Changes in Cell Surface Antigen Expression During Hemopoietic Differentiation

By C. Sieff, D. Bicknell, G. Caine, J. Robinson, G. Lam, and M. F. Greaves

Human bone marrow cells were separated on a fluorescence activated cell sorter (FACS) according to their binding of a series of monoclonal antibodies; the positive and negative fractions were cloned for erythroid burst and colony-forming units (BFU-E and CFU-E) and myeloid colony-forming units (CFU-GM), and cytocentrifuge slides were prepared for microscopy of maturing precursors. The pattern of antigen expression on hemopoietic progenitor and precursor populations has been established using antibodies defining blood group (A, I/i), HLA-associated (A, B, C, DR, DC1), lineage specific, and transferrin receptor antigens. Like monomorphic HLA-DR, the antigen defined by monoclonal antibody OKT10 is expressed on the earliest progenitors and lost during differentiation, suggesting a role in interactions regulating the differentiation of these cells. The HLA-linked DC1 determinant, in contrast to HLA-DR, is not expressed at a detectable level on progenitor cells. Although a lineage-specific early antigen has not been identified, the transferrin receptor is expressed on the majority of erythroid progenitors, but only weakly on myeloid progenitors, and may provide an approach to isolating erythroid progenitors. These and earlier studies with monoclonal antibodies against HLA-DR and glycoprotein now provide a detailed "map" of antigen expression during hemopoietic differentiation.

The development of clonal assays for hemopoietic progenitors has provided new insight into the regulation of hemopoiesis at a cellular level. Committed myeloid progenitors (CFU-GM) can be cloned in methylcellulose in the presence of colony-stimulating activity. Two classes of committed erythroid progenitor form colonies in methylcellulose in the presence of the glycoprotein hormone erythropoietin (epo), relatively mature erythroid colony-forming units (CFU-E) and less mature burst-forming units (BFU-E). Erythropoietin regulation occurs primarily at the level of CFU-E, and sensitivity to erythropoietin is most probably acquired during the transition from BFU-E to CFU-E. It is now clear that BFU-E are regulated by epo-independent mechanisms, thought to be mediated by short-range cell interactions or factors released from neighboring cells. "Burst-promoting activity" (BPA) has been attributed to T-lymphocytes or monocytes, which enhance colony formation when cocultured with BFU-E in vitro, but the identity of interacting cells is still controversial. Monoclonal antibodies may provide ideal probes for isolating hemopoietic progenitor cell populations and for identifying cell surface structures involved in regulatory interactions. When used in conjunction with the fluorescence activated cell sorter, monoclonal antibodies help define the antigenic phenotype of numerically infrequent progenitor cells assayed by colony formation. Alternatively, complement-fixing monoclonal antibodies can be used to inhibit or kill progenitor cells. These observations and other studies using nonmonoclonal reagents have revealed that human hemopoietic progenitor cells express HLA-DR antigens. HLA-DR antigens are structurally homologous to murine type II (H-2, I region, specifically I-E/C) histocompatibility antigens. I region products (i.e., Ia antigens) are involved in cell interactions and antigen recognition in immune responses. The possibility has therefore been raised that they are similarly involved in interactions important for early hemopoietic differentiation. Disturbances in regulatory interactions involving immunocompetent cells and hemopoietic stem cells may, in some patients, have important pathologic consequences, e.g., aplastic anemia.

In this article we describe the changes in expression during erythroid and myeloid maturation of four groups of cell surface antigens (see Table 1); blood group antigens (A and I/i), HLA-D-associated structures, putative erythroid, myeloid and lymphoid lineage-specific antigens, and transferrin receptors identified by monoclonal antibody OKT9 or by binding of transferrin itself.

MATERIALS AND METHODS

Normal bone marrow was obtained by aspiration from adult volunteers. Approximately 5–10 ml of marrow from 1–2 aspirations was collected into 10 ml Hank's buffered salt solution containing 200 U preservative-free heparin. The marrow suspensions were centrifuged (400 g for 40 min at 20°C) on Ficoll-Isopaque (1.077 g/cu cm) and the interface mononuclear cells collected, washed twice, and suspended in Eagle's medium plus 2% fetal calf serum (FCS).


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**Table 1. Monoclonal Antibodies Used to Investigate Antigen Expression on Hemopoietic Progenitors**

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Designation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-associated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABC monomorphic</td>
<td>WB/32</td>
<td>24</td>
</tr>
<tr>
<td>DR monomorphic</td>
<td>DA-2</td>
<td>24</td>
</tr>
<tr>
<td>DR-linked polymorphic*</td>
<td>Genox 3.53</td>
<td>24</td>
</tr>
<tr>
<td>Erythroid lineage associated</td>
<td>Glycophorin A</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Band 3</td>
<td>10</td>
</tr>
<tr>
<td>Transferrin receptors</td>
<td>(gp90 Kd dimer)</td>
<td>OKT9</td>
</tr>
<tr>
<td></td>
<td>Blood group antigen A†</td>
<td>1. MAS 016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. N 16</td>
</tr>
<tr>
<td>Others</td>
<td>Thymic + panhemopoietic</td>
<td>OKT10</td>
</tr>
<tr>
<td></td>
<td>(gp40)</td>
<td>Myeloid antigen</td>
</tr>
</tbody>
</table>

*Determinant shows linkage disequilibrium with DRw1,2,6. It is probably DC1 (also called MT1 or MB1).*
†React with A1 and A2 erythrocytes but not B or O erythrocytes.

Antibodies

Murine monoclonal antibodies were produced using cell hybridization methods as previously described. Table 1 lists the antibodies used with their laboratory of origin plus established or presumed specificity.

Human monoclonal cold antibodies to blood groups I (Stephenson) and i (Dench) were obtained from Dr. K. Shumak (Toronto General Hospital).

Rabbit anti-human transferrin (DAKO) was used to study transferrin binding in some experiments (see below).

Antibody Binding

A quantity of 20–100 × 10⁶ bone marrow cells were obtained as described and incubated for 30 min at 4°C with previously determined optimal concentrations of the different antibodies (see Table 1). In the Genox 3.53 and blood group A experiments, donors were selected whose lymphocytes or erythrocytes had been previously typed for expression of Genox 3.53 or group A determinants, respectively.

After two washes, the cells were stained with an affinity-purified F(ab')₂ preparation of goat anti-mouse antibodies that had been cross-absorbed with insolubilized human immunoglobulin and labeled with fluorescein isothiocyanate (FITC). A F(ab')₂ goat anti-human immunoglobulin was used in the I/i experiments. Transferrin binding was studied using two different methods: indirectly, by incubating bone marrow cells with transferrin (Sigma, St. Louis, Mo.), washing twice, incubating with rabbit anti-human transferrin, washing twice, and then incubating with a fluorescein-conjugated sheep anti-rabbit antibody; or directly, by conjugation of transferrin with FITC, sterilization of the conjugate by Millipore filtration (0.22 μm), and subsequent incubation of 10⁶ bone marrow cells with the conjugate for 20 min at room temperature.

After labeling, the cells were washed twice with cold Eagle's medium and held on ice until fluorescence activated cell sorting and analysis using a modified FACS-I (Becton Dickinson, Mountain View, Calif.). Cells were processed in sterile conditions using relative fluorescence intensity (vertical axis) to separate positive from negative cells (cf., Fig. 1). In some experiments, an intermediate fraction comprising weakly positive cells was also collected. Cytosin preparations were made from aliquots of the unfractionated controls and from each fraction, stained with May-Grunwald-Giemsa, and differential leukocyte counts of at least 200 cells performed.

Culture Procedures

Two unfractionated controls (unlabeled and labeled) and the positive and negative fractions were washed twice in cold alpha-medium (Flow) and 2% FCS.

**Erythroid Colonies**

The cells were cultured in a mixture containing 30% FCS (Bio-cult, Gibco, Grand Island, N.Y.), 1% bovine serum albumin (Sigma Poole, Dorset), 10⁻⁴ M mercaptoethanol, penicillin, streptomycin, 0.9% methylcellulose (Dow Colorron, Orpington, Kent), and 2–2.5 U/ml erythropoietin (Connaught Step III). The final concentration of nucleated cells was 0.5, 1, or 2 × 10⁶ ml⁻¹, although fewer cells were occasionally obtained for some fractions. Volumes of 1 ml of the mixture were plated in 35-mm tissue culture plates (Nunc Gibco, Paisley, Scotland) in duplicate, and incubated at 37°C in a high humidity 5% CO₂/95% air tissue culture incubator. CFU-E, identified by the pink-red color of the cells and comprising 1 or 2 clusters, were counted on day 7 using an inverted microscope at ×40 magnification. BFU-E, large red colonies comprising 3 or more subcolonies, were counted on day 14.

**Granulocyte-Macrophage Colonies**

Granulocyte-macrophage colonies were cultured in a mixture containing 25% FCS, 1% BSA, penicillin, streptomycin, 0.9% methylcellulose, and 5% PHA-leukocyte conditioned medium. Mixtures were plated in duplicate and incubated in the tissue culture in incubator under the same conditions as the erythroid cultures. CFU-GM, comprising translucent colonies of ≥50 granulocytes, monocytes, or mixtures of both, were counted on day 13 or 14.

**Calculation of Percentage Recovery for Each Fraction**

Recovery per 10⁵ unfractionated cells was first determined by multiplying the number of colonies per 10⁵ cells in each fraction by the proportion of cells in that fraction.

Total colony recovery was then determined by summing the colonies recovered for each fraction. The number of colonies recovered in each fraction was then expressed as a percentage of the total colonies recovered.

A similar analysis of percentage recovery of erythroid and myeloid precursors was carried out using the cytospin differential leukocyte counts.

**RESULTS**

Table 2 lists the results of representative experiments for each of the nine cell surface structures studied. For simplicity, the numbers of colonies recovered are not shown, but they can be calculated from the colony numbers and percentages listed for each fraction. The differential counts from representative experiments are listed in Table 3, and Table 4 summarizes the mean percentage positivity for each cell surface structure calculated for the entire series of experiments.
Blood Group Antigens

Expression of Group A Antigen on Hemopoietic Cells

Table 4 summarizes the results obtained in a series of experiments to determine the expression of antigen A on hemopoietic cells. Using two different monoclonal antibodies, virtually all BFU-E and CFU-E consistently separate into the positive fraction. However, only a minority of BFU-E are strongly positive (7%), but this proportion increases during differentiation to CFU-E (38%) (Table 2). In most experiments, the majority of erythroid precursors are also positive. CFU-GM show a similar pattern to BFU-E, with the
majority of progenitors in the positive fraction, and myeloid precursors show moderate to strong positivity.

Expression of Group I/i Antigen on Erythroid Cells

Results for BFU-E were variable, with 26%–69% of progenitors in the positive fraction (Table 4). The majority of CFU-E are positive (62%–83%) and I expression increases further during differentiation to recognizable precursors. Myeloid cells show little I expression.

In contrast, i antigen is not expressed on BFU-E from adult donors, is only expressed on a minority of CFU-E (1.1%–14%), but is expressed on erythroid precursors. CFU-GM and myeloid precursors show strong i expression.
Lack of Expression of a Polymorphic HLA-DR-Linked Antigen on Hemopoietic Progenitors

Previous studies have documented the expression of monomorphic (nonallelic) HLA-A, B, C determinants on BFU-E and CFU-E and monomorphic HLA-DR antigens on BFU-E. Monoclonal antibody Genox 3.53 appears to react with a polymorphic (allelic) determinant that is coexpressed (i.e., in linkage disequilibrium) with HLA-DRw1, 2, 6. In common with antimonomorphic DR antibodies, Genox 3.53 precipitates 2 noncovalently linked polypeptides (α, β), but these are distinct from DR molecules and probably correspond to separate (but HLA-D-linked) DC1 locus product.

The results of 4 experiments with Genox 3.53 are shown in Table 4 and demonstrate unequivocally that neither BFU-E (1%–6%) nor CFU-E (1%–3%) express the antigen defined by this antibody, despite its presence on blood (B) lymphocytes of the bone marrow donors. Erythroid precursors, CFU-GM, and myeloid precursors show little positivity.

**Lineage-Specific** Antigens (Table 4)

The Band 3 Antigenic Determinant Detected by Monoclonal Antibody R6A

This determinant is expressed on only a minority of erythroid progenitors, since 15% of BFU-E and 21% CFU-E sort into the positive fraction, whereas 90% of the normoblasts are positive, albeit weakly. CFU-GM are mostly negative (<20%), and there is no increase in positivity during myeloid maturation. Adult blood or marrow erythrocytes are strongly positive.

**OKM-1**

Virtually all BFU-E, CFU-E, and erythroid precursors are unreactive with OKM-1, as are the majority of CFU-GM, whereas 58%–89% of morphologically recognizable myeloid precursors are recovered in the positive fraction.

**OKT10**

Of BFU-E, 79%–99% are positive, and the proportion decreases with differentiation through CFU-E.
(53%–59%) to erythroid precursors. Although CFU-GM show a wider range of positivity (33%–81%), a similar reduction in expression is evident with myeloid maturation.

‘‘Proliferation-Associated’’ Transferrin Receptor

Monoclonal OKT9

A very wide range of positivity was found in erythroid progenitors (BFU-E 1%–78%; CFU-E 14%–96%), but in 7 of 8 experiments, there was an increase in positivity from BFU-E to CFU-E. A further increase was observed from CFU-E to erythroid precursors.

CFU-GM results in 3 experiments were also variable (6%–29% positive) with little change during maturation to recognizable precursors. However, if the myeloid recovery data are calculated separately for early (blasts to myelocytes) and late (metamyelocytes and neutrophils) precursors, virtually all the early erythroid progenitors (BFU-E 1%–78%; CFU-E 4%–96%) showed a similar pattern to that seen using the monoclonal OKT9, with the majority of early but virtually no late myeloid cells in the positive fraction (Table 3).

Transferrin Binding

The results for BFU-E differed according to the method used to label the cells. When bone marrow cells were indirectly labeled, 38%–42% of cells were positive, with 97% BFU-E in this fraction. Direct labeling with transferrin-FITC conjugate resulted in fewer positive cells (14%–28%), with fewer BFU-E recovered in this fraction (80%). Virtually all CFU-E consistently sort into the strongly positive fraction, with an 8–11-fold enrichment, whatever method is used to label the cells, and over 80% of recognizable erythroid precursors were recovered in the positive fraction.

The CFU-GM results showed a similar difference according to the labeling method used. Sixty-seven percent of CFU-GM were positive with indirect labeling, whereas, in marked contrast to the BFU-E results, only a minority of CFU-GM were positive (25%) when bone marrow cells were directly labeled. Maturing precursors showed a similar pattern to that seen using the monoclonal OKT9, with the majority of early but virtually no late myeloid cells in the positive fraction (Table 3).

**DISCUSSION**

The homogeneity, specificity, and high titer of monoclonal antibodies are ideal features for probes of cellular differentiation. However, since they detect individual antigenic determinants (e.g., configurations consisting of a few amino acids or sugars), failure to detect any particular antigen on a cell type does not necessarily imply absence of the structure with which that determinant is usually associated. This reservation is particularly pertinent to carbohydrate determinants whose expression might be linked to maturation-linked glycosylation and to serologic assays with infrequent progenitor cells, since no complementary biochemical analysis is possible at present to confirm the presence of particular cell surface structures.

Ideally, therefore, the application of monoclonal antibodies to cell differentiation studies requires a biochemical description of the antigenic determinants identified. Such information is available on some but not all of the monoclonal antibodies we have used in this study; our analysis reported here therefore concerns antigenic determinants.

Another limitation of the monoclonal antibody flow sorting approach we have used is the sometimes arbitrary distinction between positive and negative cells. Although flow cyt fluorimetry machines such as the FACS allow quantitative and sensitive recording of fluorescence signals, there are limits of antigen density on cells below which unequivocal detection is difficult or impossible. Thresholds, therefore, have to be set for

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**Table 4. Mean (Range) Percentage Positive Hemopoietic Cells for the Series of Experiments**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>BFU-E</th>
<th>CFU-E</th>
<th>Precursors</th>
<th>CFU-GM</th>
<th>Precursors</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (MAS 016 and N 16)</td>
<td>3-5</td>
<td>65.7 (15.7–99.1)</td>
<td>91.2 (67.8–99.9)</td>
<td>86.7 (73.0–94.0)</td>
<td>44.1 (11.6–98.8)</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>44.0 (26.0–69.0)</td>
<td>72.5 (62.0–83.0)</td>
<td>100</td>
<td>27.6 (27.0–28.2)</td>
</tr>
<tr>
<td>i</td>
<td>3</td>
<td>2.4 (0.4–5.0)</td>
<td>9.0 (1.1–14.0)</td>
<td>72.3 (70.5–74.0)</td>
<td>96.0 (95.9–96.2)</td>
</tr>
<tr>
<td>Genox 3.53</td>
<td>1-4</td>
<td>2.6 (1.1–6.4)</td>
<td>1.8 (1.2–2.9)</td>
<td>28.0 (9.3–34.0)</td>
<td>88</td>
</tr>
<tr>
<td>Band 3 (R6A)</td>
<td>2-3</td>
<td>15.0 (0.6–25.2)</td>
<td>21.1 (0.6–37.4)</td>
<td>89.8 (86.8–92.8)</td>
<td>19.4 (12.3–26.5)</td>
</tr>
<tr>
<td>OKM1</td>
<td>2</td>
<td>1.0 (0.6–1.3)</td>
<td>0.2 (0.1–0.3)</td>
<td>13.4 (4.5–22.0)</td>
<td>5.9 (4.1–7.1)</td>
</tr>
<tr>
<td>OKT10</td>
<td>2</td>
<td>88.5 (79.1–98.6)</td>
<td>56.3 (53.1–59.4)</td>
<td>28.0 (18.0–38.0)</td>
<td>56.6 (32.5–80.6)</td>
</tr>
<tr>
<td>OKT9</td>
<td>3-8</td>
<td>33.8 (0.4–78.0)</td>
<td>62.8 (14.0–96.0)</td>
<td>72.6 (64.0–85.0)</td>
<td>18.3 (6.0–29.0)</td>
</tr>
<tr>
<td>Transferrin</td>
<td>Indirect</td>
<td>3</td>
<td>97.4 (96.4–99)</td>
<td>99.6 (99.3–99.9)</td>
<td>92.1 (87.2–100)</td>
</tr>
<tr>
<td>Direct</td>
<td>3</td>
<td>79.9 (59.4–94.5)</td>
<td>99.6 (99.5–99.8)</td>
<td>83.6 (73.0–90.8)</td>
<td>24.8 (16.2–37.7)</td>
</tr>
</tbody>
</table>

*Number of experiments.
HEMOPOIETIC CELL SURFACE ANTIGENS

Sorting and are determined by comparison with controls (e.g., "normal" immunoglobulins). Difficulties arise because of the relatively high "background" staining of myeloid cells in bone marrow, which forces up the threshold for positivity, particularly with those monoclonals that give a broad or continuous distribution of staining (cf., Fig. 1).

Problems of interpretation can be avoided if cell sorting manoeuvres take into account weak or equivocal staining fractions as well as clear positive cells (cf., Fig. 1) and if negative cell populations are not considered as necessarily being totally devoid of the feature detected by the fluorescent antibody or probe used.

Finally, the yields from the FACS are variable; although there is no evidence for selective loss of progenitor cells, the interpretation of results clearly needs to take imperfect yield into account. We emphasize these technical reservations, since the experimental approach used in these studies is likely to be fairly extensively applied to a wide range of biologic problems involving rare cell selection.

With these reservations in mind, the experiments described here have revealed an intriguing pattern of antigen expression during erythroid and myeloid differentiation.

Blood group A antigen as detected by monoclonal antibodies MAS 016 and N16 is expressed on the earliest committed erythroid and myeloid progenitors (BFU-E and CFU-GM). Although the cut-off between strong and weak positive cells is not distinct, the data suggest an increase in strong positivity (and therefore probably antigen density) during differentiation from BFU-E to normoblasts. The wide range of positivity obtained for BFU-E and CFU-GM is probably due to the difficulty of distinguishing weak positive from negative cells. The experiment in Table 2 shows a large weak positive fraction (70%) containing the majority of BFU-E and CFU-GM, whereas in other experiments (data not shown) the weak positive fraction appeared to be much smaller (13%-16%) and the correspondingly larger negative fraction (73%-74%) contained 70%-84% of these progenitors. These results contrast with those of Fitchen et al.12 who found that cytotoxic A and B antisera had no effect on CFU-GM or BFU-E. Furthermore, Karhi et al.29 (1981), in a study involving maturing precursors only, incubated bone marrow cells with blood group A specific lectin from Vicia cracca and found blood group A activity on cells of the erythroblastic series only, first detectable at the basophilic normoblast stage. Pronormoblasts were negative. There are several possible explanations for these differences.

Although the two monoclonal antibodies used react specifically with erythrocytes carrying group A determinants34,35 and are inhibited by group A substance from saliva,36 they may not detect the A determinant(s) normally revealed by conventional human anti-A antibodies. The specificity of these two monoclonals and the determinants they detect on erythroid progenitors are perhaps best referred to at present as "A-like" (cf., Helix pomatia lectin). Cellular cytotoxicity depends not only on the class of antibody, but on cell surface antigen density as well, and if, as our results suggest, antigen density is low on BFU-E and CFU-GM, this could explain the negative results obtained by others with some cytotoxic anti-A antibodies. Karhi et al.26 showed by gel electrophoresis of solubilized erythrocyte membranes that the A determinants precipitated by Vicia cracca were present on glycoproteins mainly of band 3, with a minor component in the band 4.5 region. Erythrocytes contain blood group A activity both in glycoproteins and in glycolipids.39 It is not known whether Vicia cracca binds A activity at both these sites, but our failure to demonstrate band 3 on most erythroid progenitors with monoclonal antibody R6A suggests that the lectin may only detect glycoprotein-associated activity. Furthermore, our findings of group A antigen activity well before the appearance of band 3 (on erythrocytes) suggests that the monoclonal antibodies could be detecting glycolipid-associated A or A-like antigens, and that this activity appears earlier during maturation than that associated with glycoproteins of band 3 and band 4.5.

The majority of erythroid progenitors and precursors expressed blood group I determinants. BFU-E and CFU-E had little or no detectable expression of i antigen, but the majority of precursors (in contrast to mature adult erythrocytes) were i positive. Myeloid progenitors and precursors were also i positive, but only a minority were i positive. Since i determinants are exposed on incompletely glycosylated molecules and I antigens on the more mature molecules (glycolipids), these data probably reflect alterations in the activities of glycosyltransferase with cell maturation and differences in cell lineages with respect to their activity.

Our results for i contrast with those of O'Hara et al.,31 who used the same anti-i serum in cytotoxicity experiments and found that CFU-E in patients with anemia or solid tumors were relatively rich in i antigen expression, whereas expression in CFU-GM varied greatly from person to person. Their studies also showed a correlation of i expression with DNA synthesis. Since the mean recovery of CFU-E and CFU-GM in the experiments of Table 4 was >90% and >80%, respectively, it is unlikely that these differences are due to selective loss of progenitors in our experiments. It is possible that the difference is due to the source of bone marrow cells, since some anemic patients could
have an increase in the proportion of CFU-E in cycle. Alternatively, I antigen expression on CFU-E may be below the threshold of detection using the FACS.

We previously reported that nonpolymorphic HLA-DR antigen is expressed on the majority of BFU-E but lost during differentiation to CFU-E. Similar findings were reported also by Winchester et al., and Fitchen et al., who used complement-fixing rabbit antisera to Ia-like (presumed HLA-DR) glycoproteins. Our finding that Genox 3.53, a monoclonal antibody reactive with a polymorphic DR-linked antigen, does not bind to erythroid progenitors was therefore surprising. This monoclonal antibody is reactive with B lymphocyte typing as HLA-DRw1, 2, or 6.

Recent studies have uncovered another locus, DC (MT or MB), in linkage disequilibrium with HLA-D. Products of this locus identified by alloantisera or monoclonal antibodies are structurally similar to but different from DR. Genox 3.53 appears to recognize DC1 rather than a supertypic DRw1, 2, 6 specificity. Furthermore, while DC1 may be related to murine I-A subregion of H-2, HLA-DR molecules show structural homology with I-E/C. The reactivity of hemopoietic progenitors with the antimonomorphic DR reagent DA-2 but not anti-DC1 (Genox 3.53) could have some relevance to the possible involvement of HLA-D-linked molecules in cellular interactions regulating hemopoiesis. A more trivial explanation for the lack of reactivity of BFU-E with Genox 3.53 is that HLA-D linked (H-2, I equivalent) products, as a whole, may be expressed less than on mature B cells and at such a level that they are detected by monoclonal anti-DR reagents such as DA-2, which may recognize all family members of the type II histocompatibility antigens (HLA-D I,i linked region) but not an antipolymorphic monoclonal with more restricted activity.

OKT10 defines an antigenic determinant associated with a 40,000 molecular weight polypeptide. The latter shows some structural similarities to HLA molecules. Although first described as a “thymic” antigen because OKT10 reacts with 95% of thymocytes, it is also present on blasts of common and B-acute lymphoblastic leukemias, acute myeloid leukemias, and mitogen-activated T cells. The OKT10-defined antigen is present on the majority of BFU-E but is gradually lost during differentiation through CFU-E to erythroid precursors, approximately one-third of which are positive. Myeloid cells show a similar pattern of expression, although fewer CFU-GM are positive in comparison with BFU-E. Crawford et al. recently reported that the majority of CFU-GM bind OKT10. This pattern of expression suggests a possible role for the antigen as a receptor for proliferation regulating interactions, and we are currently testing this possibility.

The cell surface structure defined by OKT9 is a 90 Kd glycoprotein expressed mainly on proliferating cells, both malignant and normal. Transferrin receptors have similar widespread tissue distribution to OKT9, and their expression is also strongly associated with cell proliferation. Polyacrylamide gel electrophoresis (PAGE) of isolated transferrin receptors shows that they have the same general characteristics as the structure defined by OKT9. Furthermore, OKT9 can precipitate I-labeled transferrin when the latter is bound to its receptor, demonstrating that the structure defined by OKT9 is the transferrin receptor.

OKT9, however, does not inhibit the binding of radiolabeled transferrin to cells, suggesting that OKT9 and transferrin probably bind to different sites on the same molecule. The OKT9-defined antigenic determinant has rather variable expression on BFU-E, but increases during maturation through CFU-E to recognizable normoblasts. Mature erythrocytes do not express this antigen, and its pattern of expression on the erythrocyte lineage is therefore consistent with the data showing a correlation with cell proliferation (and in the erythroid lineage) with hemoglobin synthesis. A similar pattern in OKT9 binding was observed during myelopoiesis when the precursor data were analyzed for dividing and nondividing cells. Parallel changes in antigen expression with increasing granulocytic maturation have been defined previously using monoclonal antibodies and the promyelocytic cell line HL-60. Thus, when HL-60 cells are induced to mature in vitro (and concomitantly pass out of cycle) by DMSO or retinoic acid, they show diminished binding of OKT9 and transferrin and an increase in several myeloid maturation-linked antigens including the OKM1-defined determinant.

Somewhat different results were obtained when transferrin receptors were identified more directly by the binding of transferrin itself. The data indicate that a very high proportion of BFU-E, and virtually all CFU-E, express detectable transferrin receptors in comparison with the moderate and variable positivity observed with monoclonal antibody OKT9. This discordance in expression is surprising and is not usually observed when other normal and malignant cells are compared (i.e., the two labeling methods do not appear to differ substantially in sensitivity). One possible explanation of the difference could be that the transferrin receptor portion of the molecule is more exposed on the early erythroid cell surface than the determinant recognized by OKT9. It is, however, interesting that BFU-E have transferrin receptors apparently long before they require iron for hemoglobin synthesis.
Terminal maturation of normoblasts to enucleated erythrocytes is known to be associated with the loss of transferrin receptors,39 and in our experiments, no binding of OKT9 or labeled transferrin to erythrocytes was observed. Finally, with direct labeling, only a minority of CFU-GM and myeloid precursors express detectable transferrin receptors, and this correlated with expression of the determinant recognized by OKT9. The difference in transferrin receptor expression between erythroid and myeloid progenitors using transferrin-FITC conjugate offers a useful means of separating cells of the two lineages.

These data accord with the observations of Fukuda et al.40 who reported a biochemical comparison of cultured human erythroblasts and erythrocytes. They observed that erythroblasts had a strong expression of glycophorin but differed from erythrocytes in their weak or negligible expression of band 3 and their relatively low content of polyactoaminoglycans (which carry ABH and Ii blood group antigens). In addition, erythroblasts were found to have a pronounced expression of two membrane glycoproteins of 105,000 and 95,000 that were not detectable on erythrocytes. It is possible that the latter of these corresponds to the transferrin receptor as detected in our experiments.

These data, and those published earlier with monoclonal antibodies to monomorphic HLA-A, B, C, and DR determinants and glycophorin, now provide a reasonably detailed profile of cell surface antigen expression during early erythroid and myeloid (GM) differentiation. A summary and schematic view of these immunologic phenotypes is provided in Fig. 2. This is largely still a qualitative description; possible quantitative differences of antigen expression within a given “maturation compartment” and/or association with proliferative activity should be borne in mind also.

A similar analysis of lymphoid progenitors is hampered by the current lack of colony assays in vitro for these cells; using nuclear immunofluorescence with anti-terminal deoxynucleotidyl transferase as a marker for putative lymphocyte (T and B?) precursors, it has been possible, however, to similarly identify a composite monoclonal antibody-defined phenotype that is unique to these cells.37,41

Although the analysis of cell surface antigen expression during hemopoietic differentiation is still descriptive only, it may prove to be a useful tool for defining antigens important for cell interactions and a means of positively selecting hemopoietic progenitors, both for studies in vitro and possibly for marrow transplantation as well. It will also provide a basis of comparison for further classification of the acute leukemias.37

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REFERENCES

28. Shackleford DA, Mann DL, Van Rood JJ, Ferrara GB, Strominger JL: Human B-cell alloantigens DC1, MT1 and LB12 are identical to each other but distinct from the HLA-DR antigen. Proc Natl Acad Sci USA 78:4566, 1981


43. Edelman L, Rouger Ph, Dionel Ch, Garchon H, Bach JF, Reviron J, Salmon Ch: Thermodynamic and immunological properties of a monoclonal antibody to human blood group A. Immunology 44:549, 1981
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