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scores of the patient's blood was very low (16) and resembled those of newborn infants. It should be noted that in human development the I component is barely detectable at birth and develops slowly in the first 18 mo of life.

An attempt was made to determine if the cells agglutinated by anti-i antibody had a higher concentration of HbF than the supernatant cells. Seventy percent of the red cells were agglutinated by the addition of the i antibody. Blood smears from the agglutinated and supernatant cells were stained for HbF using the Kleihauer technique. No clearcut separation of cells containing HbF from those apparently lacking HbF was obtained by this method. In contrast to red cell carbonic anhydrase, which was low (as it is in fetal cells), other enzymes not characteristically decreased in fetal cells were normal. The concentration of erythrocyte glucose-6-phosphate dehydrogenase was normal (3.7 lU/mi RBC; normal 2.1-5.1), as was the concentration of erythrocyte 6-phosphogluconate dehydrogenase (l.7 lU/mI RBC; normal 1.7-2.3). Pyruvate kinase was slightly decreased (l.6 IU/ml RBC; normal 1.7-2.1).

DISCUSSION

The disorder affecting the patient described in this report had the features of an acquired aplastic anemia. Exposure to an insecticide containing parathion (known to produce aplastic anemia) was followed by anemia, leukopenia, and thrombocytopenia. A remarkable feature in this case was the excellent response to combined androgen-corticosteroid therapy, with a marked rise in both total Hb and in HbF. Variably increased concentrations of HbF in acquired aplastic anemia have occurred relatively frequently. Prior to therapy HbF concentrations up to 19 (1.4 g/dl) have been reported. There have also been reports of modest increases in HbF during androgen-steroid therapy. Shahidi et al. reported a boy who showed a rise in HbF from 0.5 g/dl (7.3 of total Hb) to 1.5 g/dl (100) following androgen-steroid therapy. Our patient had an initial concentration of HbF of 0.85 g/dl (18.8% of total Hb) and achieved a maximum of 9.6 g/dl (60.2% of total Hb) at the end of 30 wk of fluoxymesterone-prednisonetherapy. Such a marked response to androgen-steroid therapy has not been previously reported.

The increase in both total Hb as well as in the proportion of HbF was followed by a decline in both values upon cessation of therapy. This finding suggests that the initial improvement was a specific result of drug treatment. This interpretation is further supported by the second increase in the total Hb and in HbF after reinstitution of therapy.

The HbF was heterogeneously distributed among the erythrocytes of the present subject, as has been reported in the other cases of aplastic anemia. The parents of the subject had normal HbF levels, and the distribution of HbF in their erythrocytes was heterogeneous. Thus a form of hereditary persistence of fetal hemoglobin as an explanation for the increase in HbF in the propositus is not likely.

There are a few previous studies of the synthesis of globin chains in aplastic anemia. Ramot et al. reported balanced synthesis of A and non-A globins in a case of refractory hypochromic anemia. In the present case, the A to non-A chain synthetic ratio was 1:1, demonstrating a balanced globin chain synthesis.

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HbF SYNTHESIS IN APLASTIC ANEMIA

This balanced globin chain synthesis contrasts with the findings in the thalassemias and in congenital dyserythropoietic anemia. Rosa et al. described three cases of aplastic anemia with \( \frac{G}{A} \) ratios in HbF of the fetal type. In two other subjects the \( G/A \) ratio was intermediate between those of the adult and fetal types. Schroeder and Huisman studied the \( \gamma \)-chain composition in six cases of "idiopathic" aplastic anemia. The \( G/A \) ratios in three were in the newborn range, one was in the adult range, and two were intermediate. The same authors studied seven cases of aplastic anemia due to benzene poisoning and found one case with \( G/A \) ratio in the newborn range, three in the adult range, and the remainder in between. No association has been made between the nature of the \( G/A \) ratio and response to therapy. In our patient, in whom there was a response to therapy, the \( G/A \) ratio was that of the fetal type, i.e., 3:1.

Increased red cell i antigen and low levels of carbonic anhydrase enzyme have been observed in leukemias and in refractory anemias. Increases in HbF to as much as 85% of total hemoglobin associated with other "fetal" red cell characteristics have been described in cases of juvenile chronic myelogenous leukemia.

The red cells of the subject in this report demonstrated not only a marked increase of HbF but also other features characteristic of "fetal" red cells. At present there is no explanation for the apparent change in erythropoiesis from an "adult" type to a "fetal" type in aplastic anemia. Alter et al. noted transient "fetal" erythropoiesis following bone marrow transplantation. In their patients gamma chain synthesis in blood and bone marrow represented 20% of total non-\( \alpha \) globin synthesis. A high titer of i antigen was also found. In their patients all fetal red cell characteristics declined after 200 days. Our case differed since the HbF and i antigens remained high continuously during the study period of 92 wk.

Studies using the acid elution technique showed that in normal adults only a small proportion of red blood cells contains HbF. Recent studies using fluorescent antibody techniques confirmed those findings. It is possible that in our patient production of these normally occurring HbF-containing red blood cells was preferentially stimulated by androgen-steroid therapy.

ACKNOWLEDGMENT

We thank Dr. W.G. Wood for performing the fluorescent anti-F antibody technique.

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**Membrane Markers**

A polyvalent 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 NaN3 (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 54× objective on a Leitz phase-contrast epillumination 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 (SMlgG)-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 monocyctoid 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 SMlg+, Fc+, and/or SMlgG+ 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 SMlg 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 10^9/cc)</th>
<th>Abnormal Cells</th>
<th>E Rosettes</th>
<th>Fc Receptors</th>
<th>SM1gt</th>
<th>SM1gG</th>
<th>PAS</th>
<th>Dots</th>
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<td>Lbl</td>
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</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), SM1g (determined by a fluorescein-labeled polyclonal anti-human immunoglobulin), or SM1gG (determined by a fluorescein-labeled anti-human γ-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; Lbl, 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 AMMaL 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+, SM1g+, and SM1gG+ 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
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 cytophilia.

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 AML 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, polypoid, and vacuolated erythroid precur-

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*Table 3. Characterization of the Peripheral Blood in Selected Cases of Acute Myelogenous Leukemia*

<table>
<thead>
<tr>
<th>Case</th>
<th>WBC ($x 10^9$/cc)</th>
<th>Abnormal E Rosettes* (%), Fc Receptors* (%), SM1g* (%), SM1gG (%), AS-D-CE* (%), Px* (%)</th>
<th>Promyelocytes* (%), SM1g* (%)</th>
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<td>9.5</td>
<td>81 8 25 42 21 1 92 22</td>
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</tr>
</tbody>
</table>

*See footnotes to Table 1.

1. Percentage of abnormal cells positive on a smear of unseparated whole blood; AS-D-CE, AS-D-chloroacetate esterase; Px, peroxidase.

2. Percentage of promyelocytes on a smear (Wright stain) of unseparated whole blood.
LEUKAPHERESIS: C’ AND LEUKOSTASIS

Leukopak NFFL filter (1 g fiber/1 ml plasma). After 30 min 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 min or 50°C for 20 min), 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 min and then centrifuged at 10,000 g for 10 min to remove particulate matter; then 50 or 300 MI of each was incubated at 37°C for 30 min 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 50.5 ml 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; 0.05M). 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, neutropenia occurred during the first hr of NFFL in all donors. The nadir, which occurred after approximately 15 min of the NFFL run, averaged 46% (±8.3SEM) 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/min and very modest PMN count decrements.) Total blood flow rates through the filters ranged from 30 to 60 ml/min. At 15 min, 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>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100 ± 2.8</td>
</tr>
<tr>
<td>15</td>
<td>90.3 ± 2.8</td>
</tr>
<tr>
<td>30</td>
<td>89.8 ± 2.6</td>
</tr>
<tr>
<td>60</td>
<td>84.2 ± 3.6</td>
</tr>
<tr>
<td>90</td>
<td>82.1 ± 3.2</td>
</tr>
<tr>
<td>120</td>
<td>83.6 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>p (compared to baseline)</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>&lt;0.01</td>
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<td>&lt;0.001</td>
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<tr>
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<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 (× 10³/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>
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<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>
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<td>35</td>
<td>3.5</td>
<td>3</td>
<td>35</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>75</td>
<td>20</td>
<td>5</td>
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<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>
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<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>
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<tr>
<td>38</td>
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<td>13</td>
<td>84</td>
<td>81</td>
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<td>13</td>
<td>42</td>
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<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>
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* 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, 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. 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 cytophilic. We were unable to prove absolutely that the IgG is cytophilic 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 antiimmunoglobulin 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 cytophilic immunoglobulin, what is integral surface membrane immunoglobulin, and what is true antibody directed to the blast when SMIg is present on blasts.

One trivial explanation for apparent SMIg positivity would be the binding of a fluorescein-labeled antiimmunoglobulin 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 cytphilic. Proof of the cytphilic 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 cytphilic 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 cytphilic 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).
ACKNOWLEDGMENT

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REFERENCES

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Surface membrane characteristics and cytochemistry of the abnormal cells in adult acute leukemia

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