Deficiency in Epstein-Barr Virus Receptors on B-Lymphocytes of Preleukemia Patients

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ABSTRACT

Lymphocytes from eight preleukemia patients were exposed to Epstein-Barr virus (EBV) in vitro in an attempt to establish lymphoblastoid cell lines. No signs of viral infection were detected, and no cell lines were obtained. Studies using fluorescein-labeled EBV and flow cytometry revealed an unusual and consistent deficiency in EBV receptors in all patients examined. In control studies, about 15% of the unseparated lymphocytes from healthy donors bound fluorescein-labeled EBV. In spite of the lack of EBV receptors, B-lymphocytes amounted to 10 to 20% of the preleukemia lymphocyte populations, a proportion similar to that in healthy donors. When lymphocytes from preleukemic patients were first implanted with functional EBV receptors and then exposed to EBV, synthesis of EBV-determined nuclear, early, and viral capsid antigens was induced. Subsequently, several cell lines originating from preleukemic patients' lymphocytes were established. These lines are of a B-lymphocyte origin and carry EBV genome. They will provide experimental material for the molecular analysis of lymphocytic defects in preleukemia and their possible role in the transition to acute leukemia.

INTRODUCTION

The preleukemia syndrome is characterized by a variety of clinical symptoms, the most consistent among them being pancytopenia, hypercellular bone marrow, and maturation abnormalities in erythroid, granulocytic, and megakaryocytic progenitor populations (8, 13). No evidence of overt leukemia is present; however, 50 to 75% of these patients ultimately develop acute nonlymphocytic leukemia (9).

The wide range of abnormalities in preleukemia implies that the basic defect(s) responsible for the development of an acute leukemia may reside at the level of the multipotential hematopoietic stem cell. Sufficient data have been accumulated to indicate that cytogenetically aberrant clones are often present in the bone marrow of the preleukemic patients (11, 14). The karyotypic changes are nonrandom, including mainly trisomy for chromosomes 1q, 8, 9, and 21 and loss of all or part of chromosomes 5, 7, 20q, and iso-17q (11, 14). The chromosomes most often involved are Nos. 5 (17.1%), 7 (22.4%), 8 (37.1%), 17 (19.6%), and 21 (23.4%). Sometimes, more unusual chromosome features are reported, such as monosomy 8 and rearrangements involving chromosomes 11, 12, and 16 (23). Nonrandom chromosomal abnormalities of the sort described above accompany the development of most human tumors. It is almost a consensus among oncologists that genetic aberrations are major ingredients, if not the driving force, of the carcinogenesis process (2, 7, 16). Chromosomal aberrations in bone marrow cells of the preleukemic patients may thus account for the increased risk for the ultimate development of leukemia.

Current concepts regarding the differentiation of lymphoid and myeloid cells imply that both lineages originate from a common pluripotent stem cell in the bone marrow (4, 5). The pluripotent stem cell gives rise to the committed lymphoid and myeloid stem cells, and these differentiate into the circulating blood elements (4, 5). If the genetic aberrations in preleukemia happen in the pluripotent bone marrow stem cells, they may lead to a gradual expansion of myeloid and lymphoid clones. Thus, although preleukemia usually converts into acute nonlymphocytic leukemia (8, 13), one may expect to find aberrant preleukemia lymphocytes as well. Abnormalities in the latter could lead to a decreased immune activity and could predispose to the transition from preleukemia to acute leukemia. Analysis of the preleukemic lymphocytes, on the other hand, may help to evaluate the nature of the molecular defects underlying this syndrome.

The preliminary characterization of preleukemic patients' lymphocytes in our laboratory included analysis of surface markers and attempts to establish lymphoblastoid cell lines. Human B-lymphocyte cultures can usually be obtained by transforming cells with EBV (Footnote 3; Ref. 10). Only human and certain primate B-lymphocytes express EBV receptors and can serve as targets for EBV-mediated transformation (10, 22). Here, we report our inability to establish cell lines even by repeated exposure to EBV of cells derived from 8 patients with preleukemia. Subsequent studies revealed that B-lymphocytes from all 8 patients were deficient in EBV receptors. When, however, functional EBV receptors were implanted onto these cells (21), preleukemic patients' lymphocytes could then be infected and transformed by EBV; permanent cell lines were established.

MATERIALS AND METHODS

Patients. The 8 patients studied satisfied the diagnostic criteria for preleukemic syndrome of Linnaen and Bagby (9). Their peripheral blood smears showed anisocytosis, poikilocytosis, and macrocytes; marrow aspirates revealed megaloblastoid erythropoiesis and in some instances ringed sideroblasts. There was no evidence of overt acute leukemia. Bone marrow of these patients was hypercellular, with maturation abnormalities in at least one cell lineage, and included fewer than 5% blast cells. Megaloblastic anemia, secondary to vitamin B12 and folic acid deficiencies, was ruled out in each case. In several patients, levels of fetal hemoglobin were increased. The ages of the patients ranged from 19 to 83 years, and none had received any cytotoxic drugs. WBC and differentials from the preleukemic patients showed varying degrees of granulocytopenia (Table 1). One patient (Patient 1) evolved into frank

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The abbreviations used are: EBV, Epstein-Barr virus; EBNA, Epstein-Barr virus-determined nuclear antigen; FITC-EBV, fluorescein isothiocyanate-labeled Epstein-Barr virus; EA, Epstein-Barr virus-determined early antigen; VCA, Epstein-Barr virus-determined virus capsid antigen.
the remainder are clinically stable.

Acute nonlymphocytic leukemia several months after being studied, but

The infectivity of B-EBV was assessed by its ability to induce EBNA in

The virus was purified and concentrated to 0.2% of the initial volume.

(B-EBV) was obtained from supernatants of starving B-95-8 cell cultures

separated by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density

centrifugation. The virus remained biologically active, as assessed by its ability to induce

viral protein:stain ratio was 2:1. After 1 hr at room temperature, the

fluorescein isothiocyanate (Sigma Chemical Co., St. Louis, Mo.). The

labeled virus was separated from an unbound stain by 2 passages

supplemented with 20 ng of the reconstituted vesicles at 4°. After 10

tris(hydroxymethyl)methylglycine, and NaOH, pH 7.2, (fusion buffer) were

lymphocytes in 0.5 ml of buffer containing 160 mM NaCl, 20 mM N-

vesicles were used for the receptor implantation as follows; 10 x 106

previously (22). The coreconstituted hybrid Sendai virus-EBV receptor

imunoglobulin-expressing cells, which consist mostly of B-lympho-

proportion of B-cells. As shown in Table 2, the surface immu-

specific surface markers for the mature normal human B-lym-

sence of specific FITC-EBV association with the cells (Chart 1,

in preleukemia, as indicated by OKT-11 and OKT-3 positivity,

unimpaired B-cell secretory function (1). The T-cell subpopulation

RESULTS

Lack of EBV Receptors on Lymphocytes from Preleukemic Patients. All preleukemia patients examined displayed a lack of

EBV receptors on their lymphocytes, as assessed by the absence of specific FITC-EBV association with the cells (Chart 1, A and B; Table 2). Since the EBV receptors are considered as specific surface markers for the mature normal human B-lymphocytes, the preleukemia cells were analyzed for their relative proportion of B-cells. As shown in Table 2, the surface immunoglobulin-expressing cells, which consist mostly of B-lymphocytes, amounted to 10 to 20% of the preleukemic lymphocyte population, a proportion similar to that of healthy donors. The preleukemic cells were also positive for complement C3d receptors and monoclonal B-1 marker (not shown). Plasma immunoglobulin levels of the patients were within normal limits, indicating unimpaired B-cell secretory function (1). The T-cell subpopulation in preleukemia, as indicated by OKT-11 and OKT-3 positivity, ranged between 13 and 80% of the total lymphocyte population (Table 2). Unlike the lymphocytes from preleukemic patients, about 15% of the unseparated lymphocytes from healthy donors were found to bind FITC-EBV (Table 2). This is in agreement with the proportion of cells that bound the surface immunoglobulins of the patients.
Lack of EBV Receptors in Preleukemia

The preleukemic syndrome is characterized by a very heterogeneous set of signs and symptoms, most prominent among these being abnormalities in the myeloid cell population (8, 9, 13). Its etiology remains unknown. There have been as yet no cell lines originating from patients with preleukemia that could be used for the detection of possible pathogenetic molecular defects. Moreover, due to the uncertain clinical diagnosis of preleukemia, there has been no treatment aimed at preventing the conversion from the preleukemia to the full acute leukemia state.

The present work describes an abnormal B-lymphocyte feature, the deficiency in EBV receptors, in 8 preleukemic patients. In contrast, normal mature human B-lymphocytes have EBV receptors and can be transformed by the virus (6, 12, 24). The receptors, like receptors for complement (C3d), may be regarded as differentiation antigens marking a population of immunologically active B-lymphocytes. Neither lymphoid stem cells and immature B-cells nor the mature plasma cells possess EBV and C3d receptors, nor can they be infected by the virus (6, 12, 24). The absence of viral receptors on B-lymphocytes from preleukemic patients may thus indicate a deficiency in certain active immunocompetent B-cell subsets. The relevance of this observation to certain clinical manifestations in preleukemic patients, such as the reduced resistance to bacterial infection (13), is not

### Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>EBV receptor</th>
<th>Surface immunoglobulin</th>
<th>OKT-11 or OKT-3 receptors</th>
<th>Exposure of nonmanipulated cells to EBV</th>
<th>Exposure of EBV receptor-implanted cells to EBV</th>
<th>Immortalization</th>
</tr>
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<tr>
<td></td>
<td>EBNA-positive cells (%)</td>
<td>EBNA-positive cells (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
<td>20.4</td>
<td>55.2*</td>
<td>0</td>
<td>0</td>
<td>2 100 +</td>
</tr>
<tr>
<td>2</td>
<td>0.8</td>
<td>15.1</td>
<td>47.4</td>
<td>0</td>
<td>0</td>
<td>1.5 0  +</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>9.5</td>
<td>28.4</td>
<td>0</td>
<td>0</td>
<td>100 +</td>
</tr>
<tr>
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<td>0</td>
<td>10.5</td>
<td>35.3*</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
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<td>14.6</td>
<td>47.6*</td>
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<td>2 100 +</td>
</tr>
<tr>
<td>6</td>
<td>NT</td>
<td>10.7</td>
<td>75.4</td>
<td>0</td>
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</tr>
<tr>
<td>7</td>
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<td>NT</td>
<td>82.1*</td>
<td>0</td>
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<tr>
<td>8</td>
<td>0</td>
<td>9.6</td>
<td>13.3*</td>
<td>0</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>Healthy donors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15.3</td>
<td>19.0</td>
<td>83.4</td>
<td>3</td>
<td>100 +</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>15.4</td>
<td>12.9</td>
<td>67.9*</td>
<td>5</td>
<td>100 +</td>
<td></td>
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<tr>
<td>3</td>
<td>11.4</td>
<td>10.1</td>
<td>67.9</td>
<td>2</td>
<td>100 +</td>
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</table>
yet clear. Previously, it was assumed that neutropenia or granulocyte dysfunction was responsible for these infections (9). Analysis of other B-cell differentiation markers such as the intra-
cellular, surface, and secreted immunoglobulin, Fc, C-3, and
insulin receptors may show further abnormalities in the dif-
terentiation status of the preleukemia versus normal B-lymphocytes.
The presence of surface immunoglobulin taken with the absence of
EBV receptors on B-cells (Table 2) indicates that preleukemia
lymphocytes may belong to the category of lymphoplasma-
plasma cells, according to the criteria for the normal B-cell lineage
(12).

Although the lymphocytes from preleukemic patients are EBV
receptor negative, they can be infected and transformed by EBV
following EBV receptor implantation, a technique that allows
introduction of viral DNA into the cytoplasm (Table 2). Using this
approach, several cell lines originating from the preleukemia cells
were established (20). All the cell lines were of B-cell origin (20),
which indicated that the EBV-infected T-lymphocytes were elimi-
nated by the viral lytic cycle, as reported elsewhere (21). The results
of the present study confirm our previous finding that virtually all
human and animal cells can be infected by EBV, if the membrane
barrier resulting from the lack of EBV receptors is bypassed by receptor
implantation (17, 18, 20–22). Clearly, however, viral infection appears
to be followed by cell transformation only in human B-lymphocytes and,
possibly, in normal epithelial cells (17, 18). The approach described here may also be
helpful in other cases, such as Hodgkin’s disease and chronic
lymphocytic leukemia, where the establishment of B-lymphoblas-
toid cell lines by standard methods seems impossible.

The expression of EBV DNA in preleukemic patients’ lympho-
cytes differed from that in normal human cells. EBV-infected
normal human lymphocytes usually display a tight control over
the EBV life cycle, allowing only for a latent expression of viral
functions as reflected by the total block of the viral reproductive
cycle, exclusive expression of EBNA, and cell immortalization
(10). After prolonged cultivation in vitro, a small proportion (1 to
2%) of the EBV-transformed lymphoblastoid cell lines loses its
control over the viral lytic pathway, starts to express EA and
VCA, and releases small quantities of new viral particles (10). In
contrast, when the cells from preleukemic patients were im-
planted with receptors and infected by EBV, a significant pro-
portion of the cells expressed EA and VCA as early as 3 days
after the infection. Replicated viral DNA and new EBV particles
could be detected by cytohybridization and electron microscopy,
respectively, 3 to 4 weeks after the infection. Cell lines estab-
lished from preleukemic lymphocytes are now used for a regular
production of infectious EBV in our laboratory.

All the preleukemic patients tested had serum antibodies
against EBV antigens, just as do the healthy individuals who
have had infectious mononucleosis and developed a life-long
immunity to EBV (3). However, the lymphocytes from preleu-
kemic patients, unlike the cells of healthy seropositive persons,
are deficient in EBV receptors. It is likely that the individuals with
preleukemia did have EBV receptor-positive cells when they
were originally exposed to EBV. These cells might subsequently
have been lost or altered during the progression of cellular
molecular changes related to the development of preleukemia.
The resulting lymphoid stem cells of preleukemic patients may
have as yet undefined molecular defects at the level of gene
expression or its control, as reflected by the lack of EBV receptor
on the differentiated circulating B-lymphocytes and an incomplete
control of EBV infection. This confirms our hypothesis that
genetical aberrations in pluripotential stem cells in the bone
marrow of preleukemia patients (11, 14, 23) may be transmitted
to lymphoid stem cells and circulating lymphocytes. Additional
indication is our recent finding that preleukemia patients’ T-
lymphocytes have a generally low or absent natural killer cell
activity, as well as an aberrant T-regulatory cell function (1).
The newly established lymphoblastoid cell lines originating from
preleukemic patients will provide ample experimental material for
the molecular studies devoted to exploring the above hypothesis.

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