Alteration of Cell-mediated Immunity in the Mouse following Administration of 4-Nitroquinoline 1-Oxide

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SUMMARY

The effect of 4-nitroquinoline 1-oxide on the cell-mediated immune response was tested in C57BL/6 female mice. The carcinogen was administered s.c. either 30 days before or just prior to grafting the female recipients with C57BL/6 male skin. Skin graft survival times were noticeably prolonged in mice that received the carcinogen 30 days before grafting. Administration of 4-nitroquinoline 1-oxide just prior to skin grafting, however, yielded an unexpected result in that this group of mice rejected their grafts much sooner than their controls. The cause of this accelerated rejection is not presently known.

The tumors that arose following s.c. implantation of 4-nitroquinoline 1-oxide pellets were moderately to highly immunogenic and had a short latent period. In this respect they are comparable to tumors induced with the polycyclic hydrocarbon carcinogens.

INTRODUCTION

The depression of the humoral immunological system has been well demonstrated for the polycyclic hydrocarbon carcinogens (13, 17, 18, 20, 21), aminoazo dyes (1) and, more recently, urethan (9, 10). Interference with the humoral immunological system has also recently been documented for 4-NQO2 by Phillips (11) and Nahashima and Ono (8). However, a more crucial point in the induction of tumors may be whether the cell-mediated immunological system is affected following carcinogen administration.

Delayed skin graft rejection times have been demonstrated by Prehn (14), across a weak male-specific histocompatibility barrier, approximately 1 month after application of MCA. Following the application of polycyclic hydrocarbon carcinogens, Linder (6), Stjernswärd (20), and Rubin (17, 18) have shown that impairment of the homograft response was not readily demonstrated until the mean tumor latent period was reached. Similar delayed skin graft rejection times following treatment with carcinogenic aminoazo dyes (1) or urethan (4, 5, 9) have been demonstrated. This delayed graft rejection time seen following treatment with chemical carcinogens was a crucial factor in the hypothesis of Prehn (14) that interference with the immune response is one aspect associated with the tumorigenic action of chemical carcinogens.

The immunosuppressive nature of the carcinogen appears to correlate with the arial of carcinogen-induced tumors of high immunogenicity. MCA and other hydrocarbon carcinogens that produce a marked impairment of the homograft responses produce tumors of relatively high antigenicity and shorter latent periods (15, 22); whereas tumors produced following treatment with carcinogens that interfere to a lesser degree with the cellular immune response, such as urethan or film carcinogenesis, tend to produce less immunogenic tumors (13).

These experiments were performed to study the effect of 4-NQO on the cell-mediated immunological system. An attempt was also made to determine whether a correlation exists between the tumor latent period and the immunogenicity of the tumors produced following application of 4-NQO occurred.

MATERIALS AND METHODS

Carcinogen Administration. 4-NQO was suspended in 0.9% NaCl solution at a concentration of 5 mg/ml or was suspended in paraffin and impregnated onto Millipore filter strips to form pellets containing approximately 1.2 mg 4-NQO. Each mouse was given either a carcinogen-impregnated MLF s.c. or an injection of 0.1 ml of the NaCl suspension. The mice were treated with the carcinogen either on the day of grafting or approximately 30 days prior to grafting.

Skin Grafting. Split-thickness skin grafts were prepared from C57BL/6 male mice as described by Thoenes (23). Skin was excised from donor mice and, after removal of the underlying muscle and fat, a no. 9 cork borer was used to punch out 13-mm-diameter grafts. Split-thickness graft beds were cut on the C57BL/6 female recipients according to the method described by Billingham (2). Terramycin-polymyxin B powder (Charles Pfizer and Co., New York, N. Y.) was dusted onto the raw graft bed prior to application of the skin graft. Petroleum jelly-impregnated gauze (Adaptic Non-Adhering Dressing; Johnson & Johnson, New Brunswick, N. J.) was placed over the grafts which were then secured with Air Vent Tape (Johnson & Johnson). Tape and gauze bandages were removed 10 days after grafting.

Grafts were inspected 2 to 3 times a week and the time of complete rejection was scored. Mean survival times and their standard deviations were computed by the method of Litchfield (7). Statistical comparison of the data was accomplished with the Mann-Whitney U test (18).

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2The abbreviations used are: 4-NQO, 4-nitroquinoline 1-oxide; MCA, methylcholanthrene; MLF, Millipore filter pellet.

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Tumor Immunogenicity. The relative immunogenicity of tumors that arose in the 4-NQO-treated mice was calculated by a method similar to that described by Prehn and Main (16). The tumor was first passaged at least once in a thymectomized and irradiated isologous mouse (450 R) to provide adequate amounts of the tumor for the subsequent immunization in tumor immunogenicity tests and also to serve as a repository to provide the source of tumor for challenge. One group of isologous mice were inoculated with a trocar-sized piece of tumor. The tumors were allowed to grow to approximately 6 to 8 mm in diameter, and they were then excised. The control group was sham excised on the same day. Ten to 14 days following excision or sham excision of the tumors, both groups of mice were challenged with a trocar piece of the tumor. When two-thirds of either group reached an average tumor diameter of >5 mm, the tumor immunogenicity ratio was calculated by dividing the mean tumor diameter of the control mice by the mean tumor diameter of the immunized mice. The resultant quotient if larger than 1 was indicative that the tumor being tested was immunogenic; if equal to 1 the tumor was nonimmunogenic, and if less than 1 it was assumed that the tumor could elicit a tumor-enhancing effect.

RESULTS

Effect of 4-NQO on Skin Graft Rejection and Its Relationship to Time of Administration. Mice given injections of the 4-NQO suspension 30 days prior to their being grafted were highly immunosuppressed. The average time of rejection in the controls was 24 days, while in the carcinogen-treated mice it was 31 days (Table 1). This 7-day difference was statistically very significant (2p = 0.0009) with the Mann-Whitney U test.

In contrast, the result seen when the mice were given grafts immediately after carcinogen administration was not expected. We expected either to see no difference between the control and experimental groups or to at least see a trend in the direction of immunosuppression in the carcinogen-treated animals. However, the exact opposite was noted, namely immunostimulation. The grafts on the carcinogen-treated recipients were rejected faster than those of the controls. The mean survival time in the experimental groups was 17 days, and in the control group it was 22 days. These groups were significantly different when evaluated with the Mann-Whitney U test (2p = 0.015).

Relative Tumor Immunogenicity. Six tumors that arose in either C57BL/6 or C3H mice implanted s.c. with MLF containing 4-NQO in paraffin were moderately to highly immunogenic. According to the immunogenicity test described, these tumors had an antigenic ratio that ranged from 1.73 to 10.60.

Tumor Incidence and Latency. Five of 12 C3H and 6 of 14 C57BL/6 mice implanted with MLF containing 2% 4-NQO in paraffin developed tumors that histologically were fibrosarcomas. This yielded an approximate tumor incidence of 41 and 43%, respectively, over an 11-month observation period. Eight of the 11 tumors have been banked in a liquid nitrogen tissue bank and have subsequently been recovered. The mean tumor latency period was approximately 6 months.

DISCUSSION

Demonstration that cellular-mediated immunosuppression has occurred is very often extremely dependent on the type of assay system used. There has been much discussion in the literature on this point. In many experiments for which the investigators have used carcinogens with the intent of demonstrating their immunosuppressive capability, the authors have been able to demonstrate good immunosuppressive results only when the antigenic differences of the test system were weak, for example, in the C57BL/6 male to female skin graft assay or across a very weak non-H-2 difference (e.g., BALB/c X DBA/2 strain grafted onto DBA/2).

Linder (6) could demonstrate only that MCA reduced the immune response, using a skin graft assay when the test system was based on a non-H-2 difference, and then only when the mean tumor latent period was reached. He was unable to show immunosuppression with a strong H-2-different allograft test system. Prehn (12), using a male antigen isograft test system to demonstrate immunosuppression following MCA treatment, was able to show that some immunosuppression had occurred 1 month after the carcinogen treatment; in addition, he stated that previous experiments with a stronger antigenic system failed to reveal the immune depressive action of MCA. This immunosuppressive aspect of the polycyclic hydrocarbon carcinogens was one of the cornerstones in Prehn's (14) clonal selection theory of chemical carcinogenesis. The validity of the concept that chemical carcinogens are immunosuppressants has been well documented for the polycyclic hydrocarbon carcinogens (12, 17, 18, 20–22) and for urethan (4, 5, 9, 10).

The effects of 4-NQO on the immune response have been described by Phillips (11); however, she was not able to demonstrate any depression of the cell-mediated immunological phenomena. Possibly, this may be explained in that the antigenic differences in her cell-mediated test assay systems used were too strong. Another reason that may be of importance was that the assays to test for depression of the cell-mediated immunity were started long before the carcinogen was able to exert its immunosuppressive effects. That this is a crucial point at least for the hydrocarbon carcinogens has been well documented by Rubin (17, 18), Stjernswärd (20, 21), and Prehn (12). That the time period is also crucial in 4-NQO-induced immunosuppression is documented in this paper.

In the experiments in which the 4-NQO was administered just before the skin grafts were applied, an unexpected result was obtained in that the skin grafts were rejected much earlier.

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Table 1
Survival of C57BL/6 male skin grafts on C57BL/6 female recipients

<table>
<thead>
<tr>
<th>Group</th>
<th>Time of 4-NQO injection (days)</th>
<th>N</th>
<th>Survival time ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>46</td>
<td>24 ± 1.39</td>
</tr>
<tr>
<td>4-NQO</td>
<td>30</td>
<td>46</td>
<td>31 ± 1.44</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>54</td>
<td>22 ± 1.54</td>
</tr>
<tr>
<td>4-NQO</td>
<td>0</td>
<td>76</td>
<td>17 ± 1.67</td>
</tr>
</tbody>
</table>

* Mean ± S.D.; calculated by the method of Litchfield (7).
than the controls. This was quite contrary to the expected result of either no difference or a small increase in graft survival over that of the controls. The significant difference in these experiments was quite surprising and has been alluded to by others who were demonstrating the immunosuppressive capability of urethan (5) or hydrocarbon (17) carcinogens.

The mechanism for this type of action may be the depression of the humoral immune system either to a greater extent or earlier than that of the cell-mediated system. Griswold et al. (3) have shown that by using a chemical immunosuppressant they are able to reduce selectively either the humoral immune response or both the humoral and cellular responses to a specific antigenic challenge.

Phillips (11) and more recently Nahashima and Ono (8) reported that immunosuppression of the humoral immune response occurred early after the administration of 4-NQO. From the experiments reported here and those of Phillips (11) and Nahashima and Ono (8), it is perhaps possible to state that following 4-NQO administration the depression of the humoral immune response occurs much earlier than that of the cell-mediated one. The probable cause of the earlier skin graft rejections, seen in experiments in which the 4-NQO carcinogen was administered just prior to grafting, may thus be due to a selective and early depression of the humoral antibody system. The selective depression of the humoral antibody might therefore allow sufficient time for the still active cell-mediated immunological system to act, producing the earlier graft rejections.

The demonstration of depressed cellular immunity approximately 30 days after 4-NQO administration also reinforces the validity of the assumption that immunosuppression and carcinogenic activity are related, as has previously been demonstrated for the polycyclic hydrocarbon, urethan, and film carcinogens. The moderate to high levels of immunogenicity expressed by these tumors may possibly be correlated with the relatively strong immunodepression provided by the 4-NQO.

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REFERENCES

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