Myeloid and Megakaryocytic Properties of K-562 Cell Lines


Unité de Recherches sur les Anémies, Inserm U.91, Hôpital Henri Mondor, 94010, Creteil, France [A. T., P. G. P., G. V., W. V., U. T., H. R., J. B-G.]; Department of Pathology, University of Alberta, Edmonton, Alberta, Canada [P. M.]; Division of Pediatric Oncology, The Johns Hopkins Oncology Center, Johns Hopkins University, School of Medicine, Baltimore, Maryland 21205 [C. I. C.]; and Group d’Immuno-Biologie des Tumeurs, Institut Gustave Roussy, 94805, Villejuif Cedex, France [M. L.]

ABSTRACT

The expression of myeloid and megakaryocytic markers of differentiation has been studied in one K-562 cell subline, in its clones, and in the original cell line. Cytotoxicity, electron microscopy, immunofluorescence studies with a panel of polyclonal and monoclonal antibodies, and radioimmunoassays were performed on K-562 cells before and after induction with hemin, sodium butyrate, and 12-O-tetradecanoylphorbol-13-acetate. Myeloid membrane markers were present in all K-562 cell lines. Only the early granulopoietic cell surface markers were expressed in 75 to 95% of the cells, while none of the late membrane markers was detected. In contrast, neither the early (myeloperoxidase) nor late (lactoferrin) cytoplasmic markers were present. Thus, K-562 cells showed a membrane phenotype similar to that of a normal or leukemic promyelocyte but lacking myeloperoxidase.

Membrane megakaryocytic markers, such as platelet glycoprotein IIa and platelet peroxidase, were also detected in K-562 cells. However, some other early megakaryocytic markers, such as platelet glycoprotein Ib, Factor VIII-R-Ag, and platelet Factor 4, could not be detected by fluorescent labeling. Cloning of the cell line did not result in the selection of a unipotential cell line. These results could be explained by the expression of multilineage markers in a single cell. In all of the cell lines and clones, hemin slightly increased the expression of the myeloid membrane markers without any modification of the megakaryocytic markers. Sodium butyrate and 12-O-tetradecanoylphorbol-13-acetate diminished most of the myeloid markers and very significantly increased the expression of the megakaryocytic markers.

INTRODUCTION

The K-562 cell line, obtained from a patient in blast crisis of chronic myeloid leukemia (23), has many features of an erythroleukemia cell line, since all of the erythroid-specific proteins found in early erythroblasts are synthesized (13, 16, 33, 38). In addition, cell surface analysis by immunofluorescence has clearly shown that the myeloid markers identified in normal or leukemic promyelocytes are lacking in K-562 cells. This led to the study of the expression of myeloid and megakaryocytic antigens in K-562 cells.

1 This work was supported by INSERM, CRL 82-2-018 and CRL 82-2-031, and Groupement des Entreprises Françaises dans la Lutte contre le Cancer.
2 To whom requests for reprints should be addressed, at Unité INSERM U.91, Hôpital Henri Mondor, 94010, Creteil, France.
Received December 20, 1982; accepted July 3, 1983.

OCtober 1983
- antigen that is present on metamyelocytes, polymorphonuclear leukocytes, and monocytes; and (f) OK M1, which has been described previously as recognizing monocytes, granulocytes, and a subpopulation of null lymphocytes (2). All of the monoclonal antibodies were obtained as ascitic fluid except anti-My 1 and anti-My 18, which were culture supernatants. All ascitic fluid was used at 1/100 to 1/1000 dilution. Polyclonal rabbit anti-myeloperoxidase F(ab’)2 fragment coupled to fluorescein and polyclonal rabbit anti-lactoferrin F(ab’)2 fragment coupled to rhodamine were kindly supplied by Dr. D. Ross (Chapel Hill, N. C.).

**Antibodies against the Megakaryocytic Lineage.** (a) AN 51, kindly provided by Dr. A. MacMichael (Oxford, England), recognizes platelet glycoprotein Ib (28); (b) J 15, kindly provided by Dr. A. MacMichael, identifies an antigen related to the platelet glycoprotein Ib-lla complex. It binds to platelets and normal and leukemic promegakaryoblasts (39); (c) C 17, kindly provided by Dr. P. A. T. Tetteroo and P. M. Lansdorp (Amsterdam, The Netherlands), an IgG, monoclonal antibody which specifically labels the megakaryocytic lineage including promegakaryoblasts. It recognizes an antigen related to glycoprotein IIa IIIa; and (d) J 2, kindly supplied by Dr. T. Hercend (Sidney Farber Cancer Institute, Boston, Mass.), recognizes a glycoprotein common to the lymphoid cell lineage and platelets (14). This antibody labels the megakaryocytic lineage from promegakaryoblasts to platelets. All of these monoclonal antibodies were obtained as ascitic fluid. They were used at 1/100 to 1/1000 dilution.

Three rabbit polyclonal antibodies have also been used: an affinity-purified rabbit immunoglobulin against platelet Factor 4 (Dr. Levine, San Antonio, Texas); a rabbit antiserum against Factor VIII-R-Ag (Dakopatts, Copenhagen, Denmark); and a rabbit IgG against platelets that had been extensively adsorbed with lymphocytes, monocytes, granulocytes, and erythrocytes and then adsorbed on platelet proteins (43) and recovered.

**Other Antibodies.** The following mouse monoclonal antibodies against specific lymphoid antigens were also tested: OKT 6 (30), OKT 11 (41), Bl (35), and J 5 (31). An antiglycophorin A monoclonal antibody (8) was applied to 15 clones selected from K-562 on the basis of their variable hemoglobin content (37).

Controls in each experiment used an ascitic fluid containing a monoclonal antibody (IgG) recognizing a synthetic peptide (glutamic acid, L-alanine, or tyrosine) (20), or 2 mouse myeloma immunoglobulins, TEPC 183 (IgM-k) and MOPC 21 (IgG-k). In addition, J 15, the monoclonal IgM recognizing a platelet antigen, served as a control for 80 H5 and anti-My 1, the IgM antibodies against granulocytic markers of differentiation.

**Ultrastructural Studies**

Peroxidase activities were detected by 2 different techniques (3) and then processed for electron microscopy (4).

**Immunofluorescence Labeling**

Indirect fluorescent labeling assay was performed on unfixed cells except for Factor 4, Factor VIII-R-Ag, myeloperoxidase, and lactoferrin. In these last cases, cells were cytocoentrifuged and then fixed as follows. For Factor 4 and Factor VIII-R-Ag, the cells were fixed by methanol; for myeloperoxidase and lactoferrin, a buffered formol-acetone fixative (27) was used. Binding of mouse monoclonal antibodies and of rabbit antibodies was assessed using, respectively, a fluorescein-coupled goat anti-mouse immunoglobulin or a rhodamine-coupled goat anti-rabbit F(ab’)2 fragment (Cappel Laboratories, Cochranville, Pa.). Myeloperoxidase and lactoferrin were detected by direct immunofluorescence.

All of the fluorescent labeling was performed in at least 2 different experiments.

**Fluorescence-activated Cell Sorter Analysis**

Quantitative fluorescence analysis was performed with the Fluores-
Table 1

Percentage of K-562 cells labeled by monoclonal antmyeloid antibodies before and after induction

<table>
<thead>
<tr>
<th></th>
<th>K-562 (o)</th>
<th>K-562 (a)</th>
<th>K-562 (201)</th>
<th>K-562 (209)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>94 76</td>
<td>91 76</td>
<td>86 59</td>
<td>90 78</td>
</tr>
<tr>
<td>Hemin</td>
<td>91 75</td>
<td>94 88</td>
<td>90 54</td>
<td>94 89</td>
</tr>
<tr>
<td>Butyrate</td>
<td>93 33</td>
<td>88 68</td>
<td>69 34</td>
<td>70 37</td>
</tr>
<tr>
<td>TPA</td>
<td>74 27</td>
<td>88 66</td>
<td>81 34</td>
<td>85 28</td>
</tr>
<tr>
<td>80 H5</td>
<td>Anti-My 1</td>
<td>Anti-My 1</td>
<td>Anti-My 18</td>
<td>Anti-My 18</td>
</tr>
<tr>
<td>Anti-My 18</td>
<td>34 0</td>
<td>52 40</td>
<td>28 6</td>
<td>33 13</td>
</tr>
<tr>
<td>80 H3</td>
<td>12 2</td>
<td>73 52</td>
<td>28 6</td>
<td>33 13</td>
</tr>
<tr>
<td>OKM1K-562</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

Mean value of at least 2 experiments.

80 H5, anti-My 1, and 82 H1 diminished after sodium butyrate or TPA induction (Chart 1). In contrast, the expression of myeloid antigens slightly increased after hemin induction.

Radioimmunoassay. The labeling of K-562 and HL 60 cells with 80 H5 was compared by radioimmunoassay. The 2 curves were essentially similar (Chart 2). The binding was also studied on K-562 (o) and on clone 201 before and after sodium butyrate induction (Chart 3). The results showed that K-562 (o) was more inducible by sodium butyrate than was clone 201.

Cytotoxicity by 80 H5. Dilution of 80 H5 up to 10^-4 resulted in a complete lysis of K-562 cells from the 2 sublines tested.

Megakaryocytic Lineage

Ultrastructural Studies. Under all conditions of culture (with or without inducer, on the K-562 sublines or the clones), specific organelles of the megakaryocytic lineage (demarcation membranes and α-granules) could never be detected by conventional transmission electron microscopy (5). In all K-562 cell lines [K-562 (o), K-562 (a), or clones], a peroxidase activity similar to platelet peroxidase was present. This peroxidase activity was present in the endoplasmic reticulum and in the nuclear envelope. It was absent from the Golgi apparatus and in the granules. This peroxidase activity was only detected on unfixed cells. It could not be demonstrated after aldehyde fixation.

Before induction, the percentage of cells exhibiting a peroxidatic activity was extremely low (<1%). After sodium butyrate or TPA induction, the number of cells exhibiting a peroxidase activity highly increased in the 2 cell lines and in the clone 209. It was not significantly modified in clones 48 and 201. After induction, the percentage of cells with a peroxidase activity could reach up to 30% for the 2 cell lines and 90% for clone 209.

Immunofluorescent Labeling. AN 51, Factor VIII-R-Ag, and anti-platelet Factor 4 were essentially negative before and after induction (Table 2).

Four antibodies against membrane markers were positive. J 2 or C 17 labeled 0 to 3% of the K-562 cell lines. The polyclonal rabbit anti-platelet antibody labeled 50 to 100% of the K-562 cells. J 15 was only positive on a small fraction (<1%) of cells from K562 (o) cell line.

After induction by TPA or sodium butyrate, labeling with J 2 or C 17 antibodies increased up to 38%. Staining with the polyclonal rabbit anti-platelet antibody showed an increase in intensity. In contrast, labeling with J 15 was not significantly modified. No effect was observed on the megakaryocytic markers after hemin induction.

Flow cytometric analysis confirmed the results of Table 2. Labeling with C 17 and J 2 greatly increased after TPA and sodium butyrate induction, while that of J 15 slightly increased. AN 51 was always negative.

DISCUSSION

Previous studies have established that the K-562 cell line has an erythroid phenotype. All of the main erythroid-specific markers
Charts 2 and 3. Investigation of the binding of 80 H5 antibody to K-562 cells by radioimmunoassay. The experimental conditions were similar to those reported in Chart 2. •, K-562 (o), control; △, K-562 (o) butyrate; ○, K-562 (209) control; ⬤, K-562 (209) butyrate.

Charts 2 and 3. Investigation of the binding of 80 H5 antibody to K-562 cells by radioimmunoassay. The cells were subsequently incubated with 80 H5 antibody, rabbit anti-mouse IgG, and then 125I protein A. The results are expressed as cpm of 125I protein A fixed for 1 x 10^6 cells. ■, HL 60; □, K-562 (o).

Chart 2. Investigation of the binding of 80 H5 antibody to K-562 (o) and HL 60 cells by radioimmunoassay. The cells were subsequently incubated with 80 H5 antibody, rabbit anti-mouse IgG, and then 125I protein A. The results are expressed as cpm of 125I protein A fixed for 1 x 10^6 cells. ■, HL 60; □, K-562 (o).

(glycophorin, hemoglobin, spectrin, and acetylcholinesterase) are expressed in K-562 cells (1, 13, 16, 25, 33, 38, 42). However, some nonerythroid markers have been noted in the K-562 cell line (7, 12, 25, 32, 38). The present report amplifies this feature and allows a better understanding of the action of different inducers.

The present results have been achieved with the use of a panel of monoclonal and polyclonal antibodies directed against membrane or cytoplasmic components. In the 2 K-562 cell sublines and all of the clones, some myeloid and megakaryocytic markers were indeed present. Early myeloid membrane markers were strongly expressed, whereas late myeloid membrane markers could not be detected. None of these myeloid monoclonal antibodies (80 H5, anti-My-1, or 82 H1) stained normal, leukemic, or cultured erythroblasts or megakaryoblasts by fluorescent or ultrastructural immunoperoxidase labeling. Ultrastructural immunoperoxidase staining combined with cytochemistry has demonstrated that the antigen recognized by 80 H5 and anti-My-1 antibody is specific for the myeloid lineage among hemopoietic cells, is absent from myeloblasts, and appears at the promyelocytic stage of maturation.5

Technics using cytotoxicity have shown that the 80 H5 antigen
is expressed on CFU-GM (24), while anti-My-1 antigen is absent from their surface (36). Therefore, the cell surface phenotype of K-562 cells established with the monoclonal antibodies against the myeloid antigens would be identical to that of a promyelocyte. However, myeloperoxidase was not detected in K-562 cells. Two hypotheses could explain these characteristics of K-562 cells. First, a major asynchrony in the expression of the myeloid markers may be present in K-562 cells. Alternatively, since preliminary results suggest that the antigen recognized by 80 H5 and anti-My 1 is the same glycolipid (15), it may be postulated that this antigen may be expressed during the entire myeloid differentiation but may be partially or totally hidden at specific stages of maturation. In that case, K-562 cells would have the myeloid phenotype of a CFU-GM. The presence of undifferentiated leukemic cells in secondary leukemia with an identical phenotype to K-562 cells may support this hypothesis. However, it cannot be excluded that this antigen is hidden in the membranes of erythroid cells.

Our group has previously reported that a peroxidase activity similar to platelet peroxidase was present in K-562 cells (40). In the present study, the same peroxidase activity was found in K-562 (o). Surface platelet markers (glycoprotein IIIa) were also expressed in K-562 cell lines. Our results agree with those of Gerwitz et al. (12), who described the presence of some platelet membrane megakaryocytic markers. This heterogeneity in inducibility has been reported for other leukemic cell lines (9, 17, 32).

In conclusion, K-562 cells exhibit markers of 3 different hematopoietic cell lineages; however, while all of the erythroid markers were synthesized by K-562 cells, only a minority of megakaryocytic or myeloid markers was expressed by K-562 cells. Different inducers modify the phenotype of K-562 cells, but none of them leads to the expression of markers of a single-cell lineage alone.

ACKNOWLEDGMENTS

The authors would like to thank M. Segear and A. M. Dulac for typing the manuscript; M. Titiaux, A. Henri, J. Guichard, and J. P. Masse for excellent technical assistance, and Dr. R. Carmel for reviewing this manuscript. Fluorocytometry analysis was performed by the Service de cytofluorometrie analytique et séparative, Villejuif, France (Dr. C. Rosenfeld and Dr. C. Prevot).

REFERENCES


Myeloid and Megakaryocytic Properties of K-562 Cell Lines

A. Tabilio, P. G. Pelicci, G. Vinci, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/43/10/4569

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.