Surface Membrane Characteristics and Cytochemistry of the Abnormal Cells in Adult Acute Leukemia

By David S. Gordon and Marjorie Hubbard

Membrane marker and cytochemical analyses were carried out on the abnormal cells from 70 adult acute leukemia patients. Such information may (1) supplement standard morphology and serve as a basis for a new classification scheme for acute leukemia, and (2) characterize the surface membranes of granulocyte, lymphocyte, and monocyte "progenitors." Classification of acute lymphoid leukemias solely on the basis of morphology was unsatisfactory. The presence or absence of T- or B-cell markers was helpful in classifying lymphoid leukemias. Monocyte progenitors were characteristically nonspecific esterase positive and Fc-receptor and membrane-IgG positive, but poorly phagocytic. Promyelocytes and myelocytes were frequently Fc-receptor positive and consequently positive for surface immunoglobulin. Myeloblasts were characteristically Fc-receptor negative. We conclude that surface membrane markers are essential in diagnosing lymphoid leukemias and helpful in nonlymphoid acute leukemias, and that cytochemistry is essential in delineating lymphoid from nonlymphoid leukemias and in subclassifying the latter.

DEVELOPING TECHNIQUES for marking specific membrane receptors and applying these techniques to the study of lymphocytes have been quite fruitful in furthering our understanding of the structure-function relationships of these cells. The presence of membrane immunoglobulin, receptors for complement, receptors for cytophilic IgG, and receptors for sheep red blood cells are characteristic but not necessarily specific for human lymphocytes.

Receptors for complement and for cytophilic immunoglobulin are also an integral part of the cell membrane of mature granulocytes and monocytes. The ontogeny of cells of the myeloid series, at least in terms of standard morphology, is common information among clinical hematologists. However, there is little functional information available about early myeloid cells and virtually none concerning immunoglobulin receptors on granulocyte or monocyte progenitors.

In addition to providing information regarding correlates of cellular development and membrane receptors, classifying the membrane characteristics of the immature cells in acute leukemia may be important diagnostically or prognostically. For example, in the pediatric age group, the presence of T markers on acute lymphocytic leukemia blasts is clearly related to poor prognosis.

Finding specific enzymes in the cytosol of some cells but not in others (as defined by cytochemical techniques) adds valuable information to standard morphology. Specific and nonspecific esterase stains have been particularly

Submitted April 15, 1977; accepted November 10, 1977.
Address for reprint requests: David S. Gordon, M.D., Clinical Immunology Laboratory, Bureau of Laboratories, Center for Disease Control, Atlanta, Ga. 30333.
© 1978 by Grune & Stratton, Inc. ISSN 0006-4971/78/5104-0011S02.00/0
useful in the analysis of hematopoietic cells, including those present in acute leukemia.

The purpose of this report is to describe the results of our evaluation of 70 cases of adult acute leukemia by standard morphology, cytochemical stains, and membrane receptors. We hoped that with these findings we could define better the cellular lineage of the leukemic blast in questionable cases and better characterize the membrane of granulocyte and monocyte precursors.

MATERIALS AND METHODS

Patient Selection

Seventy consecutive cases of adult acute leukemia were referred to our laboratory for evaluation. All cases of chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), including CML in blast crisis, and hairy cell leukemia (as defined by standard morphology and/or cytogensetics) were excluded from this analysis.

Lymphoid leukemias, other than those with the small round cell characteristic of CLL, were included. The lymphoid leukemia sample population consisted of 12 cases with morphology typical of acute lymphoblastic leukemia. Nine other cases were included in which the abnormal cells were similar to "prolymphocytes" and the clinical picture was one of recent-onset illness with primary involvement of bone marrow and peripheral blood at presentation.

Cytochemistry

Peripheral blood (PB) smears were made, allowed to air dry, and stained with Wright stain.

Heparinized blood (10 U/ml) and bone marrow (100 U/ml) smears were fixed for 60 sec at room temperature in 10% formalin-ethanol, and intracellular peroxidase was determined by the method of Kaplow,10 using Wright stain as a counterstain. Cytochemical demonstration of AS-D-chloroacetate esterase (AS-D-CE) and nonspecific (a-naphthyl acetate) esterase (NSE) was done by the method of Yam et al.11 In our hands this combined esterase stain provided an excellent method of distinguishing between monocytes and granulocytes on the same smear. The NSE stained orange-brown and the AS-D-CE blue in the combined esterase preparation. Characteristically the NSE staining was diffuse in monocytes but appeared as a "dot" in some lymphocytes (see below). Cells were stained with periodic acid-Schiff (PAS) stain according to the method of Hayhoe et al.12

In all of the cytochemical stains the slides were scored as the percentage of the number of immature or abnormal cells present that were positive. Thus in acute myeloblastic leukemias only the myeloblasts and promyelocytes were counted; in acute lymphoblastic leukemia only the lymphoblasts; in acute monocytic leukemias the blasts and promonocytes and "mature" monocytes; in acute myelomonocytic leukemia, only the blasts, promyelocytes, promonocytes, and the "myelomonocytes;" and in lymphoma-leukemia only the poorly differentiated lymphocytes.

Cell Preparation

Heparinized PB samples were diluted (1:3) in Hank's balanced salt solution without Ca\(^{2+}\) or Mg\(^{2+}\) and were layered gently over 10 ml isosmolar Ficoll-Hypaque (specific gravity 1.077) and centrifuged at 21°C for 40 min at 400 g. Interface cells were harvested, washed three times, and incubated with latex beads (1.1 μm, Dow, Indianapolis, Ind.) for 30 min at 37°C (final concentration, 0.1%,) in RPMI-1640 containing 25%, fetal calf serum (FCS) (both from the CDC Tissue Culture Unit). The preparations were then centrifuged twice at 300 g at ambient temperature over a cushion of FCS to remove excess latex. The pellets were resuspended in RPMI-1640 buffered with 10 mM HEPES pH 7.2 (N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid; Calbiochem, La Jolla, Calif).

Recovery rates were usually > 60% of the mononuclear cell populations and consisted of monocytes (pro- and mature), lymphocytes (blasts and mature), and myeloid cells from the blast to the myelocyte/metamyelocyte stage (defined by standard Wright stain). The percentage of abnormal cells in the Ficoll-Hypaque suspensions was either equivalent to or higher than the percentage in the starting population.
Membrane Markers

A polyclonal fluorescein-labeled goat anti-human Fab’ reagent (F-GAHlg) was donated by Dr. C. B. Reimer, CDC. Ficoll-Hypaque-separated latex-marked cell suspensions (0.1 ml, 5 x 10^7/ml) were incubated at 4°C in the presence of NaN_3 (0.2%) for 30 min with an equal volume of a 1:50 dilution of the F-GAHlg (plateau dilution). After washing twice at 4°C, the latex-negative immunofluorescent-positive cells were enumerated under a 54x objective on a Leitz phase-contrast epifiilluminated fluorescent Orthoplan microscope equipped with an HBO 100-W mercury lamp.

Fc receptors were evaluated by an indirect immunofluorescent technique with heat-aggregated IgG and a fluorescein-labeled goat anti-human γ-chain reagent (F-GAHlgG, donated by Dr. C. B. Reimer, CDC). All cell preparations were also reacted with the F-GAHlgG alone both as a “control” on the Fc receptor test and in order to enumerate all the surface membrane IgG (SMIgG)-positive cells. In our hands the staining of the bound aggregates was easily distinguishable from the staining with the F-GAHlgG alone.

Fc receptors were also detected by a rosetting technique. Ox red blood cells (ORBC) were incubated with an optimal dilution of the Sephadex G-200 7S peak of a rabbit antisera to ORBC for 30 min at 37°C (EA reagent) and washed twice in RPMI-HEPES. The EA reagent was added to the test cell suspension (EA/cell, 10:1), centrifuged at 200 g at 4°C, and incubated for 30 min at room temperature. After they were gently resuspended, the preparations were either read fresh or were fixed with 0.05 ml 0.1% glutaraldehyde in phosphate-buffered saline (PBS) and centrifuged onto slides with a Shandon cytocentrifuge (Shandon-Elliott, London). Standard and cytochemical stains were used to stain the EA-marked Fc-receptor-positive cells. The criterion for positivity was ≥ 3 red cells per test cell. T-cell nonimmune E rosettes were prepared as previously described.

RESULTS

Cell Separations

Characterization of the various cells in a living cell suspension is difficult. Cytoplasmic granules (seen with transmitted light or phase-contrast microscopy), when present, allow for classification of cells as myeloid, but blasts (myeloid and otherwise) and early promyelocytes that do not have granules cannot be separated with certainty from lymphoid (particularly large ones) or nonphagocytic mononuclear cells. The identification of early monocytes in a living cell suspension is also very difficult, since nuclear convolutions are common in many cells prepared by our methods and since early and abnormal monocytes do not always ingest latex (mature monocytes are > 90% latex positive in preparations of normal blood). We therefore tabulated the percentage of latex- and "granule"-negative cells SMIg+, Fc+, and/or SMIgG+ without regard to cell size or cell morphology.

Lymphoid Leukemia

Twenty-one cases were classified as lymphoid leukemia by the standard morphology in PB, occasionally supplemented by bone marrow morphology (Table 1). Cases could be characterized as B-cell diseases (Nos. 1–6), T-cell diseases (Nos. 7–9), or neither (Nos. 10–21) by virtue of the presence of SMIg and Fc receptors, presence of E rosettes, or absence of these markers, respectively (cf. abnormal column with marker columns, Table 1).

The morphology of the cells from cases 1–7 was similar to published descriptions of acute lymphosarcoma cell leukemia and prolymphocytic leukemia. The cells were large with a prominent nucleolus and moderate amounts of cytoplasm. We prefer the term lymphoma-leukemia when describing these cells.
Table 1. Characteristics of the Peripheral Blood in Lymphoid Leukemia

<table>
<thead>
<tr>
<th>Case</th>
<th>WBC (x 10^3/μl)</th>
<th>Abnormal Cells*</th>
<th>Fc E Rosettes*</th>
<th>Fc Receptors†</th>
<th>SM IgG †</th>
<th>SM IgG‡</th>
<th>PAS †</th>
<th>Dots ‡</th>
<th>Predominant Morphology §</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.3</td>
<td>60</td>
<td>8</td>
<td>75</td>
<td>86</td>
<td>ND</td>
<td>25</td>
<td>75</td>
<td>B</td>
</tr>
<tr>
<td>2</td>
<td>95.3</td>
<td>71</td>
<td>5</td>
<td>92</td>
<td>94</td>
<td>2</td>
<td>0</td>
<td>50</td>
<td>B</td>
</tr>
<tr>
<td>3</td>
<td>82.5</td>
<td>54</td>
<td>3</td>
<td>91</td>
<td>92</td>
<td>1</td>
<td>0</td>
<td>20</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>53.6</td>
<td>66</td>
<td>7</td>
<td>76</td>
<td>83</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
<td>5.4</td>
<td>12</td>
<td>25</td>
<td>68</td>
<td>44</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>B</td>
</tr>
<tr>
<td>6</td>
<td>15.0</td>
<td>7</td>
<td>55</td>
<td>64</td>
<td>47</td>
<td>1</td>
<td>ND</td>
<td>31</td>
<td>B</td>
</tr>
<tr>
<td>7</td>
<td>93.8</td>
<td>76</td>
<td>45</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>17</td>
<td>0</td>
<td>T</td>
</tr>
<tr>
<td>8</td>
<td>150.0</td>
<td>93</td>
<td>31</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>T</td>
</tr>
<tr>
<td>9</td>
<td>226.0</td>
<td>85</td>
<td>93</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>11</td>
<td>0</td>
<td>T</td>
</tr>
<tr>
<td>10</td>
<td>62.0</td>
<td>93</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>16</td>
<td>5</td>
<td>45</td>
</tr>
<tr>
<td>11</td>
<td>55.4</td>
<td>64</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>null LL</td>
</tr>
<tr>
<td>12</td>
<td>52.8</td>
<td>40</td>
<td>6</td>
<td>12</td>
<td>15</td>
<td>12</td>
<td>9</td>
<td>56</td>
<td>null llbl</td>
</tr>
<tr>
<td>13</td>
<td>50.8</td>
<td>84</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>14</td>
<td>87.6</td>
<td>78</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>86</td>
<td>49</td>
<td>null llbl</td>
</tr>
<tr>
<td>15</td>
<td>9.5</td>
<td>83</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>null llbl</td>
</tr>
<tr>
<td>16</td>
<td>7.9</td>
<td>13</td>
<td>36</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>44</td>
<td>68</td>
<td>null llbl</td>
</tr>
<tr>
<td>17</td>
<td>2.4</td>
<td>21</td>
<td>44</td>
<td>9</td>
<td>9</td>
<td>2</td>
<td>18</td>
<td>0</td>
<td>null llbl</td>
</tr>
<tr>
<td>18</td>
<td>13.1</td>
<td>84</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>3</td>
<td>21</td>
<td>19</td>
<td>null llbl</td>
</tr>
<tr>
<td>19</td>
<td>28.0</td>
<td>82</td>
<td>12</td>
<td>6</td>
<td>7</td>
<td>3</td>
<td>15</td>
<td>0</td>
<td>null llbl</td>
</tr>
<tr>
<td>20</td>
<td>6.4</td>
<td>69</td>
<td>15</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>null llbl</td>
</tr>
<tr>
<td>21</td>
<td>31.6</td>
<td>64</td>
<td>20</td>
<td>6</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>13</td>
<td>null llbl</td>
</tr>
</tbody>
</table>

Controls†† 72 ± 8 16 ± 5 14 ± 5 3 ± 4

ND, not done.

*Percentage of abnormal cells on the Wright-stained smear.
†Percentage of latex-negative Ficoll-Hypaque-separated cells positive for E (nonimmune T sheep red blood cell rosettes), Fc receptors (determined by indirect immunofluorescence), SM IgG (determined by a fluorescein-labeled polyclonal anti-human immunoglobulin), or SM IgG (determined by a fluorescein-labeled anti-human Y-heavy chain antiserum).
‡Percentage of abnormal cells positive on a smear of unseparated whole blood; PAS, periodic acid–Schiff stain; dots, discrete dotlike staining with nonspecific esterase.
§LL, lymphoma-leukemia; llbl, lymphoblastic leukemia.
††Percentage of positive cells in the latex-negative Ficoll-Hypaque preparation from normal adult donors; mean ± SD, 150 determinations.

Though usually of B-cell type by membrane markers (cases 1–6), case 7 demonstrated that T cells could also appear morphologically as lymphoma-leukemia.

Cases 8 and 9 were typical morphologically of acute lymphoblastic leukemia, with small cells, dispersed chromatin, and rare or nonprominent nucleoli. The morphology of cells null by membrane markers (cases 10–21) also varied. Most were typical of lymphoblasts with a very high nuclear/cytoplasmic ratio and rare nucleoli (cases 12–21); others, however, (cases 10 and 11) were much more similar to the lymphoma-leukemias (Fig. 1).

As noted in Table 1, “dots” of NSE were commonly observed in the lymphoid leukemias but were not observed in the abnormal cells of any of the other cases in this study. The dot was characteristically single, discrete, and perinuclear in location (Fig. 2). Our experience with isolated fractions of normal peripheral blood indicates that the dotlike staining is not typically found in a particular subpopulation of lymphoid cells (T, B, or null cells). This discrete dotlike staining in lymphocytes is quite unlike the diffuse staining pattern with NSE in monocytes.
Fig. 1. Three immature lymphoid cells, null by membrane markers, characterized as lymphoma-leukemia cells with prominent nucleoli and large amounts of basophilic cytoplasm. Case 11. Wright stain. × 450.

Fig. 2. Two lymphoma-leukemia cells showing the single, perinuclear dot of NSE. Combined esterase stain. × 450.

Fig. 3. Ox EA-positive promyelocyte from a patient with Di Guglielmo syndrome showing strong AS-D-CE positivity. Combined esterase stain. × 450.

Fig. 4. Single bone marrow cell (arrow) from a patient with AMMsL staining with both monocytic esterase (NSE) and myeloid esterase (AS-D-CE). Combined esterase stain. × 450.
Complement Activation and Pulmonary Leukostasis During Nylon Fiber Filtration Leukapheresis

By Dale E. Hammerschmidt, Philip R. Craddock, Jeffrey McCullough, Richard S. Kronenberg, Agustin P. Dalmasso, and Harry S. Jacob

Filtration leukapheresis (NFFL) is similar to hemodialysis in that donor blood is pumped over a foreign polymeric surface and a profound neutropenia occurs early during the procedure. Suspecting complement (C)-mediated leukostasis, we studied nine leukaphereses of normal donors as well as plasma incubated with nylon in vitro. Granulocyte (PMN)-aggregating activity, identified in earlier studies with C5a, was found both in nylon-incubated plasma and in plasma from the NFFL return line. EDTA, EGTA, heat, and hydrazine-inhibition studies suggested alternative pathway C dependence, confirmed by the demonstration of C3 and factor B conversion. Gel filtration, ultrafiltration, and antiserum-inhibition studies identified C5a as the aggregating agent. Neutropenia during NFFL was more profound than could be explained on the basis of filter trapping of PMN; sequestration was inferred. A mild but steady decrement was noted in pulmonary CO diffusion during NFFL, and two of four donors tested showed elevations in closing volumes. These observations were nonspecific but consistent with the suggestion that as in hemodialysis such sequestration may occur in the lungs. NFFL-harvested PMN were poorly responsive in vitro to C-activated plasmas as chemoattractants or aggregating agents. The chemotactic defect was mimicked by deliberate preexposure of PMN to activated C. This functional impairment of NFFL granulocytes suggests that modulation of C activation during NFFL may improve the usefulness of the technique.

Acute Monocytic Leukemia (AMoL)

Six cases were classified as monocytic leukemia (cases 22–27). Pertinent data on the cytochemistry and surface membrane markers are found in Table 2. Several characteristics unique to this group of patients included marked qualitative and quantitative positivity with NSE (diffuse staining), high white cell counts, markedly elevated Fc+, SMIg+, and SMiG+ cells, and a markedly depressed number of E rosette-forming cells. In one instance (case 27) the NSE was only “moderately” positive, but in all other cases it was strongly positive. Monocytes always stained with NSE in a diffuse fashion. The peroxidase stains of the abnormal monocytes revealed some positivity (similar to mature normal monocytes), although it was qualitatively and quantitatively much less than in cells of the granulocytic series.

Although one could usually predict the cytochemical and membrane marker data from the standard morphology, there were exceptions. Case 22 on standard preparations looked more like myeloblastic than monocytic leukemia but was classified as the latter on the basis of esterase stains. As noted above, monoblasts and promonocytes phagocytized latex poorly in comparison to normal mature monocytes.

Acute Myelogenous Leukemia (AML)

We evaluated the PB preparations of 33 cases of AML, of which 12 had Auer rods. In 28 of the 33 cases at least 5%, of the abnormal cells in the peripheral blood were either peroxidase positive, AS-D-CE positive, or classified as promyelocytes with Wright stain. In most instances the percentage of the abnormal cells that were peroxidase positive was higher than the percentage of the abnormal cells that were positive for AS-D-CE or that were classified as promyelocytes by Wright stain (23 of 28 cases). In 2 of the 28 cases the percentage of promyelocytes with Wright stain was greater than the percentage AS-D-CE or peroxidase positive, and in 3 of the 28 cases the percentage of AS-D-CE-positive cells was greatest. The NSE staining in myeloblasts varied from totally absent to a weakly positive “tinge” but was easily distinguished from the moderate to strong NSE activity in monocytes.

Cells from these cases were frequently positive for Fc receptors and surface Ig, with the percentage of cells positive by both tests considerably higher than...
occasionally suffer mild respiratory symptoms as well as chills and hypotension. These similarities between hemodialysis and NFFL suggested to us that C might be activated by nylon fibers during NFFL. The results to be presented and those prelim manly reported elsewhere support this suggestion.

**MATERIALS AND METHODS**

**Studies In Vivo**

Nine normal adults (two female, seven male, ages 21 - 33 yr), after giving informed consent, underwent NFFL as previously described. A Fenwal Leukopak apparatus (Fenwal, Deerfield, Ill.) was used and hepanin (2000 U priming dose and 4000 U/hr thereafter) was administered. White blood cell counts and 200-cell differential counts were performed by standard techniques, using ethylenediaminetetraacetate (EDTA)-anticoagulated blood drawn from the afferent (donor to filter) and efferent (filter to donor) lines of the NFFL circuit.

Single-breath pulmonary diffusing capacity (DCO) was measured using a carbon monoxide/neon gas mixture. Donors were positioned identically for all determinations during a given NFFL run. Gas analysis was made in a Vanian Autogna (Model 290, Vanian, Palo Alto, Calif.), and DCO values were corrected for hemoglobin levels. Preleukapheresis values were determined in triplicate. Subsequent determinations were made in duplicate and expressed as a percentage of the mean preleukapheresis value. Determinations in which the vital capacity differed from the baseline value by more than 10% were discarded and repeated. Closing volumes, as an index of small airway function, were determined by the method of Buist and Ross and were again expressed as a percentage of the mean pre-NFFL value. As in the DCO measurements, pre-NFFL values were determined in triplicate, with subsequent determinations in duplicate.

PMN aggregometry was performed as described previously, using heparinized (2 U/ml) plasma collected during NFFL as test aggregating agents. To 0.45 ml of PMN suspension, prepared from ABO-matched normal blood, was added 50 μl test plasma in a Chrono-log Model 335 aggregometer (Chrono-log, Havercourt, Pa.). For incubation studies in vitro, 50 μl chromatographic fraction or ultrafiltrational concentrate (see below) replaced the plasma.

Statistical significance was evaluated using the Mann-Whitney U test.

**Studies of Harvested PMN**

PMN were eluted from the nylon fiber filters by mild agitation with a mixture of ABO-compatible plasma and acid citrate-dextrose (ACD) solution; they were then concentrated by room-temperature centrifugation at 750 g for 10 min as previously described. Twenty milliliters of the concentrate so obtained were centrifuged at 400 g at 4°C for 6 min and the cells resuspended in 1 - 2 ml Hanks' balanced salt solution containing 0.5 g/dl salt-poor human albumin (HBSS/A). Red blood cells were lysed by the addition of 15 ml distilled water, followed in 25 sec by restoration of isotonicity with 5 ml 3.6% NaCl. The resultant suspension was spun at 400 g for 6 min. Following resuspension in 6 ml HBSS/A, the cells were applied to the surface of a Ficoll-Hypaque solution for density separation. The remainder of the cell preparation procedure was carried out as previously described, yielding a suspension of >98%, pure PMN. These cells were compared with similarly prepared normal cells for their ability to aggregate when exposed to activated C (zymosan-activated plasma [ZAP]).

Chemotaxis was measured by our modification of the method of Boyden, using Escherichia coli broth or a 1:200 dilution of ZAP in phosphate-buffered saline (pH 7.4) as the chemotactic attractant. Test suspensions were adjusted to 10,000 PMN/cu mm, and results were expressed as a percentage of the mean values for normal cells (preleukapheresis cells obtained from the same donors) run simultaneously. All determinations were done at least in quadruplicate and were read in a coded, blind fashion.

**Studies of Nylon-incubated Plasma (NAP)**

For the preparation of NAP, fresh whole blood was drawn through a siliconized needle into a chilled plastic syringe, transferred to chilled centrifuge tubes without anticoagulant, and immediately spun at 10,000 g for 10 min at 4°C. The resulting plasma, with added hepanin (2 U/ml), was placed into a sterile plastic test tube containing sterile nylon fibers from a

would have been expected from the numbers of monocytes and B lymphocytes present. Furthermore, cells with granules were also positive for Fc receptors, SM1g, and SM1gG. Additionally, examination of preparations rosetted with the EA reagent and stained with combined esterase showed that the myeloblast was Fc-receptor negative, whereas promyelocytes, myelocytes, and metamyelocytes were frequently Fc-receptor positive. An example from Di Guglielmo syndrome of this phenomena can be seen in Fig. 3. Therefore the most likely explanation of the SM1g positivity was the presence of cytophilic Ig in Fc receptors on the membrane of granule-negative (by phase-contrast microscopy) early myeloid cells. The heavy chain class of the SM1g was usually γ, which also suggested cytophilic.

In 6 of the 33 cases (Table 3, cases 29-33) there was a marked dichotomy between the number of SM1g-positive and Fc-receptor-positive cells. Furthermore, the membrane Ig was frequently not IgG. We therefore believe that where the SM1g was high and the Fc receptor low we may have observed a true antibody response to the myeloblast.

**Acute Myelomonocytic Leukemia (AMMoL)**

We evaluated seven cases in which two populations of abnormal cells (NSE+ and AS-D-CE+) were present in the same preparation. In five of the cases we found abnormal cells that were AS-D-CE positive and moderately to strongly positive with NSE. An example of single cells staining with both the “monocytic” esterase (NSE) and the myeloid esterase (AS-D-CE) is seen in Fig. 4. We classified cases as AMMoL only when two populations of abnormal cells were present and/or when individual abnormal cells double stained.

Membrane marker data on these preparations were similar to those of AMoL in the monocytic cells and AML in the myeloid cells. Some myelomonocytes (double-staining individual cells) did have Fc receptors as defined by EA rosettes. Patients with such cells also tended to have higher presenting white blood cell counts than those with pure AML. The membrane marker and cytochemical data are summarized in Table 4.

**Di Guglielmo Syndrome**

Three cases of Di Guglielmo syndrome (erythroleukemia) were studied. The classical features of megaloblastic, polyloid, and vacuolated erythroid precur-
LEUKAPHERESIS: C' AND LEUKOSTASIS

Leukopak NFFL filter (1 g fiber/1 ml plasma). After 30 mm incubation at 37°C, plasma was removed from the fibers. Other plasmas were prepared by incubating without nylon or by pretreating the heparinized plasmas with heat (56°C for 30 mm or 50°C for 20 mm), hydrazine (final concentration 10 mM), disodium EDTA (final concentration 10 mM), or magnesium EGTA (final concentration 10 mM) before incubation with nylon.

Chromatographic fractions were prepared from NAP in a descending, gravity-fed column of Sephadex G-200 (bed volume, 491 ml; height, 1 m; void volume, 192 ml) (Pharmacia, Uppsala, Sweden). To prevent artifactual C activation by the column gel, 1/10 volume of 100 mM disodium EDTA was added to the plasma after nylon incubation. NAP was ultrafiltered using Diaflo XM-50 (nominal molecular weight cutoff 50,000 daltons) and PM-b (mol wt cutoff 10,000 daltons) membranes in a magnetically stirred Aminco Model 52 ultrafiltration cell (Aminco, Lexington, Mass.) at 4°C under 30-psi N2. The XM-50 filtrate from 32 ml NAP was concentrated to 4 ml against a PM-b membrane to yield a concentrate of the plasma components in the 10,000-50,000-dalton range.

Anti-human C3a antiserum (rabbit, lot No. 2852B) and anti-human CS antiserum (goat, lot No. 3980E) (Behring Diagnostics, Sommerville, N.J.) were freed of complement by heating at 56°C for 30 mm and then centrifuged at 10,000 g for 10 mm to remove particulate matter. Then 50 or 300 IL of each was incubated at 37°C for 30 mm with 0.5 ml of the chromatographic fraction or 1.0 ml of the ultrafiltrational concentrate, respectively, to be tested. The resulting preparations were tested as aggregating agents by adding 50 μl of each to 0.45 ml normal PMN suspension in the aggregometer.

Immunoelectrophoretic assay of C3 and factor B conversion was carried out in 1.5% agarose containing 5 mM EDTA in veronal buffer (pH 8.6: MO.05). Anti-human C3 was prepared in rabbits by immunization with purified human C3. Anti-human factor B was obtained commercially (Behring Diagnostics).

RESULTS

Studies In Vivo

As noted previously,56 neutropenia occurred during the first hr of NFFL in all donors. The nadir, which occurred after approximately 15 mm of the NFFL run, averaged 46% (±8.3 SEM) of the preleukapheresis neutrophil count. (Nadir PMN counts ranged from 4% to 86 of the pre-NFFL value; the decrement correlated roughly with the blood flow rate. The relatively high value for mean nadir PMN counts may be explained by the inclusion of two donors with flow rates of 30 ml/mm and very modest PMN count decrements.) Total blood flow rates through the filters ranged from 30 to 60 ml/mm. At 15 mm, therefore, between 450 and 900 ml of blood (approximately 8-16 of total blood volume) had been filtered, an amount that could account for a maximum decrement of 16% in neutrophil count by filter trapping alone (assuming 100% trapping of PMN). Sequestration elsewhere was inferred.

Table 1. Pulmonary Diffusion Capacity in Donors Undergoing NFFL

<table>
<thead>
<tr>
<th>Minutes of Leukapheresis</th>
<th>Mean DCO value (percent of baseline) ± SEM</th>
<th>p (compared to baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100 ± 2.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>15</td>
<td>90.3 ± 2.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>30</td>
<td>89.8 ± 3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>60</td>
<td>84.2 ± 3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>90</td>
<td>80.2 ± 4.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>120</td>
<td>83.6 ± 3.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>150</td>
<td>82.1 ± 3.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Time zero is defined as the time at which blood first completes the leukapheresis circuit and begins to be reinfused into the donor.
†Mean baseline (preleukapheresis) DCO was 39.8 ± 4.8 (SEM) ml/mm Hg in the nine donors depicted.

Table 4. Characterization of the Peripheral Blood in Acute Myelomonocytic Leukemia

<table>
<thead>
<tr>
<th>Case</th>
<th>WBC (x 10⁹/cc)</th>
<th>Abnormal Cells* (%)</th>
<th>E Rosettes* (%)</th>
<th>Fc Receptors* (%)</th>
<th>SMig* (%)</th>
<th>SMigG* (%)</th>
<th>NSE† (%)</th>
<th>AS-D-CE† (%)</th>
<th>Double I (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>178.5</td>
<td>75</td>
<td>3</td>
<td>52</td>
<td>61</td>
<td>45</td>
<td>35</td>
<td>16</td>
<td>28</td>
</tr>
<tr>
<td>35</td>
<td>3.5</td>
<td>3</td>
<td>35</td>
<td>37</td>
<td>37</td>
<td>75</td>
<td>20</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>91.1</td>
<td>57</td>
<td>5</td>
<td>56</td>
<td>69</td>
<td>61</td>
<td>43</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td>37</td>
<td>122.0</td>
<td>86</td>
<td>0</td>
<td>15</td>
<td>61</td>
<td>55</td>
<td>91</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>38</td>
<td>55.2</td>
<td>62</td>
<td>13</td>
<td>84</td>
<td>81</td>
<td>73</td>
<td>49</td>
<td>34</td>
<td>21</td>
</tr>
<tr>
<td>39</td>
<td>14.2</td>
<td>61</td>
<td>13</td>
<td>42</td>
<td>54</td>
<td>35</td>
<td>77</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>134.0</td>
<td>66</td>
<td>9</td>
<td>30</td>
<td>15</td>
<td>18</td>
<td>47</td>
<td>30</td>
<td>0</td>
</tr>
</tbody>
</table>

* See footnotes to Table 1.
† See footnotes to Table 2.
‡ Individual cells positive for both NSE and AS-D-CE.

DISCUSSION

With significant advances in the treatment of acute lymphocytic leukemia, particularly in childhood,18 misdiagnosis of ALL as acute myelocytic leukemia is of concern. In our study "dots" of nonspecific esterase in abnormal cells always indicated a lymphoid origin; they are therefore of diagnostic value. Most of our cases of lymphoid leukemia had dots of NSE and/or coarse granules of PAS-positive material (19 of 21).

Classifying abnormal lymphoid cells strictly on the basis of morphology has been suggested.19 These classification systems do not directly correlate with surface membrane characteristics. We have shown, as have many others, that acute lymphocytic leukemia lymphoblasts can be either null or T by membrane analysis and that T cells can have round or cleaved nuclei and null cells cleaved or round nuclei. Null cells can also look like either lymphoma-leukemia cells or lymphoblasts. On the other hand, we have not as yet seen typical lymphoblasts with either surface membrane Ig or Fc receptors (Table 1, cases 8, 9, 12-21).

We strongly favor classifying lymphoid leukemias by membrane markers in conjunction with standard morphology for three reasons. First, the presence of a given membrane marker mirrors the functional state of the cell as morphology alone cannot. Secondly, preliminary data suggest that leukemic lymphoid (lymphoblast or lymphoma-leukemia) cells, null by membrane markers, are for the most part terminal deoxynucleotidyl transferase (TdT) positive (kindly per-
formed by Dr. John Hutton, University of Kentucky, Lexington, Ky.) irrespective of the morphology. T lymphoblasts are also TdT positive, whereas B and T lymphoma-leukemia cells are TdT negative. More extensive studies may document the interrelationship of this enzyme and surface membrane constituents. Third, the prognosis of childhood ALL apparently correlates with the membrane characteristics of the lymphoblast, and the same may be true of adult lymphoid leukemia.

Wright or Giemsa preparations have been and remain the major diagnostic tool in acute leukemia. Peripheral blood and bone marrow smears from 33 of our cases were coded and independently evaluated by four experienced morphologists who then assigned one diagnosis to each case. In 11 of these cases (33%), there was disagreement as to whether a case was lymphoid or nonlymphoid (data not shown). Cytochemical techniques established the diagnosis in 6 of 11 cases but were not helpful in the other 5.

We assigned the diagnosis of AML to a few cases on the basis of standard morphology (case 28, Table 3) even though the cytochemistry was not definitive. On the other hand, we feel a strong argument can be made for allowing a diagnosis of AML, AMMoL, or AMoL only when the cytochemistry is definitive. Clearly, if clinical relevance can be shown for more rigorous classification schemes using cytochemical techniques (as may occur with improvements in the therapy of leukemia) then such classifications will be more extensively used by hematologists.

Though acute monocytic leukemia of the Schilling type is usually simple to classify by standard morphologic criteria, results with NSE, particularly when accompanied by membrane receptor evaluations, define some “unclassifiable” cases of acute leukemia as monocytic. With better diagnostic acumen and more effective therapy it may become apparent that the prognosis in this group of patients is different from other nonlymphoid acute leukemias.

In our study the isotype of the immunoglobulin on the abnormal cells in AMoL was usually γ(IgG); this suggests that the immunoglobulin may be cytoplphilic. We were unable to prove absolutely that the IgG is cytoplphilic because we did not succeed in stripping (by trypsin or overnight culture in media free of human serum) the membrane in these cells without significant loss of viability. Others have encountered the same difficulty.

A number of investigators utilized antimmunoglobulin reagents to evaluate suspensions of acute leukemia cells for surface membrane Ig, and some suggested that the presence of immunoglobulin on blasts is a positive prognostic sign. Our data suggest that it is very difficult to identify what is cytoplphilic immunoglobulin, what is integral surface membrane immunoglobulin, and what is true antibody directed to the blast when SM1g is present on blasts.

One trivial explanation for apparent SM1g positivity would be the binding of a fluorescein-labeled antimmunoglobulin reagent through its own Fc piece to Fc receptors on the test cells. Using rabbit antisera, Winchester et al. and our group explored this phenomena in some detail. In this study, for several reasons, we feel this is not confusing our interpretation. First, we specifically limited our reagents to those made in goats, and we consistently found much less nonspecific binding with a variety of goat reagents to cellular test material (unpublished observations). Second, two goat reagents, polyvalent anti-Ig and
heavy chain-specific anti-IgG were used in parallel in all evaluations. If one goat reagent was binding nonspecifically, the other should have been also, since they were stored and handled in the same manner. As can be seen in Tables 2-4, this “parallelism” was not observed. Furthermore, our values for Fc receptors (receptor for Ig) and SM1g/SM1gG behaved as entirely independent variables, a result inconsistent with nonspecific binding of Ig to Fc receptors.

As noted previously, abnormal young myeloid cells in AML (probably promyelocytes) can have Fc receptors and immunoglobulin on their surface. Where the percentage of Fc receptors is high in a cell suspension and the isotype of the immunoglobulin is γ, it is likely that most if not all the Ig is cytophilic. Proof of the cytophilic nature of the Ig rests in part on the observation that viable cells cannot regenerate the surface Ig after membrane stripping. We have not been able to strip satisfactorily the membrane of immature myeloid cells recovered from the vapor phase of liquid nitrogen without significant loss of viability. We are now studying prospectively this phenomenon on fresh cells.

Membrane immunoglobulin positivity of cells in AML may usually be attributed to cytophilic Ig; however, we evaluated several patients where the presence of true “antiblast” antibody was more likely (Table 3). The cell preparations from these patients showed high levels of SM1g and low levels of Fc receptors. The low levels of the Fc receptors and the non-γ isotype of the SM1g (e.g., not SM1gG) indicated that the Ig was probably not cytophilic and was either integral surface membrane Ig or anti-“blast” antibody. We favor the latter possibility; if the SM1g were an integral part of the membrane, a probable lymphoid origin of the abnormal cells would be suggested, and the cytochemical results did not indicate a lymphoid lineage (i.e., negative PAS and no dots of NSE; data not shown). Our prospective membrane stripping experiments will be crucial in sorting out the possibilities, since integral surface membrane Ig ought to be regenerated in culture after stripping, whereas antiblast antibody would not be regenerated.

Functional, structural, and biochemical studies of the Fc receptor on lymphocytes have been of great interest to immunologists recently. Considerably less is known about the characteristics of Fc receptors on monocytes and granulocytes and how they compare with Fc receptors on lymphocytes. The role of the Fc receptor in phagocytosis by monocytes and granulocytes is well appreciated, but this function is not pertinent to the nonphagocytic Fc-receptor-positive lymphocytes. However, monocytes, lymphocytes, and granulocytes under certain experimental conditions can mediate antibody-dependent cell-mediated cytotoxicity (ADCMC), presumably through Fc-receptor-antibody-target cell interaction. Whether or not the Fc receptor on early myeloid cells is functional in phagocytosis and/or ADCMC needs to be explored.

It has been suggested that the Fc receptor and the serologically detectable products of the immune response gene loci (Ia antigens) on the surface of murine lymphocytes are identical or very closely linked. Conflicting data, however, have been published. An important follow-up to this study will include the correlation of our observations on the presence of Fc receptors on early myeloid cells with the description of Ia-like antigens on human lymphocytes and acute leukemia blasts (including myelogenous leukemia).
MEMBRANE RECEPTORS IN ACUTE LEUKEMIA

ACKNOWLEDGMENT

We wish to thank Dr. Charles B. Reimer for supplying reagents, Dr. John J. Hutton for analysis of terminal transferase, all members of the Division of Hematology, Emory University School of Medicine for referral of patients, and Dr. W. Ralph Vogler, Dr. James Keller, and Edith Jacobs, Emory University School of Medicine, for reviewing the morphology on select cases.

REFERENCES


26. Winchester RJ, Fu SM, Hoffman T, Kunkel HG: IgG on lymphocyte surfaces; Technical problems and the significance of a
Surface membrane characteristics and cytochemistry of the abnormal cells in adult acute leukemia

DS Gordon and M Hubbard

Updated information and services can be found at:
http://www.bloodjournal.org/content/51/4/681.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml