CONCISE REPORT

Malignant Monoblasts Can Function as Effector Cells in Natural Killer Cell and Antibody-Dependent Cellular Cytotoxicity Assays

By Peter Hokland, Marianne Hokland, and Jørgen Ellegaard

This is the first report describing natural killer (NK) and antibody-dependent cellular cytotoxicity (ADCC) of malignant monoblasts. Pure acute monoblastic leukemia was diagnosed in bone marrow aspirations from two patients by use of conventional cytochemical methods as well as multiple immunologic techniques including detection of ALL antigens and terminal transferase. The malignant cells were subsequently found to be potent effectors in NK and ADCC assays. Addition of partially purified α-interferon to the in vitro cultures was found to have an enhancing effect on NK activity, whereas no modulation was seen in ADCC. These findings are discussed in the light of our present knowledge of lymphoid NK cells.

Both patients responded poorly to protocol treatment for AML (daunorubicin 45 mg/sq m for 3 days and cytosine arabinoside 100 mg/sq m for 7 days), and they died within 3 mo after their first admission to the hospital.

Immunologic Markers

Rosette formation with unsensitized, AET-treated sheep erythrocytes (E-rosettes) and with trypsinized sheep erythrocytes (SRBC) coated with a hyperimmune rabbit anti-SRBC IgG antiserum (EA-rosettes) was performed as previously described.4 Rosette formation with complement-coated zymosan particles was performed as described by Huber and Wizigl.2 Detection of surface membrane immunoglobulins (SmIg) was done after overnight incubation of the cells at 37°C to avoid detection of cytophilic IgG using a polyclonal rabbit anti-human IgG serum (Dakopatts, Copenhagen, Denmark, code no. F 1009). Late phagocytosis was performed as previously described.4 Detection of the common ALL antigen (CALLA) and the human thymocyte antigen (HTA) was done as two-layer fluorescence tests using a rabbit anti-mouse IgG (Seralab, Sussex Downs, UK, cat. no. AES 084) as a second layer. As first layers a monoclonal mouse anti-CALLA antibody (kindly provided by Dr. J. Ritz)4 and a monoclonal mouse anti-HTA antibody (Seralab code no. MAS 036) were used. Detection of TdT was performed as described by Minowada.5 As positive controls for the CALLA and TdT assays the null cell lines NALM-1 and Reh were used; for the HTA assay RPMI 8402 T-ALL cell line was used (kindly provided by Dr. J. Minowada).

Cytotoxicity Assays

Isopaque-Ficoll purified leukemic cells from sternal marrow aspirations were used as effectors. Target cell lines used in the NK assay were the K 562 CML line, the RAJI lymphoblastoid line, and the MOLT-4 T-ALL line. They were grown in continuous culture in medium RPMI 1640 supplemented with 10% fetal calf serum. In the ADCC assay, the P815 mouse mastocytoma line was used as target cell. It was maintained by sequential intraperitoneal transfer in DBA mice (purchased from Bomholtgaard, Ry, Denmark). The target cells were labeled with 60 μCi Na125I (Amersham, UK) for 1 hr at 37°C and washed 3 times before use.

Cultures were set up in final volumes of 1 ml in 10 × 70 mm test tubes in effector to target cell ratios varying from 25:1 to 100:1 with and without addition of 1000 international units of purified human leukocyte interferon (α-IFN, kindly provided by Dr. K. Berg) and, in the ADCC assay, with and without the addition of a rabbit anti-P815 IgG antibody in a final dilution of 1:2500. After 4-hr incubation at 37°C the cultures were harvested and the radioactivity

Support in part by the Danish Medical Research Council Grant 512-20482 and by the Danish Cancer Society Grant 64/79.

A CUTE MYELOID LEUKEMIA can be subdivided into granulocytic, monocytic, and erythrocytic varieties.1 Among these acute monoblastic leukemia (AMoBL) has lately emerged as a distinct entity, partly based on morphological criteria, and partly because of its different response to treatment and the bad prognosis.23 Whereas the leukemic cells from such patients have been well characterized cytochemically by their diffuse cytoplasmic staining pattern for nonspecific esterase activity,4,5 little is known about their immunologic surface marker phenotype and their functional capacities in vitro.

We present convincing evidence demonstrating that leukemic cells from two AMoBL patients have the ability to function as effector cells in both the antibody-dependent cellular cytotoxicity (ADCC) assay and in the natural killer (NK) cell assay.

MATERIALS AND METHODS

Patients

Two white males (A.J. aged 68 and A.N. aged 69) presented with strikingly similar symptoms. They were both severely anemic and thrombocytopenic with diffuse lymphadenopathy. In addition, one of them (A.J.) had hepatosplenomegaly, while the other (A.N.) had leukemic skin nodules on arms and legs. Both patients had WBC exceeding 100,000/cu mm with more than 90% cells resembling monoblasts in MGG-stained smears. Similar cells were found to dominate in bone marrow smears as well as in lymph node biopsies. The cytochemical pattern of the malignant cells was in both cases as follows: periodic acid-Schiff (PAS) negative, alpha naphyl acetate esterase (ANAE) weakly positive, acid phosphatase (AP) negative, and nonspecific esterase (NSE) positive showing a diffuse reaction that could be completely inhibited by addition of sodium fluoride.

From the University Department of Medicine and Hematology, Aarhus Amtssygehus, Aarhus, Denmark.

Address reprint requests to Dr. Peter Hokland, Med. afd. II, Aarhus Amtssygehus, 8000, DK-Denmark.

© 1981 by Grune & Stratton, Inc.
0006-4971/81/5705-0024$01.00/0
MALIGNANT MONOBLASTS AS NK/ADCC EFFECTORS

Table 1. Expression of Immunologic Markers on Malignant Monoblasts

<table>
<thead>
<tr>
<th>Case</th>
<th>E-RFC</th>
<th>Zy-C-RFC</th>
<th>EA-RFC</th>
<th>Smlg</th>
<th>Latex</th>
<th>CALLA</th>
<th>TdT</th>
<th>HTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.N.</td>
<td>0.5%</td>
<td>13%</td>
<td>7%</td>
<td>0.5%</td>
<td>16%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>A.J.</td>
<td>0.5%</td>
<td>33%</td>
<td>26%</td>
<td>0.5%</td>
<td>18%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

in the supernatants counted in a gamma counter. The formula used to calculate specific lysis was:

\[
\% \text{Specific release} = \frac{\text{cpm Experimental} - \text{cpm Spontaneous}}{\text{cpm Maximal} - \text{cpm Spontaneous}} \times 100
\]

RESULTS

As mentioned in Materials and Methods, the results from the cytochemical staining profile indicated that the malignant cells from both patients were monoblasts. This indication is supported by the immunologic marker analysis shown in Table 1. Presence of both mature and malignant lymphocytes in effector cell suspensions could thus be safely excluded by the negative results in the tests that measure the number of mature and immature T cells (E-rosettes, HTA antigen) B cells (Smlg), and leukemic null lymphocytes (CALLA and TdT). In contrast, significant proportions of the cells from both patients possessed Fc-IgG and complement receptors and were able to phagocytize latex particles.

In Table 2, the NK potentials of the leukemic bone marrow cells from the patients are shown. It will be seen that the two patients exhibited significant lysis against two sensitive cell lines (K 562 and MOLT-4) as well as against a less sensitive cell line (RAJI). Furthermore, IFN, which is known to enhance lymphoid NK cells, also augmented the NK activity mediated by the leukemic monoblasts.

Evidence in Table 3 shows that the malignant monoblasts also had the potential to lyse antibody-coated P815 cells. In contrast to what was seen in the NK test, IFN had no effect on the ADCC.

DISCUSSION

To our knowledge, this is the first report describing NK activity by malignant cells of myeloid derivation. Apart from adding a new perspective to our knowledge of the in vitro potentials of leukemic cells, the data also add new information concerning the definition of natural killer cells, which are usually defined as lymphoid cells which, without previous sensitization of the donor, are able to kill tumor cell lines in a 4-hr \(^{51} \text{Cr-release assay.}\)

Intense research into the properties of these cells has shown that they can be found in both T and null lymphocyte fractions, but another line of evidence has indicated that promonocytes from mouse bone marrow can act as both ADCC and NK effectors.

Though indirect evidence has indicated that a subset of E-rosette-forming cells reacts with a monoclonal antibody defining a monocytoid antigen, the human parallel of the mouse promonocytes has not yet been found. Indeed, promonocytes would probably be difficult to purify from normal human donors, but we

Table 2. NK Activity of Malignant Monoblasts

<table>
<thead>
<tr>
<th>Case</th>
<th>E/T Ratio</th>
<th>K 562 - IFN</th>
<th>+ IFN</th>
<th>RAJI - IFN</th>
<th>+ IFN</th>
<th>MOLT-4 - IFN</th>
<th>+ IFN</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.N.</td>
<td>25:1</td>
<td>10.1 ± 0.7†</td>
<td>15.3 ± 0.9</td>
<td>3.0 ± 0.2</td>
<td>7.1 ± 0.9</td>
<td>14.1 ± 0.2</td>
<td>21.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>50:1</td>
<td>25.7 ± 0.8</td>
<td>29.8 ± 0.6</td>
<td>9.8 ± 0.3</td>
<td>16.2 ± 0.8</td>
<td>23.5 ± 0.6</td>
<td>32.1 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>100:1</td>
<td>44.6 ± 0.7</td>
<td>51.0 ± 1.1</td>
<td>17.0 ± 1.0</td>
<td>22.0 ± 0.5</td>
<td>48.0 ± 1.3</td>
<td>55.0 ± 0.6</td>
</tr>
<tr>
<td>A.J.</td>
<td>25:1</td>
<td>4.7 ± 0.3</td>
<td>11.0 ± 0.7</td>
<td>1.3 ± 0.3</td>
<td>5.6 ± 0.7</td>
<td>13.3 ± 0.4</td>
<td>16.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>50:1</td>
<td>18.2 ± 0.8</td>
<td>26.0 ± 1.0</td>
<td>5.9 ± 0.5</td>
<td>11.7 ± 0.5</td>
<td>19.0 ± 0.5</td>
<td>27.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>100:1</td>
<td>31.3 ± 0.9</td>
<td>40.7 ± 0.6</td>
<td>10.9 ± 0.2</td>
<td>16.3 ± 0.4</td>
<td>34.4 ± 0.7</td>
<td>42.4 ± 0.4</td>
</tr>
</tbody>
</table>

*Addition of 1000 IU α-IFN/ml.
†Mean ± SD of triplicate values.

Table 3. ADCC Activity of Malignant Monoblasts

<table>
<thead>
<tr>
<th>Case</th>
<th>E/T Ratio</th>
<th>P815 - Ab - IFN</th>
<th>+ IFN*</th>
<th>+ Ab† - IFN</th>
<th>+ IFN</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.N.</td>
<td>25:1</td>
<td>1.0 ± 0.3†</td>
<td>26.8 ± 0.4</td>
<td>30.9 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50:1</td>
<td>2.6 ± 0.4</td>
<td>53.3 ± 1.0</td>
<td>51.3 ± 0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100:1</td>
<td>4.0 ± 0.7</td>
<td>46.5 ± 0.9</td>
<td>47.9 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>A.J.</td>
<td>25:1</td>
<td>3.5 ± 0.7</td>
<td>26.0 ± 1.3</td>
<td>22.6 ± 0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50:1</td>
<td>4.3 ± 0.5</td>
<td>34.1 ± 1.0</td>
<td>33.0 ± 3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100:1</td>
<td>5.0 ± 0.6</td>
<td>36.3 ± 0.8</td>
<td>38.7 ± 2.0</td>
<td></td>
</tr>
</tbody>
</table>

*Addition of 1000 IU α-IFN/ml.
†Addition of an anti-P815 antiserum (final dilution 1:2500).
‡Mean ± SD of triplicate values.
believe that the malignant cells from the two patients presented in this report are the results from a stop in the cell differentiation at the level at which NK activity is expressed. The NK potential of the monocytic cells seems to be further documented by the ability of α-IFN to enhance the lysis, since such a modulation seems to be selective on NK cells.13

The ADCC activity of monocytoid cells is more well established, especially in systems using human erythrocytes as target cells,18,19 but this is the first report describing ADCC activity in such immature cells as monoblasts. It could be argued that the ADCC activity seen here was exerted by mature Fc-IgG+ monocytes contaminating the bone marrow suspension, but since the cytocentrifuged EA-rosette-positive cells were demonstrated to exhibit a nonspecific esterase staining pattern very similar to that of the EA-rosette-negative cells (data not shown), this explanation seems unlikely to us. Though the effect of α-IFN on ADCC has been described as either negligible10 or enhancing,20 the fact that no effect was seen here is consistent with an earlier report from our laboratory.13

The reason why the NK potential of the monocytic cell lineage subsides (mature monocytes are very weak NK cells in 4-hr chromium-release assays) while the ADCC activity is retained, is not clear. Likewise, investigations concerning a possible relationship between the E-rosette-positive cells reacting with an antimonocyte antibody and the malignant monoblasts defined here can probably first be resolved when NK-specific antigens like those defined for mouse NK cells (e.g., Asialo GM1 and Ly-521) are found on human cells.

ACKNOWLEDGMENT

We thank Dr. J. Ritz for J-5 antibody, Dr. F.J. Bollum for the TdT reagents, Dr. J. Minowada for the RPMI 8402, MOLT-4 and NALM-1 cell lines, Lise Lotte Sørensen and Karin Meyer for technical assistance, Lise Hauge for secretarial assistance, and the Department of Pathology, Aarhus Amitsygehus, for performing the esterase staining.

REFERENCES

Malignant monoblasts can function as effector cells in natural killer cell and antibody-dependent cellular cytotoxicity assays

P Hokland, M Hokland and J Ellegaard