Type II Interferon Induction and Passive Transfer Depress the Murine Cytochrome P-450 Drug Metabolism System

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Induction of type II interferon by sensitization of mice with Mycobacterium tuberculosis strain BCG and challenge with tuberculin resulted in a depression of the cytochrome P-450 drug metabolism system of the liver. The degree of depression was significantly greater than in mice that were only sensitized to BCG. Cytochrome b5 levels were not affected. In addition, the level of the depression of the cytochrome P-450 system correlated with the levels of type II interferon induced. Passive transfer of exogenous type II interferon preparations also significantly depressed the cytochrome P-450 system. Passive transfer of mock interferon or of normal serum had no effect.

Several studies have indicated that application of inducers of interferon to animals results in specific depression of the cytochrome P-450 drug metabolism system of the liver (11, 12). In addition, induction of interferon also resulted in decreased metabolism of the drug phenytoin (10).

All of the interferon inducers to date, including tilorone-hydrochloride and polyriboinosinic-polycytidylic acid, are potent inducers of type I or "classical" interferon. Recently a new type of interferon that is a product of an immune response, type II interferon, has been described (15). Interferons have been shown to have several activities that are not directly antiviral, including regulation of immune responses (5). Type II interferon has been shown to be significantly more potent in regulation of immune responses than type I interferon (13).

The effects of interferon inducers on the cytochrome P-450 system may involve interaction with the immune response (3, 14). Since type II interferon is such an active immunoregulatory agent, it was of interest to determine the effects of type II interferon on the cytochrome P-450 drug metabolism system. The results of the present study suggest that induction of type II interferon by sensitization of mice with Mycobacterium bovis strain BCG and challenge with tuberculin results in depression of the cytochrome P-450 system. In addition, passive transfer of exogenous type II interferon preparations to normal mice also resulted in depression of the cytochrome P-450 system of recipient mice.

MATERIALS AND METHODS

Mice. Randomly bred 6- to 8-week-old female Swiss/Webster mice were supplied by Laboratory Supply, Inc., Indianapolis, Ind.

Interferon production. Mouse type II interferon was produced in vivo by intravenous injection of 50 mg of tuberculin (Jensen-Salsbery Labs, Kansas City, Mo.) into mice sensitized 3 to 4 weeks previously with M. bovis strain BCG (15). Type II interferon activity was in the serum. The interferon produced in this manner, which was used both in induction and passive transfer experiments, was characterized as type II interferon due to the following characteristics: pH 2 lability, 56°C stability, species specificity, and lack of neutralization by an antibody directed against type I interferon (15). Mock-type II interferon was produced by inoculation of 50 mg of tuberculin into nonsensitized mice.

Interferon assay. Antiviral activity was determined by means of a plaque reduction assay utilizing the Indiana strain of bovine vesicular stomatitis virus on murine L929 cells (13). The interferon titer corresponded to the reciprocal of the greatest dilution of test sample that reduced virus plaques by 50%. One interferon unit equals 0.88 National Institutes of Health no. G-002-904-511 reference standard units in this assay.

Preparation of microsomes. The mice were killed by cervical dislocation, and their livers were excised and weighed. Thereafter all procedures were carried out in the cold (0 to 4°C). The livers were homogenized in isotonic KCl solution using a Dounce homogenizer and were then centrifuged for 20 min at 9,000 × g. The microsomal fraction was prepared by centrifuging the 9,000 × g supernatant at 105,000 × g for 1 h. The microsomal pellet was suspended in KCl. All microsomal preparations were used the day they were prepared. Microsomal protein contents were determined by the method of Lowry et al. (6).

Enzyme and spectral measurements. The reaction mixture used to determine the activity of aminopyrine N-demethylation contained the following constituents in 5 ml of 0.15 M KCl-0.2 mM phosphate buffer (pH 7.4): nicotinamide adenine dinucleotide phosphate, 2 μmol; glucose 6-phosphate, 20 μmol; magnesium chloride, 10 μmol; semicarbazide, 37.5 μmol; glucose 6-phosphate dehydrogenase, 2 units; amino-
pyrine, 25 µmol; and 3 mg of microsomal protein. Formaldehyde produced by the N-demethylation of aminopyrine was determined by the method of Nash (7). Reactions were allowed to progress for 15 min before assay. All spectrophotometric readings were performed in a Cary model 219 spectrophotometer. Cytochromes P-450 and b5 were determined spectrophotometrically by the method of Omura and Sato (8).

Statistical analysis. Student's t test and Duncan's new multiple-range test were used as a test of the null hypothesis (2). Data used in the determination of the Michaelis constants were analyzed with the aid of a computer program written by Cleland (1).

RESULTS

Effects of treatments on body weight, spleen, liver weight, and microsomal proteins. When mice were intravenously sensitized with 10^9 to 10^7 BCG and examined 3 weeks later, body weight was decreased 10%, spleen weight was increased 100%, liver weight was increased 80%, and no effect was observed on microsomal protein content. Mice that were sensitized with BCG, challenged with tuberculin (inducing type II interferon), and examined 18 to 24 h later had patterns of increases and decreases of the above parameters identical to those of mice only sensitized with BCG.

Challenge of normal mice with tuberculin had no effect on body, spleen, and liver weight and on microsomal protein content when the mice were examined 18 to 24 h after challenge. Passive transfer of type II interferon, mock interferon, or normal serum also had no effect on body, spleen, and liver weight and microsomal protein content of recipient mice.

Effects of tuberculin and BCG administration on cytochrome P-450 system. Intravenous administration of 50 mg of tuberculin had no effect on aminopyrine N-demethylase, cytochrome P-450, and cytochrome b5 levels when the mice were examined 18 h after tuberculin administration (Table 1). Sensitization of mice with 10^6 to 10^7 BCG resulted in a significant decrease in aminopyrine N-demethylase and cytochrome P-450 levels, but had no effect on cytochrome b5 levels when the mice were examined 3 weeks after sensitization (Table 1). Results shown are a representative experiment.

Effects of type II interferon induction on cytochrome P-450 system. When mice were sensitized with 10^6 to 10^7 BCG and challenged with 50 mg of tuberculin 3 weeks later, effectively inducing high levels of type II interferon, aminopyrine N-demethylase and cytochrome P-450 levels were significantly depressed as compared to mice only sensitized to BCG for 3 weeks (Table 2). The parameters were examined 18 h after tuberculin challenge. Cytochrome b5 levels were not affected by induction of type II interferon (Table 2).

The dosage of tuberculin used to induce type II interferon was serially decreased in mice sensitized with BCG. The degree of depression of aminopyrine N-demethylase and cytochrome P-450 levels correlated with the levels of interferon induced, i.e., the greater the levels of type II interferon induced, the greater the depression of the parameters studied (Table 3). Results shown are representative experiments.

Effects of induction of type II interferon on the apparent kinetic constants of aminopyrine metabolism. When mice were sensitized with 10^6 to 10^7 BCG and challenged with 50 mg of tuberculin 3 weeks later, effectively inducing high levels of type II interferon, aminopyrine N-demethylase and cytochrome P-450 levels were significantly depressed as compared to mice only sensitized to BCG for 3 weeks (Table 2). The parameters were examined 18 h after tuberculin challenge. Cytochrome b5 levels were not affected by induction of type II interferon (Table 2).

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Effects of induction of type II interferon on the apparent kinetic constants of aminopyrine metabolism.

**Table 1. Effects of tuberculin and BCG on the cytochrome P-450 drug metabolism system**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Aminopyrine N-demethylase</th>
<th>Cytochrome P-450</th>
<th>Cytochrome b5</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3</td>
<td>909.7 ± 193.3</td>
<td>0.77 ± 0.07</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>Tuberculin (50 mg)</td>
<td>3</td>
<td>925.6 ± 43.0</td>
<td>0.70 ± 0.03</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>BCG</td>
<td>3</td>
<td>574.6 ± 29.6</td>
<td>0.37 ± 0.03</td>
<td>0.14 ± 0.02</td>
</tr>
</tbody>
</table>

* Nanomoles of formaldehyde formed per milligram of protein per hour ± standard error.
* Nanomoles per milligram of protein ± standard error.

**Table 2. Effects of induction of type II interferon on the cytochrome P-450 drug metabolism system**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Aminopyrine N-demethylase</th>
<th>Cytochrome P-450</th>
<th>Cytochrome b5</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCG</td>
<td>4</td>
<td>356.6 ± 48.8</td>
<td>0.54 ± 0.06</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>BCG + tuberculin</td>
<td>4</td>
<td>196.2 ± 17.7</td>
<td>0.28 ± 0.03</td>
<td>0.10 ± 0.02</td>
</tr>
</tbody>
</table>

* Nanomoles of formaldehyde formed per milligram of protein per hour ± standard error.
* Nanomoles per milligram of protein ± standard error.
* Interferon induced.

Statistically significant decrease (P < 0.05).
TABLE 3. Dose response of the effect of induction of type II interferon on the cytochrome P-450 drug metabolism system

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Aminopyrine N-demethylase</th>
<th>Cytochrome P-450</th>
<th>Interferon titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCG only</td>
<td>4</td>
<td>207.4 ± 11.2</td>
<td>0.24 ± 0.02</td>
<td>≤10</td>
</tr>
<tr>
<td>BCG + tuberculin</td>
<td>4</td>
<td>217.8 ± 8.9</td>
<td>0.29 ± 0.04</td>
<td>30</td>
</tr>
<tr>
<td>BCG + tuberculin</td>
<td>50</td>
<td>162.6 ± 7.3</td>
<td></td>
<td>1,235</td>
</tr>
</tbody>
</table>

* Nanomoles of formaldehyde formed per milligram of protein per hour ± standard error.

** Nanomoles per milligram of protein ± standard error.

P < 0.05 versus BCG + tuberculin.

P < 0.02 versus BCG.

P < 0.001 versus BCG + tuberculin.

nopyrine N-demethylation. The Michaelis constants of aminopyrine N-demethylation by microsomes from untreated, BCG-treated, and BCG + tuberculin-treated (interferon-induced) mice suggest that there may be a qualitative change in aminopyrine N-demethylase of mice in which type II interferon has been induced (Table 4). Corresponding $V_{\text{max}}$ values from the three groups show that BCG treatment decreased the amount of enzymatic activity; however, induction of type II interferon caused an even larger decrease in activity (Table 4).

Effect of exogenous type II interferon preparations on the cytochrome P-450 system. Passive transfer of 6,000 units of type II interferon preparations resulted in a significant decrease in aminopyrine N-demethylase and cytochrome P-450 levels of the recipient mouse (Table 5). Cytochrome $b_5$ levels were not affected by passive transfer of type II interferon preparations. Passive transfer of equivalent volumes of normal serum or of mock interferon did not affect levels of aminopyrine N-demethylase, cytochrome P-450, and cytochrome $b_5$ of recipient mice (Table 5). All parameters were examined 18 h after receipt of the last dosage of interferon or other preparation. Results shown are a representative experiment.

**DISCUSSION**

The induction of type II interferon in mice appears to depress the cytochrome P-450 drug metabolism system of the liver. The degree of depression is correlated to the titer of interferon induced. In addition, passive transfer of type II interferon preparations also results in depression of the cytochrome P-450 system. This decrease appears to be specific for the cytochrome P-450 system, since cytochrome $b_5$ levels are not affected.

As has been previously reported for inducers of type I interferon (11, 12), sensitization with BCG and induction of type II interferon resulted in minimal effects on body weight and microsomal protein levels; however, in the present study, both liver and spleen weights were dramatically increased. This increase may have been due to the development of infectious foci of BCG in those organs (9).

BCG, Corynebacterium parvum, and other agents that affect the immune response have been shown to decrease the cytochrome P-450 system (3, 14). Since many inducers of type I interferon, such as polyriboinosinic-polyribocytidylic acid, can directly affect the immune response, previous workers have suggested that the effects of the agents on the cytochrome P-450 system may be the result of an interaction of the inducing agents with the immune response not involving interferon (10). The results of the present study indicate that although BCG sensitization depresses the cytochrome P-450 system, induction of type II interferon in BCG-sensitized animals further depresses the cytochrome P-450 system. In addition, the degree of depression correlated with the level of interferon induced, and passive transfer of type II interferon preparations containing no BCG to normal animals also resulted in depression of the cytochrome P-450 system. These data suggest that interferon may be directly involved in suppressing the cytochrome P-450 system.

Nevertheless, the effects of interferon on the cytochrome P-450 system may still involve interaction with the immune response. Interferon is a very potent immunoregulatory agent (13), and type II interferon preparations are as much as 100 times more potent in their immunoregulatory activities than type I interferon (13). Therefore, the effects of interferon on the drug metabolism system may involve mediation of...
the immune response by interferon. This is especially supported by the ability of passively transferred type II interferon (the more potent immunoregulatory interferon) to depress the cytochrome P-450 system, whereas equivalent amounts of passively transferred type I interferon have no effects (unpublished data).

It must be noted that when interferon is induced, several other lymphokines, or mediators of cellular immunity, are also induced (15). The lymphokines, including migration inhibitory factor, are also found in type II interferon preparations, which have not been completely purified to date (15). These lymphokines may also play some role in the effects of interferon on the cytochrome P-450 system.

In view of current clinical trials involving interferon (4), it is important to note that interferon can affect the cytochrome P-450 drug metabolism system. Coadministration of interferon with a drug could lead to prolonged retention of the drug, possibly resulting in either beneficial or deleterious effects.

ACKNOWLEDGMENTS

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LITERATURE CITED