The Effect of Respiratory Carcinogenesis on Systemic Humoral and Cell-mediated Immunity of Syrian Golden Hamsters

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SUMMARY

The effect of intratracheal instillation of benzo(a)pyrene and its noncancerogenic analog benzo(e)pyrene on the systemic humoral and cell-mediated immune response of Syrian golden hamsters was evaluated. Hamsters treated with the carcinogen had a transient suppression of the splenic plaque-forming cell response to sheep erythrocytes, compared with analog-treated controls. The numbers of direct (immunoglobulin M) and indirect (immunoglobulin G) plaque-forming cells were suppressed at the 9th week of treatment and then recovered to control levels. No suppression in the cell-mediated immune response, as assessed by the rejection of Chinese hamster skin grafts, was found.

INTRODUCTION

The induction of bronchogenic carcinomas in Syrian golden hamsters by the i.t. instillation of benzo(a)pyrene and its noncarcinogenic analog benzo(e)pyrene on the systemic humoral and cell-mediated immune response of Syrian golden hamsters was evaluated. The hamsters treated with the carcinogen had a transient suppression of the splenic plaque-forming cell response to sheep erythrocytes, compared with analog-treated controls. The numbers of direct (immunoglobulin M) and indirect (immunoglobulin G) plaque-forming cells were suppressed at the 9th week of treatment and then recovered to control levels. No suppression in the cell-mediated immune response, as assessed by the rejection of Chinese hamster skin grafts, was found.

MATERIALS AND METHODS

Animals. Male Syrian golden hamsters (Sprague-Dawley, Madison, Wis.), 10 weeks of age, were used for the carcinogenesis studies. Male Chinese hamsters (Chick Line, Vineyard, N. J.), 25 g, were used as donors for skin heterografts.

The animals were housed 5/cage and were given food and water ad libitum.

Carcinogen. Preparations of BaP (Aldrich Chemical Company, Milwaukee, Wis.) hand ground in a mullite mortar with an equal quantity of Type R 3098 ferric oxide (Fe2O3) (Charles Pfizer, Inc., New York, N. Y.) were obtained from the Illinois Institute of Technology Research Institute, Chicago, Ill. Prior to use, the BaP-Fe2O3 preparation was suspended in sterile 0.85% NaCl solution to contain 5 mg BaP plus 5 mg Fe2O3 in each 0.2-ml aliquot of the suspension. BeP was provided by Dr. Marcia Litwack, National Cancer Institute. This noncarcinogenic analog of BaP, which has been reported to be a weak tumor initiator in mice when used with a strong promoter (9), was hand ground with Fe2O3 and prepared as described above for i.t. instillation. Hamsters, anesthetized by i.p. injection of sodium brevitol (Elk Lilly and Company, Indianapolis, Ind.), received 10 weekly i.t. instillations of the carcinogen or analog, according to the method described by Saffiotti et al. (8).

PFC Determinations. SRBC, collected in anticoagulant citrate dextrose were aged for 1 week, washed 4 times in 0.85% NaCl solution at 4° and resuspended in 0.85% NaCl solution to give a 10% suspension of erythrocytes. Animals were given i.p. injections of 0.5 ml of this suspension. The plaque-forming response of IgM and IgG antibody-producing cells to SRBC in the spleen was determined 7 days after the injection of antigen by the method described by Dresser and Wortis (3). Rabbit anti-hamster IgG (Microbiological Associates, Bethesda, Md.) was used to develop the indirect plaques.

Skin Grafts. A 10- x 15-mm full-thickness skin graft was transplanted onto the back of the animal just posterior to the shoulder. Grafting and dressing were performed by the method described by Billingham (1). Plaster casts were removed from the animals after 7 days to remove sutures and to determine the status of the graft. Casts were then reapplied so as to prevent damage to the graft but still permit daily observation. Scab formation following ulceration of the graft was taken as the end point of heterograft rejection. Results were expressed as mean survival time.

RESULTS

Humoral Immune Response to SRBC. To evaluate the effect of BaP on the systemic immune response to SRBC, carcinogen-treated, analog-treated, and untreated animals were analyzed periodically during the 10-week course of carcinogen administration, as well as monthly during the following 6 months. Three animals/group for each time
had a mean 142 IgM PFC per 10^6 spleen cells, while those receiving BeP formed 168 PFC per 10^6 spleen cells. No significant suppression was noted at 6 weeks. After 9 carcinogen treatments, there was a significant suppression of IgM PFC response (208 PFC per 10^6 spleen cells versus 455 PFC per 10^6 spleen cells). No suppression occurred once carcinogen treatment was stopped at 10 weeks.

A similar pattern of suppression was noted when comparing the IgG PFC response (Table 2). No suppression was noted until the 9th week of treatment. Animals receiving carcinogen formed 182 IgG PFC per 10^6 spleen cells while those receiving the noncancinogenic analog formed 437 IgG PFC.

The data plotted in Chart 1 represent the IgM and IgG PFC response of carcinogen-treated animals expressed as the percentage of those obtained from animals treated with the noncarcinogenic analog BeP. The suppression declined to about 40% of control at the 9th week and then recovered to normal levels.

Cell-mediated Immune Response to Chinese Hamster Skin Grafts. The effect of BaP treatment on graft survival time can be seen in Table 3. No suppression in the cell-mediated immune response was noted. Grafts placed on animals in all treatment groups had a mean survival time of 14 to 16 days.

DISCUSSION

The immunodepressive capability of the hydrocarbon carcinogens has been demonstrated in several laboratories (6, 7, 10-12, 14). In some cases, this depression appeared to be long lasting and to include interference with both humoral and cellular immunity. Studies using MCA administered to mice reduced the number of antibody-producing spleen cells after immunization with sheep erythrocytes (10). The immunodepression was rapid and long; at 2 days after exposure to carcinogen, the number of PFC decreased by 50% and remained depressed for a period corresponding to the latency period prior to the appearance of tumor. In
were suppressed during carcinogen treatment. No effect was observed on cell-mediated immunity as measured by direct and indirect plaque-forming cell response to SRBC. A suppression of both direct and indirect PFC was not noted in the spleen until the 294th day of exposure, while the indirect PFC response was markedly suppressed by the 120th day.

The experiments reported here demonstrate that i.t. instillation of BaP led to a transient suppression of both the direct and indirect plaque-forming cell response to SRBC. A transient suppression in the splenic direct PFC response of hamsters during the course of 7,12-dimethylbenz(a)anthracene treatment has been reported by Szakal and Hanna (15). No consistent depression of the indirect PFC response to SRBC was found. The authors stated, however, that the indirect assay in hamsters led to a 50% suppression of direct plaques. To correct for this observation, we first enumerated the direct plaques before the addition of anti-IgG and complement to develop the indirect plaques. This modification resulted in the observation that the indirect PFC response to SRBC was also suppressed in BaP-treated hamsters.

The i.t. instillation of BaP had no apparent effect on the ability of Syrian hamsters to reject Chinese hamster skin grafts. This finding supports that of Demoise et al. (2), who found that allogeneic tumor cells failed to grow in mice treated with i.t. MCA.

The results of this investigation have provided some insight into the possible effects of respiratory carcinogenesis on the immune response of hamsters. Recent evidence has suggested that the immunosuppressive effects of the hydrocarbon carcinogens may be dose related (13, 14). Low levels of MCA led to the development of tumors in mice with no observed immunodepression. This suggests that the assessment of active immune responses after relatively high doses of chemical carcinogens may provide some insight into the effects of immunosuppressive chemical compounds but may not provide useful information concerning the role of the immune response in the development of neoplasia. Assessment of more subtle changes in lymphoid and phagocytic cell function caused by low-level exposure to chemical carcinogens may provide more meaningful information.

ACKNOWLEDGMENTS

The technical assistance of Laura Campolito is gratefully acknowledged.

REFERENCES


Table 3

Mean survival time of Chinese hamster skin grafts on BaP-treated Syrian golden hamsters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Wk 2</th>
<th>Wk 6</th>
<th>Wk 9</th>
<th>Wk 12</th>
<th>Wk 16</th>
<th>Wk 20</th>
<th>Wk 24</th>
<th>Wk 34</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaP</td>
<td>14.7 ± 1.3*</td>
<td>14.7 ± 0.9</td>
<td>15.7 ± 0.9</td>
<td>15 ± 1</td>
<td>15.5 ± 0.6</td>
<td>15 ± 0.6</td>
<td>14.7 ± 0.9</td>
<td>14.6 ± 0.3</td>
</tr>
<tr>
<td>Bep</td>
<td>16 ± 0.6</td>
<td>13.3 ± 0.3</td>
<td>16 ± 0.6</td>
<td>15 ± 0.6</td>
<td>15 ± 0</td>
<td>14.7 ± 0.9</td>
<td>15.3 ± 0.3</td>
<td>15 ± 0</td>
</tr>
<tr>
<td>Untreated</td>
<td>14.9 ± 0.6</td>
<td>14 ± 0.6</td>
<td>14 ± 0.6</td>
<td>16 ± 0.6</td>
<td>15.3 ± 0.9</td>
<td>14.7 ± 1.2</td>
<td>14.5 ± 0.5</td>
<td>15.5 ± 0.5</td>
</tr>
</tbody>
</table>

* Mean ± S.E.
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