressed in relation to the number of target cells (A) required for the same reduction (absorbing capacity = A/B x 100).

**Morphology.** Air-dried smears of tumor cells were stained with May-Grünwald-Giemsa stain.

**RESULTS**

**Characterization of Rat Leukemias and Lymphomas by Membrane Markers.** The reactivity of membrane markers used on normal lymphoid cells is shown in Table 1. By using these membrane markers, the leukemias and lymphomas studied could be divided into 6 groups (Table 2). Group 1 represents MuLV-induced lymphomas which were of thymic origin and had a lymphoblastic appearance. All lymphomas in Group 1 were killed by ALS, anti-DBLA-6, anti-Thy-1.1, and anti-T sera, and showed rosette formation with GpRBC. None of them were killed by anti-B serum, nor did they have surface immunoglobulin and complement receptors. Group 2 represents MuLV-induced extrathyemic lymphomas. These lymphomas also had a lymphoblastic appearance and were morphologically indistinguishable from the lymphomas in Group 1. They were killed by ALS and anti-DBLA-6 sera but not by anti-B serum. None of them demonstrated surface immunoglobulin. However, a great difference was found between the lymphomas in Groups 1 and 2 with respect to other membrane markers. None of the lymphomas in Group 2 were killed by anti-Thy-1.1 and anti-T sera, nor did they have receptors for GpRBC. However, WFT-22, WFT-24, and WRT-5 demonstrated rosette formation with YC particles. Groups 3 and 4 represent BNU-induced leukemias which have undifferentiated blast cell morphology. These leukemias were killed by anti-DBLA-6 and anti-Thy-1.1 sera but anti-T and -B sera failed to react with them. Furthermore, surface immunoglobulin and receptors for GpRBC and complement were never demonstrable on them. The only difference
between Groups 3 and 4 was that the leukemias in Group 3 were killed by ALS, while the leukemia in Group 4 was not. Group 4 includes only one leukemia, KNL-13. Group 5 represents BNU-induced leukemias which were cytologically granulocytic and had azurophilic granules. Complement receptors were demonstrated, but all other markers were negative in these leukemias. Leukemias which gave negative reactions with all membrane markers used constituted Group 6. They had erythroblastic morphology. ENU-induced leukemia AL-604 resembled an immature erythroblast. F-MuLV-induced leukemia WFT-28E and -29E showed a maturation tendency toward normoblast.

Quantitative Absorption Test of Anti-Thy-1.1 and Anti-T Sera. Although anti-Thy-1.1 serum did not cause significant lysis in all the lymphomas in Group 2, there is a possibility that they express a low amount of the Thy-1.1 antigen which was undetectable by means of the cytotoxicity test. When 0.1 ml of aliquots of 1:16 diluted anti-Thy-1.1 serum was absorbed with $5 \times 10^7$ cells from WFT-22, -24, and -25, the cytotoxic activity on normal thymocytes was completely removed (data not shown). A quantitative absorption test was next performed to investigate the relative amount of Thy-1.1 antigen in WFT-24 (Group 2) as compared to WGT-14 (Group 1) and DBLA-6 (Group 3). As shown in Chart 1, it was demonstrated that WFT-24 possesses less than 12% of the Thy-1.1 antigen as compared to WGT-14 and DBLA-6. A similar experiment was performed using anti-T serum. One-tenth ml of aliquots of 1:6 diluted anti-T serum was absorbed with various numbers of cells from WGT-13, -15, and -20 (Group 1), WFT-22, -24, and -25 (Group 2), DBLA-1, -6, and -9 (Group 3), and normal thymocytes. The residual cytotoxicity of anti-T serum was tested on thymocytes. As shown in Chart 2, WGT-13, -15, and -20 were not killed by anti-T sera from WFT-22, -24, and -25.
induced by MuLV's. MuLV's selectively infect and transform hematopoietic cells. Neonatal injection of G-MuLV and LLVF exclusively induces thymic lymphoma in the rat, while that of F- and R-MuLV predominantly induces extrathymic lymphoma (6–8). Although thymic lymphoma cells and extrathymic lymphoma cells are morphologically indistinguishable, characterization of them by using a panel of membrane markers clearly demonstrated that they belong to a quite different entity of lymphoma. All thymic lymphomas which have T-cell antigen and receptors for GpRBC, indicating that these lymphomas have developed along the T-cell differentiation pathway, were classified into Group 1. The fact that Group 1 lymphomas and normal thymocytes share the T-cell antigen and T-cell marker suggest that thymocytes are the target cells of G-MuLV and LLVF rather than that extrathymic lymphocytes are transformed and develop clonal expansion after coming into the thymus. All extrathymic lymphomas studied have several common characteristics with respect to membrane markers and were classified into Group 2. They lack definite T- and B-cell markers, but 3 lymphomas demonstrated complement receptors. In addition, the finding that Group 2 lymphomas have a very low amount of Thy-1.1 antigen compared to Group 1 lymphomas may provide an important clue to the origin of these lymphomas. Recently, Hunt et al. (4) reported that immature B-cell precursors possess a very low amount of Thy-1 antigen in the rat. Therefore, the presence of complement receptor and a low amount of Thy-1.1 antigen on Group 2 lymphoma cells might reflect their development from the B-cell differentiation pathway. BNU-induced leukemias were divided into 3 groups (Groups 3, 4, and 5). Group 3 and Group 4 leukemias have similar morphological and immunological characteristics, i.e., undifferentiated blast cell morphology, absence of T- and B-cell markers, and positive reaction with anti-DBLA-6 and anti-Thy-1.1 sera. A major difference is that Group 3 leukemias were killed by ALS, but Group 4 leukemia KNL-13 was not. Moreover, the cytotoxic activity of ALS on DBLA-6 cells was not removed by KNL-13 cells. KNL-13 is a leukemia which is positive only for anti-Thy-1.1 serum among the well-defined membrane markers used. Recently, Goldschneider et al. (2) reported the presence of the Thy-1 antigen on the rat pluripotent stem cell. Therefore, the phenotype of KNL-13 may reflect the characteristics of the pluripotent stem cell from which it derived. The expression of lymphocyte antigen on Group 3 leukemia places them further along the maturational scale than KNL-13. We propose that KNL-13 can be classified as a “stem cell” leukemia and Group 3 leukemias as “lymphoid stem cell” leukemias by above-mentioned morphological and phenotypic characteristics. We have arranged each group which commonly reacted with anti-DBLA-6 and anti-Thy-1.1 sera into a tentative scheme of lymphoid development based upon their immunological and morphological characteristics as follows: Group 4 → Group 3 → Group 2 or 1. Leukemias belonging to Group 5 and 6 are classified as a granulocytic leukemia and an erythroleukemia, respectively.

Recently, the Thy-1 antigen has been thought to be a useful marker for the study of hemopoiesis in the rat. In the present work, it was shown that Thy-1 antigen is also a useful marker for the characterization of neoplastic cells derived from hema-

DISCUSSION

Conventional hematological staining techniques were of limited value in classifying lymphomas and poorly differentiated leukemias into subgroups in the rat because of very few differences in specific morphological characteristics. Membrane marker analysis permitted further characterization of these neoplastic cells. However, it is especially important to use a panel of membrane markers when one studies leukemia or lymphoma cells because neoplastic cells may undergo surface change and express new markers or conversely lose some normal membrane properties. In the present study, 5 antigenic markers and 3 surface markers were used for the characterization of 20 rat leukemias and lymphomas.

All lymphomas used in the present work were originally
topoietic cells, especially in the classification of lymphomas and morphologically unclassified leukemias. These Thy-1-positive "non-T non-B" leukemias in Groups 3 and 4 may provide a clue as to the origin of non-T non-B leukemias and lymphomas found both in humans and mice.

REFERENCES

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