Prevention of viral-chemical co-carcinogenesis in vitro by type-specific anti-viral antibody

(specific neutralizing antibody/in vitro transformation/model systems for transformation/transformation inhibition)

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ABSTRACT Low passage Fischer rat embryo cultures, which are normally very resistant to transformation by 3-methylcholanthrene but are highly susceptible when chronically infected with the Rauscher murine leukemia virus, were completely protected from transformation by methylcholanthrene when treated with neutralizing antibody specific for the leukemia virus prior to and during treatment with methylcholanthrene. Sister cultures were not protected by neutralizing antibody specific for the B-tropic radiation leukemia virus. This demonstrates clearly a definite type-specific role for Rauscher murine leukemia virus in the 3-methylcholanthrene transformation system in rat cells.

We have previously described a Fischer rat embryo cell line that at a low passage level (<60) requires either the addition of an exogenous type-C RNA virus or treatment with a halogenated pyrimidine prior to treatment with a chemical carcinogen in order to transform for occurrence (1, 2). At passage levels higher than 60, chemicals known to be carcinogenic in animals do on occasion transform the cells without preinfection by type-C RNA virus. These high passage cells appear to be negative for infectious type-C RNA virus; however, the gs-1 antigen of RaLV (the endogenous type-C RNA rat virus) is often expressed after transformation (2), and the rat type-C virus can be induced in both the low and high passage cells by 5-iodo-2'-deoxyuridine (1, Shif, personal communication). We have recently shown that two agents that inhibit this induction by iododeoxyuridine, namely, streptorixin (3) and cordycepin (4), protect the rat cells from transformation by the polycyclic hydrocarbon 3-methylcholanthrene. We report here that low passage Fischer rat embryo cultures chronically infected with the Rauscher murine leukemia virus (RLV) are protected from transformation by 3-methylcholanthrene if treated with neutralizing antibody specific for RLV; the cultures were not protected by neutralizing antibody specific for the B-tropic radiation leukemia virus (RdLV) (5), which is Cross-like in its neutralizing antigenic determinants.

MATERIALS AND METHODS

Reduction in plating efficiency of the chronically infected Fischer rat embryo cells relative to a control serum was used to determine the toxicity of the antisera. Five hundred cells in 5 ml of complete growth medium (Eagle’s minimal essential medium in Earle’s salts supplemented with 5% dialyzed calf serum, 5% fetal bovine serum, 2 mM L-glutamine, 0.1 mM nonessential amino acids, and a mixture of 100 units of penicillin and 100 ug of streptomycin per ml) were added to each 60-mm plastic dish (Lux). After a 4-hr incubation period at 37°C (to allow cell attachment) the medium was decanted and replaced with a fresh medium now containing serial, 2-fold dilutions of antisera or control serum. Five days later the cells were fixed and stained (Giemsa) and macroscopic colonies were counted.

Both F119 and F115 are sister cultures of Fischer rat embryo cells (F111) that had been inoculated at subculture 2 with RLV and then subcultured separately. These cultures are chronically infected. They routinely produce 10⁸ to 10⁹ infectious particles per ml of unfiltered supernatant medium and have previously been shown to be readily transformed by 3-methylcholanthrene and other carcinogenic chemicals. Duplicate cultures were seeded at a concentration of 100,000 cells per ml in 25-cm² plastic flasks in a complete medium without antisera. Four hours later the medium was decanted and replaced with a medium now containing the various dilutions of antisera or control sera. The next day the medium was decanted and replaced with a fresh medium containing either control sera, antisera, or 0.5 µg/ml of 3-methylcholanthrene alone, or combinations of control sera or antisera and the methylcholanthrene. Three days later the cultures were washed five times with a balanced salt solution and the medium was replaced with fresh medium containing either control sera or antisera, but not methylcholanthrene. Two days later and weekly thereafter, the cultures were subdivided 1:2 in the absence of both methylcholanthrene and antisera.

Transformation was determined by the appearance of spindle-shaped cells lacking contact inhibition and growth orientation and by the formation of macroscopic colonies in semisolid agar (6).

The presence of supernatant virus at the time of treatment with 3-methylcholanthrene and at each subculture thereafter was determined by an assay for RNA-dependent DNA polymerase (7). The percentage of cells producing virus was determined with the infectious center assay described by Pincus et al. (8), with F111 as the feeder layer.

Experiment 1. F119 at subculture 50 was treated, prior to and during treatment with 3-methylcholanthrene, with a 1:50 dilution of either normal rabbit serum or a RLV-neutralizing antisera produced in rabbits (antisera provided by Dr. R. V. Gilden). The RLV antisera at dilutions of 1:160 and 1:320 were previously shown to reduce focus formation of an MSV(RLV) pseudotype virus by 91% and 59%, respectively.

Abbreviations: RLV, Rauscher murine leukemia virus; RdLV, radiation leukemia virus.
Table 1. Inhibition of virus and prevention of transformation with type specific antisera—experiment 1

<table>
<thead>
<tr>
<th></th>
<th>F119&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Rabbit anti-RLV</th>
<th>Control rabbit serum</th>
<th>Fetal bovine serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1:50 dilution)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute plating efficiency (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.2 14.8 15.2</td>
<td>15.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative plating efficiency (%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>87 97</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymerase activity at time of methylcholanthrene treatment&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0 10,104 4,772</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphological transformation&lt;sup&gt;e&lt;/sup&gt; (passage no.)</td>
<td>-(P&lt;sub&gt;1&lt;/sub&gt;)  +(+P&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>+(P&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>+(P&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>+(P&lt;sub&gt;3&lt;/sub&gt;)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Fischer rat embryo cells chronically infected with RLV.

<sup>b</sup> Absolute plating efficiency is the percent of cells forming macroscopic colonies per 500 cells inoculated onto a 60-mm plastic dish (average of three dishes).

<sup>c</sup> Relative plating efficiency is the absolute plating efficiency relative to the control, which is adjusted to 100%.

<sup>d</sup> DNA-dependent DNA polymerase activity is the cpm of [3H]dTMP incorporated per ml of tissue culture fluids less F111 control cpm. A negative reading is listed as 0.

<sup>e</sup> Antisera and control sera added 24 hr prior to, during, and for 48 hr after treatment with 0.5 μg of 3-methylcholanthrene.

Experiment 2. F115 at subculture 50 was treated, prior to and during treatment with 3-methylcholanthrene, with dilutions of antisera produced in goats to either mouse cell grown RLV or mouse grown Kaplan radiation leukemia virus (RadLV) (1:240, 1:480, 1:960, and 1:1920). The goat antisera against RLV at dilutions of 1:400 and 1:800 had previously been shown to reduce foci formation of the MSV(RLV) pseudotype by 100% and 18%, respectively. The RadLV antisera gave significant neutralization of MSV(RLV) only at dilution 1:50, but did neutralize the homologous virus at dilutions of 1:400 and 1:800. Thus, with respect to RLV, the RadLV antisera had an 8-fold higher titer than the RadLV antisera. An additional pair of cultures was treated with RLV antisera (1:960 dilution) only after treatment with methylcholanthrene.

RESULTS

Experiment 1

Rabbit antisera against RLV at a dilution of 1:150 completely inhibited the measurable production of RNA-dependent DNA polymerase (reverse transcriptase) in the F119 cultures while reducing the plating efficiency by only 13% (Table 1). At a dilution of 1:25 the specific antisera reduced the plating efficiency by 60%. Control rabbit serum was also toxic at a dilution of 1:25 (40% reduction in plating efficiency), but was relatively nontoxic at a dilution of 1:50 and did not inhibit virus production. The RNA-dependent DNA polymerase levels of the cultures treated with the RLV specific antisera (1:150) returned to original levels one subculture after removal of the antisera. Duplicate cultures treated with 0.5 

Table 2. Inhibition of virus and prevention of transformation with type specific antisera—experiment 2

<table>
<thead>
<tr>
<th></th>
<th>F115&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Goat anti-RLV</th>
<th>Goat anti-RadLV</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute plating efficiency (%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.6 12.4 15</td>
<td>16.8 ND</td>
<td>12.6 14.4 15.6</td>
<td>16.4 16</td>
</tr>
<tr>
<td>Relative plating efficiency (%)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>35 78 94</td>
<td>105 ND</td>
<td>79 90 98</td>
<td>103 100</td>
</tr>
<tr>
<td>Absolute % of cells producing virus at time of methylcholanthrene treatment&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND 0</td>
<td>1.33 7.1 ND</td>
<td>3.4 2.8 10.9</td>
<td>8.7 10.3</td>
</tr>
<tr>
<td>Relative plaque-forming units (%)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>ND 0</td>
<td>12 73 ND</td>
<td>26 24 103</td>
<td>86 100</td>
</tr>
<tr>
<td>Polymerase activity at time of methylcholanthrene treatment&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0 0</td>
<td>64 8,366 4,877</td>
<td>1,005 904 2,361</td>
<td>9,523 11,841</td>
</tr>
<tr>
<td>Growth in agar (P&lt;sub&gt;3&lt;/sub&gt;)&lt;sup&gt;h&lt;/sup&gt;</td>
<td>ND</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Morphological transformation&lt;sup&gt;i&lt;/sup&gt; (passage no.)</td>
<td>ND -(P&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>-(P&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>+(P&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>+(P&lt;sub&gt;3&lt;/sub&gt;)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Fischer rat embryo cells chronically infected with RLV.

<sup>b</sup> Goat added on subculture after treatment with 0.5 μg of methylcholanthrene (this column only).

<sup>c</sup> Absolute plating efficiency is the percent of cells forming macroscopic colonies per 500 cells inoculated onto a 60-mm plastic dish (average of three dishes).

<sup>d</sup> Relative plating efficiency is the absolute plating efficiency (%) relative to the control, which is adjusted to 100%.

<sup>e</sup> Number of colonies formed.

<sup>f</sup> Relative plaque-forming units, number of cells producing virus relative to the control, which is adjusted to 100%.

<sup>g</sup> RNA-dependent DNA polymerase activity, cpm of [3H]dTMP incorporated per ml of tissue culture fluids less F111 control cpm. A negative reading is listed as 0.

<sup>h</sup> Goat antisera added 24 hr prior to, during, and for 48 hr after treatment with 0.5 μg of methylcholanthrene.

<sup>i</sup> Anti-RLV, antisera to Rauscher murine leukemia virus; anti-RadLV, antisera to Kaplan radiation leukemia virus; ND, not done.
Experiment 2

Using the procedure for infectious center assay (8), it was found that about 10% of the F115 cells were actually producing virus at any one time (Table 2). At a dilution of 1:960, the difference in relative plating efficiency between RLV and RadLV antisera was only 4% (94% as compared to 98%), but the number of cells able to produce virus averaged only eight per dish after treatment with the anti-RLV as compared to 68 with the anti-RadLV. At a dilution of 1:480 of the goat anti-RLV, the overall plating efficiency was reduced by 22%, but the percentage of cells producing virus was reduced to near 0. Even though the percentage of cells producing virus at any one time was reduced by treatment with specific antibody, cloning experiments showed that every cell still had the ability to produce virus. Dilutions of goat antisera against RLV of 1:480 or 1:960 also reduced supernatant virus and protected the cells from transformation by methylcholanthrene. Cultures still had a normal morphology at the termination of the experiment 15 subcultures after treatment (Fig. 2). In addition, cells from both cultures produced no foci when inoculated into the semisolid agar, nine subcultures after treatment. In contrast, cells treated with methylcholanthrene and then one subculture later treated with goat antisera against RLV at a dilution of 1:960 or cultures treated at the time of methylcholanthrene treatment with a dilution of goat antisera against RLV of 1:1920 became phenotypically transformed by subculture 7 and grew in the semisolid agar when inoculated at subculture 9. All of the cultures treated with the

μg of 3-methylcholanthrene incorporated in media containing fetal bovine serum or fetal bovine serum plus control rabbit serum showed multiple areas of transformed cells seven subcultures after methylcholanthrene treatment. In contrast, the F119 control cultures and sister cultures treated with the RLV-specific antisera prior to and during treatment with methylcholanthrene were still not transformed 15 subcultures after treatment (Fig. 1).

Fig. 1. Inhibition of 3-methylcholanthrene-induced transformation by rabbit antisera against RLV. (A) F119 P50 control (P +12); (B) F119 P50 + rabbit anti-RLV 1:50 + 0.5 μg of methylcholanthrene (P +12); (C) F119 P50 + 0.5 μg of methylcholanthrene (P +7); (D) F119 P50 + rabbit control serum 1:50 + 0.5 μg of methylcholanthrene (P +7).


Fig. 2. Inhibition of 3-methylcholanthrene-induced transformation by goat antisera against RLV. (A) F115 + goat anti-RLV 1:480 + 0.5 μg of methylcholanthrene (P +14); (B) F115 P18 + goat anti-RLV 1:960 + 0.5 μg of methylcholanthrene (P +14); (C) F115 P18 + 0.5 μg of methylcholanthrene + goat anti-RLV 1:960 (one subculture after the removal of methylcholanthrene) (P +14); (D) F115 P18 + goat anti-RadLV 1:240 + 0.5 μg of methylcholanthrene (P +14).
goat antisera produced against the RadLV (dilutions of 1:240, 1:480, 1:960, or 1:1920), as well as the F119 control, were transformed by methylcholanthrene and formed macroscopic colonies in the semisolid agar.

**DISCUSSION**

We have previously shown that our low passage Fischer rat embryo cells (F111) require preinfection with a type-"C" RNA virus in order to be transformed by chemical carcinogens (9). The effect is not purely synergistic in that addition of the virus one subculture or more after treatment with a chemical carcinogen does not result in transformation. Virus must be present and actively replicating at the time of chemical treatment (10). We now strengthen these observations by inhibiting chemically induced transformation by specific neutralizing antibody. There are several possible explanations to account for our observations that the addition of antisera specific for the exogenous virus inhibit transformation:

(i) In the population there is only a small percentage of cells able to produce virus, and it is this population that is the most susceptible to transformation. The specific antibody functions by selectively destroying this population. However, this is not the explanation because even though the percentage of cells producing virus at any one time is low, every clone still has the ability to produce virus (N. Mishra, personal communication).

(ii) We are selectively destroying transformed cells by sensitizing them to chemical toxicity or by direct action of the antibody. However, this is not the explanation since both control and transformed cells are equally sensitive to antibody toxicity.

(iii) The antibody may be inhibiting cell division (thus making the cells insensitive to transformation by the chemical carcinogen). We know that this is not the explanation since in the presence of specific antibody there is still an average 5-fold increase in cell number over a seven-day period.

There are two possible mechanisms that would account for our data:

(i) Those cells actively producing virus during the period of carcinogen treatment are the most susceptible to transformation (the virus either providing oncogenic information or combining with and switching on endogenous oncogenic information). The specific inhibition of this select population by the specific antibody would thus be responsible for the inhibition of transformation.

(ii) There is a minimal threshold for virus expression below which there is no transformation; in this case, the specific antisera would reduce virus replicating in dividing cells below the necessary threshold. Either of these models is supported by our observations that specific antibody must be present at the time of carcinogen treatment. When the specific antibody was added subsequent to carcinogen treatment, there was no protection under the conditions of the present study.

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