Antigenic Characteristics of Lymphomas Induced by Radiation Leukemia Virus (RadLV) in Mice and Rats

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SUMMARY

Injection of radiation leukemia virus (RadLV) into newborn W/Fu inbred rats results in a high incidence of lymphomas indistinguishable from those induced by this virus in mice. Plasma from rats with primary RadLV-induced lymphomas exhibited a host range of leukemogenic activity in mice similar to that of mouse-derived RadLV. The antigenicity of the rat RadLV-induced lymphomas is indicated by their transplantation behavior and by their ability to induce the production of cytotoxic antibodies. These tumors cannot be transplanted into adult syngeneic rats unless these hosts are pretreated with doses of X-rays known to depress the immune response. The serum of W/Fu adult rats immunized with syngeneic RadLV lymphomas is specifically cytotoxic for all of the rat and most of the mouse tumors induced by RadLV. The cytotoxicity of the rat antiserum is strongly absorbed by rat and mouse RadLV-induced lymphomas. Rat anti-RadLV sera also exhibit virus-neutralizing activity, as measured by inhibition of the leukemogenic activity of RadLV in mice. Cytotoxic and absorption tests performed with specific rat antisera show complete cross-reactivity between RadLV and Gross lymphoma antigens. In addition, rat and mouse RadLV-induced lymphomas absorb the specific cytotoxicity of mouse anti-Gross serum. These data demonstrate that RadLV and Gross viruses induce identical or very closely related antigens in the transformed cells. The presence of the RadLV cell-associated antigen in most radiation-induced mouse lymphomas supports the view that RadLV may be an etiologic factor in these tumors.

INTRODUCTION

Radiation leukemia virus (RadLV), extracted from radiogenic strain C57BL mouse lymphomas, has been found to induce the same type of tumor in several strains of mice known to be susceptible to X-ray leukemogenesis. It has therefore been postulated that the induction of leukemias in mice by X-rays may be mediated, after its “activation,” by this virus which apparently preexists in susceptible mice in a latent state (12). The cell-free transmission of lymphomas induced by other physical (5), chemical (8, 15, 18), and hormonal (11) agents suggests that RadLV or a similar viral agent may be a common denominator in the etiology of mouse lymphomas resulting from exogenous carcinogens.

In recent years, immunologic studies have yielded significant new information concerning the identification, taxonomy, and natural history of mouse leukemia viruses. Specific antigens in the cells of the lymphomas induced by most of these viruses have been defined and classified (13). Among them, the antigen associated with the Gross virus is of particular interest due to its widespread occurrence in strains of mice characterized by a high incidence of “spontaneous” lymphomas (14).

The present study was undertaken in order to determine whether lymphomas induced by RadLV are also characterized by the presence of a specific antigen and, if so, to establish its serologic relationships with the other known antigenic systems in mouse leukemia. Our attempts to prepare a cytotoxic antiserum in C57BL mice, specific for RadLV lymphomas, have been unsuccessful despite prolonged immunization with histoincompatible or histocompatible lymphomas induced by RadLV, probably reflecting a state of tolerance in this strain. Recent work by Geering et al. (3) has shown that inbred rats provide a more suitable system for the production of antiserum to the G (Gross) antigen than does the mouse system. Therefore, we undertook a similar approach for immunologic studies on RadLV. A preliminary report of our findings has been presented elsewhere (2).

MATERIALS AND METHODS

Animals

Mice were males and females of the inbred C57BL/Ka, BALB/c, and C3H/Km strains obtained from the production colonies of this laboratory.

Rats used in these experiments were the W/Fu inbred rats which are descendants of littermate breeding pairs kindly provided by Dr. Lloyd J. Old. The inbreeding of these animals has been maintained in this laboratory by sister-brother mating.

Virus

For the infectivity experiments, RadLV was obtained from RadLV-induced C57BL/Ka lymphomas following a method already described (12) or from plasma of rats with primary and transplanted lymphomas induced by RadLV. These
plasmas were pooled and clarified by centrifugation at 7,000 rpm for 10 minutes. The supernatant was then spun at 100,000 × g for 1 hour in a Beckman model L-2 ultracentrifuge and the obtained pellet resuspended in phosphate-buffered saline to approximately one third of the original volume of plasma.

Lymphomas

Details of the methods of induction of mouse lymphomas by X-rays and by RadLV have been given in previous reports from this laboratory (10, 12). E δ G2, a transplanted C57BL lymphoma induced by Passage A Gross virus, was obtained from Dr. Lloyd J. Old. Lymphomas were induced in W/Fu rats by neonatal intrathymic and intraperitoneal inoculation of cell-free extracts from RadLV-induced C57BL lymphomas. Serial transplantation of the mouse tumors was carried out by injection of cell suspensions intraperitoneally into young normal histocompatible mice. Cell viability was routinely checked in control tubes. Since adult W/Fu rats rejected concentrated viable cell suspensions of syngeneic RadLV-induced lymphomas, the passage lines of these tumors were established in adult W/Fu rats that had been exposed to total body X-ray doses of 400 to 450 R shortly before intraperitoneal inoculation of 50—100 × 10^6 viable cells.

Preparation of Antisera

Suspensions of viable cells were prepared from enlarged thymuses, spleens, and lymph nodes of rats bearing primary or transplanted RadLV-induced lymphomas. W/Fu rats more than 3 months of age were immunized with an initial subcutaneous inoculum of 10 × 10^6 viable cells, followed by inocula of about 100 × 10^6 cells every 10—15 days. Serum was obtained by tail bleeding 7—10 days after the second and subsequent immunizations, pooled, and stored at —70°C.

Two different Gross antisera were obtained from Dr. Lloyd J. Old. One was produced in W/Fu × BNf1 rats immunized against W/Fu lymphomas induced by Gross virus from C58 mice. The other was produced in C57BL/6 mice immunized with a transplanted AKR spontaneous lymphoma (K36).

Serologic Methods

Cytotoxic Tests. The cytotoxic test in vitro was performed according to the technic of Gorer and O’Gorman (4) as modified by Boyse et al. (1). Cell suspensions were prepared in Medium 199 by mincing the normal or leukemic lymphoid tissues with fine, curved scissors. After allowing the brei to settle, the cell suspensions were filtered through four layers of gauze, washed twice at low speed centrifugation, and resuspended to a final concentration of 5 × 10^6/ml cells. When the proportion of dead cells exceeded 15 percent by the trypan blue method, the cell suspension was discarded. Volumes of 0.05 ml of each cell suspension were placed in small test tubes containing equal volumes of progressive two-fold dilutions of heat-inactivated antiserum. Each tube then received 0.05 ml of complement (guinea pig serum diluted 1:3). After shaking, the tubes were incubated at 37°C for 40 minutes. The results were read by adding 0.1 ml of a 0.20 percent saline solution of trypan blue to each tube and determining the percentage of stained cells. The antiserum dilution tube containing approximately 50 percent of dead cells was considered the endpoint. Tubes containing either antiserum or complement alone were simultaneously incubated, and only those with a cell viability higher than 85 percent in these control tubes were evaluated.

Absorption Test. The in vitro procedure used was essentially that described by Old et al. (14). Cell suspensions were prepared in the same manner as for the cytotoxic test and were packed by centrifugation at 2500 rpm for 10 minutes in small test tubes. After removal of the supernatant, one volume of packed cells was mixed with an equal volume of heat-inactivated antiserum diluted to two two-fold dilutions below its endpoint (determined previously by direct cytotoxic test on a selected standard positive lymphoma). The mixture was incubated for 1 hour at room temperature and for 30 minutes in the cold with occasional resuspensions. After centrifugation at 2500 rpm for 10 minutes, the antiserum was carefully removed and titrated by serial dilution for cytotoxicity against the selected standard positive lymphoma. In each test three controls were included, one consisting of nonabsorbed antiserum, another with antiserum absorbed with a known positive leukemia, and the third with antiserum incubated with a known negative tissue. Table 1 shows examples of absorption experiments and illustrates the criteria employed for scoring the results. We considered absorption to be strong (+) when the residual cytotoxic activity of the absorbed antiserum (average percentage of killed cells in the first two dilution tubes) was less than 30 percent of that of the unabsorbed antiserum; absorption was termed weak (±) when this percentage was between 30 and 70; and absorption was scored as negative (−) when it was 70 percent or more.

RESULTS

Leukemogenic Effect of RadLV in W/Fu Rats

Cell-free extracts of RadLV-induced C57BL lymphomas were injected intrathymically and intraperitoneally into 11 newborn W/Fu rats. Nine of these animals developed lymphomas after a relatively short latent period (Table 2). No lymphomas have been observed among 15 rats injected intrathymically with saline and kept under observation for more than 14 months. The rat tumors were similar to those induced by RadLV in mice; macroscopically the thymus was the organ preferentially, and in some cases exclusively, involved by the leukemic process. Histologically, the tumors were classified as lymphocytic and lymphoblastic lymphosarcomas. Injection of concentrated viable cell suspensions from rat RadLV-induced lymphomas into young C57BL mice was not followed by leukemic growth, ruling out the possibility that these tumors resulted from the multiplication of mouse leukemic cells inadvertently transmitted to the newborn rats with the cell-free extracts.

Pellets obtained by ultracentrifugation of plasma from rats bearing RadLV-induced lymphomas contained abundant Type C virus particles, as revealed by electron microscopic examination (F. Gibbs, J. F. Ferrer, and H. S. Kaplan, unpublished...
W/Fu recipients were pretreated with a single, total-body, X-ray dose of 400 to 450 R. Consequently the serial transplantation of these tumors was established in similarly irradiated adult hosts. The failure of the rat RadLV-induced lymphomas to grow in adult rats of the same strain made it possible to immunize these hosts by the repeated inoculation of viable leukemic cells. Under these conditions, no iso- or hetero-antibodies can be induced. Table 3 summarizes the results of in vitro cytoxic tests performed with the immune sera pooled from several rats. Nine different RadLV-induced W/Fu lymphomas were examined as primary tumors or after a few transplant generations. All were sensitive to the cytotoxicity of the immune sera, and most of them exhibited high titers. To check the specificity of these reactions, 3 different pools of lymphoid cells from the spleens and lymph nodes of adult W/Fu rats were tested. Since serial passage of the rat lymphomas was carried out in irradiated hosts, two of these lymphoid cell pools were from rats that had previously received the same dose of X-rays as the recipients of the transplanted lymphomas. No cytotoxic effect was observed with any of these control lymphoid cell pools. Sixteen lymphomas induced by RadLV in mice were also tested after they were established in serial transplantation; 14 were positive, although their titers were generally lower than those of the rat lymphomas. No cytotoxic effect was observed with any of these control lymphoid cell pools.

Transplantation of RadLV Rat Lymphomas

Attempts to transplant 8 different primary lymphomas by the intraperitoneal injection of concentrated viable cell suspensions (usually more than 80 X 10^6 unstained cells with the trypan blue test) into adult W/Fu recipients were not successful. In contrast, tumor growth was readily obtained when the W/Fu recipients were pretreated with a single, total-body, X-ray dose of 400 to 450 R. Consequently the serial transplantation of these tumors was established in similarly irradiated adult hosts.

After a few passages, some of these lymphomas were tested again for transplantability into nonirradiated recipients. Only one of them grew progressively in 1 out of 4 animals. Since W/Fu rats are highly inbred, these results strongly suggest that the cells of these tumors possess a new transplantation antigen. Similar transplantation behavior of the lymphomas induced by Gross virus in W/Fu rats has been reported (3).

In Vitro Serologic Studies with RadLV Antiserum Prepared in Rats

The failure of the rat RadLV-induced lymphomas to grow in adult rats of the same strain made it possible to immobilize these hosts by the repeated inoculation of viable leukemic cells. Under these conditions, no iso- or hetero-antibodies can be induced. Table 3 summarizes the results of in vitro cytoxic tests performed with the immune sera pooled from several rats. Nine different RadLV-induced W/Fu lymphomas were examined as primary tumors or after a few transplant generations. All were sensitive to the cytotoxicity of the immune sera, and most of them exhibited high titers. To check the specificity of these reactions, 3 different pools of lymphoid cells from the spleens and lymph nodes of adult W/Fu rats were tested. Since serial passage of the rat lymphomas was carried out in irradiated hosts, two of these lymphoid cell pools were from rats that had previously received the same dose of X-rays as the recipients of the transplanted lymphomas. No cytotoxic effect was observed with any of these control lymphoid cell pools. Sixteen lymphomas induced by RadLV in mice were also tested after they were established in serial transplantation; 14 were positive, although their titers were generally lower than those of the rat lymphomas. Lymphoid cells pooled from spleens and lymph nodes from normal C57BL adult mice were negative.

The distribution of the antigen interacting with the rat antiserum was further studied in in vitro absorption experiments. Table 4 shows that all of the W/Fu RadLV-induced lymphomas completely removed the specific cytoxic activity of the rat antisera. In contrast, pools of lymphoid cells from thymuses, spleens, and lymph nodes of nonleukemic rats were not active. Eight individual leukemias induced by RadLV in mice, including 1 negative and 2 weakly sensitive in the direct cytoxic tests, were also tested by the absorption technic, and all were positive. Cells from pooled thymuses of young adult C57BL mice had no effect in four separate experiments.
Spleen and lymph node cells from the same animals were also negative in three experiments. In the fourth a weak but distinct absorption was observed.

Antigen Relationships between RadLV and Gross Lymphomas

The results of cytotoxic tests and absorption experiments with RadLV and G antisera produced in rats are presented in Table 5. Most of the lymphomas listed in this table were examined with the two antisera simultaneously in parallel tests. All of the mouse and rat RadLV lymphomas studied gave positive reactions in direct cytotoxic tests with the Gross antisera, with titers similar to those observed when the corresponding tumors were tested with the RadLV antisera. Likewise, the cytotoxicity of the RadLV antisera for Gross lymphomas was comparable to that of the Gross antisera. This cross-reactivity in the cytotoxic tests was fully confirmed in the absorption experiments. In fact, each type of lymphoma removed the specific cytotoxicity of the corresponding antisera as well as that of the antisera prepared against the other lymphoma type. In addition, among radiation-induced lymphomas, those that were negative or positive with one antisera were respectively negative or positive with the other.

The possibility was considered that other antigenic determinants, detected by the mouse and not by the rat typing system, could differentiate the two types of lymphomas. How-
Occurrence of the RadLV Antigen among Radiation-induced Tumors

In connection with the possible role of RadLV in X-ray leukemogenesis, it was of interest to assess the presence in radiogenic lymphomas of the antigen associated with this virus. Sixteen different lymphomas arising in C57BL or BALB/c mice after exposure to X-radiation were tested in early transplant generations. Eleven (68 percent) had the RadLV antigen, as revealed by their ability to remove partially or totally the specific cytotoxic activity of the rat RadLV antiserum (Table 7).

Neutralization of the Leukemogenic Activity of RadLV by Specific Antiserum

Another experiment was set up in order to determine whether the rat anti-RadLV serum was capable of interfering with the leukemogenic activity of RadLV. Graded concentrations of the virus preparation were mixed with an equal volume of either heat-inactivated rat antiserum, heat-inactivated normal rat serum, or saline. After incubation for 45 minutes at room temperature, the mixtures were injected intraperitoneally and subcutaneously into newborn C57BL/Ka mice. The results summarized in Table 8 indicate that the rat antiserum has strong neutralizing activity for RadLV.

DISCUSSION

Of the numerous viruses known to cause leukemia in mice, the Gross agent and RadLV are of particular interest because they appear to be widely distributed in this species. Immunologic studies have shown that the antigen associated with the Gross virus is present in normal and leukemic tissues of mouse strains with a high incidence of "spontaneous" lymphomas, as well as in a number of leukemias and solid tumors of low incidence mouse strains (14, 16). The fact that most
Antigens of Radiation Leukemia Virus

However, on the basis of such criteria as morphology, target organ selectivity, pathogenicity, and, presumably, mode of transmission, RadLV and Gross virus share many characteristics in common (9, 12). The experiments reported here show that the pathogenic and immunologic behavior of RadLV in rats is also similar to that described for the Gross virus (3). Like the Gross virus, RadLV is highly leukemogenic when inoculated into newborn W/Fu rats. The tumors induced by both viruses seem to possess a new transplantation antigen as indicated by their failure to grow in histocompatible adult rats. A specific antigen, common to the lymphomas induced by RadLV in mice and rats, can also be demonstrated in in vitro tests with cytotoxic antisera from W/Fu rats immunized with syngeneic lymphomas. Cytotoxic tests and absorption studies with specific rat and mouse antisera show that this antigen is indistinguishable from the Gross lymphoma antigen.

It has been suggested that in virus-induced lymphomas the specific antigen reacting with cytotoxic antibodies represents components of the viral coat incorporated into the cell membrane (3). If this is true, the cross reactions between Gross and RadLV lymphomas observed in the present study would imply that the corresponding viruses also share surface antigens. Our results, indicating that the anti-RadLV rat sera contains virus-neutralizing antibodies, provide a more direct approach for studies on the relationships of the envelope antigens of RadLV and the Gross virus. In this connection it should be noted that rat anti-Gross sera exhibit neutralizing activity against both the Gross and the RadLV pseudotypes of MSV (7). Since the envelope of these agents is apparently provided by the helper viruses, it seems reasonable to anticipate that the Gross and RadLV leukemia viruses will also show cross-reacting antigens in neutralization tests. Another antigenic similarity between these two viruses is that RadLV contains an internal soluble antigen present in the Gross as well as in all other mouse leukemia viruses studied to date (6).

On the other hand, Igel et al. (7) have prepared mouse complement-fixing (CF) antibodies against a sedimentable RadLV-associated antigen that do not react significantly with Gross or other mouse leukemia CF antigens (7). Thus, although RadLV and the Gross virus are immunologically closely related, they appear not to be identical. It would be of interest to study whether nonshared antigenic determinants, such as those revealed by the mouse CF antisera, account for the contrasting strain preferences of these two viruses.

In contrast to the immunologic reactivity of rats to the cell-associated RadLV antigen, no cytotoxic antibodies have been detected yet in C57BL mice repeatedly inoculated with rat or mouse RadLV lymphomas. Since RadLV seems to be vertically transmitted in this strain of mice (9), it is probable that these animals are naturally tolerant to the RadLV-associated antigen detected in cytotoxic tests. Conversely, the strong immunologic response of rats to RadLV lymphomas, together with their known resistance to X-ray leukemogenesis (3), may indicate that they do not normally harbor RadLV. The apparent failure of C57BL mice to produce anti-RadLV cytotoxic antibodies is in striking contrast to the strong CF antibody response observed by Igel et al. (7) in these animals after immunization with extracts of RadLV-induced lymphomas. Further work on the nature and relationship of the anti-

Table 7

<table>
<thead>
<tr>
<th>Origin of the radiogenic lymphomas</th>
<th>Degree of absorption</th>
<th>Total positive/total no. tested</th>
</tr>
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<tr>
<td>C57BL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BALB/c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
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</table>

Absorption in vitro of the specific cytotoxicity of the rat radiation leukemia virus (RadLV) antiserum with radiation-induced lymphomas.

Residual cytotoxic activity tested on standard sensitive mouse or rat RadLV-induced lymphomas.

Table 8

<table>
<thead>
<tr>
<th>Dilution of virus</th>
<th>Virus incubated with:</th>
<th>Net No. of mice</th>
<th>No.</th>
<th>Percent</th>
<th>Average latent period (days)</th>
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</thead>
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<tr>
<td>$10^0$</td>
<td>Saline or normal rat serum</td>
<td>18</td>
<td>11</td>
<td>61</td>
<td>168</td>
</tr>
<tr>
<td>$10^1$</td>
<td>Immune rat serum</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$10^{-1}$</td>
<td>Saline or normal rat serum</td>
<td>22</td>
<td>12</td>
<td>55</td>
<td>161</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>Immune rat serum</td>
<td>13</td>
<td>1</td>
<td>7</td>
<td>326</td>
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<tr>
<td>$10^{-2}$</td>
<td>Saline or normal rat serum</td>
<td>9</td>
<td>3</td>
<td>33</td>
<td>165</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>Immune rat serum</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Neutralization of radiation leukemia virus (RadLV) leukemogenesis by the rat RadLV antiserum.

Strains of mice are susceptible to radiation leukemogenesis suggests that RadLV, which appears to be the causative factor of radiation induced lymphomas, is also prevalent in this species. The natural occurrence of RadLV and Gross viruses points to them as prime candidates for a central role in the etiology of murine leukemias. Accordingly, the relationship between these two agents acquires a particular significance.

The distinction between RadLV and Gross virus has been established on the basis of the differences in their host ranges. Although strain C57BL mice are the most susceptible hosts for RadLV, they are relatively resistant to the Gross virus. Conversely, the Gross virus exhibits maximal leukemogenic activity in strain C3H mice, in which RadLV is very weakly active (9). These findings closely paralleled recently reported studies on the "rescue" of the mouse sarcoma virus (MSV) genome by superinfection of MSV-induced tumor cells in vitro with mouse leukemia viruses. MSV pseudotypes produced by superinfection with RadLV are active in strain C57BL, but not in Swiss mice, whereas the Gross MSV pseudotype exhibits the opposite host selectivity (7).
gens detected by the cytotoxic and CF technic is needed in order to clarify this apparent discrepancy. One possibility to be considered is that, as opposed to the antigen interacting with cytotoxic antibodies, the RadLV CF antigen is acquired by the virus only after its "activation." Thus, C57BL mice, naturally harboring "inactive" RadLV, would not become tolerant to this antigen and, in consequence, would be readily immunized against it by extracts of lymphomas induced by "activated" RadLV. Another explanation for the lack of tolerance of C57BL mice to the CF antigen is that this antigen is one of the internal components of RadLV and is therefore normally masked from the host's recognition. However, this antigen would be unmasked as a consequence of virus rupture occurring during the preparation of the lymphoma extracts.

Most of the radiation-induced lymphomas of strain C57BL and BALB/c mice tested in the present work contained the RadLV antigen detected by the specific rat cytotoxic antisera. These findings agree with previous results from this laboratory showing that infective RadLV can be isolated reasonably consistently from primary radiogenic C57BL lymphomas (9) and substantiate the view that RadLV may be involved in the mechanism of radiation leukemogenesis. However, the possibility cannot be excluded that the presence of RadLV in these tumors is the consequence of superinfection and is thus without etiologic significance. It has been shown that leukemic cells can become superinfected with unrelated leukemogenic viruses and acquire the antigens of these agents (17). Since the RadLV rat antisera seem to have high titers of virus-neutralizing antibodies, the participation of RadLV in radiation leukemogenesis can now be investigated more critically by the passive immunization of C57BL mice with rat antisera at appropriate intervals after exposure to X-irradiation.

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