Effect of Actinomycin D on Immune Antibody, Normal Antibody, and Complement

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Received for publication 3 September 1965

ABSTRACT

The present work was undertaken to study the effect of actinomycin D on the immune response when administered simultaneously with antigen and to determine the effect of actinomycin D on naturally occurring antibody levels. Since it has not been established unequivocally that naturally occurring antibodies arise partly as a result of normal physiological maturation or solely by immunization (Hook et al., Nature, in press), the results obtained might contribute to the elucidation of this problem. In addition, the effect of actinomycin D on complement and Cx-reactive protein (CxRP) levels was determined with amounts of the antibiotic that suppressed the primary immune response to an antigen deliberately injected into the animals.

MATERIALS AND METHODS

Immunization schedules. White female rabbits (about 2.7 kg each) were divided into five groups. Group I rabbits received intravenous injections of ovalbumin (twice crystallized, Worthington Biochemical Corp., Freehold, N.J.), 30 μg on day 1, 60 μg on day 3, and 120 μg on day 5. Group II rabbits received the same amount of ovalbumin, but, in addition, were injected intraperitoneally with 85 μg of actinomycin D (obtained through the courtesy of Elmer Alpert, Merck Sharp and Dohme Research Laboratories) each day on days 1 to 5. Group III rabbits received only the actinomycin D in 85-μg amounts each day on days 1 to 5. Group IV rabbits were injected intravenously with 25 μg of endotoxin of Salmonella typhosa O901 (product of Difco, prepared by the Westphal method) on day 1 and 50 μg of this substance on day 3. Group V rabbits were injected similarly with the endotoxin and also with 85 μg of actinomycin D each day on days 1 to 5.

All animals were bled before injection on day 1, bled again on day 8 (3 days after the final injection), and finally bled on day 10, except that half of the animals in group III were bled on days 1, 6, and 8 instead of 1, 8, and 10.

Prior to day 10, there were two deaths among the seven rabbits in group II, two deaths among the six rabbits in group IV, and one death among the six rabbits in group V. It appeared, therefore, that appreciable lethality was associated with the doses of endotoxin and actinomycin D used.

Serological procedures. The antibody response to ovalbumin was determined by a quantitatively stand-
ardized complement-fixation method (6), by use of ovalbumin at an amount found to be optimally re-active (2.5 μg). The immunological response to the endotoxin was determined by the titration of bactericidal antibody against the organism from which the endotoxin was derived, by a quantitatively standardized photometric assay (5). Appropriate control tests indicated that actinomycin D, which may have been present in the test sera, exerted no detectable bactericidal effect. Complement was assayed by the same method used for the standardization of complement in the complement-fixation test (6), and represents the complement titer. CxRP was determined by the precipitin reaction resulting from the mixture of equal volumes of the test sera and anti-CxRP (Mann Research Laboratories) in a capillary tube.

**RESULTS**

The primary anti-ovalbumin response in those rabbits injected with ovalbumin plus actinomycin D, compared to those injected with ovalbumin alone, was prevented temporarily, as indicated by the results on day 8, and was reduced at day 10, which is 5 days after the completion of the anti-

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**TABLE 1. Complement-fixing antibody titers in response to injections of ovalbumin**

<table>
<thead>
<tr>
<th>Day of test</th>
<th>Group I (ovalbumin alone)</th>
<th>Group II (ovalbumin and actinomycin D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preimmunization, day 1</td>
<td>&lt;2.5†</td>
<td>&lt;2.5†</td>
</tr>
<tr>
<td>Postimmunization, day 8</td>
<td>5.9</td>
<td>&lt;2.5†</td>
</tr>
<tr>
<td>Postimmunization, day 10</td>
<td>14.1</td>
<td>8.7</td>
</tr>
</tbody>
</table>

* Results are given in terms of geometric mean titers of four rabbits in group I and of five rabbits in group II.
† No detectable antibody was found in any of the sera from these rabbits.

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**TABLE 2. Bactericidal antibody titers against Salmonella typhosa O901 in rabbits subjected to actinomycin D alone, S. typhosa endotoxin alone, and S. typhosa endotoxin plus actinomycin D**

<table>
<thead>
<tr>
<th>Day of test</th>
<th>Group III (actinomycin D alone)</th>
<th>Group IV (endotoxin alone)</th>
<th>Group V (actinomycin D plus endotoxin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preimmunization, day 1</td>
<td>132</td>
<td>295</td>
<td>224</td>
</tr>
<tr>
<td>Postimmunization, day 8</td>
<td>174</td>
<td>36,300</td>
<td>11,300</td>
</tr>
<tr>
<td>Postimmunization, day 10</td>
<td>116</td>
<td>29,500</td>
<td>9,120</td>
</tr>
</tbody>
</table>

* Results are given in terms of geometric mean titers of four or five animals in each group.

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**TABLE 3. Complement and CxRP levels in rabbits injected with actinomycin D (group III)**

<table>
<thead>
<tr>
<th>Day of test</th>
<th>Complement titer*</th>
<th>CxRP†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment, day 1</td>
<td>98</td>
<td>0</td>
</tr>
<tr>
<td>Post-treatment, day 6</td>
<td>174</td>
<td>3.0</td>
</tr>
<tr>
<td>Post-treatment, day 8</td>
<td>162</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* Geometric mean results from four animals.
† Expressed as millimeters of precipitate.

gen injections (Table 1). Actinomycin D also suppressed the bactericidal antibody response to *S. typhosa* endotoxin (comparison of groups IV and V in Table 2), although a very substantial response may be noted at day 8, only 3 days after cessation of the administration of the antibiotic. Yet it did not lower the level of naturally occurring bactericidal antibody in those animals not deliberately injected with endotoxin. In fact, administration of actinomycin D without the antigenic stimulus of the endotoxin resulted in a slight rise, from a mean titer of 132 to 174, in bactericidal antibody against *S. typhosa* (Table 2). Similarly, hemolytic complement levels were increased in animals subjected to actinomycin D, and CxRP made its appearance in those animals (Table 3).

**DISCUSSION**

The effect of actinomycin D in causing a delay in the formation of anti-ovalbumin in rabbits is in agreement with previous results indicating that actinomycin D may delay the immune response of rats to sheep erythrocytes and β-galactosidase (9). These results are compatible, therefore, with the premise that DNA-dependent messenger RNA is synthesized during the induction phase of antibody formation and that actinomycin D specifically inhibits this synthesis.

The appearance of anti-ovalbumin at day 10 (Table 1), despite the administration of actinomycin D, is easily explained. Since the administration of actinomycin D ceased after the 5th day in our experiments, the antibiotic became, therefore, increasingly less available for binding with newly synthesized DNA, and messenger RNA production may have begun by the 10th day, with subsequent synthesis of anti-ovalbumin. Of considerable interest is the fact that the same amounts of actinomycin D which effected a significant suppression of a primary immune response against ovalbumin did not cause a decline in the levels of normal antibody against unrelated *S. typhosa*. On the contrary, the mean antibody titer was 30% higher after administra-
tion of the antibiotic. Antigenic differences between *S. typhosa* and ovalbumin are probably not contributory to this result, since actinomycin D also exerted a significant diminution of the antibody response to *S. typhosa* endotoxin (titer of 11,300 compared to 36,300 at day 8, and 9,120 compared to 29,500 at day 10) when the endotoxin was deliberately injected.

These observations suggest that there may be differences in the mechanism of formation of normal antibody and those antibodies elicited as a result of the deliberate injection of an antigen. Whatever cells or processes may be involved in the production of normal antibodies, it is clear that they are relatively refractory to actinomycin D compared to those mechanisms involved in antibody synthesis resulting from the deliberate injection of antigen. On the other hand, actino-
mycin D would not be expected to exert a suppressive effect on normal antibody formation if the messenger RNA molecules involved in the formation of such antibodies were present and available at the time of injection of the actinomycin D. Moreover, observations have been made that some messenger RNA is relatively stable (16 hr) and not rapidly degraded (1). Of course, the dosage of actinomycin D in our experiments was rather large, prolonged for 5 days, and sufficed to prevent the appearance of antibody to ovalbumin at day 8, so that unusual stability would be required for an explanation on this basis. Resolution of this question obviously requires studies on the turnover rate of messenger RNA involved in the synthesis of antibody. Whatever the true explanation, however, normal antibody is also relatively resistant to X irradiation compared to antibody formation elicited as a result of deliberate immunization (3, 4), and similar factors may be involved.

The effect of actinomycin D in causing increased levels of complement activity, and the appearance of CxRP (Table 3), indicate that the effects of this antibiotic, direct and indirect, may be quite diverse. The appearance of CxRP may be taken merely as an index of tissue injury. Similarly, enhanced levels of complement are associated with an acute-phase reaction (2). The adjuvant effect of actinomycin D, noted by Claman and Bronsky, may be related also to these phenomena. Other adjuvants, such as endotoxin and Freund's adjuvant, also give rise to C-reactive protein or CxRP (7). In summary, actinomycin D, which is capable of suppressing a primary antibody response, may, on the other hand, elicit a rise in other substances concerned with immunity, such as normal antibody and complement.

**Acknowledgment**

This investigation was supported by Public Health Service grant AI-05454 from the National Institute of Allergy and Infectious Diseases.

**Literature Cited**