The Coating Reaction of the Herpes-type Virus Isolated from Malignant Tissues with an Antibody Present in Sera

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SUMMARY

Herpes-type viruses recovered from established cell lines were coated by homologous rabbit antisera as visualized by electron microscopy. These chronically infected cell lines were derived from malignant human tissues. Cross reaction was detected among the herpes-type viruses isolated from African and American patients afflicted with neoplastic disorders. This group of viruses are not related to herpes simplex virus as revealed by the coating and the serum neutralization tests. Patients with neoplastic disorders have a high incidence of the coating antibody. The coating antibody is also present in sera of normal adults and to a lesser extent in normal children.

INTRODUCTION

Herpes-type viruses have been observed in continuous cell lines established from biopsy specimens of tumor tissue from patients afflicted with Burkitt's lymphoma (6, 20), and from lymph nodes, bone marrow, or peripheral blood leukocytes of patients with different neoplastic disorders (Refs. 16, 17, and E. Jensen, to be published).

The virus isolates tested were not identified with well-known herpes group agents (8, 9). The infectivity of the virus in vivo and in vitro has not yet been demonstrated.

The reaction between virus and antibody has been studied using the electron microscope for the erythroblastosis virus (5), poliovirus and bacteriophage (2), vaccinia virus (3), influenza virus (4, 12), warts and polyoma viruses (1, 18), adenovirus (19), murine leukemia viruses (14, 15), and more recently for the EB-3 herpes-type virus (10).

The present report describes electron microscope observations of interaction between the herpes-type viruses and their antisera prepared in rabbits as well as with human sera originating from patients with different neoplastic disorders. The work was done in an attempt to demonstrate a pattern to the reaction and to yield some information suggesting possible virus-disease relationship. Sera of individuals without clinical symptoms of the disease were screened for this type of interaction for comparison.

MATERIALS AND METHODS

The virus utilized in this study was derived mainly from chronically infected cell lines that were established from several Burkitt tumors. These include Epstein's EB-3 line and a line designated P3-J in our laboratory which was received from Professor R. J. V. Pulvertaft. Herpes-type viruses recovered from other cell lines established from American patients were also used in this study. These lines include Jensen's F-125, F-132, F-151, and F-152. F-125 was established from an axillary lymph node of a patient with carcinoma of the esophagus; F-132 was established from a lymph node of a patient with reticulum cell sarcoma. F-151 was established from a lymph node of a leukemic patient, and F-152 was established from a brain tissue of a child who died of acute lymphocytic leukemia (E. Jensen, to be published).

All cell lines were propagated in Eagle's minimal essential medium supplemented with 20 percent fetal calf serum. The presence of the herpes-type virus was monitored by electron microscopy.

Virus Recovery. Most of the herpes-type virus was found to be associated with the cells although a small amount was present in the tissue culture growth fluids.

The virus was recovered from the cells following the procedure described by Toplin and Schidlovsky (21), with the exception that the sonicated cells were not digested with proteolytic enzyme.

The Kaplan strain of herpes simplex virus was received through the courtesy of Dr. Manaker of the National Cancer Institute. The virus was propagated in primary rabbit kidney cells and was separated from the growth fluid by centrifugation at 30,000 × g for one hour in a Spinco model L centrifuge. The virus recovered in this manner was concentrated 50-fold.

Test Sera

Immune Sera. Rabbit immune sera for the EB-3 and P3-J viruses were prepared by the injection of 0.1 ml of the virus mixed with equal amount of Freund's adjuvant in each of the four foot pads followed by intramuscular injection of 0.5 ml of the virus alone at 2 and 3 weeks later. The rabbits were bled 10 days after the last injection. The immune sera were absorbed with calf serum to remove antibodies against the residual calf serum used in the growth of cells. Completeness of absorption was checked by gel diffusion. This was followed by three absorptions with 1 × 10⁷ cells from normal human spleen cell line per 1 ml of serum. The removal of cellular antibodies could be checked by using electron microscopy to demonstrate absence of clumping of the cellular
Heterologous sera that caused clumping of cellular debris did not show evidence of interaction with virus particles. All sera were heat-inactivated at 56°C for 30 minutes.

Herpes simplex immune serum was prepared in rabbits by intravenous inoculation of 0.5 ml of 50-fold virus concentrate with an infectivity titer of 10^{6.7} ID_{50}/ml on four successive weeks. Rabbits were bled 10 days after the last injection.

**Human Sera.** A number of sera were obtained to test their reaction with the Burkitt virus. Human sera were obtained from the following donors: African children with Burkitt tumors, American children with acute lymphocytic leukemia, patients with Hodgkin's disease, several patients with different neoplasms, and adults and children without any symptoms of neoplastic disease. The blood typing for each of the American sera for A, B, and the Rh factor was obtained from hospital records.

The A and B human isoagglutinins present in human sera were removed by absorption with human red blood cells. One ml of serum was absorbed with 1 ml of 50% packed washed human A and B red blood cells. Complete removal of A and B isoagglutinins was ascertained before use of a serum specimen. These isoantibodies were removed to rule out any reaction between these antibodies and the isoantigens that might adhere to the virus capsids as a contaminant.

The ability of serum from a Burkitt lymphoma patient to coat the herpes-type virus could be removed by absorption of the serum with a pellet of purified P3-J virus sedimented by high-speed centrifugation. For this purpose one ml of the serum diluted 1:20 was absorbed three times with pellets of the sedimented virus each estimated to contain 10^8 to 10^9 virus particles per 1 ml.

**Virus Coating.** The coating phenomenon was demonstrated by mixing 0.2 ml of 1:2 dilution of serum with 0.2 ml of the virus. The mixture was incubated for 30 minutes at room temperature and overnight at 5°C. The contents were then transferred to 12-ml plastic Spinco centrifuge tubes. The tubes were filled completely with 0.05 M sodium citrate in order to dilute the serum protein because an excess interferes with the negative staining of virus particles (14). The tubes were centrifuged in a Spinco model L centrifuge using the No. 40 rotor at 30,000 x g for one hour. The supernates were removed by aspiration and the pellets were resuspended in 0.2 ml of 0.05 M sodium citrate. The virus was dispersed by aspirating with a tuberculin syringe fitted with a 27-gauge needle.

For negative staining, one part of the virus suspension in a watch glass was mixed with two parts of a 2% potassium phosphotungstic acid. The film side of a carbon-coated grid was drained on a filter paper, air dried, and observed in the electron microscope.

**RESULTS**

**Rabbit Antisera.** The reaction of the herpes-type virus with homologous rabbit antisera resulted in the deposition of a layer of a finely structured substance on the surface of the viral capsid (Fig. 1A). This layer, roughly 200 Å thick and intermeshed with and often obscuring the capsomeres, will be referred to here as the viral "coat." This coat is not to be confused with the viral "envelope" which is a membranous sac of varying size loosely enclosing one or more virus particles. The reaction was not obtained with normal rabbit sera (Fig. 1B) nor with antisera prepared against density gradient bands of a cell homogenate from a virus-free cell line established from a lymphoma patient (7). The reaction occurred only with naked virus particles. Enveloped particles remained uncoated regardless of the type of the coating serum. The coating reaction was not accompanied by agglutination of the virus particles regardless of serum dilution.

**Antisera prepared against the herpes-type viruses obtained from cell lines derived from African lymphoma patients also reacted with the viruses recovered from other cell lines that have been established from American patients with neoplastic disease (F-125, F-132, F-151, F-152). The herpes simplex virus rabbit immune serum which coated the homologous virus failed to coat the P3-J virus. Results of the cross-reactions observed between the lymphoma derived herpes-type viruses of different origins and selected rabbit immune sera are summarized in Table 1.

None of the immune sera prepared against the EB-3 and P3-J viruses coated the herpes simplex virus. A herpes simplex immune rabbit serum and a human herpes simplex convalescent serum that did not neutralize the herpes simplex virus as measured by the serum neutralization test also failed to coat the same virus. These sera did not neutralize 100 TCID_{50} of the virus when tested at 1:5 dilution. Anti-herpes simplex virus serum which had a neutralizing titer of 1:640 coated the herpes simplex virus but failed to coat the viruses from EB-3, P3-J, F-125, and F-132 cell lines. Sera that had a neutralizing titer of less than 1:40 against the herpes simplex virus did not coat the virions.

**Human Sera.** Each of 19 sera of African children afflicted with Burkitt tumor produced a thick coat around the P3-J virus. Four of these sera when tested on F-125 virus which was recovered from an American patient gave a positive coating reaction. The coating of P3-J virus also was observed after it was incubated with sera of patients with Hodgkin's disease and other neoplasms, as well as with commercial pooled human serum.

The ability of the sera drawn from apparently normal individuals and from patients afflicted with different neoplastic disorders to react with virus from the P3-J cell line was determined. The results are shown in Table 2. Fifteen sera from 20 children

### TABLE 1

<table>
<thead>
<tr>
<th>Immune serum</th>
<th>Virus tested</th>
<th>Coating reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-P3-J</td>
<td>P3-J</td>
<td>Positive</td>
</tr>
<tr>
<td>Anti-P3-J</td>
<td>EB-3</td>
<td>Positive</td>
</tr>
<tr>
<td>Anti-P3-J</td>
<td>F-151</td>
<td>Positive</td>
</tr>
<tr>
<td>Anti-P3-J</td>
<td>F-152</td>
<td>Positive</td>
</tr>
<tr>
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<td>F-125</td>
<td>Positive</td>
</tr>
<tr>
<td>Anti-P3-J</td>
<td>F-132</td>
<td>Positive</td>
</tr>
<tr>
<td>Anti-EB-3</td>
<td>EB-3</td>
<td>Positive</td>
</tr>
<tr>
<td>Anti-EB-3</td>
<td>P3-J</td>
<td>Positive</td>
</tr>
<tr>
<td>Anti-P3-J</td>
<td>Herpes simplex</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-herpes simplex</td>
<td>Herpes simplex</td>
<td>Positive</td>
</tr>
<tr>
<td>Anti-herpes simplex</td>
<td>P3-J</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* Rabbit antisera.
with acute lymphocytic leukemia coated the virus and five sera from 19 normal children without history of neoplastic disorders also reacted positively. Six out of six sera from children afflicted with different neoplasms (lymphosarcoma, neuroblastoma, Ewing’s rhabdomyosarcoma, and Wilms’ tumor) and five of six sera from children with Hodgkin’s disease likewise coated the virus particles.

The results obtained with the sera of adults are shown in the lower part of Table 2. Sera from most adult patients with neoplastic disease were positive for virus-coating activity. Ten out of the sera from 16 normal adults reacted with the virus from P3-J cell line. Five lots of pooled commercial human sera reacted with viruses from P3-J and F-125 cell lines and with herpes simplex virus.

Coating the P3-J virus was independent of the reaction with the herpes simplex virus. Some of the human sera that coated the P3-J virus failed to coat the herpes simplex virus. Other individual human sera that contained the herpes-simplex-virus-neutralizing antibodies and coated the herpes simplex virus failed to coat the P3-J virus.

A number of sera were tested to determine the highest dilution which would coat the P3-J virus. Two sera from Burkitt patients had a titer of 1:128, while a serum from a child with lymphocytic leukemia had a titer of 1:16. Two samples from patients with Hodgkin’s disease of long standing had titer of 1:64 and 1:16. One pooled commercial human serum had a titer of 1:32.

### DISCUSSION

The antigen-antibody reaction between the herpes-type virus and homotypic antisera resulted in coating the virus. This reaction was similar to that described for other viruses and more recently for the EB-3 virus by Henle (10). The observations reported here strongly suggest that the coating reaction observed when some human sera are incubated with the P3-J virus is the result of an antibody reaction with specific viral antigens. This reaction was found to occur with naked but not with enveloped particles. This finding supports the conclusion that the antigens on the virus capsid differ from those on the envelope. A similar conclusion concerning the origin of the herpes virus envelope was reached by Watson (22), who stated that the envelope contains no virus-specific antigens.

The composition of the viral coat may be interpreted from favorable images as a tight fibrillar mesh work; however, no individual antibody fibers have been recognized with sufficient certainty on the surface of the capsid. In contrast to the results of Henle et al., we did not observe the agglutination of the virus particles. The coating phenomenon in our hands appears to be a sensitive detector of an antigen-antibody reaction.

The reaction between rabbit antisera and viruses which were recovered from cell cultures of lymphomas of African origin (P3-J and EB-3) and viruses isolated from cultured cells from American patients (F-125, F-132, F-151, F-152) indicates that these two groups of herpes-type viruses are either similar or share a common antigenic component. This finding was strengthened by the fact that sera from patients suffering with Burkitt’s lymphoma reacted with the herpes-type viruses isolated from American patients.

The coating activity which was observed in human sera was not associated with the A, B, M, N, and Rh iso-anti-bodies. Absorption of human sera with red blood cells to remove the A and B isoagglutinins did not affect the ability of these sera to coat the herpes-type virus. The coating activity of the serum was found in the purified globulin fraction. When the serum of a Burkitt lymphoma patient was absorbed with pellets of purified P3-J virus, the coating activity was removed, suggesting an association with an antibody.

Our results which confirmed the findings of Epstein, Henle, and Rabson indicate that the viruses isolated from neoplastic tissues differ from the herpes simplex virus despite their similar morphology. This conclusion is based on the following findings: (a) antisera prepared against the EB-3 and P3-J viruses neither coated the herpes simplex virus nor showed neutralizing antibodies when titered by the serum neutralization test; (b) herpes simplex virus immune serum did not coat the herpes-type viruses isolated from neoplastic tissues; (c) human sera which coated one virus did not necessarily coat the other.

Some specimens tested which failed to coat the P3-J virus were antisera to Rauscher and Moloney leukemia viruses, antiserum to Reo 3 virus, sera from 3 dogs afflicted with leukemia, and antihuman globulin.

Although the number of human sera tested for the coating antibody is limited, it can be seen from the results presented here that the incidence of the coating antibody in individuals with neoplastic disorders is high. All of the sera from 25 children afflicted with Burkitt lymphoma or with other solid tumors, contained the coating antibody. Two adult patients afflicted with cancer did not contain the coating antibody. One of these was in the terminal stage of cancer.

The presence of the coating antibody in normal adults would indicate that one or a group of antigenically related viruses is quite prevalent within the population. The coating antibody was less prevalent in children without a history of neoplastic disorders than in normal adults. The incidence of the coating anti-
body in normal adults and children is in agreement with the figures published by Henle using the immune fluorescent technic (13).

The observations made indicate that the virus-coating reaction may prove useful in broader studies encompassing groups of individuals with non-neoplastic disease in an attempt to elucidate further the significance of the results obtained in this and other laboratories engaged in studies with these herpes-type viruses.

The frequency with which the herpes-type viruses have been detected in cultures of tissues from patients with neoplastic disease required that particular attention be directed to the determination of their significance, if any, in such disease. A limited amount of data has been presented in support of the coating reaction as a tool to be applied in the further study of these viruses. At this time, no information is available linking these agents etiologically with any pathologic condition in man. Higher serum titers observed for lymphoma patients may reflect a response to an increased antigenic stimulus resulting from the activation of occult infection in a host under stress. However, it is interesting to note that serum titers for coating activity of 1:128 were observed for sera from Burkitt lymphoma patients while titers of 1:16 were obtained with serum from children with acute lymphocytic leukemia. Further study is being conducted to determine whether antigenic differences between herpes-type viruses isolated from cultures of human neoplastic tissue can be detected by cross-reacting titers of the coating antibody.

ACKNOWLEDGMENTS

We wish to thank Drs. Donald Pinkel and John Smith, St. Jude Hospital, Memphis, Tennessee, for supplying us with sera from leukemia children. We are indebted to Drs. Bryan, Dalton, Fink, and Manaker, Department of Viral Oncology, National Cancer Institute, for their helpful suggestions and the review of the manuscript.

REFERENCES

Fig. 1. A, the reaction of P3-J virus with specific rabbit immune serum, coated; B, P3-J virus mixed with normal rabbit serum, naked control. × 150,000.
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