Effect of Corynebacterium acnes on Interferon Production in Mice

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Received for publication 6 March 1972

Exposure to Corynebacterium acnes, the most prominent member of our normal skin flora, produces stimulation of lymphoid tissue and certain reticuloendothelial system functions, as well as the immune response. Alteration of the host response is extended by these studies to include changes in the pattern of interferon production in response to a representative group of inducing agents. Serum interferon levels induced by the injection of endotoxin in mice are enhanced, whereas interferon production after injection of Newcastle disease virus, Chikungunya virus, and polyinosinic:polycytidylic acid is depressed in animals inoculated with viable or nonviable C. acnes organisms.

Anaerobic diphtheroids, such as Corynebacterium parvum, have been shown to have stimulatory effect on the reticuloendothelial system in experimental animals. This effect is characterized by enlargement of the liver and spleen, increased phagocytic activity as measured by carbon clearance, and an enhanced immune reactivity (11, 15-17). Similar effects have been noted with C. acnes, an anaerobic diphtheroid which is a prominent member of the normal skin flora (P. A. Farber and R. F. Smith, Bacteriol. Proc., p. 83, 1969).

(It was recently proposed that C. acnes and C. parvum be dropped from the genus Corynebacterium on the basis that they share too few properties with the other members of the genus [1] and be placed in the genus Propionibacterium.) Injection of either viable or heat-killed organisms in mice produced hepatic and splenic hypertrophy. Animals exposed to C. acnes also produced greater amounts of antibody as compared to untreated controls when they were challenged with sheep red cells. Anaerobic diphtheroids appear to have a stimulatory effect on both macrophage and lymphoid elements of the reticuloendothelial system (18). Evidence that these cells are an important source of circulating interferon (9, 12, 20, 22) suggested that the ability of C. acnes to influence their activity might modify production of this antiviral substance.

MATERIALS AND METHODS

C. acnes, from the American Type Culture Collection, was grown on brain-heart-infusion agar slants in Brewer jars. After 5 to 7 days of growth, the organisms were suspended and washed twice in phosphate-buffered saline. The concentration of the inoculum was adjusted to 5 X 10⁶ organisms per ml. Heat-killed organisms were prepared by incubating the inoculum at 60°C for 1 hr. Female CD-1 mice, 6 to 8 weeks of age, were injected intravenously or intraperitoneally with 0.2 ml of the C. acnes suspension. Seven to 14 days later, these animals, along with uninfected controls, were injected intraperitoneally with virus, bacterial lipopolysaccharide (endotoxin), or poly I:C.

Virus. Newcastle disease virus (NDV), Herts strain, grown in the allantoic cavity of embryonated eggs titered 2 X 10⁸ plaque-forming units (PFU) per ml. Vesicular stomatitis virus, Indiana strain, was obtained from the American Type Culture Collection. Stock virus pools were grown in L-929 cells and titered approximately 2 X 10⁶ PFU/ml. Chikungunya virus (CV) was obtained from Philip Russell, Walter Reed Army Medical Center. Stock virus was prepared from the brains of infected suckling mice, made into a 10% suspension, and assayed by the plaque method in primary chick embryo fibroblasts. Virus pools used for induction of interferon production titered approximately 2 X 10⁶ PFU/ml.

Interferon inducers. Bacterial lipopolysaccharide (endotoxin), prepared according to the Westphal phenolic extraction method from Escherichia coli O111 B4, was obtained from Difco Laboratories. Animals were inoculated with 100 μg by the intraperitoneal route.

Polyinosinic:polycytidylic acid (Poly I:C [P-L Laboratories]), dissolved in phosphate-buffered saline, was inoculated by the intraperitoneal route at a dosage of 100 μg/mouse.

Interferon assay. Groups of five animals were bled at each sampling time; the sera were harvested, pooled, and assayed as previously described (14) by the 50% plaque-inhibition technique employing vesicular sto-
matitis virus as the challenge virus in L-929 cells obtained from the American Type Culture Collection. Each assay was standardized by incorporation of an internal laboratory mouse serum interferon standard which was in turn standardized with the international standard mouse interferon preparation obtained from the National Institutes of Health. One unit of interferon by our assay system equalled approximately 1.4 units of the international mouse standard.

RESULTS

The effect of *C. acnes* on the capacity of the host to produce interferon was determined by defining the interferon response to a representative group of inducing agents including bacterial endotoxin, two viruses, and a synthetic polynucleotide. These inducing agents were selected because they stimulated an interferon response which was markedly suppressed (NDV), partially inhibited (CV), unaffected (Poly I:C), or enhanced (endotoxin), by whole-body X-irradiation (4–7).

In preliminary experiments, animals infected with viable *C. acnes* organisms were found to have an altered capacity to produce interferon in response to a series of representative inducing agents. To eliminate the possibility that the observed effects were the result of replication of viable organisms, these experiments were carried out with nonviable, heat-killed preparations of organisms. The experiments were carried out between 7 and 14 days after inoculation of heat-killed *C. acnes*. This time was selected to be beyond the usual period when "tolerance" or "hyporeactivity" would be expected to be observed after injection of bacterial organisms. Furthermore, this coincided with the time of maximum cellular response as evidenced by peak hepatosplenicmegaly (14 to 16 days) in response to an inoculation of the nonviable *C. acnes* preparation.

The effect of the inoculation of 10⁹ heat-killed *C. acnes* organisms was documented in each experiment. Representative spleen and liver weights, as well as peripheral white-blood-cell counts from noninfected control and *C. acnes* inoculated animals, are summarized in Table 1. Animals infected with *C. acnes* demonstrated striking hepatosplenicmegaly and elevated white-blood-cell counts.

Endotoxin. In an initial series of experiments, the effect of *C. acnes* on the interferon response to endotoxin was determined. Seven days after inoculation with approximately 10⁹ heat-killed *C. acnes* organisms, animals were injected with 100 μg of *E. coli* endotoxin. The results of a representative experiment are shown in Fig. 1. Pretreatment with *C. acnes* significantly increased endotoxin-induced interferon response. There was both an enhancement in the peak serum response, 1,200 units/ml compared with 100 in the controls, and an increased duration of the response. Interferon was detectable in the serum of *C. acnes*-exposed animals as long as 8 hr after injection of endotoxin, whereas none was detected in controls after 4 hr.

Prior infection with other microorganisms, e.g., mycobacterium, has been recognized to enhance the host reaction to endotoxin (21, 23, 24). The enhancement of both the interferon response and susceptibility to the lethal effect of endotoxin was determined in mice inoculated with *C. acnes*. The results of one experiment are summarized in

<table>
<thead>
<tr>
<th>Table 1. Effect of inoculation of adult mice with heat-killed Corynebacterium acnes*</th>
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<tbody>
<tr>
<td><strong>Day</strong></td>
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<tr>
<td><strong>Control</strong></td>
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<td>11</td>
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<td>14</td>
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</table>

* Mean spleen weights at 14 days, in groups of five animals, were: control group, 134 mg (060–175); *C. acnes* group, 465 mg (372–550). Mean liver weights at 14 days were: control group, 2.3 (2.2–2.4); *C. acnes* group, 4.6 g (3.2–5.3).
Table 2. At all three dose levels (25, 50, and 100 μg), the experimental group of animals succumbed to a nonlethal inoculum of endotoxin for controls. Thus, the enhancement of interferon production was associated with an increase in susceptibility of the animal to the lethal effect of endotoxin.

**Virus.** In contrast to the stimulatory effect of *C. acnes* on the induction of interferon by endotoxin, the interferon response to NDV and CV was depressed. In these experiments, the viruses were inoculated by the intraperitoneal route at 7 to 14 days after infection with nonviable *C. acnes*.

The suppression was most pronounced when NDV was utilized as the inducing agent. The results from one of four similar experiments are illustrated in Fig. 2. At 6 hr after inoculation, the interferon level in the serum was 2,400 units/ml in control animals compared with 400 in the experimental group. When interferon production was stimulated by CV, a less striking suppression was observed. The data from one representative experiment are presented in Fig. 3.

**Table 2. Mortality after injection of endotoxin in *Corynebacterium acnes*-treated and control mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose of endotoxin (μg)</th>
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<th>50</th>
<th>100</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td><em>C. acnes</em></td>
<td></td>
<td>4/6</td>
<td>5/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

* Number dead/number injected.

**DISCUSSION**

Stimulation of the reticuloendothelial system by *C. acnes* has a differential effect on interferon production in mice. Interferon formed in response to endotoxin is enhanced whereas the serum interferon levels induced by NDV and CV are depressed in animals pretreated with *C. acnes*. These
findings bear striking similarities to those reported in mice subjected to sublethal X-irradiation. In the latter system, the level of NDV-induced interferon in the serum is markedly reduced, whereas the interferon response to endotoxin is either not affected or is strikingly enhanced (7). At the outset, it appears that these observations are paradoxical—C. acnes and X-irradiation have opposite effects on the reticuloendothelial system. C. acnes causes an increase in the number of lymphoid cells and also increases their synthetic capabilities as reflected in enhancement of antibody production (Farber and Smith, Bacteriol. Proc., p. 83, 1969), whereas X-irradiation results in the destruction of lymphocytes and inhibition of antibody synthesis. It has been suggested that the more radioprotected macrophage may be the source of endotoxin-interferon, whereas the radiosensitive lymphocyte synthesizes NDV-interferon. One interpretation of our data would be that the stimulatory effect of C. acnes on phagocytosis by macrophages may increase uptake of the virus by reticuloendothelial system cells, thus altering the interferon response by changing the pattern of virus clearance from the blood. The same effect could be achieved by X-irradiation in which the lymphocytes are destroyed. In the absence of lymphocytes, the more radioprotected macrophages may take up a greater percentage of the NDV but produce less interferon.

Another interesting aspect of the observed effect of C. acnes on interferon production in vivo is the contrast with the effect of other adjuvants such as BCG or Freund’s complete adjuvant (6, 13, 19, 23, 24). BCG also produces an increased reactivity to endotoxin as manifested both by an enhanced interferon production (23) and an increased sensitivity to the lethal effect (21). In contrast, however, the production of interferon following inoculation of NDV is not affected in BCG-infected mice (23). On the other hand, Mendelson (13) has shown that mineral oil increases the production of interferon by peritoneal leukocytes exposed to CV or vaccinia virus. Under in vivo conditions, De Clercq and co-workers (6) have found enhanced interferon production and increased resistance to vesicular stomatitis virus in mice inoculated with Freund’s adjuvant. Thus, although C. acnes and Freund’s adjuvant may similarly enhance the immune response, their effects on interferon production are strikingly different.

These data lend additional support to the evidence that reticuloendothelial system cells, or lymphatic tissue, or both, are involved in the production of interferon in vivo. The data further suggest that processes or infections which affect the reticuloendothelial system may alter the host’s capacity to synthesize interferon on exposure to viral or synthetic inducers. Recently, evidence from two experimental models in our laboratory have supported this concept. Glasgow and Bullock (8) have shown that an Mycobacterium leprae murium infection in mice suppresses the interferon response to CV. Similar results have also been observed in animals infected with Eperythrozoon coccoides (10). In both models, an enhancement of the interferon response to endotoxin was also observed. The presumably nonspecific enhancement of interferon production stimulated by endotoxin in animals infected with viable C. acnes or inoculated with nonviable preparations of organisms raises the question whether the enhanced response observed on second inoculation of C. acnes does in fact represent a manifestation of cell-mediated immunity. This observation suggests that the capacity to respond to certain inducers (endotoxin, C. acnes) may be altered by a previous experience, e.g., inoculation with C. acnes or BCG which nonspecifically stimulates an altered state of reactivity in reticuloendothelial system cells resulting in an enhanced or suppressed response on subsequent exposure to interferon-inducing agents. For this reason, we do not believe that the enhanced production of interferon in mice on second exposure to that same organism is satisfactory evidence for the interpretation that the response is due to cell-mediated immunity.

The implication of these observations is further enhanced by the recent development of C. parvum as an agent for immunotherapy in certain human malignancies (2). If interferon is a contributing
factor in host resistance to viral infection, then susceptibility to viruses may be further enhanced in patients receiving this agent for therapy of their malignant disease.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grant AI 10207 from the National Institute of Allergy and Infectious Diseases, and a grant-in-aid from Temple University. We thank Bunnie Schwartz, Patricia Dwyer, and Jennifer Fischbach for technical assistance.

LITERATURE CITED