Immunocompetence of Leukemic Murine Lymphoblasts: Ultrastructure, Virus and Globulin Production

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SUMMARY

Direct and indirect immunofluorescence studies of a mouse lymphoma cell line with antisera specific for the 5 major classes of mouse immunoglobulins demonstrated the capacity of the cells to produce immunoglobulins γ1 and γ2a. Similar studies with specific monkey and rabbit anti-Rauscher sera demonstrated the presence of Rauscher viral antigens in the same cell line. Ultrastructural investigations confirmed the viral infection and showed the presence of typical murine leukemia virus. These results indicate that the murine lymphoma cell line 818 has the capacity both to produce immunoglobulins and to carry or replicate a leukemogenic virus at the same time. Implications of these findings regarding the known association between immunological diseases and neoplasias of the reticuloendothelial tissue are discussed.

INTRODUCTION

While numerous investigators have reported immunoglobulin synthesis in established cell lines deriving from normal and malignant human lymph nodes, from Burkitt lymphoma patients, and from peripheral blood cells, no similar information has been reported concerning lymphoid cell cultures of mouse origin carrying proven oncogenic viruses (5, 7, 15, 16, 23–26). This report concerns immunological and ultrastructural studies on a long-term culture of a mouse lymphoma carrying the Rauscher mouse leukemia virus.

MATERIALS AND METHODS

Culture Methods. The mouse lymphoma cell line 818 was initiated in August 1965 from the mince of leukemic lymph nodes of a Timco Swiss mouse which had developed lymphocytic leukemia 8 months after inoculation with fluids from a tissue culture line (479) infected with Rauscher virus (19). From the beginning, numerous round, lymphocyte-like cells have predominated. As reported elsewhere, when injected subcutaneously the cells grew into huge lympho-

sarcomatous tumors without gross evidence of generalization (19, 20). Control cells used in this study consisted of mouse cell lines 479 and 491, both of which have also been previously reported (19). The pattern of growth of these cells has been mainly fibroblastic, although line 479, initially infected with Rauscher virus, became malignant after 2.5 years in culture and contained numerous giant multinucleated cells (20). Active production of infective virus particles in culture line 479 has occurred continuously, unlike culture 491, in which only a small number of particles resembling mouse leukemia virus have been observed occasionally; the possibility of contamination was considered (20). In our laboratory, the cultures have been maintained in Ham’s F-10 medium supplemented with 20% fetal calf serum. Medium was changed 3 times weekly. For immunofluorescence studies, the cultures were grown on slides or coverslips in Petri dishes in a 5% CO2 atmosphere.

Immunofluorescence Methods. The coverslips and slides with cells attached were washed 3 times in 0.9% NaCl solution buffered at pH 7.2, air dried, and fixed in acetone for 10 min at room temperature. Direct and indirect immunofluorescence techniques were used for the demonstration of viral antigen and immunoglobulin production. In all instances fluorescein isothiocyanate was used as labeling material. Blocking was performed for all preparations by the usual technique. Impression smears were made from the subcutaneous tumors and stained similarly. The following antisera were used: rabbit anti-Rauscher serum, unlabeled; normal rabbit serum, unlabeled; sheep antiserum to rabbit γ-globulin, unlabeled, and sheep antirabbit γ-globulin, labeled (from Difco Laboratories, Detroit, Mich.); goat antiserum to mouse γ-globulin, unlabeled, goat antimus mouse γ-globulin, labeled, rabbit antiserum to goat γ-globulin, unlabeled, and rabbit antigoat γ-globulin, labeled (Hyland Laboratories, Los Angeles, Calif.); monkey anti-Rauscher γ-globulin, labeled (Bionetic Research Laboratories, Falls Church, Va.); rabbit antimonouse γ1 serum, unlabeled, goat antimonouse γ2a serum, unlabeled, rabbit antimouse γ2b serum, unlabeled, rabbit antimonouse IgA serum, unlabeled, and rabbit antimonouse IgM serum, unlabeled (Dr. W. Hyman, National Cancer Institute, Immunological Reference Center).

The rabbit anti-Rauscher sera were prepared according to the method of Yoshida et al. (27). The sera were then absorbed with sheep and mouse red blood cells and also with mouse plasma and liver. The rabbit and goat antisera against the 5 different types of mouse immunoglobulins were obtained from Dr. W. Hyman at National Cancer Institute.
Immunoglobulin Reference Center and were all absorbed with ascitic fluids from mice bearing plasma cell tumors. The absorption data as submitted by the National Cancer Institute Immunoglobulin Reference Center is as follows:

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Absorbing material</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-γ1 (R161A)</td>
<td>γ2a</td>
</tr>
<tr>
<td>anti-γ2a (G2911A)</td>
<td>γ1, γ2b</td>
</tr>
<tr>
<td>anti-γ2b (R126A)</td>
<td>γ1, γ2a</td>
</tr>
<tr>
<td>anti-IgA (MR 31/32A)</td>
<td>γ1, γ2a, γ2b, IgM</td>
</tr>
<tr>
<td>anti-IgM (MR 7/8A)</td>
<td>IgA</td>
</tr>
</tbody>
</table>

**Electron Microscopy.** Attached monolayers of the cultured cells were fixed in 2.5% glutaraldehyde and postfixed in 1% osmium tetroxide. Suspended cells were sedimented to a pellet by low-speed centrifugation and fixed similarly. All specimens were dehydrated in acetone and embedded in Epon 812 (13). Sections were cut with the MT-1 Porter-Blum ultramicrotome and stained with uranyl acetate and lead citrate (12, 17). All preparations were examined with a Siemens Elmiskop IA at 80 kV.

**RESULTS**

By both direct and indirect immunofluorescent techniques the mouse lymphoma cell line 818 showed cytoplasmic fluorescence when exposed to anti-Rauscher sera (Table 1). Fluorescence was confined to a narrow rim of the cell cytoplasm and was present in less than 50% of the cells (Fig. 1A). No reaction occurred when normal rabbit serum was used instead of Rauscher immune serum, and application of the fluorochrome alone was without effect (Fig. 1B). Blocking by unlabeled rabbit anti-Rauscher sera and unlabeled sheep antirabbit γ-globulin, followed by exposure to the corresponding labeled globulin, resulted in a marked decrease of fluorescence. When the same techniques were applied to tumor impression smears from an infected mouse, similar results were obtained. With the tumor smears, however, practically 100% of the cells showed cytoplasmic fluorescence. Slight cytoplasmic fluorescence in approximately 20% of the cells was noted with control line 479, while no reaction at all occurred with either the direct or indirect method when control mouse line 491 was used (Table 1).

As shown in Table 2 and Fig. 2A, the mouse lymphoma line reacted with antimouse γ-globulins in both direct and indirect tests. The reactions could be blocked, and labeled rabbit antigoat γ-globulin alone did not cause fluorescence (Fig. 2B). No fluorescence was observed when the control lines were used.

**Table 2**

<table>
<thead>
<tr>
<th>Mouse line 818 carrying Rauscher virus</th>
<th>Mouse line 479</th>
<th>Mouse line 491</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indirect test: goat antiserum to mouse γ-globulin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Indirect test: goat antiserum to mouse γ-globulin</td>
<td>+</td>
<td>done</td>
</tr>
<tr>
<td>Labeled rabbit antigoat γ-globulin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Direct test: labeled goat antiserum</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Direct test: labeled goat antiserum</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Direct test: labeled goat antiserum</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

When antimouse globulin fractions were used in the indirect tests, cytoplasmic fluorescence occurred with anti-γ1 and anti-γ2a, but not with anti-IgM, anti-γ2b, and anti-IgA (Fig. 3A and B). The positive reactions could be blocked. Ultrastructural studies showed "C" type viral particles outside the cells and within inclusions in the cytoplasm in a major proportion of cells examined in each electron microscopy field (Fig. 4A and B). In the area comparable to that stained with anti-Rauscher and antimouse γ-globulin, characteristic viral budding from the cell membrane was also observed (Fig. 4C).
DISCUSSION

Previous publications have already provided information on the origin as well as on the oncogenicity of the 818 mouse cell line (19, 20). It has also been reported previously that cell-free filtrate of line 818 tissue culture fluids failed to cause leukemia (19). The presence of the Rauscher virus in this cell line, however, is confirmed in the present study by direct and indirect immunofluorescence techniques utilizing monkey and rabbit anti-Rauscher γ-globulin. In the reaction with the Rauscher antiserum there is only cytoplasmic fluorescence, while Fink and Malmgren (8) demonstrated nuclear and cytoplasmic fluorescence in similar studies utilizing Rauscher virus-infected tissues of BALB/c mice (Fig. 1A). Although most of the cells in tumor impression smears fluoresced after exposure to anti-Rauscher sera, the number of reactive cells in culture was smaller. The electron microscope studies of the 818 cell line showed the typical “C” type murine leukemia virus both within cytoplasmic inclusions and outside the cells. The characteristic “budding” of the maturing viral particles from the cell membrane was also observed (Fig. 4C) (1). Earlier studies by Szakacs et al. (22) also showed numerous “C” type viral particles in the cytoplasm and in the intracellular spaces of the in vivo-grown line 818 cells.

The positive results obtained with the immunofluorescence method confirm the observation that the line 818 cells produce immunoglobulin. The reactions with the antimouse globulin fractions were positive with anti-γ1 and anti-γ2a, which suggests that the cell line is probably polyclonal, since at least 2 types of immunoglobulins are being produced (Figs. 3A and B) (6). The fact that these reactions could be blocked further support the conclusions.

Since our original observation, in vitro immunoglobulin production by lymphoid cell lines of human origin has been demonstrated by several investigators (6, 7, 15, 16, 23–26). The presence of viruses in some of those cultures is also well documented (2–4, 9, 11, 21, 24). A herpes virus-like particle, the Epstein-Barr virus, has often been found in long-term cell cultures obtained from patients with Burkitt lymphoma, acute leukemia, lymphoma, and Hodgkin’s disease, and even from normal individuals (2–4, 9, 21). While the serological studies of Henle et al. (10) strongly suggest a close relationship, if not identity, between the latter virus and the causative agent of infectious mononucleosis, the exact nature and especially the oncogenic potential of the Epstein-Barr virus remains to be elucidated (11). Rarely, an occasional human long-term lymphoid culture has been reported to contain a small number of particles within cytoplasmic inclusions bearing some resemblance to the “C” type leukemogenic virus found in the mouse (25). It has not as yet been shown that any of the human lymphoid cell lines thus far established have the capacity both to produce immunoglobulins in vitro and to carry or replicate a proven oncogenic virus. In this regard, the mouse lymphoma line reported here is unique, since it does indeed have the capacity to synthesize virus particles antigenically identical with the Rauscher leukemia virus and immunoglobulins at the same time.

The capacity of the 818 mouse lymphoma line to produce both virus and immunoglobulins is especially significant considering the often observed association of neoplasias of the reticuloendothelial and hematopoietic tissues with autoimmune diseases in both man and mouse. Recently, a link between a leukemogenic virus and the development of both neoplasia and autoimmune disease in NZB/B1 mice has been reported (14). A similar association was reported as early as 1962 (18). At that time the following 4 observations were made. Injection into newborn Swiss mice of lymphoid cells deriving from in vitro culture of a mesenteric lymphosarcoma of a Swiss mouse caused runt disease. Splenic cells from runted animals, in turn, caused runting or lymphosarcomatous growth at the site of inoculation. A few animals that had recovered from severe runting succumbed later to generalized lymphatic leukemia. Filtrate-induced generalized lymphatic leukemia in infant mice was associated with runting and hemolytic anemia.

Not uncommonly in man, dysproteinemia, hemolytic anemia, and other immunological disorders accompany and occasionally precede (as in Sjögren’s syndrome) certain types of lymphoma and leukemia. Investigations with the murine cell line 818 demonstrate that neoplastic lymphoid cells can be immunologically functional while at the same time harboring and actively producing an oncogenic virus. An intriguing question is the possible relationship between the virus and the antibodies produced by these cells. Does the production of immunoglobulins result from an interaction between the transforming virus and the neoplastic cell, so that the globulins act as specific antibodies directed against the viral antigens? Should this be the case, total or partial inactivation of the virus particles could result, with obvious implications concerning the success of virus isolations from mouse or human lymphoid cell lines. Moreover, a hapten role for cells harboring the virus and its related antigens could be conceived, since it is possible that many different parenchymatous tissues can carry the viral particles. Under such circumstances, some of the immunological disorders previously mentioned could result from direct immune attacks by the antiviral antibodies produced by the neoplastic lymphoid cell.

REFERENCES

Immunocompetence of Leukemic Murine Lymphoblasts


Fig. 1. Cytoplasmic fluorescence of cells from line 818. X 400. A, indirect technique with rabbit anti-Rauscher serum. B, normal rabbit serum used instead of Rauscher immune serum.

Fig. 2. Cytoplasmic fluorescence of cells of line 818. X 400. A, indirect test with unlabeled sheep antimouse serum and labeled rabbit antisheep γ-globulin. B, block of indirect test with unlabeled sheep antimouse serum and unlabeled rabbit antisheep γ-globulin, followed by exposure to labeled rabbit antisheep γ-globulin.

Fig. 3. Cytoplasmic fluorescence of cells of line 818. X 400. A, indirect test with unlabeled rabbit antimouse γ1 serum and labeled sheep antirabbit globulin. B, indirect test with unlabeled goat antimouse γa2a serum and unlabeled rabbit antigoat globulin.

Fig. 4. A, electron micrograph of lymphoid cells illustrating mature (arrow) and budding (double arrow) virus-like particles in the intercellular space. X 32,000. B, virus-like particles with electron-dense nucleoids frequently found aggregated in cytoplasmic inclusions. X 32,000. C, budding of the cytoplasmic particles into the extracellular space. The plasma membrane seems to form the envelope around the virus. X 32,000.
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