A Greatly Simplified Method of Establishing B-Lymphoblastoid Cell Lines

Hiroko Tohda, Atsushi Oikawa, Toshio Kudo, and Takehiko Tachibana

ABSTRACT

Ten lymphoblastoid cell lines were established by Epstein-Barr virus-induced transformation directly from 0.04 to 0.15 ml of peripheral whole blood of one patient with xeroderma pigmentosum and four normal healthy adults. All these lines expressed B-lymphocyte characteristics. The advantages of this method are: (a) only a few drops of blood are required for establishing a permanent line; (b) damage and loss of cells in separation procedures are minimal; and (c) the method is simple, reliable, and applicable, if desired, to any patient, even babies.

INTRODUCTION

In a previous paper (13) we reported a convenient method of establishing permanent lymphoblastoid cells from xeroderma pigmentosum patients, in which the leukocyte fraction was separated from peripheral blood and transformed by EBV. In this study the method was further simplified by omitting the separation procedure, and thus only a few drops of whole blood were necessary to obtain the cell line. The present method is better than the previous method (13), especially when only a limited amount of blood is available. This paper describes the procedure and evidence that the established lines have the characteristics of B-lymphocytes.

MATERIALS AND METHODS

Blood Donors. Peripheral blood samples were obtained from a xeroderma pigmentosum patient (XP18SE) and 4 normal healthy adults (N2, N5, N6, and N7). In one case (NL2-WE) the sample was obtained from an ear lobe.

Virus. The culture filtrate from a B95-8 marmoset cell line was used as a source of virus, as described in a previous paper (13).

Culture Medium. Roswell Park Memorial Institute Medium 1640 (Nissui Seiyaku Co., Tokyo, Japan) supplemented with 20% heat-inactivated fetal bovine serum (Grand Island Biological Co., Grand Island, N. Y.), 2 mm L-glutamine, and 50 μg kanamycin per ml was used.

Detection of EBNA. EBNA was detected by the anticomplement immunofluorescence test according to the method of Reedman and Klein (8). Daudi and MOLT-4F cells were used as EBNA-positive and EBNA-negative controls, respectively.

Detection of Immunoglobulin. Surface immunoglobulin and cytoplasmic immunoglobulins were assayed with fluorescein isothiocyanate-conjugated monospecific rabbit anti-human IgM, IgG, and IgA (Behringwerke, Marburg-Lahn, West Germany) as described by Jondal and Klein (2) and Hinuma and Grace (1), respectively.

Detection of Membrane Receptors. Membrane receptors were examined by measuring rosette formation according to the method of Tachibana and Ishikawa (11). Indicator cells used were as follows: (a) EAC' (sheep erythrocytes sensitized with the IgM fraction of rabbit anti-sheep erythrocyte serum and incubated with fresh human serum (6)) for the detection of C3b receptor (9). (b) EACm (sheep erythrocytes (1×10⁹/ml) sensitized with the IgM fraction of rabbit anti-sheep erythrocyte serum and incubated with fresh C5-deficient mouse serum (1:10) at 37° for 120 min) for the detection of C3d receptor (9); (c) E(i) (neuraminidase-treated sheep erythrocytes) for the detection of sheep erythrocyte receptor on T-cells.

Peroxidase Stain. The cytochemical peroxidase reaction was performed as described by Kaplow (3).

RESULTS

Establishment of Lymphoblastoid Cell Lines. From 1 to 3 drops of heparinized peripheral whole blood were diluted with 2.5 ml of culture medium, mixed with 0.2 or 0.4 ml of the filtrate containing EBV in Falcon plastic culture flasks (50 ml), and incubated at 37°. Every 3 or 4 days, 2 to 3 ml of fresh culture medium were added to the cultures. Within 1 to 3 weeks after EBV infection, transformed cells were recognized microscopically as proliferating foci embedded in a thick layer of erythrocytes (Fig. 1), and eventually lymphoblastoid cell lines were established. In this way all 10 trials were successful, and the resulting cell lines with their characteristics are listed in Table 1.

Characteristics of Established Cell Lines. Morphologically, the established cell lines were indistinguishable from each other and also from cell lines obtained from a leukocyte-enriched fraction as described in the previous paper (13).

All the cell lines carried EBNA and cytoplasmic IgM. Some of them had cytoplasmic IgG or IgA in addition to IgM. Six of 9 cell lines examined had surface IgM, although...
Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Amount of blood (ml)</th>
<th>Time before transformation (days)</th>
<th>Cytoplasmic IgM</th>
<th>IgG</th>
<th>IgA</th>
<th>Surface IgM</th>
<th>EAC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>EAC&lt;sup&gt;m&lt;/sup&gt;</th>
<th>EN&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>XPL18-W1</td>
<td>0.04</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>97, 86</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>XPL18-W2</td>
<td>0.08</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>95, 79</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>XPL18-W3</td>
<td>0.12</td>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>92, 75</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>NL2-W2</td>
<td>0.10</td>
<td>14</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>96, 90</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>NL2-WE</td>
<td>0.04</td>
<td>19</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>99, 93</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>NL5-W3</td>
<td>0.12</td>
<td>12</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>97, 88</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>NL6-W3</td>
<td>0.12</td>
<td>14</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>94, 90</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>NL7-W1</td>
<td>0.05</td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>99, 98</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>NL7-W2</td>
<td>0.10</td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>98, 98</td>
<td>4.6</td>
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<tr>
<td>NL7-W3</td>
<td>0.15</td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>95, 95</td>
<td>2.3</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Symbols preceding hyphens, donors; W and numbers after hyphens, "whole blood" and number of drops used; E, from ear lobe.

<sup>b</sup> Cells were examined for receptors for EAC<sup>a</sup>, EAC<sup>m</sup>, and EN. Numbers represent percentages of cells forming rosettes with the indicator cells. We examined 300 cells of each cell line.

<sup>c</sup> NT, not tested.

DISCUSSION

In the method described in this paper, lymphoblastoid cell lines were established from 1 drop of peripheral whole blood by EBV-induced transformation, without previous purification of the lymphocytes. According to the report of Katsuki et al. (4), 1 drop (40 μl) of the blood should contain more than 500 surface IgM-bearing B-lymphocytes, the target cells for EBV. Thus the volume of whole blood used in our method could be reduced still further if necessary.

Consistent with EBV-induced transformation used, all the established cell lines were confirmed to originate from B-lymphocytes by demonstrating that they contained cytoplasmic immunoglobulins and the surface receptors for C3b and C3d fragments of the third component of complement and that they did not have sheep erythrocyte receptor, which is a characteristic of T-lymphocytes (2). From the negative results on peroxidase staining, it is unlikely that they originated from monocytes or granulocytes (14).

Transformation of B-cells by EBV in vitro is influenced by cellular components in the blood; namely, macrophages have an enhancing effect (7, 10), while T-lymphocytes, especially those from adult donors, have an inhibitory effect (12). In the present method the number of target cells in transformation culture was much less than that in the conventional method, which uses a lymphocyte-enriched fraction, but the reliability and the time required for establishing cell lines were almost the same as in the conventional method. Possible reasons for this are: (a) damage to target cells due to separation of cells was avoided; (b) blood was diluted 20- to 70-fold with culture medium in the transformation culture, so that the inhibitory action of T-lymphocytes would be lowered; and (c) loss of macrophages due to their adhesion to the glass wall during cell separation was minimal, favoring transformation. It is not known whether the presence of a great number of erythrocytes is favorable or not.

Since a very small amount of blood is required in the present method, B-lymphoblastoid cell lines can be established from blood samples of practically any clinical case, such as those discussed in the previous paper (13).

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REFERENCES


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