The Epstein-Barr Virus (EBV) in Burkitt's Lymphoma and Nasopharyngeal Carcinoma*

WERNER HENLE, M.D.† AND GERTRUDE HENLE

Division of Virology, The Children's Hospital of Philadelphia, Philadelphia, PA 19146 and School of Medicine, University of Pennsylvania Philadelphia, PA 19104

ABSTRACT

Evidence is presented for an oncogenic potential of Epstein-Barr virus (EBV). Information is also given on how EBV-specific test procedures might aid in the diagnosis of Burkitt's lymphoma and nasopharyngeal carcinoma and might provide information on the prognosis of the patients and the success of therapy.

One might question whether or not this presentation should be included in a seminar on the laboratory diagnosis of cancer. The answer depends on whether or not one accepts the mounting evidence for an etiologic involvement of the Epstein-Barr virus (EBV) in Burkitt's lymphoma (BL), the most frequent malignancy of African children, and in nasopharyngeal carcinoma (NPC) of adults in many parts of the world.

EBV, a member of the herpes group of viruses, has a world-wide distribution based on antibody surveys. Primary EBV infections at an early age either remain silent or usually cause such mild illnesses that they are not readily distinguishable from many other minor viral diseases of childhood. With advancing age, primary EBV infections increasingly induce typical infectious mononucleosis (IM) and, indeed, the virus was shown to be the cause of this disease. The evidence for this relationship will not be discussed here since it has been reviewed recently.5,6

IM is a lymphoproliferative disease which has earned the epithet of a self-limited leukemia since, in its early stages, it may be confused with such a malignancy. It thus is not inconceivable that EBV-stimulated lymphoid cells might escape, on rare occasions, control by host defenses and cause true malignancies. In fact, EBV is, as a rule, not completely eliminated after the primary infection but establishes a permanent carrier state in the lymphoreticular system.

Under low socio-economic conditions, such as prevailing in African regions of high BL incidence, primary EBV infections

---

* Supported by Research Grant CA 04568 and Contract PH 43-66-477 within The Virus Cancer Program, National Cancer Institute, U. S. Public Health Service.
† Career Award 5-K6-22, 683, National Institutes of Health, U. S. Public Health Service.
occur with few exceptions within the first three years of life. Under improved socio-economic conditions, primary EBV infections are increasingly delayed to older age groups; ultimately, however, nearly everybody becomes infected and, with it, a permanent carrier of the virus. These observations suggest that BL might develop, under rare circumstances, as a relatively early consequence of primary EBV infections, a possibility now explored in a prospective serologic study of the disease in East Africa. In contrast, NPC, being mainly a tumor of adults, would have to be a late, rare consequence of persistent viral carrier states. If these considerations were valid, other factors undoubtedly must contribute to induction of these malignancies by EBV. These factors could be immunologic defects, genetic predisposition, environmental co-carcinogens, or preconditioning by other viral or parasitic infections.

The evidence for an etiologic association of EBV with BL has been reviewed recently and, therefore, only the most pertinent and recent references will be cited. The evidence can be divided into four categories:

(1) Fingerprints of EBV were found in nearly every biopsy examined; that is (a) viral DNA was demonstrated in amounts equivalent to multiple EBV genomes per cell by hybridization of cellular DNA with \(^3\)H-labelled viral DNA or c-RNA transcribed therefrom, and (b) EBV-determined cell membrane antigens (MA) were detected by immunofluorescence in the majority of live biopsy cells unless the cells were coated by the patient’s antibodies blocking the antigenic sites and (c) an EBV-associated nuclear antigen (EBNA), comparable to the T antigens in cells transformed by papovaviruses or adenoviruses was demonstrated by an anticomplement immunofluorescence technique in practically every cell of given biopsies.

In view of these results, it is not surprising that all continuous cultures initiated with biopsy cells invariably contain EBV. Such lines are presently indistinguishable from continuous lymphoblast cultures established from peripheral leukocytes of IM patients or healthy carriers of the virus. They are divided into “producer” and “non-producer cultures” depending on the continuing presence or absence of small percentages of lymphoblasts which synthesize virus particles, or at least viral capsid antigens (VCA) and/or early viral antigens (EA). All cells, whether or not from producer or non-producer lines and regardless of their origins, were shown to contain EBNA which thus appears to be the earliest expression of the viral genomes they carry. Injection of cells from such lines into immunoincompetent or immunosuppressed animals was found to cause fatal, metastasizing tumors.

(2) EBV, whether or not derived from producer cultures of BL, IM or other origins, was shown to transform lymphoid cells from susceptible donors, cord blood or fetal organs in vitro into permanently growing lymphoblasts. Lines so established have the same morphologic appearance and range of properties as the continuous cultures initiated with BL cells or IM leukocytes discussed previously. These findings indicate that EBV has an oncogenic potential.

(3) Early attempts to induce tumors in non-human primates and other animals by injection of EBV-containing materials failed owing, among other reasons, to species barriers or specific immunity since some primate species were found to acquire antibodies to EBV under natural conditions. However, fatal lymphoproliferative malignancies were induced recently in several marmosets within five to six weeks after injection of EBV derived originally from an IM patient and in one owl monkey within 14 weeks after injection of cul-
EBV IN BURKITT’S LYMPHOMA AND NASOPHARYNGEAL CARCINOMA

TABLE I

EBV-Related Antigen-Antibody Systems

<table>
<thead>
<tr>
<th>Antibodies Versus</th>
<th>Test Cells from Producer (P) or Non-producer (NP) Lines</th>
<th>Test Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>VC A - Viral capsid antigens</td>
<td>P - Acetone-fixed smears</td>
<td>Indirect IF*3</td>
</tr>
<tr>
<td>MA - Cell membrane antigens</td>
<td>P - Live</td>
<td>Blocking of direct IF*2</td>
</tr>
<tr>
<td>EA - Early antigens</td>
<td>NP - Abortively EBV-infected</td>
<td>Indirect IF*4</td>
</tr>
<tr>
<td>D - Diffuse component</td>
<td>Acetone- or methanol-fixed smears</td>
<td></td>
</tr>
<tr>
<td>R - Restricted component</td>
<td>Acetone-fixed smears</td>
<td></td>
</tr>
<tr>
<td>S - Soluble antigens</td>
<td>NP, extracts</td>
<td></td>
</tr>
<tr>
<td>EBNA - Nuclear antigen</td>
<td>NP - Acetone-methanol-fixed smears</td>
<td>C' fixation15 Anti-C' IF16</td>
</tr>
<tr>
<td>Infectious virus particles</td>
<td>NP - Live</td>
<td>Neutralization (no inhibition of colony formation17</td>
</tr>
</tbody>
</table>

*Immunofluorescence

†Epstein, M. A., personal communication.

tured BL cells from a producer line.† These findings, if confirmed and shown to be a direct and specific effect of EBV, offer the prospect of animal models for study of the oncogenicity of EBV.

(4) All African BL patients were found to have antibodies to EBV as did most of appropriate control children. However, striking differences between the two groups became evident by comparing titers and the spectra of antibodies to the various EBV-related antigens listed in table I. Every patient had anti-VCA, often at high titers, so that the geometric mean was eight-fold higher than that of controls of which a few had no anti-VCA. Similar quantitative differences were noted with respect to anti-MA and EBV-neutralizing antibodies. Even more striking is the fact that control children rarely had anti-EA and, if so, mostly anti-R at very low titers. In contrast, many BL patients had high anti-R titers. Some had additional or only anti-D titers. Anti-R cannot be detected in the presence of brilliant staining by anti-D.

In longitudinal serologic studies of BL patients, changes in the spectra and titers of antibodies were found referable to preceding or subsequent changes in clinical status and, in turn, to provide prognostic information. Loss of anti-MA preceded in several patients by several months recognition of recurrent tumors. Patients in remission who had no anti-EA or who showed steadily declining anti-R titers were those who became long-term survivors.⁸ Over 35 patients who have survived, to date, 5 to 10 years without evidence of disease showed these patterns. In contrast, patients in remission who maintained, or in time developed high anti-R and/or anti-D titers had usually multiple, ultimately fatal tumor recurrences. The great majority of patients who died six or more months after initial admission had high...
anti-R and often also anti-D titers at the
time of death. Since D and R are intra-
cellular antigens which, furthermore, are
not expressed detectably in the tumor, the
corresponding antibodies serve merely as
indicators of other adverse conditions such
as a simultaneous presence or development
of tumor enhancing antibodies or declines
or loss of cell mediated immunity.

Evidence for an etiologic association of
EBV with NPC also has been steadily in-
creasing. It can be summarized thus:

(1) Biopsies were shown to contain reg-
ularly viral DNA at concentrations similar
to those found in BL specimens. While the viral genomes could be restricted
to the lymphoid elements of the tumor,
recent studies on frozen tumor sections in-
dicated that they might be present mainly
in the carcinoma cells: (a) in situ hy-
bridization experiments with labelled
viral c-RNA followed by autoradiography
showed that the isotope was concentrated
in what appeared to be carcinoma cells and
(b) tests for the presence of EBNA yielded
suggestive staining. Unfortunately, the car-
cinoma cells do not survive in culture to
permit confirmation of these results under
less complex conditions.

(2) Since no cells other than those of the
lymphoid series have been transformed by
EBV, it is unlikely that NPC is a direct
consequence of primary infections as sug-
gested also by the seroepidemiologic data.
However, viable hybrid cells were obtained
in vitro by para-influenza virus-induced fu-
sion of epithelial cells with lymphoblasts
from producer lines. The hybrid cells re-
tained EB viral genomes during prolonged
cultivation as evident from demonstration
of viral DNA sequences in cellular DNA
extracts and EA synthesis in some of the
cells following derepression of the genomes
by exposure of the cultures to 5-iododeox-
uridine (IdU) or 5-bromodeoxuridine
(BrdU). It is tempting to speculate that
such hybrid cells might arise during para-
influenza or other lytic virus infections in
the postnasal space of EBV carriers by
fusion of epithelial cells with EBV genome-
carrying lymphoblasts that might be pres-
ent at the site at the right time. This hy-
pothesis might be testable in non-human
primates.

(3) All NPC patients were found to
have antibodies to EBV, but the titers and
spectra of antibodies were related to the
stage of the disease; i.e., the total tumor
burden. In Stage I, in which no lymph
node involvement is noted, the anti-VCA
titers were at most slightly higher than in
healthy control populations and anti-EA
was usually absent. With progress of the
disease to Stages II to V accompanied by
lymph node invasion at increasingly more
distant sites from the primary tumor and
ultimately wide-spread metastases, the geo-
metric mean anti-VCA titers increased
stepwise to a finally 8-fold higher level
than observed in Stage I patients. Also
anti-EA; i.e., dominantly anti-D, became
detectable in Stage II and reached high
titers in later stages. Conversely, after suc-
cessful eradication of the tumor, the anti-
VCA titers declined to lower levels and
anti-D often became non-detectable again.
These results denote that progression of the
disease is reflected by rising antibody titers
and that the success of therapy can be
monitored serologically, especially by ob-
servation of the anti-D reactivity.

The experience with the BL and NPC
patients has shown that longitudinal sero-
logic studies may provide information on
the progression of the diseases, the likeli-
hood of relapses and the effectiveness of
therapy. Tests for EBV-related antibodies
might therefore be of some value in the
therapeutic management of patients. From
a diagnostic point of view, EBV-specific
test procedures hardly seem needed since
the diagnosis of these two malignancies is
established on clinical and histological
grounds. However, problems have arisen
with respect to BL. Biopsies of two recent African patients failed to reveal EBV-DNA or EBNA.§ Cells from these biopsies contained, however, a nuclear antigen detectable by the anti-complement immunofluorescence technique with sera from patients with acute myelogenous leukemia. If this antigen were, like EBNA, the fingerprint of a virus, it would appear that BL could be induced occasionally by a virus other than EBV. This could explain why a considerable proportion of American or European patients with a diagnosis of BL had no antibodies to EBV, or anti-VCA titers not exceeding those generally found in controls.10,13 The diagnosis of some of the Caucasian patients studied in this laboratory was probably incorrect since the tumor arose at sites not observed in African cases, or did not respond significantly to therapy and, in fact, was reclassified in several instances at autopsy as another malignancy. Other Caucasian patients conformed, however, in every aspect to African cases, including EBV-related serology. Search for EBNA and other as yet unidentified or unknown nuclear antigens might serve in the future to establish probable causes of this type of tumor.

Also NPC in Caucasians appears to differ to some degree from Chinese or East African cases.|| While all patients have shown high anti-VCA titers, some American or Swedish patients showed low or no anti-D titers. This could denote that in Caucasians the tumor is generally recognized at an early stage or that it is frequently of the invasive type in which lymph node involvement is limited or absent. Chinese patients with the invasive type of NPC had low or no detectable levels of anti-D even when classified as having Stage III or IV disease.9

The differences observed would limit the usefulness of EBV-specific serology in Caucasian NPC patients.

References


§ Klein, G., personal communication.
|| Henderson et al, de Schryver et al, to be published.