Effect of Lactate Dehydrogenase Virus on Chemically Induced Mouse Lung Tumorigenesis

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ABSTRACT

Lactate dehydrogenase virus is the third in a series of viruses which have been examined for the capacity to alter chemically induced mouse lung tumorigenesis. This virus was given to strain A mice by i.p. injection either 4 weeks before, on the same day as, 4 weeks after, or 8 weeks after the s.c. injection of urethan (either 0.25 or 1.0 mg/g). The pulmonary adenoma response to urethan was suppressed in all of the lactate dehydrogenase-infected mice, with a maximum suppression of 30 to 40% when the virus was given simultaneously with or 4 weeks after urethan. As with murine sarcoma virus and reovirus, it is postulated that this suppression of chemically induced mouse lung tumorigenesis is due to virally induced alterations in the immune response of the mouse to chemically induced tumors.

INTRODUCTION

To explore the role of viral infection in chemical carcinogenesis, our laboratory undertook an investigation of the capacity of a series of 3 unrelated RNA viruses to modify the pulmonary adenoma response of strain A mice to chemical carcinogens. The first virus studied was M-MSV, a C-type RNA virus with oncogenic activity (10). The second virus tested was Reo-3, a nononcogenic, double-stranded RNA virus found in humans and a wide variety of other animal species (11).

The third and last of the series of viruses that we have investigated as modifiers of chemical carcinogenesis is LDV. This is a nononcogenic virus which has the capacity to modify the malignancy of both transplantable (1, 4, 9, 12) and virally induced (7, 12) tumors. In this report, we present the results of our study of LDV and summarize the results obtained with all 3 viruses tested as modifiers of chemical carcinogenesis.

MATERIALS AND METHODS

Viral Titration and Proof of Infection. Stock LDV (obtained through the Office of Programs, Resources, and Logistics, National Cancer Institute) was titered by use of a previously described assay (5). Tenfold dilutions of the virus stock were inoculated i.p. into groups of 5 male Ha/ICR Swiss mice (L. C. Strong Research Foundation, San Diego, Calif.) (0.1 ml/mouse). Four days after inoculation, blood was collected from these mice and 5 untreated mice by retroorbital bleeding into heparinized tubes, and plasma was obtained by centrifugation.

The LDH levels in these plasma samples were determined by a colorimetric reaction (5). Twofold dilutions of the plasma were prepared in 0.1 M phosphate-buffered saline, pH 7.4, and 24 ml of each dilution were placed in a well of a microtiter plate (Cooke Laboratory Products, Cooke Engineering Co., Alexandria, Va.). Three drops of reaction mixture (8 mg sodium pyruvate, 100 mg NADH, and 100 ml 0.1 M phosphate-buffered saline, containing 0.138 M NaCl, 0.027 M KCl, 0.008 M Na2HPO4, and 0.0015 M KH2PO4, pH 7.4) were added to each well, and the microtiter plate was incubated at 37°C for 1 hr. One drop of color reagent (20 mg (2,4-dinitrophenyl)hydrazine, 7.5 ml concentrated HCI, and 92.5 ml distilled water) was added to each well; after 15 min of shaking at room temperature, 1 drop of 5 N NaOH was added to each well. After 5 more min of shaking, each well of the microtiter plate was scored for decolorization.

To obtain proof that the strain A mice used in the carcinogenesis study were in fact infected with LDV, plasma samples were obtained from 5 LDV-inoculated and 5 untreated mice by retroorbital bleeding. The lungs and spleens of 3 of the LDV-inoculated and untreated mice were also excised, homogenized, and subjected to a Freon virus purification procedure (3). The plasma, lung, and spleen samples from LDV-injected and control mice were then injected i.p. into Ha/ICR Swiss mice (0.1 ml/mouse, 2 mice/sample). Plasma was obtained from these mice and from 5 untreated Ha/ICR Swiss mice by retroorbital bleeding 4 days after inoculation, and the LDH levels in serial dilutions of these plasma were determined by the colorimetric assay described above (5).

Pulmonary Adenoma Bioassay. Six-week-old male A/St mice (L. C. Strong Research Foundation) were placed in groups of 30. Five groups of mice received s.c. injections of urethan (0.25 mg/g) (Matheson Gas Products, East Rutherford, N. J.) dissolved in 0.9% NaCl solution, and 5 groups received injections of urethan (1.0 mg/g). One group of mice at each urethan dose level received i.p. injections of 105 LDH-elevating units of LDV either 4 weeks before, simultaneously with, 4 weeks after, or 8 weeks after urethan injection. One group of mice at each urethan dose level was retained as a non-virally infected control. Additional controls included a group of mice given s.c. injections of 0.9% NaCl solution only and a group of mice given i.p. injections of 105 LDH-elevating units of LDV only.

Sixteen weeks after urethan exposure, all mice were sacrificed, and the excised lungs were placed in Telyesniczky's fluid (100 ml 70% ethanol, 5 ml formalin, and 5 ml glacial acetic acid) for 3 days. The lungs were then examined under a Spencer dissecting microscope (×10), and the number of surface adenomas was counted. A few surface nodules were...
examined histologically to confirm the typical morphological appearance of the adenoma. The frequency of lung tumors in each LDV-infected group was statistically compared with that in the appropriate uninfected group by Student’s t test.

RESULTS

Proof of Infection. The results presented in Table 1 demonstrate that the strain A mice that received injections of LDV were in fact infected. The plasma samples from LDV-injected strain A mice produced a clear increase in plasma LDH in the Ha/ICR test mice as compared with test mice that received injections of plasma samples from control strain A mice. Lung and spleen samples from LDV-injected mice also produced an increase in LDH in test mice, but this increase was not as pronounced.

It should be noted that the plasma LDH levels in the Ha/ICR Swiss mice that received injections of control strain A mouse plasma were essentially identical to the plasma LDH levels in untreated Ha/ICR Swiss mice. This provides evidence that the strain A mice used in this investigation were LDV free prior to injection of LDV.

Pulmonary Adenoma Bioassay. In mice exposed to the low dose of urethan, LDV injection inhibited the lung tumor response somewhat when infection occurred 4 weeks before, 4 weeks after, or 8 weeks after urethan exposure (Table 2). This inhibition was more pronounced when LDV infection occurred 4 weeks after urethan injection of plasma samples from control strain A mice. Lung and spleen samples from LDV-injected mice also produced an increase in LDH in test mice, but this increase was not as pronounced.

DISCUSSION

The results obtained suggest a suppressive effect of LDV infection on the pulmonary adenoma response of strain A mice to urethan. The finding that LDV inhibits the lung tumor response when administered 4 weeks after urethan injection suggests that the suppressive effect of virus infection may be due to interference with lung tumor progression rather than interference with the induction of pulmonary adenomas by urethan.

<table>
<thead>
<tr>
<th>Virus exposure</th>
<th>Carcinogen treatment</th>
<th>% of alteration in lung tumor incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-MSV</td>
<td>MCA</td>
<td>−41c</td>
</tr>
<tr>
<td>−14 days</td>
<td>Urethan</td>
<td>−42d</td>
</tr>
<tr>
<td>−14 days</td>
<td>MCA</td>
<td>−40c</td>
</tr>
<tr>
<td>−14, 0, +7 days</td>
<td>Urethan</td>
<td>−35f</td>
</tr>
<tr>
<td>+14 days</td>
<td>Urethan</td>
<td>−46f</td>
</tr>
<tr>
<td>−7, 0, +7, +14 days</td>
<td>Urethan</td>
<td>+20g</td>
</tr>
<tr>
<td>Reo-3</td>
<td>Urethan</td>
<td>−22d</td>
</tr>
<tr>
<td>−6 days</td>
<td>Urethan</td>
<td>−39d</td>
</tr>
<tr>
<td>0 days</td>
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<td>−31d</td>
</tr>
<tr>
<td>+4 wk</td>
<td>Urethan</td>
<td>−20d</td>
</tr>
<tr>
<td>+8 wk</td>
<td>Urethan</td>
<td></td>
</tr>
</tbody>
</table>

As a summary of our research, it can be noted that both M-MSV and Reo-3 also suppressed the pulmonary adenoma response to chemical carcinogens (Table 3). In the case of M-MSV, it was suggested that this was due to an enhanced immune response, produced either by a virally induced increase in lung tumor cell antigenicity or by virally induced stimulation of the immune response of the host (10). With Reo-3, it was suggested that interferon, induced by exposure to reovirus, suppressed the synthesis of blocking antibodies and enhanced the cell-mediated immune response (11).

Immune mechanisms may also be invoked to explain the LDV results. LDV infection produces a short but pronounced increase in circulating interferon (2). Thus, this virus may act like reovirus (11). The effect of LDV may also be explained by a direct interaction between the virus and the immune system of
the host. The ability of LDV to decrease the susceptibility of mice to transplantable plasmacytoma (4) has been ascribed to LDV-induced suppression of the immune system of the host (6, 8), followed shortly thereafter by a rebound of the immune system, resulting in an enhancement of the immune response to the tumor. It is possible that the same type of rebound effect resulted in LDV-induced suppression of the pulmonary adenoma response.

Thus, with all 3 viruses tested by our laboratory, the pulmonary adenoma response to chemical carcinogens was suppressed. In each case, this suppression may be explained by virally induced alterations in the immune response of the host. However, further work using other viruses, as well as nonviral immunogens in this model system, would be necessary to substantiate this explanation.

ACKNOWLEDGMENTS

The authors are indebted to Linda S. Terry and Stephen H. Parker for their excellent technical assistance.

REFERENCES

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