Review Article

IMMUNOLOGIC DIAGNOSIS AND MONITORING OF HUMAN ACUTE LEUKEMIAS

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Immunologic Diagnosis and Monitoring of Human Acute Leukemias

A Review

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THE HUMAN LEUKEMIAS are currently identified and classified by morphologic and histochemical criteria.[1,2] Supplementary studies utilizing electron microscopy,[3,4] biochemistry,[5,6] and cytogenetics[7,8] may improve our classification methods. Recent advances in immunology have improved our ability to examine the surface structures of importance in the acute leukemias. Studies of cell surface characteristics by immunologic means may lend a new dimension to the classification of leukemias and at the same time improve our understanding of the disease process itself.

Leukemic cells can be phenotyped by the use of a panel of surface markers (Table 1). This review will examine surface characteristics of human leukemias cells that have begun to influence our clinical approach to the disease. A number of surface features detected by immunologic means are shared by nonleukemic cells of known lineage. These features have provided insight into tissues of origin of the acute leukemias and have fostered a more accurate classification of the acute leukemias, based on their probable tissues of origin. While some markers have gained widespread popularity, others that we will describe are less popular but may be of equal importance in the understanding of response to therapy.

Antigens that distinguish leukemic from nonleukemic cells in blood and bone marrow are reviewed but are less well understood. Their potential value lies in the immunodiagnosis both initially and at subsequent follow-up of patients under treatment.

Standard techniques for determining surface characteristics will be summarized in the initial sections, and methodologic problems and pitfalls will be emphasized. Application of these techniques to the hospital laboratory and the clinical setting has influenced our selection of the material discussed.

Cell Surface Markers Shared by Nonleukemic Tissues

Because acute leukemias are initiated predominantly in stem cells or immature hemopoietic precursors, they express an immature phenotype that does not always correspond to their normal adult counterpart. Nevertheless, before discussing the immunologic profile of each variety of acute leukemia, it is of value to consider the cell surface phenotype of normal adult mononuclear cells (see Table 2). Information on the enzymatic marker terminal deoxynucleotidyl transferase (TdT), the cholera toxin, and Epstein Barr virus receptors are not included in this review. TdT as a hematopoietic cell marker has recently been extensively reviewed.[9]

Cell Surface Antigens

Immune-Associated Antigens: In humans, the ma-

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Table 1—Membrane Antigens and Receptors Used to Phenotype Human Leukemic Cells

Antigen or receptor	Assay system	Comments	References
Ia antigens	Cytotoxicity C-dependent IIF test using allo-, heteroantiserums, mouse A-TH anti-A-TL anti-I^k, mouse monoclonal antibodies	Leukemic cells usually positive except T ALL	18, 19, 20, 21, 22
Monocyte-macrophage antigen	IIF test, C-dependent cytotoxicity using heteroantiserums	Specific for monocyte-macrophage series	36
Myelomonocyte antigen (M)	IIF test or C-dependent cytotoxicity using heteroantiserums, monoclonal antibodies	Shared by monocytes and granulocytes	29, 40, 41, 42
"T" antigens	Cytotoxicity C-dependent, IIF test using heteroantiserums, mouse monoclonal antibodies against thymocytes or peripheral T-cells	Defines pre-T ALL (E^-, T^+)	45-48, 61-64
"i" antigen	C-dependent cytotoxicity using strong allo-anti-i	May differentiate lymphoblasts from myeloblasts	72, 73
B-differentiation antigen	Rabbit antiserum raised against monkey B cells	Unique B cell antigen	74
SIg	Direct or IIF test using fluorescein-conjugated anti-Ig	Specific for B cells, if absorption via Fc receptor is avoided	77
Cyt IgM	Immunofluorescence	Defines pre-B ALL (SIg^-, Cyt IgM^+)	86
Fc receptors		Descriptive, nondiscriminating marker	
-IgG-FcR	Agg Hu IgG, EA rosette (IgG antibody), FITC-protein A, PAP Ig	Tests of varying sensitivity	92-95
-IgM-FcR	EA rosette (IgM antibody)		88
Complement receptors		Descriptive, nondiscriminating marker	
-EAC	Rosette assay		112
-ZyC	Rosette assay		114-115
SRBC receptors	Spontaneous rosette (E rosette)	Most useful T-cell marker	120-122
Mouse-RBC receptors	Spontaneous rosette (M rosette)	Detects a subpopulation of B cells	118
ALL antigen	IIF test using heteroantiserums, mouse monoclonal antibodies	Probably normal early differentiation antigen of the lymphoid lineage	146, 155, 157, 160
Thy-leukemia-associated antigen(s)	Heteroantiserums, mouse monoclonal antibodies against T-leukemic cells		59, 161, 170, 171
AML antigen(s)	IIF, C-dependent cytotoxicity assay using allo-, heteroantiserums		146, 155, 159, 175

IIF = indirect immunofluorescence assay; ZyC = zymosan particles coated with complement; PAP Ig = peroxidase anti-peroxidase Ig.

jor histocompatibility genes map within the HLA complex of chromosome 6,[10] and genes in the HLA-D region control the expression of antigens that appear to be homologous with murine immune-associated (Ia) antigens (DR, p 28, 33, B cell allo-antigens).[11] These antigens are composed of two covalently linked glycosylated polypeptide antigens of 28,000 and 33,000 daltons (28,000 and 35,000 for murine Ia antigens),[12,13,14] and they constitute the D-related (DR) system of HLA antigens. Recent serologic data[15,16] strongly suggest that other human gene products can be determined by HLA loci (other than HLA-A,B,C and D/DR loci) by analogy to murine H-2 I regions, where at least three H-2 I loci control B cell specificities (I-A, I-C, I-E).[17] Even though the anti-Ia serums probably contain antibodies against several distinct cell surface structures, the term "Ia" is retained and is used to designate these structures collectively.

Human Ia antigens can be demonstrated on the surface of various cells using human alloantiserums,[18] rabbit heteroantiserums,[19] murine alloantiserums directed against antigen defined by the I^k region of the murine H-2 complex, (mouse A-TH A-TL anti-I^k alloantiserums)[20,21] and mouse monoclonal antibodies.[22]

Table 2—Cell Surface Phenotype of Human Blood Mononuclear Cells

Antigen or receptor	Blood cells				
	T cell	B cell	Null cell*	Mono-cytes	Granu-locytes
Ia	−†	+	±	+	−
HuMA	−	−	±[27]	+	−
M	−	−	−	+	+
T	+	−	−	−	−
i	+	+	ND	+	+
BDA	−	+	ND	−	−
SIg	−	+	−	−††	−
IgG FcR	±‡	±‖	±	+[250]	+[252]
IgM FcR	±§	±¶	±[249]	±‡‡	ND
CR	−	±	±**	+[250]	±§§
SRBC	+	−	−	−	−
MRBC	−	+	−	−	−
ALL	−	−	−	−	−
H-Thy-L	−	−	ND	ND	ND
AML	−	−	ND	−	−

+ = > 95% positive; − = < 5%; ± = subset reactive; ND = not determined.

* Also called third cell population, unclassified lymphoid cells, or "L" cells. Includes NK and K cells. Considerable heterogeneity; majority are Ia⁺, IgG FcR⁺, E⁻, SIg⁻[27], some with low affinity E_{sheep} rosettes.

† Activated T cells may be Ia⁺.[31-34]

‡ T-suppressor cells: IgG-FcR.

§ T-helper cells: IgM-FcR.

‖ Exact percentage of B cells bearing IgG FcR is a controversial issue.

¶ After incubation of cells at 37 C for 6-24 hours in IgM-free medium.[248]

** Approximately 50% express CR1 receptor.

†† Negative if Ig adsorption through Fc receptor is avoided.

‡‡ Only after neuramidase treatment.[251]

§§ Negative for CR2 receptor.

Human Ia molecules are highly polymorphic, carrying both "private" and "public" determinants. The human Ia "public" determinants can be detected by heteroantiserums raised in rabbits immunized against glycoproteins of 28,000–33,000 mol wt derived from B cells,[23] by mouse A-TH A-TL anti-I^k alloantiserums,[20,21] and monoclonal antibodies against monomorphic DR determinants.[22] However, since human alloantiserums[18] and monoclonal antibodies against polymorphic DR determinants[22] recognize "private" determinants, a panel of antiserums should be used to determine the presence of Ia antigens as a given normal or neoplastic cell population.

Because of their reproducibility and relative simplicity, the microdroplet cytotoxicity method[24] and the indirect immunofluorescence assay are commonly used to determine the presence of Ia antigens on the cell surface. Ia antigens are serologically detectable on B lymphocytes, macrophages,[25,26] a subset of null cells,[19,27] undifferentiated bone marrow stem cells, endothelial cells, spermatozoa,[28] and activated peripheral T cells. There is a maturation-linked expression of Ia antigens with a progressive loss during granulocyte differentiation.[29] The antigen is absent on promyelocytes,[30] CML cells,[29] thymocytes, and nonactivated peripheral blood T lymphocytes.[19] Normal human T cells can express Ia antigens on their surface after alloantigenic or mitogenic activation[31,32] and during the course of graft-versus-host disease.[33] Elevated levels of Ia⁺ peripheral blood T cells were observed in a high proportion of patients with rheumatoid arthritis, systemic lupus erythematosus, and various types of infections (infectious mononucleosis).[34] Ia⁺ T cell acute lymphoblastic leukemia (ALL) have been reported only exceptionally.[35]

These recent findings suggest that the detection of Ia antigens on the cell surface may not clearly discriminate between T and B lymphocytes.

Human Monocyte Macrophage Antigen: Antiserums have been raised in rabbits against peritoneal fluid macrophages and after absorption with tonsil cells appeared to define a specific antigen of the human monocyte macrophage series.[36] That human macrophage antigen (HuMA) is present on virtually every phagocytic mononuclear cell from blood, spleen, and bone marrow. Cells of the myeloid series do not stain in indirect immunofluorescence with the antiserums. However, small nonphagocytic cells are reactive with anti-HuMA serums, especially in the bone marrow and spleen.[36] These cells are probably members of the "third lymphocyte" population described by Frøland and Natvig.[37]

Myeloid-Specific and Myelomonocyte Antigens: Antiserums specific for human myeloid antigen(s) have been raised in rabbits and, after extensive absorption and purification procedures, appeared to be specific for cells of the myeloid lineage (no reaction with monocytes).[38]

Other investigations have failed to produce an antiserum strictly restricted to the myeloid cells.[39] Antiserums raised against myelomonocyte and monocytic leukemia cells[29,40] and granulocytes[41] have defined a cell surface antigen common to granulocytes and monocytes, as well as their progenitor cells and malignant counterparts. The sharing of membrane antigen(s) by granulocytes and monocytes has also been noted by other investigators using monoclonal antibody to human monocytes.[42]

Such antiserums are not routinely used in the immunologic evaluation of acute leukemia, despite their potential clinical value.

T Cell Antigenic Determinants: Antiserums with specificity for T lymphoid cells are theoretically attractive as diagnostic reagents[43,44] but remain difficult to prepare. However, several successful attempts to produce T cell antiserums have been reported.[45-48]

These heteroantiserums directed towards "human T cell determinants" have been prepared by the use of a variety of immunization schedules, absorption procedures, and methods for assay; they have been raised in rabbits, horses, and monkeys; the antigens used have included human and monkey thymocytes, soluble extracts of thymocytes, thymoma cells, brain cells, leukemic lymphoblasts bearing E-rosette markers, and peripheral lymphocytes from normal individuals or patients with X-linked agammaglobulinemia.[49-52] Virtually all the heteroantiserums require extensive absorption with a variety of cells, including allogeneic cultured B cell lymphoblastoid lines, B cell chronic lymphocytic leukemia (CLL), bone marrow cells, erythrocytes, or fetal cells, to render them specific.

Although traditionally considered as reacting against human T cell antigens, it is readily apparent from serologic and biochemical studies that these antiserums recognize different antigens and presumably different subpopulations of cells. For instance, the constituent antibody in the heterologous antiserum raised against brain-associated T cell antigen is an antibody against the glycolipid asialo GM_1, which is associated with ALL cells (null or T cell type) but not with other lymphoid neoplasias and hematologic disorders.[53] Some heteroantiserums detect T cell antigenic determinants shared by thymocytes, peripheral T cells and T-ALL cells,[54-56] and others react with an antigen shared by thymocytes and T-ALL cells but absent from peripheral T cells.[57,58]

Other investigators have begun to define the differentiation antigens of human T cell subpopulations, using heterologous antiserums absorbed with autologous B cells alone, or B cells plus cells of different T cell ALL.[59,60] Using these reagents, they have identified distinct subsets of T cells, designated TH_2^+ and TH_2^-. The normal human peripheral blood T cell compartment is composed of 80% TH_2^- and 20% TH_2^+ cells; human suppressor T cells are TH_2^+, whereas helper T cells are TH_2^-.

Successful production of monoclonal antibodies reacting with human peripheral T cell antigens[61,62] and human thymocyte antigens of different specificities[63,64] have been recently described. Further production and characterization of monoclonal antibodies to normal lymphocyte molecules should foster the immunologic and biochemical dissection of the human T-cell. associated antigens.

Despite their ill-defined antigenic specificities, various heteroantiserums detecting "T cell antigenic determinants" have been helpful in the phenotypic characterization of human acute leukemias.

i Antigen: The i antigen was originally described on fetal and cord red blood cells[65] but has since been described on a variety of nonerythroid cells, both normal and malignant. Cells with i antigen detectable include lymphocytes,[66] polymorphonuclear leukocytes,[67] monocytes,[68] fibroblasts, fetal lung tissue, HeLA cells, and cells from normal or malignant gastrointestinal mucosa.[69-71]

The i antigen has been described on leukemic blast cells in acute myeloblastic leukemia (AML) and ALL[72,73] as well as on leukemic lymphocytes in CLL.[72] Quantitation of i antigen has shown that much less is present on myeloblasts than on lymphocytes or lymphoblasts.[73] This difference in i antigen levels is best observed in cytotoxicity testing using potent anti-i-alloserums and may be useful in differentiating acute lymphoblastic from acute myeloblastic leukemia.[73]

B-Differentiation Antigen: A recently described antiserum prepared in rabbits against monkey B cells detects a B-differentiation antigen (BDA) on cells of the B lymphocyte line distinct from Ia antigens.[74] The BDA is present on all pre-B cells, B lymphocytes, plasma cells,[74] B-lymphoblastoid lines, and B lymphoid malignancies, but is not found on other types of blood cells, qualifying BDA as a unique B cell antigen.

Surface Immunogloblin and Intracytoplasmic IgM

Surface immunoglobulin (SIg) refers to immunoglobulin synthesized by the lymphocyte and then expressed on the cell membrane in contrast to immunoglobulin absorbed passively or via the Fc receptor onto the surface. SIg seems to be the only marker that is truly specific for B cells in man, although SIg may be greatly diminished or absent early in their differentiation pathway.[75] Some laboratories have described surface Ig on T cells, using specialized techniques,[76] but this has not been seen with the use of the immunofluorescence assays. This issue remains controversial, since T cell IgM and IgG Fc receptors may explain the presence of SIg bound to T cells.

SIg is detected with the use of anti-immunoglobulin antibodies conjugated to fluorochromes, radioisotopes (^{125}I), peroxidase, or particles (polymer spheres). Direct immunofluorescence tests using rhodamine or fluorescein-conjugated antihuman immunoglobulins are more commonly used.[77]

Several technical problems have been shown to be associated with the immunofluorescence staining procedure. Serum IgG may be passively absorbed to cells that are not producing their own surface immunoglobulin, particularly in hypergammaglobulinemia and hyperviscosity states. Heterologous anti-Ig antibodies may bind to cells via the cell surface Fc receptor, IgG anti-Ig reagent has a tendency to form small aggregates that have a high affinity for IgG Fc recep-

tors, and fluid-phase plasma Ig may form immune complexes with the anti-Ig reagent at the cell surface. The immune complexes generated can then bind to Fc receptors on non-B cells. These different mechanisms of nonspecific SIg staining result in a spuriously high percentage of Ig-bearing cells.[78]

Recognition of these difficulties led to the development of several alternatives to circumvent the nonspecific uptake of anti-Ig reagents or serum Ig. These include enzymatic stripping or incubation of cells at 37 C for 2–24 hours to allow shedding of cytophilic IgG,[79] pretreatment of cells with mild acid buffer,[80] use of the $F(ab^1)_2$ portion of detecting antibodies,[78] and use of goat antibodies because of their low affinity for human IgG Fc receptors.[81] A conjugate of protein A and fluorescein is commercially available that may be used to fluorescence-label cell-bound anti-Ig while simultaneously blocking the Fc portion of the reagent.[82]

Reliability is dependent on the sensitivity and specificity of the antiserums used. The specificity of commercial reagents is not always assured, and each lot of antiserum obtained commercially must be subjected to assessment of sensitivity and specificity.

If the percentage of potential B leukemic cells is low, differentiation from monocytes may present a problem: monocytes frequently bear SIg via the Fc receptor site, form EAC rosettes via a complement receptor site, and thus "masquerade" as B cells. Monocyte contamination can be appreciated by the routine use of cytochemical markers with naphthol acetate and/or phagocytic markers with latex particles and diminished by removal of phagocytic cells with iron filings.

Polyspecific antiserums against all immunoglobulin classes and a full panel of monospecific antiserums against human heavy chains (γ, α, μ, δ, ε) and light chains (κ, λ) should be used to determine the monoclonality of B cell leukemias.

The determination of cytoplasmic IgM is essential to the demonstration of cells with the pre-B phenotype (SIg^-, intracytoplasmic (Cyt) IgM^+). This assay is difficult, requiring special equipment to detect minimal immunofluorescence. The detection of intracytoplasmic immunoglobulin requires an acetone, methanol, or ethanol fixation of test cells in order to render the plasma membrane permeable to fluorescent anti-immunoglobulin probes.[83] Cytoplasmic immunoglobulin varies with developmental changes of B cells. Pre-B cells have a scant amount of cytoplasmic IgM distributed in a lacy pattern,[84] whereas B lymphocytes have a thin rim of Ig confined to the plasma membrane,[85] and plasma cells display a rich and diffuse pattern or cytoplasmic Ig.[86]

Receptors

Fc receptors: Four types of Fc receptors (FcR) are now recognized, one each for the Fc portions of IgG, IgM, IgE, and IgA.[87–91]

The most popular methods for identification of IgG FcR are rosetting techniques[92,93] using antibody sensitized human (HuEA), sheep (ShEA), or σx (OxEA) erythrocytes-antibody complexes (EA), and immunofluorescent techniques using heat-aggregated IgG of human origin (agg HuIgG).[87] IgG FcR may also be demonstrated by a fluoresceinated staphylococcus protein A[94] or soluble peroxydase antiperoxydase Ig complexes.[95] The literature on IgG FcR suggests that no single test is optimal for the demonstration of all IgG Fc receptors. There is evidence that lymphocyte receptors that bind EA complexes differ from those that bind agg IgG.[95] Agg Hu IgG and OxEA seem to have the broadest range of sensitivity for the IgG FcR of different cell types. HuEA and OxEA best demonstrate T cell IgG FcR and react less well with B lymphocytes.[97] However, the B cell IgG FcR has more avidity than T cells for agg Hu IgG. These recent findings suggest a qualitative heterogeneity in the IgG FcR present in B cells, as compared with the IgG FcR present on non-B cells.[96,98] IgG FcR are present on B cells,[99] suppressor T cells,[100,101] the third population of lymphocytes lacking specific B or T markers,[102] and macrophages and granulocytes.[103] IgM FcR are readily detected by rosette assay with IgM-sensitized OxEA.[88] IgM Fc receptors are difficult to detect on freshly isolated cells because of high avidity for free plasma IgM. To promote shedding of receptor-bound IgM, cells are cultured overnight in medium lacking IgM. IgM FcR are apparently restricted to helper T cells[101] and weakly present on a proportion of B cells.[104,105]

The presence of Fc receptors on lymphoid and nonlymphoid cells limits the value of the Fc receptor detection in cell identification. Moreover, the presence of antileukemic antibodies on the cell surface may induce false-positive results. They should therefore be regarded as descriptive rather than discriminating markers.

Complement Receptors: Lymphocytes have been shown to contain 2 different types of complement receptors (CR), the CR_1 (detecting C4b, C3b) and CR2 receptors (detecting C3d).[106] These two types of CR are structurally distinct and are located on separate molecules within the lymphocyte membrane.[107,108] The CR_1 receptor is shared with erythrocytes, granulocytes, and monocytes; and the CR2 receptor, only with monocytes.[109] Granulocytes and mononuclear phagocytes have a third type of CR (CR3) that is distinct from lymphocyte CR2.[110] B cells express both

CR1 and CR2 receptors, whereas only the CR1 receptor is expressed on third population cells.[111] Suppressor and helper T cells do not seem to express CR on their surface,[111] but CR have been described on certain E-rosette-forming cells.[112]

Most investigators have assayed for CR using sheep erythrocytes (SRBC) coated with IgM rabbit antibody (A) to sheep erythrocyte and nonlytic human or mouse complement (C).[113] Fresh serum from C5-deficient mice is the most common source of complement for this test.[77] It is essential that purified IgM be used to sensitize the red cells, since IgG-antibody-coated red cells may bind to lymphocyte Fc receptors; 19 S anti-red-blood-cell preparations are commercially available and appear to be suitable. Human erythrocytes should not be used to prepare EAC assays, because of the presence of CR1 receptor on their surface. If SRBC are used, incubation is carried out at 37 C to prevent the formation of E rosettes with T cells, and controls using E and EA preparations should be included.[77]

Another approach to identifying complement receptor-bearing cells has involved the use of zymosan beads (polysaccharide extract of yeast cell wall) coated with human or mouse complement.[114,115] Zymosan particles activate the complement system by the alternative pathway, thus generating C3b on their surfaces.[116] This technique has considerable advantages over the classical erythrocyte-antibody-complement (EAC) assay. Large amounts of indicator particles can be prepared all at once and kept frozen at −70 C. Any source of complement can be used, since the zymosan particles are not lysed; zymosan activates the complement system so that antibody is not necessary; controls using E and EA preparations are avoided, and the final cost of the assay is considerably lower. Zymosan particles coated with complement can be standardized for conducting longitudinal studies. Standardization of EAC reagent is difficult because of the biologic variability of red cells, IgM molecules, and complement.

Because particle-bound C3b is rapidly degraded by serum enzymes, complement-coated erythrocytes or zymosan particles prepared by incubation with *whole* serum detect primarily CR2 receptor. It is possible to assure specificity for CR1 receptor by sequential addition of purified human components to sheep erythrocytes sensitized with IgM antibody,[117] but this is not of clinical interest.

The CR assay like the Fc receptor assay is descriptive but nonspecific.

Erythrocyte Receptors: Erythrocytes from several animal species bind spontaneously to human lymphocytes, forming rosettes.[118-124] Mouse and macaca erythrocytes have been shown to adhere to B cells,[118,119] whereas sheep, rhesus monkey, and goat red blood cells adhere to T cells.[120-124] The SRBC rosette method has been generally accepted as a technique for the identification of both normal and neoplastic human T lymphocytes.

This method is inherently simple, but a number of procedures have been introduced to increase the stability of rosettes. One can gain enhancement by performing the test in 10% human serum in 25% fetal calf serum,[77,125] in the presence of 9% Ficoll or in 5% dextran.[126] Pretreatment of sheep erythrocytes with neuraminidase[127] or 2-aminoethylisothiouranium (AET)[128,129] leads to larger, more stable rosettes and more rapid attainment of plateau values. The use of neuraminidase-pretreated SRBC may induce B lymphocytes to form E rosettes, rendering the methods nonspecific.[130] Similarly, the use of AET-treated SRBC to phenotype ALL increases the number of samples reacting with SRBC as well as the proportion of rosette-forming blast cells in each sample.[131] These findings suggest that any increase in sensitivity achieved through chemically altered erythrocytes is offset by a decrease in specificity.

"Active rosettes" are formed after preincubation of lymphocytes with fetal calf serum, followed by the addition of SRBC, brief centrifugation, and immediate suspension and reading.[132]

A range of other technical factors may influence the results, for example, the ratio of lymphoid cells to sheep erythrocytes, the total concentration of cells, centrifugal force, the temperature and time of incubation during the rosette-forming period, the technique of resuspension, and the method used for visual counting.

The evaluation of formation of E rosettes should be conducted at 4 C (E^{+4}) and 37 C (E^{+37}), because about 75% of T-ALL are thermal stable (more than 40% rosettes formed at 37 C), in contrast to the thermal lability of normal circulating T lymphocytes.[133-135] The determination of E^{+4} and E^{+37} may clarify some cases falling in the intermediate range of 20–40% E^{+4}-rosette-forming cells.

Mouse RBC rosettes are not routinely used as B cell markers. The mouse RBC receptor should be considered as a transient differentiation marker that may be lost by maturing B cells.[136] Some neoplastic proliferation of B cells, such as prolymphocytic leukemia and chronic lymphosarcoma-cell leukemia are negative for mouse rosette formation.[137]

Several human cell types form SRBC rosettes, including fibroblasts and parenchymal cells from liver, lung, and parathyroid.[138] This lack of specificity must be kept in mind when poorly differentiated neoplasms

are studied. In practice, the capacity of a given leukemic cell population to bind SRBC and form rosettes remains a distinctive property of leukemias with the T phenotype.

Leukemia-Associated Antigens

Studies from several investigators using cell-mediated immune assay systems[139-142] and/or antiserums to human blast cells or their extracts[143-150] have clearly suggested the existence of cell surface antigens characteristic of acute leukemias.

Remission lymphocytes underwent blast transformation upon culture with inactivated autochthonous leukemic cells,[139,141] suggesting the presence of "foreign" antigens on the surface of leukemic cells. Lymphocytes from HLA-identical siblings reacted in mixed lymphocyte culture (MLC) to leukemic cells from identical siblings but not to remission leukocytes.[140] Some laboratories have reported circulating autoantibodies against leukemic cells[143,148] and the presence of immunoglobulins on the surface of blast cells.[151-153] Metzgar et al were able to detect immunoglobulins on the leukemic cell surface, and antibody activity to LAA was found in the eluates prepared from these cells. The specificity of eluted antibodies was similar to that defined by a nonhuman primate antiserum to leukemia-associated antigens (LAA).[154]

Production of Antiserums to LAA

Antiserums to human leukemic blast cells have been raised in mice,[155] rabbits,[156-158] and nonhuman primates.[146] Antiserums have been derived from patients receiving immunotherapy with leukemia-cell vaccine[159] and by somatic hybridization techiques.[160,161] The antileukemic heteroantiserums and mouse monoclonal antibodies have been shown to distinguish leukemic blast cells from remission or normal white cells in both peripheral blood and bone marrow. The development of heteroantiserums that are operationally specific for leukemic antigens has been difficult. Leukemic cells may express highly immunogenic normal surface antigens, particularly histocompatibility antigens, including Ia antigens, so that the immunized animal recognizes these normal structures in preference to specific antigens. Approaches to this problem have included a number of immunologic strategies. Baker et al raised antiserums to leukemic myeloblasts in mice rendered tolerant with cyclophosphamide to remission cells[144,155] or platelets[162] from the same patients. Greaves et al raised antiserums to leukemic null lymphoblasts in rabbits by covering the injected blast cells with rabbit antibodies to normal human blood cell antigens.[156] Mohanakumar et al have immunized chimpanzees and rhesus monkeys with acute leukemic cells.[146] Since absorptions with normal tissues and unrelated leukemic cells may be necessary in these heteroantiserums, the titer and the quantity of antiserums may be limited. Immunization of animals with antigen(s) obtained through solubilization, purification, and extraction procedures has improved antiserum specificity.[58,163,164]

The hybridoma technique of Kohler and Milstein[165] may aid in the search for "specific" antiserums against human leukemia-associated antigens. In this technique, the immunization of mice with whole cells, cell extracts, or even purified antigen, followed by *in vitro* hybridization of the murine antibody-synthesizing spleen cells with mouse myeloma cells, may lead to development of clones against particular leukemic antigens. The antiserums produced are monospecific and require no absorptions. Large quantities of antibodies can be produced *in vitro* by cloned cell lines or *in vivo* by mouse peritoneal transplants and immortalized in tissue culture or in frozen storage. The hybridoma technique requires considerable technical expertise and constant attention if one wishes to avoid a number of pitfalls. Failure of fusion, overgrowth of cells, superinfections, and cell death are some of the technical problems encountered. Large-scale screening procedures are mandatory and time-consuming. Maintenance of long-term clones requires considerable space, technical help, and special facilities. In addition, the monoclonal antibodies produced may be directed against quite a small portion of the potentially interesting compounds and may be inactive in cytotoxic assays.

Antiserums to LAA have been used in the description, isolation, and partial biochemical characterization of at least three different LAA: the common acute lymphoblastic leukemia-associated antigen (cALL or ALL), the human thymus leukemia-associated antigen (H-Thy-L), and the acute myeloblastic leukemia-associated antigen (AML).

ALL-Associated Antigen

ALL-associated antigen has been defined by heteroantiserums raised in mice,[155] rabbits,[156] and nonhuman primates,[146] and by the hybridoma technique.[160] Antiserums to ALL cells raised in tolerant mice retained exclusive activity against ALL cells after absorption with AML cells.[155] Nonhuman primates immunized with ALL cells produced antiserums detecting an antigen[5] common to ALL and CLL cells.[166] Greaves et al[156] raised antiserums in rabbits to ALL cells coated with rabbit antihuman lymphocyte antibodies. The absorbed antiserums detected a non-T, non-B ALL leukemia-associated antigen. Rabbit an-

ti-ALL serum also reacts with malignant cells from a variable proportion of patients with acute undifferentiated leukemia, T-ALL, Ph¹(Philadelphia chromosome)-positive ALL and Ph¹-positive CML in blast crisis.[167] The ALL antigen defined by rabbit antiserum has been shown to be a normal early differentiation antigen of the lymphoid lineage,[35] since studies utilizing the fluorescence-activated cell sorter (FACS) detected antigenic activity in a number of nonleukemic lymphoid tissues.[168]

Recently, successful production of monoclonal antibodies to ALL antigen was reported.[160] Monoclonal anti-ALL antibody failed to react with normal hematopoietic cells and reacted exclusively with common ALL or chronic myelocytic leukemia blast crisis (CML-BC), lymphoid type.[160] Immune precipitation experiments showed that both the rabbit and hybridoma anti-ALL antibodies were reactive with a glycoprotein with a molecular weight of 95,000–100,000 daltons, suggesting that the two antiserums are recognizing the same antigen.[160,169]

Human Thymus Leukemia-Associated Antigen(s)

Human thymus leukemia-associated antigens (H-Thy-L) have been defined by nonhuman primate[170,171] and rabbit[58] heteroantiserums and by hybridoma monoclonal antibodies.[161] With a radioimmunoassay (RIA), increased levels of a H-Thy-L antigen have been detected in leukemic cells and in plasma, mainly in T-ALL cells but also in some E⁻-ALL, AML, acute myelomonocytic leukemia (AMML), acute undifferentiated leukemia (AUL), and CML-BC.[172] Neither healthy subjects nor patients with nonleukemic diseases had increased quantities of H-Thy-L antigen determined by RIA in peripheral white cells. Chechick et al have recently identified this antigen as a thymic isozyme of adenosine deaminase of a molecular weight 45,000 daltons.[173] This is of particular interest, since the mean adenosine deaminase activity of T cell lymphoblasts has been found to be sufficiently greater than the mean of null lymphoblasts to be useful for distinguishing between these two major groups.[174]

Levy et al[161] produced hybridoma monoclonal antibodies detecting a H-Thy-L antigen expressed preferentially on T cells, T-ALL-derived cell lines, and some null ALL cells. By contrast, B cell leukemias, B-lymphoblastoid cell lines, and normal and malignant myeloid cells contain either low or undetectable amounts of this antigen. Preliminary biochemical characterization showed this antigen to be associated with a polypeptide of 28,000 daltons,[161] suggesting that rabbit anti-H-Thy-L and monoclonal anti-H-Thy-L may have different specificities.

AML-Associated Antigen(s)

Antibodies to myeloblastic leukemia-associated antigen(s) have been raised successfully in rabbits,[175] nonhuman primates,[146] and mice.[155] Antiserums have also been derived from patients receiving immunotherapy with leukemia-cell vaccines.[159]

AML-associated antigens are less clearly defined than ALL and H-Thy-L antigens, and may include differentiation, maturational cell cycle restricted antigens, viral, or other nonleukemic antigens.

Nonhuman primates immunized with AML cells or their extracts produced antiserums detecting an antigen(s) common to AML and CML cells,[146] but not reacting with ALL or CLL cells. Antiserums have been raised to AML cells from individual patients in mice rendered tolerant with cyclophosphamide to remission leukocytes from the same individual.[155] Mice and rabbit anti-AML serums absorbed with ALL cells retained measurable cytotoxicity against AML cells but not against ALL and normal cells.[155,175] Human alloantiserums against myeloblastic-leukemia-associated antigen have been obtained from patients with acute myeloblastic leukemia who had received immunotherapy with allogeneic AML cells.[159] Reactivity of these alloantiserums could be demonstrated against leukemic myeloblasts and not against leukemic lymphoblasts, remission cells, or nonleukemic cells. Preliminary biochemical characterization indicates that myeloblastic-associated leukemia antigen might be a glycoprotein of 75,000–80,000 daltons.[176]

The analysis of cell surface glycoproteins of various murine and human leukocytes using a combination of selective radiolabeling and polyacrylamide slab gel electrophoresis[177-179] has revealed that normal leukocytes express different surface glycoprotein patterns, depending on cell type, stage of maturation, and functional differentiation. Leukemic cells from patients with different types of human leukemia have been shown to display characteristic and distinguishable surface glycoprotein and glycolipid profiles.[53,180] Such analysis of cell surface structures offers a new tool for the differential diagnosis and classification of human hematopoietic malignancies and provides a basis for the search for leukemia-associated surface molecules.

Immunodiagnosis of Acute Leukemias

Techniques such as Romanowsky stains of properly made films and cytochemical reactions remain important as diagnostic tools in leukemia, but immunologic techniques bring additional objectivity to the analysis of the blast cell population. Cell surface phenotyping

Table 3—Cell Surface Phenotype of Human Acute Leukemic Cells

	Lymphoid					Myeloid				CML-BC	
	Non-B, Non-T	Pre-T	T	Pre-B	B	M1-M2	M3	M4	M5	Lymphoid	Myeloid
Ia	+	±	−‡	+	+	+	−	+	+	+	+
HuMA	−	−	−	−	−	±‖	ND	+	+	−	−
M	−	ND	−	ND	−	+	ND	+	+	−	+
"T"	−	+	+	−	−	−	−	−††	−	−	−
"i"	+	ND	ND	ND	ND	±¶	ND	ND	ND	ND	ND
BDA	−	ND	ND	+	+	−	ND	−	−	ND	ND
Sig*	−	−	−	−	+	−	−	−	−	−	−
Cyt IgM	−	−	−	+	−	−	−	−	−	−	−
FcR	±	±	±	±	±	±	±	±	±	±	±
CR	±	±	±	±	±	±	±	±	±	±	±
SRBC	−	−	+	−	−	−	−	−	−	−	−
ALL	±†	±	−§	±	−	−**	ND	−	−	±	−
H-Thy-L	−	ND	+	−	−	−	−	−	−	−	−
AML	−	−	−	−	−	+	+	+	ND	−	+

+ = > 90% positive; − = < 10% positive; ± = variable; ND = not determined.

* Leukemias other than B cell ALL may appear to be Sig+ through passive or active adsorption of immunoglobulins.

† 85% of childhood ALL, 55% of adult non-B, non-T ALL are positive for ALL antigen.[253]

‡ Exceptionally positive for Ia.[35]

§ T ALL positive for ALL antigen are probably exceptional.[35, 190]

‖ AML may express up to 30% of HuMA+ cells.[231]

¶ Very weak expression in comparison to ALL.[73]

** Rare cases of AML are ALL+.[35]

†† AMML (M4) and CML BC positive for a T antigen when determined by simian antiserums.[254]

can provide a more precise method for distinguishing different types of leukemic cells (Table 3). Identification of differentiation antigens on leukemic blast cells that are present on nonleukemic cells of known tissues of origin have been used to refine our understanding of blast cell lineage. The leukemia-associated antigens described in the above section may improve our diagnostic acumen and influence the classification of leukemias seen clinically. In the following section, we review the contribution of the immunologic phenotype to the classification of the acute leukemias.

Acute Lymphoblastic Leukemias

Acute lymphoblastic leukemia (ALL) is a heterogeneous disease as defined by clinical, morphologic, cytochemical, and immunologic criteria.

A wide range of clinical responses to therapy observed over the past two decades has suggested that ALL is a heterogeneous disease. Early extensive evaluation of clinical and laboratory data identified a number of prognostic factors, such as age, initial white count, and the presence or absence of a mediastinal mass.

The French-American-British (FAB) group has proposed three subtypes of ALL, based on morphologic criteria, eg, the amount of cytoplasm, predominance of nucleoli, the basophilia of the cytoplasm, and other characteristics.[1] Even when blast cells express the L3 morphology (Burkitt type),[181] it is difficult, if not impossible, by cytomorphology alone to predict whether lymphoblasts are T, B, or null, and to predict constantly the clinical course and prognosis. Blast cell surface markers and morphologic features appear to be independent prognostic variables in childhood ALL.[182]

Cell surface phenotyping using the differentiation markers described in the above section has been useful in producing a clinically significant classification that is reproducible and can be carried out in most hospital settings. Immunologic diagnosis of ALL should be based on the study of the bone marrow (BM) blasts or both BM and peripheral blood cells, especially if the leukocyte count is less than 10,000/cu mm.[183] The presence of circulating normal T lymphocytes may lead to conflicting results with respect to the nature of the leukemic process. Three major categories have now been described that carry clinical significance:[47,184,185] the non-B, non-T cell type and the T and B cell variants. Application of the additional differentiation and functional markers has led to subclassification of these types, and further clinical studies are in progress to test these categories for clinical relevance. Five categories of ALL can now be clearly distinguished with the use of immunologic phenotyping of blast cells from peripheral blood or bone marrow.

Non-B, Non-T Cell ALL: Seventy-five percent of ALL patients have blast cells that are negative for con-

ventional B and T markers.[186,187] The leukemia in this group has been generally termed non-B, non-T ALL, or null or common ALL, without any further distinction. Some workers prefer to subdivide the non-B, non-T ALL into common cell type and null (or unclassified) cell type,[186,188] based on the reactivity with anti-ALL serums.

Current evidence indicates that the non-T, non-B subgroups of ALL are immunologically heterogeneous and can express multiple phenotypes. The most frequent immunologic profile is SIg^-, Ia^+, ALL^+, E^-, T^- (thymocyte). This typing pattern represents a favorable prognostic factor for children with ALL.[189] Its absence may increase the likelihood of a poor response to therapy, whether or not high-risk clinical features are present. Some cells do not express either Ia or ALL (SIg^-, Ia^-, ALL^-, E^-, T^-).[190] Patients with ALL^+ antigen lymphoblasts have a more favorable prognosis than those without the ALL antigen.[186,191,192]

About 20% of non-B, non-T ALL cells with the phenotype SIg^-, Ia^+, ALL^+, E^-, T^- have cytoplasmic IgM and must be considered as pre-B ALL instead of non-B, non-T ALL.[193]

Using indicator erythrocytes coated with a high concentration of antibody, IgG Fc and IgM Fc receptors are observed on non-B non-T lymphoblasts.[194] As leukemic lymphoblasts express Fc receptors regardless of their differentiation markers, the presence of Fc receptors does not appear to be specific for a particular immunologic type of leukemic lymphoblast.

Non-B, non-T ALL cells can display C receptors on their surface, and sometimes in an exclusive way. It has been suggested that null ALL cells expressing exclusively CR are associated with an unfavorable prognosis[195] and a higher frequency of mediastinal involvement.[196-198] More recently, studies have shown that patients with CR^+, Ia^- lymphoblasts are clinically similar to patients with T cell ALL (usually CR^+, Ia^-) and may represent T-derived ALL lacking sheep erythrocyte receptors (pre-T ALL).[199] These patients may be mistakenly grouped with the more common and prognostically favorable common cell ALL if only SRBC and SIg markers are assessed. Esbar and co-workers observed that null cell patients that expressed neither FcR or CR had a significantly longer median duration of remission than those patients that expressed either one or the other marker.[200]

The non-B, non-T cell ALL seen in adults differs clinically and morphologically from the more common childhood type. The cells in adults are usually L2 (FAB classification) and show less reactivity with rabbit anti-ALL.[185] The remission rate in adults is lower than in children, there is earlier relapse, and the median duration of survival is much shorter.

Childhood common ALL tends to present with a low white blood cell count and to attain a high remission rate and a longer duration of the first complete remission in contrast to B and T ALL. Non-B, non-T cell leukemia has the best prognosis of the three major groups.[201]

Pre-T Cell ALL: Malignancies that arise early in T cell differentiation are characterized by the absence of sheep erythrocyte (E^-) receptors and the presence of "T" antigens (T^+). They may or may not express normal differentiation antigens such as Ia and ALL antigens.[190] The clinical significance of these leukemias (SIg^-, $Ia^\pm$, $ALL^\pm$, E^-, T^+) is not well established: some investigators are of the opinion that both E^+, T^+, Ia^- and E^-, T^+, Ia^- cases respond poorly to therapy,[202,203] and others believe that pre-T ALL behave more like non-B, non-T cell leukemias.[204]

T Cell ALL: T cell leukemia (SIg^-, Ia^-, ALL^-, E^+, T^+) is limited to those cases that are positive for SRBC receptor and "T" or H-Thy-L antigen.

The blast cells show T cell features in roughly 20–25% of patients with ALL.[186] The incidence of T-ALL may vary from series to series, according to the criteria used to label the leukemic cells as T-derived. In fact, the presence of T antigens sometimes contrasts with the poor ability of the blast cells to form E rosettes, and these pre-T ALL have been considered to be T cell ALL without any further distinction. Pre-T ALL cases probably account for 20% of T-derived ALL.[186,190]

The most frequent phenotype of T ALL cells is SIg^-, Ia^-, ALL^-, E^+, T^+. Ia^+ T ALL is uncommon but has been occasionally reported.[35,190] The ALL antigen may be weakly detectable in 10 per cent of cases of T ALL, although many investigators consider T ALL to be strictly ALL-antigen-negative,[190] particularly with the use of monoclonal antibody to human ALL antigen.[160] Several reports indicate that diseases of the T (E^+, T^+) phenotype are often CR-positive.[2] Reinherz and co-workers[207] have observed patients with T cell ALL who are either TH_2-antigen-positive or TH_2-negative, and despite the limited number of patients studied, the clinical presentation and course of children with TH_2^+ T ALL appeared to differ from children with TH_2^- T ALL. Broder et al[208] have described a patient with T cell leukemia associated with marked hypogammaglobulinemia, in whom malignant T cells represented a clonal expansion of progenitors of suppressor T cells. Saxon et al[209] reported a case of T ALL whose cells were expressing Fc portions of IgM and IgG. The patient's T cells have been shown *in vitro*

to provide either helper or suppressor activity. This study demonstrates neoplastic transformation in a committed T cell capable of further differentiation into the distinct pathways for help or suppression.

Studies of E rosette formation at 4 C (E^{+4}) and 37 C (E^{+37}) suggest that the difference in thermal stability may have prognostic significance. The median duration of remission for the thermolabile group is longer than the thermostable group, but this difference has not yet reached statistical significance.[210]

T-lymphoblastic leukemia has well-defined hematologic, cytochemical, and clinical features.[187,211,212] The cellular morphologic character of malignancies of the T phenotype is often of the convoluted type.[205,213,214] There is a strong acid phosphatase reaction in most cases of T ALL with E^+ or E^+ CR^+ surface profile,[215] in contrast to the weak or negative reaction in null and B ALL. Myeloblasts (M1 of the FAB classification) occasionally give a similar acid phosphatase reaction localized to the Golgi zone, and must be differentiated from T ALL cells.[186]

Correlative clinical studies in individuals with ALL have shown that T ALL patients are predominantly older children and adolescents (increased male:female ratio) who characteristically present with supradiaphragmatic lymphadenopathy (often with mediastinal mass or pleural effusion or both).[216,217] Rapid progression to the central nervous system is fairly common, especially in patients with mediastinal involvement. Both the disease-free interval and overall survival are shorter in this group than in patients with non-B, non-T ALL. However, median duration of remission may improve with the addition of alkylating agents such as cyclophosphamide or an anthracycline such as adriamycin to the maintenance regimen.[218] It has been postulated that ALL associated with a mediastinal mass is a more advanced stage of mediastinal lymphoblastic lymphoma,[219] but this has not been firmly established. Because of the heterogeneous phenotype expression of T ALL cells, the clinical heterogeneity between different T ALL cases is not surprising.[220] The elucidation of subsets that constitute the T cell population in man will, we hope, provide data for reliable subclassification of T cell ALL and provide a means of understanding the clinical variability arising within T cell malignancies.

Pre-B Cell ALL: ALL of pre-B cell phenotype is characterized by lymphoblasts containing small amounts of cytoplasmic IgM but undetectable surface immunoglobulin.[221] These cells were reported to have Ia and ALL antigens, and they can display Fc receptors for IgG as well complement receptors. About 20% of patients with non-B, non-T ALL have blasts with pre-B cell characteristics.[193] This leukemia can also occur during the blastic phase of CML.[222]

The clinical and laboratory characteristics of pre-B cell leukemia in childhood have recently been summarized by Crist et al.[223] Their data suggest that the pre-B cell and non-B, non-T cell forms of ALL are closely related entities. In contrast, 4 of 6 patients described by Brouet et al[193] had tumoral disease; one patient died during the induction phase, and another relapsed 3 months after the onset of remission. Further studies with larger numbers of patients are necessary to delineate the prognosis of this new subgroup of ALL and to work out possible therapeutic implications.

B Cell ALL: ALL of B-cell origin constitutes about 2% of all ALL cases.[47] B leukemic cells bear membrane-bound immunoglobulin molecules (SIg^+). These leukemic cells usually synthesized monoclonal surface IgM with little or no surface IgD, in contrast to the presence of both IgM and IgD at the surface of B-derived cells.

The most frequent phenotype of B-cell ALL is SIg^+, Ia^+, ALL^-, E^-, T^-. Ia antigens seem to be invariably present on the surface of these blasts. Leukemic B ALL cells may or may not carry Fc or complement receptors on their surface.

This group has a very poor prognosis, even poorer than T ALL.[191,224] Although the L3 morphologic characteristics were originally described to represent the leukemic phase of an underlying Burkitt or poorly differentiated lymphoma of monoclonal B-cell type, Koziner et al have found it related to cell lineages other than B.[181] Although most of the L3 leukemias are neoplasias of B lymphocytes, other lineages may also express this particular morphologic character.

Acute Nonlymphoblastic Leukemias

Immunologic techniques have been extensively applied to the evaluation of ALL cells, but AML cells have received much less attention. Most AML categories (M1 to M6) can be identified on the basis of morphology and cytochemistry, especially cytochemical reactions for myeloid enzymes. More attention to the immunologic phenotype of AML is warranted, since distinguishing ALL and AML is of considerable clinical importance.

Acute Myeloblastic Leukemia (M1, M2): The distinction between M1 (AML) and L2 (ALL) type leukemic cells may be difficult if one uses only morphologic criteria, but cytochemistry studies are often helpful. However, AML cells may stain weakly with myeloperoxydase (or Sudan black B) stains or may be confused with nonleukemic early myeloid cells on this ba-

sis. In this situation, immunologic studies may help to determine the correct diagnosis. Because AML and non-B, non-T ALL cells share many markers in common (both are SIg^-, Ia^+, E^-, $FcR^\pm$, $CR^\pm$), the battery of immunodiagnostic reagents should include one or more of the antiserums defining the ALL antigen, the myeloblastic-associated antigen(s) or the myelomonocytic antigen(s), and the i antigen.

The antimyeloid serums described by Roberts and Greaves have been found to react with normal and leukemic cells of myeloid lineage but not those of lymphoid lineage.[29] Similarly, a recent study has reported that 16 of the 17 AML tested and none of the CLL tested react with a myeloid antiserum (M^+ cells).[41] These anti-M serums have potential use in the diagnosis and classification of leukemia.

Appropriately diluted anti-i alloantiserums have been found to be strongly cytotoxic to lymphoblasts (50% kill in dilutions up to 1:2560) but weakly cytotoxic to myeloblasts. (50% kill in dilutions up to 1:2).[72,73]

In the majority (approximately 90% of cases of AML) the myeloblasts are Ia-antigen-positive.[35] FcR and CR are expressed in a variable proportion of AML cells.[225] AML cells often express SIg probably because of adsorption of IgG to Fc receptor. Trypsin treatment or overnight culture in serum-free media should remove extrinsic SIg, but trypsin treatment may result in a significant loss of cell viability.[226,227]

The most frequent phenotype of AML cells is therefore M^+, Ia^+, ALL^-, T^-, E^-, SIg^-, $FcR^\pm$, $CR^\pm$.

Promyelocytic Leukemia (M3): The presence of large coalescent sudanophilic granules and bundles of Auer rods (faggots) are so characteristic of this particular form of acute leukemia that diagnosis can be made reliably by cytomorphologic criteria.[1]

An atypical form characterized by minimal rather than excessive granulation has been described[228] and is likely to be misdiagnosed as an atypical monocytic leukemia. The determination of Ia antigen may help to differentiate between these two forms of acute leukemias, the M3 type being Ia-negative.[30]

Acute Myelomonocytic and Monocytic Leukemias (M4, M5): The diagnosis of AMML or acute monocytic leukemia (AMoL) is based largely on clinical and morphologic features. Markedly elevated serum and urine muramidase levels[6] and strong staining of cytoplasmic granules for alpha-naphthyl acetate esterase[229] support this diagnosis.

Immunologic studies do not contribute substantially to the diagnosis of AMoL and AMML. These leukemias have a monocyte–macrophage differentiation antigen (HuMA) on their surface.[230] This marker is present on almost all cases of AMoL, the majority of AMML, and a minority of AML cells, but is absent from ALL cells.[230]

Blast Crisis of Chronic Myelocytic Leukemia

The terminal phase of CML, known as blast crisis, may be characterized by an increasing proportion of blast cells in the bone marrow and peripheral blood. Although clinically this phase resembles AML, morphologic, cytochemical, biochemical, and immunologic studies have indicated that the blastic phase of CML is heterogeneous.[231-233] In addition to the classical myeloblastic or myelofibrotic proliferation, lymphoblastic, promyelocytic, megakaryoblastic, and erythroblastic transformation have been described.[234] Because of different responses to chemotherapy, the distinction between myeloblastic and lymphoblastic types is of clinical importance.

Immunologic studies are effective in distinguishing myeloid and lymphoid CML blast crises.[35] The phenotype of the "lymphoid" blast cells in Ph′ positive CML is identical antigenically and enzymatically to the typical non-B, non-T ALL seen in children. The immunologic profile of myeloid blast crisis cells is identical to the typical *de novo* AML seen in adults. The phenotype expression of the CML cells during the chronic phase is ALL^-, Ia^-, M^+ strong and differs from the phenotype expressed by CML BC cells of lymphoid phenotype (ALL^+, Ia^+, M^-) or myeloid phenotype (ALL^-, Ia^+, M^+ weak). Blastic crisis of pre-B cell phenotype (SIg^-, Cyt IgM^+) has also been described.[235]

Immunologic Monitoring of Acute Leukemia

Complete clinical remission is achieved by chemotherapy in most children and adults with acute leukemia.[236,237] Once hematologic remission is achieved, the next therapeutic objective is to prevent the recurrence of leukemia. However, the presence of some residual leukemic cells not detectable by sequential morphologic evaluation of the bone marrow may reduce the length of the first remission and consequently reduce the chances of long-term survival. The persistence of resistant leukemic cells is supported by several observations, including the reappearance of previously documented cytogenic abnormalities at the time of relapse,[238] virus-related reverse transcriptase in "normal" remission cells,[239] the development of transient leukemic-type growth patterns in marrow cultured during remission,[240] the detection of "inhibitory activity" in the marrow of a large number of patients with acute leukemia in complete clinical remis-

sion,[240] and the detection of leukemia-associated antigens.

Progress in the development of effective maintenance chemotherapy may be aided by the development of sensitive methods for detecting residual leukemia.[240-243]

The serologic detection of LAA-bearing cells during the remission period is a promising tool for this task.

Techniques of Immunomonitoring

Cells can be assessed serologically for the presence of LAA by a number of techniques, including complement-dependent microcytotoxicity, indirect immunofluorescence, and radioimmunoassay.

Complement-dependent assays carry the hazard of a variable nonspecific leukemic cell kill by rabbit complement. Immunofluorescence testing can markedly improve the sensitivity of the assay, particularly with the use of a fluorescence-activated cell sorter that can detect reactive cells at a frequency of less than 1%. RIA is also very sensitive for detecting cell-bound antigen but can also be applied to soluble antigen circulating in plasma.[244] The enzyme-linked immunosorbent assay (ELISA) can be as specific and sensitive as the RIA and has the advantages of requiring inexpensive equipment and utilizing stable, nontoxic reagents. The development of monoclonal antibodies may further improve specificity.

Non-B, Non-T Cell ALL

Following induction chemotherapy, the number of ALL$^+$ cells regularly falls below the detection level of 0.5% with the use of the FACS.[245] Serial testing of marrow cells may detect reappearance of ALL$^+$ cells before or simultaneously with morphologic relapse;[245] however, a number of exceptions have been noted.

In some cases, the rapid appearance of leukemic blast cells in relapse occurred without any prior detectable increase in ALL$^+$ cells above the 0.5% threshold. In patients with postchemotherapy lymphocytosis, weakly stained ALL$^+$ cells may reappear transiently without any subsequent relapse. Previously positive ALL cells may lose the ALL marker antigen, and consequently the emergence of ALL$^-$ cells in relapse cannot be identified immunologically. Monoclonal antibodies to human ALL antigen may obviate some of these difficulties, since they apparently do not react with postchemotherapy regenerating bone marrow cells.[160] RIA for ALL antigen in plasma has been recently developed.[244] Elevated level of plasma ALL antigen was associated exclusively with ALL patients, but the level of the antigen did not necessarily parallel the blast cell count. Determination of plasma ALL antigen by RIA might be a reliable indicator of imminent relapse, but this remains to be established.

T Cell ALL

Increased quantities of human thymus leukemia antigen have also been detected by RIA in the plasma and on the cells of patients with different forms of acute leukemia, including AML.[171] Neither healthy subjects nor patients with nonleukemic diseases had increased quantities of H-Thy-L antigen in peripheral white cells. Even though it is not specific for T cell leukemias, H-Thy-L could be useful as a sensitive probe for peripheral blood leukemic cells in certain patients. Studies are now in progress to evaluate the significance of plasma levels of H-Thy-L, quantitated by RIA, as a means of monitoring the state of disease in patients with increased quantities of antigen in leukemic cells or plasma.

Acute Myeloblastic Leukemia

Serial immunologic monitoring of marrow cells may provide early evidence for the onset of relapse of AML. Using an indirect immunofluorescence technique, Baker et al [246] have demonstrated that sequential evaluation of patients in remission using mouse anti-AML serums allows detection of imminent relapse an average of 3.7 months prior to morphologic relapse in the bone marrow. As in the studies with rabbit anti-ALL serums some patients relapsed morphologically without increased prior immunoreactivity being detected, and one patient has continued in complete remission for 2 years despite intermittently high immunologic reactivity. Peripheral blood samples have not been evaluated with this technique.

Simian anti-AML serums have been used to monitor the leukemic state of a patient with AMML.[166] Forty percent seropositive cells were noted in the circulation at a time when no blasts were observed in peripheral blood smears.

The detection of ALL antigen in plasma suggests that surface antigen is shed from blast cells *in vivo*.[244] The supernatant from short-term cultures of leukemic myeloblasts[247] contains soluble leukemic antigen, and efforts are under way to determine whether this occurs *in vivo* as well.

Serologic detection of LAA may alter our definition and concepts of leukemic remission and relapse and could influence patterns of treatment. Further clinical investigation is required to test this hypothesis.

Summary and Future Directions

The immunologic characterization of the leukemic

cell surface has become a useful tool for the identification and classification of the human leukemias. The techniques and resulting phenotypes discussed in this review should still be considered as supplying supportive information to morphologic and cytochemical techniques currently in use. Cell phenotyping has nevertheless reached sufficient clinical importance that hematology or immunology laboratories should now be prepared to perform the tests necessary for classification. At the present time, the clinical laboratory has access to techniques demonstrating surface and cytoplasmic immunoglobulins, sheep red blood cell receptors, Fc receptors, complement receptors, and Ia antigens. In the near future we feel that standardized, commercially available monoclonal antibodies will be equally important. Monoclonal antibodies to ALL antigens,[160] H-Thy-L antigen,[161] peripheral T cell antigens,[61,62] thymocyte antigens,[63,64] monocyte antigen,[42] and Ia antigen[22] have been described as of this writing.

The production of further polyspecific or monoclonal heteroantiserums, the development of RIA and ELISA tests to utilize these reagents, and the correlation of results obtained with clinical course and response to treatment will increase the impact of this field on clinical hematology in the foreseeable future.

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