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Research Article

The bone marrow-derived (B) lymphocyte can be identified by the presence of easily detectable surface immunoglobulin and a receptor for antigen-antibody-complement complexes (EAC′). Monocytes and macrophages also bear a receptor for EAC′ and in addition possess a receptor for red cell-IgG complexes (EA). Thymus-derived (T) lymphocytes bear neither of these receptors. The cells of 15 patients with leukemia and 19 human lymphoblastoid cell lines were examined for the presence of the EAC′ and EA receptors. Of the human leukemias studied, only the cells from the patients with chronic lymphatic leukemia (CLL) possess the EAC′ receptor. The EA receptor could not be demonstrated on CLL cells; hence, CLL cells bear the lymphocyte EAC′ receptor and by this criteria represent B lymphocytes. 12/19 of the cell lines studied could be classified as B lymphocytes by the presence of the EAC′ receptor and absence of the EA receptor. 2/19 cell lines possessed both the EAC′ and EA receptors and thus resemble the monocyte. 5/19 cell lines had no detectable receptor for EAC′ or EA. The approach presented in this study for the classification of leukemias and cell lines as to their B lymphocyte, T lymphocyte, or monocyte origin may have useful diagnostic and therapeutic implications.

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Receptors for Complement and Immunoglobulin on Human Leukemic Cells and Human Lymphoblastoid Cell Lines

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ABSTRACT The bone marrow-derived (B) lymphocyte can be identified by the presence of easily detectable surface immunoglobulin and a receptor for antigen-antibody-complement complexes (EAC′). Monocytes and macrophages also bear a receptor for EAC′ and in addition possess a receptor for red cell-IgG complexes (EA). Thymus-derived (T) lymphocytes bear neither of these receptors. The cells of 15 patients with leukemia and 19 human lymphoblastoid cell lines were examined for the presence of the EAC′ and EA receptors. Of the human leukemias studied, only the cells from the patients with chronic lymphatic leukemia (CLL) possess the EAC′ receptor. The EA receptor could not be demonstrated on CLL cells; hence, CLL cells bear the lymphocyte EAC′ receptor and by this criteria represent B lymphocytes. 12/19 of the cell lines studied could be classified as B lymphocytes by the presence of the EAC′ receptor and absence of the EA receptor. 2/19 cell lines possessed both the EAC′ and EA receptors and thus resemble the monocyte. 5/19 cell lines had no detectable receptor for EAC′ or EA. The approach presented in this study for the classification of leukemias and cell lines as to their B lymphocyte, T lymphocyte, or monocyte origin may have useful diagnostic and therapeutic implications.

INTRODUCTION

Lymphoid cells of the immune system can be divided into two functional compartments. The thymus-derived T[1] lymphocyte appears to be responsible for cell-mediated immunity; the bone marrow-derived B lymphocyte is responsible for antibody production (1). These two populations of cells can be distinguished by the presence of different membrane receptors and differentiation antigens. In the mouse, the T lymphocyte can be identified by the presence of the θ-iso-antigen (2); in man, no satisfactory marker of the T lymphocyte has as yet been identified. The B lymphocyte, in most species studied, can be identified by the presence of easily detectable surface immunoglobulin (3). Most B lymphocytes also bear a recently described receptor for antigen-antibody-complement complexes (EAC′); the complement component involved has been shown to be a modified C_3 (4). Cells of the monocyte-macrophage series also play an important role in the immune response and they too bear a receptor for EAC′ which is similar to the B lymphocyte receptor (5). In some species the EAC′ receptor of monocytes can be distinguished from the EAC′ receptor of B lymphocytes because of the requirement of the former for the Mg^{++} ion for the attachment of EAC′; in humans however, the requirement for the Mg^{++} ion does not distinguish (6) the EAC′ receptor of monocytes from the EAC′ receptor of B lymphocytes. In addition, monocytes and macrophages bear a receptor for IgG; this receptor is detected by using red cell-IgG complexes (EA) (7). This monocyte EA receptor is not found on normal lymphocytes or on lymphocytes stimulated with phytomitogens in vitro (8).

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[1] *Abbreviations used in this paper:* B lymphocytes, bone marrow-derived lymphocytes; CLL, chronic lymphatic leukemia; EA, red cell-IgG complexes; EAC′, antigen-antibody complement complexes; SRBC, sheep red blood cells; T lymphocytes, thymus-derived lymphocytes.

TABLE I
Receptors on Normal Cells

Cell	Binding properties of the cells with		
	IgM EAC′	IgG EA	IgM EA
B lymphocyte	+	0	0
T lymphocyte	0	0	0
Monocyte	+	+	0

The presence or absence of these various receptors on cells of unknown type may provide information as to their origin. Previous studies have demonstrated the presence of surface immunoglobulin on the majority of cells from patients with chronic lymphatic leukemia (CLL) (9–11) and on a number of lymphoblastoid cell lines derived from patients with Burkitt lymphoma (12). Hence, both the CLL lymphocyte and the Burkitt lymphoma cell line possess some of the properties of the B lymphocyte population. Studies from our laboratory have demonstrated the lymphocyte EAC′ receptor on the L_2C lymphatic leukemia cell of the guinea pig (13) and the monocyte EA receptor on a number of mouse leukemias and lymphomas (14). In the present report, we examine the cells of 15 patients with leukemia and 19 human lymphoblastoid cell lines for the presence of EAC′ and EA receptors.

METHODS

Leukemic cells were obtained either by gelatin sedimentation of peripheral blood (15) or by leukapheresis. Some samples of leukemia cells were frozen in Eagle's medium supplemented with 20% fetal calf serum and 10% dimethyl sulfoxide and then stored in liquid nitrogen. These cells were rapidly thawed before study and were washed twice in RPMI-1640. Viability was 80–90% as determined by trypan blue dye exclusion. The lymphoblastoid cell lines were derived from a variety of patients (Table III) and maintained as single cells in suspension cultures in Eagle's minimal essential media supplemented with 20% fetal calf serum, penicillin, and streptomycin. All cultures were fed twice weekly. Immediately before study the cell lines were washed twice in medium RPMI-1640 without serum.

Lymphocytes were obtained from the peripheral blood of normal volunteers by a modification of the Hypaque-Ficoll technique (16). In most cases, the blood was allowed to sediment in 5% dextran, and the white cell-enriched supernate was used for the Hypaque-Ficoll centrifugation rather than whole blood. The preparations obtained from peripheral blood were 80-90% lymphocytes; the remainder of the cells were mostly monocytes. The yield of lymphocytes was always greater than 80%. In some cases the cells obtained from the Hypaque-Ficoll sedimentation were treated with carbonyl iron (see below).

Rabbit IgG and IgM antibodies to sheep red blood cells (SRBC) were prepared as previously described (17); they were isolated by gel filtration on Sephadex G-200 columns followed by sucrose gradient ultracentrifugation. Sensitized sheep erythrocytes (EA) were prepared by mixing the erythrocytes with the isolated IgG or IgM antibodies using the optimal concentration of antibody for maximal complement-mediated hemolysis at 37°C. Antigen-antibody-complement complexes (IgM EAC′) were prepared by the addition to the IgM EA of an equal volume of fresh mouse serum (as a source of complement) diluted 1/10 in veronal-buffered saline with optimal amounts of calcium and magnesium and 0.1% gelatin. After a 30 min incubation at 37°C, the cells were washed twice with medium and resuspended at a cell concentration of 1×10^6/ml in RPMI-1640.

Normal peripheral blood lymphocytes, leukemia cells, or cell line cells were suspended at a concentration of 2×10^6/ml in RPMI-1640. 0.4 ml of the cell suspension was added to 0.4 ml of the EAC′ or EA suspensions in 1-ml plastic tubes and gently rotated at 37°C for 30 min. A drop of the cell suspension was then examined in a hemacytometer chamber and the number of white blood cells with three or more SRBC adherent to their surface (rosettes) was determined. After lysis of the RBC with 2%acetic acid, the number of white blood cells in the suspension was counted and per cent of white cells forming rosettes determined.

The cell suspensions were examined with an IGM EAC′ complex to detect the lymphocyte or monocyte EAC receptor. An IgG EAC′ complex is an unsuitable reagent for the detection of the monocyte EAC′ receptor because EA IgG sites within the IgG EAC′ complex would also bind to the monocyte EA receptor. A receptor for a heterologous IgM EA complex has not been identified on either lymphocytes or monocytes; in the present study, none of the normal lymphocytes, leukemia cells, or the cell line lymphocytes showed binding of IgM EA. The expected binding properties of the reagents used for the cells bearing receptors are shown in Table I.

RESULTS

The peripheral blood lymphocytes of 10 normal volunteers as isolated by the Hypaque-Ficoll gradient technique were examined for the binding of IgM EAC. Marked variation in the percentage of white blood cells forming rosettes was seen with a range of from 10–25%. It should be noted that these lymphocyte suspensions were contaminated with 5–20% monocytes and some of the cells which formed rosettes with IgM EAC′ were probably monocytes. The addition of EDTA to the medium to a final concentration of 0.01 M did not decrease the percentage of rosettes; this observation confirms the findings of Nussenzweig, Bianco, Dukor, and Eden (6) that the human monocyte EAC′ receptor does not require the presence of the Mg^{++} ion for the binding of EAC′ to occur under these experimental conditions. In order to obtain pure populations of lymphocytes, the mononuclear cells of five normal volunteers obtained from the Hypaque-Ficoll gradient were allowed to phagocytize carbonyl iron[2] and the monocytes which had phagocytized the iron removed by passage of the cell suspension through tubing wrapped around the poles of a magnet. The preparations

[2] Lymphocyte-separating reagent, Technicon Instruments Corp., Tarrytown, N. Y.

TABLE II
Receptors on Leukemic Cells

Patient	Diagnosis	Peripheral WBC count	Predominant cell type used for study	Cells forming rosettes with IgM EAC′	Cells forming rosettes with IgG EA
		mm^3		%	
R. H.	Chronic lymphatic leukemia	16,300	82% lymphocytes 18% polymorphonuclear cells	25–30	ND*
K. N.	"	48,000	90% lymphocytes	90	0
F. S.	"	55,000	90% lymphocytes	80	0
G. L.	"	37,000	92 % lymphocytes	80	0
P. L.	"	14,300	80% lymphocytes 20% polymorphonuclear cells	88	ND
C. U.	"	92,000	96% lymphocytes	80–85	0
D. H.	"	100,000	97% lymphocytes	50–60	0
R. D.	"	35,000	88% lymphocytes	90	0
G. O.	Acute lymphatic leukemia	27,800	95% lymphoblasts	2	ND
C. R.	"	114,000	100% immature lymphoblasts	0	ND
A. L.	"	60,000	75% lymphoblasts, 10% normal lymphocytes, 15% polymorphonuclear cells	0	ND
G. D.	"	50,000	90% lymphoblasts	0–1	0
E. D.	Acute myelogenous leukemia	140,000	90% myeloblasts	3	ND
M. C.	"	14,000	60% polymorphonuclear cells, 30% myeloblasts, 10% lymphoblasts	5	ND
B. L.	Chronic myelogenous leukemia	42,000	93% polymorphonuclear cells, 6% lymphoblasts	10	ND

* Not done.

obtained in this fashion were greater than 98% lymphocytes as judged by morphology after Giemsa staining. The percentage of lymphocytes forming rosettes with IgM EAC′ in these preparations ranged from 3–10%.

Table II illustrates the binding properties of the cells from 15 patients with leukemia. 25–90% of the cells from eight patients with CLL demonstrated rosette formation with IgM EAC′. None of the cells of the six patients with CLL studied bound the IgG EA complex. This result indicates that most chronic lymphatic leukemia cells bear the lymphocyte EAC′ receptor and therefore by this criteria represent B lymphocytes. The cells from the four patients with acute lymphatic leukemia, two patients with acute myelogenous leukemia, and 1 patients with chronic myelogenous leukemia, failed to form a significant number of rosettes with IgM EAC′.

The binding properties of the 19 lymphoblastoid cell lines are shown in Table III. Group I (12 lines) demonstrated 80–100% of the cells forming rosettes with IgM EAC′. Cells from some of these lines also showed a small percentage of rosettes with IgG EA. Two cell lines (group II) showed 80–90% of the cells binding IgM EAC′ and 80–100% of these cells also binding the IgG EA reagent. These two lines therefore resemble the monocyte which possesses both the IgM EAC′ and IgG EA receptors. Five of the cell lines (group III) demonstrated only a low percentage of cells forming rosettes with the reagents used. These lines lack both the EAC′ and EA receptors. No correlation is seen between the presence or absence of the EAC′ or EA receptors and the diagnosis of the patient from whom the cell line was derived, the HL-A type of the donor, the presence of Epstein-Barr (EB) virus, or the secretion of immunoglobulin.

DISCUSSION

The normal immune response involves three populations of cells; the T lymphocyte, the B lymphocyte, and the monocyte-macrophage. In most species studied, the B lymphocyte can be identified by the presence of easily detectable surface immunoglobulin. In the mouse (4) and the guinea pig (13), most, if not all immunoglobulin bearing lymphocytes bear a receptor for EAC′. In man, from 10–35% of peripheral blood lymphocytes

TABLE III

Properties of Human Lymphoblastoid Cell Lines

Line	Date initiated	Diagnosis/source	Presence of EB virus*	Immunoglobulin production*	HL-A antigens‡	Cells forming rosettes with IgM EAC′	Cells forming rosettes with IgG EA
						%	
Group I							
IM-4	4/67	Burkitt lymphoma / Biopsy	+	Negative	1, 2, 8, A4, A6	80	0
IM-9	8/67	Multiple myeloma / bone marrow	+	M, G, λ	1, 2, A9, FISK, A6	100	0
IM-10	5/69	Multiple myeloma / peripheral blood	−	ND	ND	90	0–5
8205	?	Chronic myelogenous leukemia / peripheral blood	+	G, κ	2, 8, A6, LC17	100	0
8866	1965	Acute myelogenous leukemia / peripheral blood	−	G, κ, weak M	2, 3, 7, A6	80–90	0
HUP-1	12/66	Mycosis fungoides / lymph node	+	G, κ, weak M	8, A6	100	0
HUP-2	4/67	Spherocytic anemia / spleen	+	G, M, κ	1, 2, 5, A4	100	0
4265	1965	Chronic myelogenous leukemia / peripheral blood	+	G, M, κ	2, A9, 7, A6, LC17	90	10–20
Raji	7/63	Burkitt lymphoma / Gum biopsy	−	Negative	3, 5	100	10–20
F230	?	Breast carcinoma / pleural effusion	−	Negative	ND	70–90	5–10
LY-28	?	Nasal pharyngeal carcinoma / Biopsy	+	ND	ND	90–100	0–10
Onesmus	?	Burkitt lymphoma / Biopsy	+	ND	ND	80	0–5
Group II							
IM-1	8/66	Lymphoma / gum biopsy	+	G, M, κ	1, 3, 7, A4, A6	80–90	100
F265	?	Normal adult / peripheral blood	−	ND	ND	100	100
Group III							
8235	1965	Same as 8205	+	G, κ	2, 8, A6, LC17	0	0
8226	1965	Myeloma / peripheral blood	−	λ, faint M, A	ND	10	0
1788	?	Normal adult / peripheral blood	ND	ND	ND	10	0
HR-1K	?	Burkitt lymphoma / Biopsy	+	ND	ND	0	1–5
MAKU	?	Burkitt lymphoma / Biopsy	+	ND	ND	0	25–30

* Unpublished observations of Dr. D. Buell.

‡ Unpublished observations of Dr. G. N. Rogentine.

have been reported (9–11, 18) to bear surface immunoglobulin. In the present report, we have demonstrated that from 3–10% of normal human peripheral blood lymphocytes form rosettes with EAC′. An iden-

tical per cent of EAC′ positive cells were recently reported by Pincus et al.[3] Thus, in humans not all immunoglobulin bearing lymphocytes may bear the EAC′ receptor. In contrast to the peripheral blood lymphocytes of normals, a much higher percentage of the cells from eight cases of chronic lymphatic leukemia demonstrated binding of the EAC′ complex. This result supports the immunofluorescent studies of other investigators (9, 10) which demonstrate easily detectable surface immunoglobulin on the majority of CLL cells. Thus, the CLL lymphocyte possesses two of the functional properties of the normal B lymphocyte population.

Human lymphoblastoid cell lines have been derived from patients with a variety of hematological disorders as well as from normal individuals. The cell lines obtained from patients and from normal individuals are indistinguishable in terms of morphology, presence of EB virus, or presence and secretion of immunoglobulin (19). Morphologically, these cell lines are characterized by a heterogeneity of cell type; they are predominantly lymphoblasts, but also contain small numbers of plasmacytoid cells and macrophage-like cells. In the present study, we have demonstrated that 12 of the 19 cell lines studied bear the receptor for EAC′ which is found on B lymphocytes; two of the cell lines bear both the EAC′ and EA receptors and thus resemble the monocyte. Five of the cell lines did not bind EAC′ or EA. There was apparently no relationship in this study between the disease of the patient and the characteristics of the cell line obtained from the patient. For example, plasma cells are known (4) not to possess the EAC′ receptor; however, two of the cell lines derived from patients with multiple myeloma (Im-9, Im-10) demonstrated binding of EAC′. This lack of relationship is not unexpected since out of the large number of cell lines available for study, there are only two (20, 21) well-documented lines with a clear relationship between the properties of the cell line and the clinical state of the individual from whom these lines were obtained.

Of additional interest is the observation that no correlation could be seen between the secretion of immunoglobulin and the presence of the EAC′ receptor. However, the B lymphocyte which bears immunoglobulin may not secrete detectable quantities of immunoglobulin. Conversely, the secretion of immunoglobulin by those cell lines which do not bear the EAC′ receptor may be due to the presence of a small number of plasmacytoid cells which secrete immunoglobulin but do not bear the EAC′ receptor. Cell line Raji which we have found to bear the EAC′ receptor has recently been shown to lack any cell associated immunoglobulin by a highly sensitive Farr technique (22). This finding suggests that the receptor for EAC′ on the normal B lymphocyte is not surface immunoglobulin. Perhaps the Raji cell line is derived from lymphocytes at a stage of differentiation when they do not bear surface immunoglobulin, but do bear the EAC′ receptor.

The cells of the four patients with acute lymphatic leukemia did not show rosette formation with EAC′. The leukemic cell populations and the cell lines which did not bind EAC′ or EA may represent populations of cells which are derived from T lymphocytes of from undifferentiated stem cells which do not bear these receptors. Studies[4] of mouse leukemias and lymphomas have shown that some bear the θ-iso-antigen and are therefore probably derived from T lymphocytes. The cells from only a small number of patients with myeloid leukemias were studied and they also did not demonstrate binding of EAC′. Since receptors for both EAC′ and EA have also been described on some polymorphonuclear cells (5), more cases of myeloid leukemia should be investigated for the presence of these receptors. In fact, Nishioka (23) has reported that the cells from some chronic myelogenous leukemia patients do bind EAC′.

Our results differ from those of Michlmayr and Huber (24) who were unable to detect the EAC′ receptor on CLL cells and from Nishioka (23) who was unable to detect the EAC′ receptor on a large number of Burkitt lymphoma lines. Michlmayr and Huber demonstrated that 20% of normal peripheral blood lymphocytes bore the EAC′ receptor, yet most of the cases of CLL studied demonstrated a lower number of EAC′-binding cells. A number of possible explanations can be offered for this discrepancy. First, the higher number of EAC′-positive cells in normal human peripheral blood reported by these investigators as compared with our study may be due to the presence in their preparations of significant numbers of monocytes or polymorphonuclear leukocytes which can form rosettes with IgM EAC′ in the presence of 0.01 M EDTA. Second, the most likely explanation for their failure to find CLL cells binding EAC′ is their use of human serum diluted 1/40 or 1/80 as a source of complement. In our study, mouse serum which is not lytic was used at a concentration of 1/10 as the source of complement. Although no detailed study of the C_3 reactivity on a molecular basis in different species is available, it is possible that a 1/40 or 1/80 dilution of human serum supplies fewer reactive C_3 molecules than a 1/10 dilution of mouse serum. In his studies of cell lines

[3] Pincus, S., C. Bianco, and V. Nussenzweig. Submitted for publication.

[4] Shevach, E. M., J. Stobo, and I. Green. 1972. Immunoglobulin and θ bearing murine leukemias and lymphomas. *J. Immunol.* **108**: 1146.

Nishioka used (23) 300 immune adherence U of guinea pig C_3 per sheep erythrocyte to prepare the EAC′ complexes. From his earlier studies (25) in which a direct comparison of C_3 hemolytic units and immune adherence units is made, it appears that 300 immune adherence U are equivalent to a 1/1000 dilution of guinea pig serum. Thus, here again the number of C_3 sites may have been insufficient for formation of rosettes.

Further detailed study of the lymphocytes and monocytes from patients with a wide variety of malignant, infectious, and immunologic diseases for both qualitative and quantitative deficiencies of the immunoglobulin and complement receptors as well as the B or T cell origin of their lymphocytes should prove rewarding. Indeed, a deficiency of the monocyte EA receptor has recently been reported in some patients with the Wiskott-Aldrich syndrome (26).

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