Immunological Reactions to Tumor-associated Antigens in Burkitt’s Lymphoma and Other Lymphomas

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Summary

Evidence for immune response to Burkitt’s tumor and other lymphomas is reviewed. The common tumor-associated antigens detected in some assays and the natural reactivity to some of these antigens are analogous to the findings in virus-induced tumors in experimental animals. Delayed hypersensitivity reactions to autologous tumor extracts were observed in Burkitt’s lymphoma, in Hodgkin’s disease, and in other lymphomas. Patients with leukemia and lymphoma, but not carcinomas, also reacted with extracts of tumor-derived lymphoid cell lines. Studies of in vitro cell-mediated cytotoxicity against autologous tumor cells, using the 51Cr release assay, also gave evidence for reactivity against tumor-associated antigens. In humoral and cell-mediated cytotoxicity assays against lymphoid tissue culture cells, widespread natural reactivity was found. The nature of the antigens detected in these assays is discussed, particularly the possible relationship to Epstein-Barr virus. Delayed skin reactivity to the extracts of lymphoid cell lines was found to correlate with elevated Epstein-Barr virus antibody titers.

Many investigators have suggested that Burkitt’s lymphoma is caused by, or at least closely associated with, a virus (3, 16, 44). Most of the attention has been focused on the EBV and the immune reactions against EBV-associated antigens. Other immune reactions to antigens on Burkitt’s lymphoma and to tissue culture cell lines derived from the tumor have been described, but their relationship to EBV is not clear (5, 6, 9, 21, 32, 41).

Immunological Reactions to Antigens on Experimental Virus-induced Tumors

A better understanding of the nature of the different antigens found in Burkitt’s and other lymphomas and the immune response to these antigens could provide more insight into the etiology of these diseases. Extensive studies have been performed on the antigens associated with virus-induced tumors in experimental animals [reviewed by Herberman (18)], and these may serve as guides to the study of tumor-associated antigens in man. It is becoming increasingly clear that virus-induced tumors may contain a complex variety of antigens. These antigens include those associated with virus particles themselves, virus-induced antigens, fetal antigens, and individually specific antigens. The viral and virus-induced antigens are common to many or all tumors induced by the particular virus, as opposed to the antigenic individuality of tumors induced by chemical carcinogens. Therefore, the finding of common antigens in tumors of unknown cause in experimental animals or in man has suggested that they might be caused by a virus. However, there are several problems with this extrapolation. Tumors induced by chemicals may contain common antigens (14, 38). Fetal antigens (42) and tissue-associated antigens may be common to a variety of virus-induced and non-virus-induced tumors.

In addition to common antigenicity, natural immune reactivity to tumor-associated antigens may be a clue to a virus etiology. Antibodies to cell surface antigens of Gross leukemia virus-induced tumors have been found in the sera of some untreated mice, in strains with a low incidence of spontaneous leukemias (1). The finding of natural cytotoxic antibodies directed against the PC antigen on mouse plasma cell tumors (20) provided an important clue to the close association of a particular C-type virus to mouse myelomas (T. Aoki and M. Potter, personal communication). Natural cell-mediated immunological reactivity with virus-induced leukemias in mice and rats has also been found to occur (M. Nunn, J. Djeu, D. Lavrin, and R. B. Herberman, unpublished observations). Such findings provide some rationale for the implication of a virus when natural antibodies (35) or cell-mediated reactivity (30) to human tumor-associated antigens are found.

Antibodies to Burkitt’s Lymphoma and Tissue Culture Cell Lines

A variety of antibodies have been described that react with Burkitt’s lymphoma cells or with tissue culture cell lines derived from the tumors. Antibodies to EBV have been extensively studied, but we will not discuss these, since they have been well covered by others in this Symposium. Klein et al. (27), using an assay for membrane immunofluorescence, found antibodies in the sera of Burkitt lymphoma patients and some African controls, which reacted with

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1 Presented at the International Symposium on Human Tumors Associated with Herpesviruses, March 26 to 28, 1973, Bethesda, Md. This work was supported in part by Contracts NIH-69-2160 and NIH-NCI-72-3227 from the National Cancer Institute.

2 Presented by.

3 The abbreviation used is: EBV, Epstein-Barr virus.
suspensions of autologous and allogeneic tumor cells. This
group subsequently found that these antibodies also reacted
with tissue culture cell lines derived from Burkitt's lym-
phoma or from other lymphoid cells, which were EBV
positive (26). The antigens detected appeared to be induced
by, or at least closely associated with, infection by EBV
(29).

Herberman and Fahey (21) found that many sera con-
tained cytotoxic antibodies that reacted with tissue culture
cell lines derived from Burkitt's lymphoma. Fass and
Herberman (5) showed that these antibodies also lysed
Burkitt's lymphoma biopsy cells. As was found in immuno-
fluorescence studies for EBV (15) and membrane (27)
antigens, patients with Burkitt's lymphoma had a signifi-
cantly higher incidence of cytotoxic antibodies and also
higher titers than control groups (22). The presence of these
antibodies in the sera of some normal children and the rise
in incidence and titer with increasing age was quite consist-
ent with the animal studies mentioned above, i.e., that a
virus, or other environmental factors, could have caused the
antibody production. However, the occurrence of cytotoxic
antibodies did not correlate with that of antibodies to EBV
or to Klemin's membrane antigen (7). In addition, studies of
the specificity of the reaction indicated that it was not
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Mann et al. (32) have produced antibodies in rabbits to an
isolated, soluble membrane fraction from the Raji cell line,
derived from a Burkitt's lymphoma. This antibody had
some specificity for blast cells from patients with acute
lymphocytic leukemia. It was also found to react with
human embryonic kidney cells when they were infected with
Rauscher leukemia virus, a murine C-type RNA virus
rather than a herpesvirus (31). The presence of an antigen in
a Burkitt cell line, possibly associated with an RNA
leukemia virus, is intriguing, and it will be important to
define further the specificity of this antigen and its impli-
cations for viral oncology.

In Vivo Cellular Immunity to Tumor Cells and to Tissue
Culture Cell Lines

Cell-mediated immunity to tumor-associated antigens has been studied in vivo by delayed hypersensitivity reac-
tions to extracts of autologous tumor cells and normal lymphocytes (2, 6). Reactivity to tumor extracts correlated
with the clinical state of the patients. Before treatment, with
tumor present, only 1 of 16 patients gave positive reactions.
In remission, about one-half of the patients gave positive
reactions to autologous tumor extracts. Those patients with
positive reactions remained in remission significantly longer than those with negative reactions. Seven patients with
positive reactions subsequently relapsed and then had
negative reactions. Upon reinduction of remission, positive
reactions were again seen.

Positive skin reactions have also been elicited in patients
with Hodgkin's disease and other lymphomas by autologous
tumor extracts (R. B. Herberman and P. H. Levine,
unpublished observations). It is not yet clear whether
reactivity correlates with clinical status in these patients.

The antigens detected in these studies appear to be tumor
associated, since comparable extracts of normal cells usu-
ally did not produce positive reactions. The relation to
virus is not clear. J. L. Ziegler and I. Magrath (unpublished
observations) have looked for common antigenicity, by
performing skin tests in Burkitt's lymphoma patients with
allogeneic tumor extracts. No positive reactions have been
seen. These negative cross-reactions indicate that virus-
associated antigens are probably not responsible for the
reactions to autologous extracts. In contrast, skin tests with
allogeneic extracts in patients with acute leukemia have
given positive results (R. B. Herberman, D. Char, and B.
Leventhal, unpublished observations). Some patients with
acute lymphocytic leukemia have also been skin tested with
extracts of Burkitt's tumor, and negative results were
obtained (R. B. Herberman and D. Char, unpublished
observations).

We have recently performed a series of skin tests with
membrane extracts prepared from human lymphoid cell
lines. The cells used were derived from Burkitt's lymphoma
(Raji, Maku, and Onesmus), from nasopharyngeal carci-
noma (Ly-28), and from normal lymphocytes (F-265).
Patients with leukemia (R. B. Herberman, D. Char, and B.
Leventhal, unpublished observations), Hodgkin's, and other
lymphomas (T. Anderson, P. Schein, P. L. Levine, and R.
B. Herberman, unpublished observations), and carcinomas
(T. C. Alford and R. B. Herberman, unpublished observa-
tions) have been tested. A summary of the results obtained
thus far is given in Table 1. A considerable number of
positive reactions were obtained with the extracts derived
from Burkitt's or other tumors when tested in patients with
leukemia or lymphoma. In contrast, patients with carci-
nomas, known to be reactive to other skin test antigens, were
completely unreactive to these extracts. The specificity of
the reactions was further demonstrated by the tests with
F-265, which was grown under the same conditions and was
morphologically identical to the other lines. Only 2 positive
reactions were seen in the 52 tests performed with this
extract. The nature of the antigens detected on the cell lines
is not clear. It is unlikely to be related to EBV, since Raji
and F-265 are both virus-negative cell lines (both con-
tain the EBV genome). In addition, a positive skin test was
obtained in a patient with no detectable antibodies to EBV.
The reactivity of lymphoma patients to the cell lines de-
rived from tumor cells correlated with the stage of dis-
ease, with Stage IV patients significantly less reactive.

In Vitro Assays for Cellular Immunity

Cell-mediated immunity to Burkitt's lymphoma has been
studied in a lymphocyte stimulation assay (41). The lymph-
ocytes of some patients with Burkitt's lymphoma under-
went blast transformation upon incubation with autologous tumor cells. However, the significance of these reactions is not clear, since lymphocyte stimulation has also been produced by autologous lymphoid cells derived from normal lymphocytes (11, 13, 25). It has been suggested that the reactions were in response to blast antigens, rather than to EBV or tumor-associated antigens (11). The EBV can, however, also produce stimulation of lymphocytes of EBV-immune individuals (8).

Cellular immunity to antigens on lymphoma and acute leukemia cells has also been studied by a $^{51}$Cr release assay (23, 34, 39). The results of tests against autologous target cells are summarized in Table 2. About 40% of the tests against lymphoma and leukemia target cells have been positive. The reactions appeared to be against tumor-associated antigens, since there were no positive reactions against autologous normal target cells. In Hodgkin's disease, all reactions thus far have been negative. Some allogeneic patients and also some normal individuals have had positive reactivity against lymphoma and leukemia target cells. This natural reactivity against common antigens again raises the question of virus-associated antigens.

The $^{51}$Cr release assay has also been used to study reactivity against lymphoid cell lines (34, 40). The pattern of reactivity with this assay has been quite different from that seen with the skin tests. High reactivity was seen against the F-265 cell line, as well as against the cell lines derived from Burkitt's and other tumors. Almost all normal individuals tested have had positive reactions against F-265. Lymphocytes from patients with immunodeficiencies or on chemotherapy have shown little or no reactivity. Also, with some cancer patients, reactivity was below the normal range. Thirteen of 19 patients with lymphoma gave reactions less than 40% of the normals (33). The data indicate that this reactivity may be a possible measure of the immunological competence of cancer patients and reflect the ability of their lymphocytes to give cytotoxic reactions. The nature of the antigens detected on the tissue culture cell lines is not clear. They do not appear to be due to the state of differentiation of the cells, to fetal bovine serum proteins, or to HL-A antigens. The pattern of reactivity has been similar to that of the cytotoxic antibodies discussed earlier. Again, the occurrence of widespread, natural reactivity could be an indication of sensitization by an environment agent, such as a virus. The cellular reactivity has not correlated with cytotoxic antibody (34) and different antigens may be involved. The antigen detected also does not appear to be related to EBV. Cell lines lacking EBV expression, such as F-265, were quite susceptible to lysis. Also, cellular reactivity has not correlated with EBV titers (see below).

Hewetson et al. (24) obtained different results, when they tested the lymphocytes of Burkitt's lymphoma patients and

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### Table 2

Lymphocyte cytotoxicity reactions against autologous target cells (19)

<table>
<thead>
<tr>
<th>Attacking lymphocytes</th>
<th>Tests positive*</th>
<th>Tumor</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8/20 (40)</td>
<td>0/30</td>
<td>0/22</td>
</tr>
<tr>
<td>Acute lymphocytic leukemia</td>
<td>6/19 (32)</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>Acute myelogenous leukemia</td>
<td>4/10 (40)</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>Lymphoma</td>
<td>0/6 (0)</td>
<td></td>
<td>0/8</td>
</tr>
<tr>
<td>Hodgkin's disease</td>
<td>22/43 (51)*</td>
<td>2/14</td>
<td></td>
</tr>
</tbody>
</table>

* Cytotoxicity in experimental group significantly higher than that of controls; p < 0.05.

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### Table 1

Delayed skin reactions to membrane extracts of lymphoid tissue culture cell lines

<table>
<thead>
<tr>
<th>Patients tested</th>
<th>Raji</th>
<th>Maku</th>
<th>Onesmus</th>
<th>Ly-28</th>
<th>All tumor-derived cell lines</th>
<th>F-265</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodgkin's lymphoma</td>
<td>2/2 (100)*</td>
<td>2/5 (40)</td>
<td>5/6 (83)</td>
<td>1/1 (100)</td>
<td>10/14 (71)</td>
<td>0/3</td>
</tr>
<tr>
<td>Burkitt's (American) lymphoma</td>
<td>3/4 (75)</td>
<td>0/1 (0)</td>
<td>1/4 (25)</td>
<td>1/3 (33)</td>
<td>5/12 (42)</td>
<td>1/2</td>
</tr>
<tr>
<td>Other lymphomas</td>
<td>3/6 (50)</td>
<td>0/3 (0)</td>
<td>4/8 (50)</td>
<td></td>
<td>7/17 (41)</td>
<td>1/9</td>
</tr>
<tr>
<td>Total lymphomas</td>
<td>8/12 (67)</td>
<td>2/9 (22)</td>
<td>10/18 (55)</td>
<td>2/4 (50)</td>
<td>22/43 (51)*</td>
<td>2/14</td>
</tr>
<tr>
<td>Acute lymphocytic leukemia</td>
<td>9/31 (29)</td>
<td></td>
<td></td>
<td></td>
<td>9/31 (29)</td>
<td>0/21</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>1/1 (100)</td>
<td>1/1 (100)</td>
<td></td>
<td></td>
<td>2/2 (100)</td>
<td>0/1</td>
</tr>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>0/1 (0)</td>
<td>0/1 (0)</td>
<td></td>
<td></td>
<td>4/4 (100)</td>
<td>0/4</td>
</tr>
<tr>
<td>Total leukemia</td>
<td>13/36 (36)</td>
<td>1/1 (100)</td>
<td>1/1 (100)</td>
<td></td>
<td>15/38 (39)*</td>
<td>0/27</td>
</tr>
<tr>
<td>Carcinomas</td>
<td>0/10 (0)</td>
<td>0/1 (0)</td>
<td>0/1 (0)</td>
<td></td>
<td>0/12 (0)</td>
<td>0/11</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, % positive.
* Reactivity to tumor derived cell lines significantly greater than that to F-265; p < 0.01; Reactivity significantly greater than that of carcinoma patients to same extracts; p < 0.05.

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of normal individuals against lymphoid cell lines, in a colony inhibition assay. They detected little cytotoxic reactivity, unless the lymphocytes were stimulated in vitro with cell lines (9, 10). The apparent differences from the ⁵¹Cr release assay may be due to methodological factors or to different antigens being detected.

**Correlations among Some of the Assays**

Several studies have been performed in which more than 1 assay of immunity was performed in the same patients. These comparisons among the assays offer an opportunity to determine whether similar immune responses are being measured, or whether there are basic differences in the immune response to the same antigens or in the reactions to several antigens. On the humoral side, extensive comparisons have been made. Although both the viral and membrane immunofluorescence tests measure EBV-associated antigens, the correlation between the antibodies in a variety of sera has not been complete (28). The occurrence of cytotoxic antibodies did not correlate with either of the immunofluorescence antibodies (7).

We have examined the correlation of skin test reactions to extracts of cell lines with the reactivity in the ⁵¹Cr release cytotoxicity assay (Table 3). Both skin test-positive and -negative individuals had a normal degree of cytotoxic reactivity against F-265. Skin reactivity did, however, correlate with the presence of high EBV titers in the sera. The nature of the relationship between these tests remains to be determined. The lymphocyte cytotoxicity reactions did not correlate with EBV titers or, as noted earlier, with cytotoxic antibodies.

**Discussion**

The differences in results obtained with the various in vitro assays and with the skin tests emphasize the complexity of the immune response to tumor-associated antigens. Lymphoma cells appear to have several different cell surface antigens and intracellular antigens associated with them. Some of these antigens are induced by the EBV or may actually be part of the virus particles. As suggested by the work of Mann et al. (31), there may also be antigens related to other types of viruses. In addition, a series of other antigens, which may not be virus associated, may also be present. Several of these antigens are common to most Burkitt’s lymphoma cells or to the lymphoid cell lines. Although this finding is consistent with induction by a virus, there are other possible explanations. Some of the antigens recognized in the lymphocyte stimulation assay may be related to the blast-like state of differentiation of the cells. The antigens could be considered differentiation antigens, or they might be found to be fetal antigens. The antigens detected by cytotoxic antibodies are normal cell surface antigens, which are greatly increased in quantity on Burkitt’s lymphoma cells and on the lymphoid cells. It is much more difficult to characterize the specificity of cell-mediated immune reactions, and the cause for the skin-reactive antigens and for the antigens detected in the ⁵¹Cr release assay remains unknown. We have recently developed an inhibition assay in our laboratory that should allow more precise determination of the specificity of the antigens detected in the lymphocyte cytotoxicity assay (34, 37).

The failure to detect common antigens by skin tests with extracts of Burkitt’s lymphoma was surprising, but this does not speak against the viral etiology of the disease. In addition to their common antigens, virus-induced mouse mammary tumors have been shown to also contain individually specific antigens (36, 43).

The occurrence of widespread natural reactivity to some of the antigens on lymphoma cells and on the lymphoid cell lines needs to be explained. Sensitization by exposure to an oncogenic virus is certainly a possible cause, and one which apparently accounts for much of the natural reactivity to virus-induced leukemias in mice and rats. However, sensitization by bacteria or other environmental agents can also produce immune reactivity against the cells, based on antigenic cross-reactivities. This is a likely explanation for the cytotoxic antibodies. In addition, some of the normal reactivity may be a reflection of the immunological surveillance mechanism of the host for reacting with altered cells.

It will be essential to sort out the interrelationships of the various immunological assays and to determine what relationship each one has to the etiology of Burkitt’s lymphoma or of other tumors. Until these factors are clearly understood, it will be hazardous to interpret the results of any particular immunological assay as reflecting the etiological agent for the disease.

**References**


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**Table 3**

<table>
<thead>
<tr>
<th>Correlation of skin tests to extracts of cell lines, lymphocyte cytotoxicity against F-265 cells, and EBV titers, performed in the same patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of tests positive/total no. of tests</strong></td>
</tr>
<tr>
<td><strong>⁵¹Cr cytotoxicity within normal range</strong></td>
</tr>
<tr>
<td>Skin tests positive⁷</td>
</tr>
<tr>
<td>Skin tests negative⁵</td>
</tr>
<tr>
<td>High EBV titer⁶</td>
</tr>
<tr>
<td>Low EBV titer</td>
</tr>
</tbody>
</table>

* Level of cytotoxicity against F-265 at least 40% of the mean of normal individuals tested at same time.
* Anti-viral capsid antigen titer greater than 80 and/or anti-early antigen test positive.
* Positive skin reaction to at least of the extracts of cell lines derived from tumors.
* High EBV titers occurred significantly more frequently in skin test positive individuals; p < 0.05 (2 × 2 contingency table analysis).
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41. Sjörsward, J., Clifford, P., Singh, S., and Svedmyr, E. Indications of


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