Protective Effect of Immunization with Polyinosinic-Polycytidylic Acid Complexed with Methylated Bovine Serum Albumin against Friend Leukemia Virus in Mice

Alain Fourcade, Charlotte Friend, Fanny Lacour, and J. Gilbert Holland

SUMMARY

Mice hyperimmunized with polyinosinic-polycytidylic acid complexed with methylated bovine serum albumin were protected against challenge with Friend leukemia virus. The virus was inoculated at the time when antibodies reacting to the polynucleotide structure had reached an appreciable level. A sparing effect of approximately 1.5 logs in infectivity was afforded the immunized mice. Our results indicate that the polynucleotide complexes may provide a promising means of immunizing against the oncornaviruses.

INTRODUCTION

Synthetic double-stranded polynucleotide complexes with MBSA as a protein carrier are capable of eliciting specific antibodies in rabbits (10) and in mice (5). They have also been shown to be antigenic without carrier in NZB mice or with Freund's adjuvant in other strains of normal mice (16). The immunological aspects of the polynucleotides have been reviewed recently (6).

Since antibody levels are enhanced when poly(I)-poly(C) is adsorbed to MBSA or mixed with Freund's adjuvant (12), the present study was undertaken to determine whether immunization with this double-stranded polynucleotide would offer nonspecific protection against an oncogenic virus. Since DBA/2J mice were found to be good responders to poly(I)-poly(C)-MBSA and are highly susceptible to FLV, the experiments were carried out with these inbred animals. The results described herein demonstrate that hyperimmunization with poly(I)-poly(C)-MBSA affords a significant measure of protection against FLV infection.

MATERIALS AND METHODS

DBA/2J mice approximately 2 months old were obtained from The Jackson Laboratory, Bar Harbor, Maine. The polynucleotides polyinosinic and polycytidylic acid, purchased from Miles Laboratories, Inc., Elkhart, Ind., were further purified by extraction with water-saturated phenol, dialysis against 0.15 M NaCl, and precipitation with cold ethanol. The double-stranded complex poly(I)-poly(C) was prepared as previously described (11).

The mice were immunized according to the following schedule. A mixture of 40 µg of poly(I)-poly(C) and 40 µg of MBSA emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) was inoculated i.p. once a week for 4 weeks. The 5th inoculation of poly(I)-poly(C)-MBSA 1 week later and the 6th 3 weeks thereafter were injected i.v. The mice tolerated this course of immunization with no sign of toxicity. One week after the final immunization inoculation, 20 mice were bled and the antibody content of each serum sample was determined. The remaining immunized mice were challenged by i.p. inoculation of 10-fold dilutions of a filtrate of FLV prepared from leukemic spleens as were nontreated mice of the same age.

For antibody assays, poly(I)-poly(C)-3H and poly(A)-poly(U)-3H were purchased from Miles Laboratories. Antigen-binding capacities of the serum of individual mice to each of the radioactive polynucleotide complexes were determined. The reaction mixture, containing 170 µl of phosphate-buffered saline (0.14 M NaCl-0.015 M phosphate buffer, pH 8.1), 20 µl of 3H-labeled antigen (20 µg/ml; specific activity, 40,000 to 60,000 cpm/µg), and 10 µl of immune serum, was incubated first for 30 min at 37° and then for 18 to 20 hr at 4°. One volume of cold 70% saturated ammonium sulfate was added. After 1 hr at 4°, the mixture was spun down by centrifuging at 12,000 x g for 10 min. The supernatant was discarded; the inside of each tube carefully wiped, avoiding 1.5 cm at the bottom; and the precipitate was dissolved in 0.3 ml of 0.1 N NaOH. After the addition of 10% trichloroacetic acid, the precipitate was poured onto a GF/B Whatman fiber glass filter, rinsed with 5% trichloroacetic acid and ethanol, and dried before being placed in a scintillation counter. The results are calculated...
as percentages of radioactivity recovered in the precipitate as compared to the total input and expressed as μg of antigen bound per ml of serum.

Two series of experiments were performed, one to determine antibody binding capacity and the other to determine whether or not the susceptibility of the immunized mice to FLV infection had been altered. All mice were immunized with the same preparation of poly(I)-poly(C)-MBSA and tested 7 days after the last injection of the immunogen.

RESULTS

Analysis of the serum of each of the immunized mice revealed antibodies reacting specifically with the polynucleotide structure. Each of the antisera bound similarly to the immunogen poly(I)-poly(C) as well as to another polynucleotide complex, poly(A)-poly(U) (Table 1). The level of binding activity of normal untreated DBA/2J mice never exceeded 0.4 μg/ml.

Table 2 gives the results of 2 separate experiments comparing the titer of FLV in control and immunized mice. In both experiments, a sparing effect in the immunized mice was evident at each virus dilution injected and there was approximately a 1.5-log difference in infectivity. The survivors remained free of disease for 6 months at which time the experiments were terminated.

DISCUSSION

Our observations suggest that the resistance to FLV infection exhibited by the poly(I)-poly(C)-immunized animals was related to the antibody induced by the synthetic double-stranded polynucleotide, since the serum of each mouse had antibody directed against the immunogen. The possibility that this nonspecific antibody may be capable of binding to virus nucleic acid is suggested by the fact that antisera to poly(I)-poly(C) precipitates avian myeloblastosis virus RNA (11).

The results also emphasize the importance of the time interval between the administration of the immunogen and the challenge with virus. While either minor beneficial effects against FLV (9) and murine erythroblastosis virus (15) or enhanced oncogenicity of murine sarcoma virus (3) had been observed previously in mice treated with poly(I)-poly(C) shortly before and shortly after infection, our mice were not challenged with FLV until after the immunization course was completed and the antibodies to the synthetic double-stranded polynucleotide complex were demonstrable. That nonspecific antibody may indeed provide a sparing effect against the oncornaviruses is supported by earlier findings that mice infected with Rauscher virus after immunization with 5 weekly injections of complete Freund’s adjuvant in combination with virus-unrelated antigens had increased survival rates as compared to the controls, although all ultimately succumbed to leukemia (13). On the other hand, a single inoculation of complete Freund’s adjuvant 10 days before or 3 days after inoculation with Rauscher virus caused an acceleration of leukemia (14). Bacillus Calmette-Guérin vaccination has also been shown to prolong life and decrease mortality of FLV-infected mice (8, 9).

The ability of poly(I)-poly(C) to induce interferon was also checked. Assays kindly performed by Dr. I. Gresser indicated that interferon production was transient and occurred in the 1st 48 hr after the inoculation of poly(I)-poly(C)-MBSA. By Day 4, as well as at the time the mice were inoculated with virus, interferon was no longer detectable. Further evidence indicating that interferon was not involved was obtained from previous studies demonstrating that immunization with poly(I)-poly(C) of NZB/NZ mice (16) and of rabbits (2) had an inhibitory effect on interferon stimulation. Finally, stimulation of humoral or cellular responses could also be excluded as a mechanism in the sparing effect against FLV, since the dose used [40 μg

![Table 1](image1.png)

<table>
<thead>
<tr>
<th>Antigen bound (mg/ml serum)*</th>
<th>Poly(I)-poly(C)-immunized mice</th>
<th>Poly(I)-poly(C)</th>
<th>Poly(A)-poly(U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19.8</td>
<td>26.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>19.4</td>
<td>28.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>18.6</td>
<td>20.6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>17.4</td>
<td>26.8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>15.4</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td>6</td>
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<td>19.4</td>
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<td></td>
</tr>
<tr>
<td>10</td>
<td>18.8</td>
<td>13.6</td>
<td></td>
</tr>
</tbody>
</table>

* Binding capacity of normal DBA/2J serum <0.4 μg/ml.

![Table 2](image2.png)

<table>
<thead>
<tr>
<th>Virus dilution (no. positive/no. inoculated)</th>
<th>DBA/2J mice</th>
<th>50% lethal dose</th>
<th>Log difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>DBA/2J mice</td>
<td>10^-1</td>
<td>10^-2</td>
</tr>
<tr>
<td>1 Control Immunized</td>
<td>5/5</td>
<td>5/6</td>
<td>7/7</td>
</tr>
<tr>
<td>1 Control</td>
<td>4/5</td>
<td>3/5</td>
<td>1/5</td>
</tr>
<tr>
<td>2 Control Immunized</td>
<td>6/6</td>
<td>6/6</td>
<td>7/7</td>
</tr>
<tr>
<td>2 Control</td>
<td>6/8</td>
<td>5/7</td>
<td>0/5</td>
</tr>
</tbody>
</table>
Poly(I)-poly(C)] had been shown to be ineffective in enhancing these phenomena (1, 4).

Although hyperimmunization with poly(I)-poly(C)-MBSA was ineffective in providing protection to mice challenged i.c. with encephalomyocarditis virus (F. Lacour and J. Huppert, unpublished results), the results of the present study suggest that it may be helpful in diminishing the effects of the murine oncornaviruses. The use of polynucleotide complexes as immunogens to induce immunity should be considered, since they are well tolerated and offer little risk.

REFERENCES


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