Importance of Early Gamma Interferon Production in Propionibacterium acnes-Induced Resistance to Toxoplasma gondii Infection in Mice

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ABSTRACT. Treatment of mice with heat-killed Propionibacterium (P.) acnes conferred transient protection against Toxoplasma infection. To investigate the mechanism of this nonspecific resistance, the production of gamma interferon (IFN-γ) by P. acnes-injected mice was evaluated in comparison with that by noninjected controls upon infection with Toxoplasma. Mice pretreated with this bacterium produced significantly more IFN-γ than that produced by control mice up to 24 hr of infection. A single injection of anti-IFN-γ MAb on day 0 but not later than day 3 of infection resulted in a total abrogation of the resistance conferred by P. acnes. Likewise, daily injection of cyclosporin A (Cs-A), a potent inhibitor of T cell function, during the first 3 days of Toxoplasma infection severely exacerbated the infection, in accordance with a marked suppression of the early IFN-γ production. In contrast, the administration of Cs-A for 3 consecutive days starting at day 4 had no significant consequence on P. acnes-induced anti-toxoplasma resistance, while it reduced greatly the ability of P. acnes-injected mice to produce IFN-γ in the later phase of infection. Moreover, no significant increase in mortality and suppression of IFN-γ production was noted in mice receiving anti-asialo GM1 antibody. These results suggest that the early IFN-γ production by T cells is an essential event for the establishment of P. acnes-induced anti-toxoplasma resistance in mice.

KEY WORDS: gamma interferon, nonspecific resistance, Propionibacterium acnes, Toxoplasma gondii.


Treatment of mice with Propionibacterium (P.) acnes (formerly designated Corynebacterium parvum) has been shown to enhance their resistance to infection with certain bacterial and protozoal organisms, including Toxoplasma (T.) gondii [1, 2, 19]. Swartzberg et al. [19] reported that the resistance conferred by killed P. acnes against Toxoplasma appeared to be a lesser degree of protection and transient compared with that acquired by a primary infection with the homologous protozoan. Despite a large number of studies dealing with the specific immunity to infection with Toxoplasma, comparatively little information is available on the mechanisms underlying such nonspecific resistance induced by P. acnes in mice.

It is well documented that gamma interferon (IFN-γ) plays a crucial role in host resistance to infection with Toxoplasma, presumably by virtue of its ability to activate macrophages [17, 18]. There is evidence that peritoneal macrophages from mice injected with P. acnes are activated to kill Toxoplasma in vitro [19]. Based on these findings, we considered it of interest to investigate the effect of treatment of mice with P. acnes on IFN-γ response during Toxoplasma infection. In this report, we provide evidence that endogenous IFN-γ produced by T cells in the early stage of Toxoplasma infection plays an important role in modulating the host defence of mice pretreated with heat-killed P. acnes.

MATERIALS AND METHODS

Mice: Six- to 7-week-old female ICR-JCL mice were purchased from a commercial breeder (CLEA Japan Inc., Tokyo) and maintained in our laboratory under conventional condition.

Bacteria, protozoa and experimental design: P. acnes (ATCC 11827) was grown in GAM broth (Nissui Pharmaceutical Co., Ltd., Tokyo) at 37°C for 3 days. The bacterial cells were harvested, washed three times with 0.01 M phosphate-buffered saline (PBS, pH 7.2) by centrifugation, suspended in PBS and killed by heating at 60°C for 1 hr. Then the organisms were resuspended in PBS at the desired concentration (wet weight), and 0.5 ml of each of the suspensions was injected intraperitoneally (i.p.) into mice (8 to 10 weeks old). At different days after the injection, mice were injected i.p. with 2.5 × 10⁶ tachyzoites of the S-273 strain of T. gondii, and the mortality was scored daily for evaluation of the protection. The 50% lethal dose (LD₅₀) of this strain was 2,500 tachyzoites for normal mice [15].

Treatment with immunosuppressive drugs: Cyclosporin A (Cs-A, Sankyo Pharmaceutical Co., Ltd., Tokyo) was dissolved in PBS, and 0.5 ml of the solution (2 mg/mouse) was injected i.p. into mice for 3 consecutive days.

λ-Carrageenan (Wako Pure Chemical Industries, Osaka) was suspended in PBS, and 0.5 ml of the suspension (4 mg/mouse) was injected i.p. at 24 hr and at 2 hr before Toxoplasma infection (day 0 of infection).

Depletion of natural killer (NK) cells: To deplete NK cell activity in vivo, 1 mg of rabbit anti-asialo GM1 globulin (Wako) was given intravenously (i.v.) in a volume of 0.2 ml PBS on day −1 of infection. Normal rabbit globulin was injected as control.

Depletion of endogenous IFN-γ: Rat anti-mouse IFN-γ monoclonal antibody (MAB) was produced by the R4-6A2 hybridoma [7] growing as ascites fluid in the peritoneal cavity of pristane-primed KSN nude mice. A globulin fraction of the ascites was prepared by 50% (NH₄)₂SO₄.
precipitation method followed by exhaustive dialysis against PBS. The IFN neutralizing titer (unit/ml) of the partially purified MAb was determined in a biological assay [15] and defined as the reciprocal of the dilution of MAb which neutralizes 50% of the antiviral activity of 20 U of recombinant murine IFN-γ (rMuIFN-γ, Genzyme Corporation, Boston, U.S.A.). One mg of the MAb had a neutralizing titer of $1.4 \times 10^6$ against rMuIFN-γ. To deplete endogenous IFN-γ, each mouse was injected i.p. with 1 mg of anti-mouse IFN-γ MAb in a volume of 0.2 ml PBS. Control mice received normal rat globulin.

Preparation of samples for IFN-γ assay: At different times after infection with *Toxoplasma*, mice were anesthetized with ether and blood was collected by cutting the axillary artery. The serum was then separated individually by centrifugation. After bleeding, spleens were aseptically removed from the mice and crushed in RPMI-1640 medium (Nissui) to prepare 10% homogenates (wt/vol) with a glass homogenizer. The homogenates were then frozen and thawed three times and clarified by centrifugation. The serum and spleen extract were stored at −80°C until assayed for IFN activity.

Assay for IFN-γ: The IFN-γ assay was carried out by a double-sandwich enzyme-linked immunosorbent assay (ELISA) according to the method described by Curry *et al.* [3]. Anti-mouse IFN-γ MAb produced by the hybridoma R4-6A2 and rabbit polyclonal anti-rMuIFN-γ antisera [12] were used for the ELISA. The IFN-γ titer (U/ml) was estimated from the standard curve, which was constructed from the results obtained by using graded doses of rMuIFN-γ.

Statistical analysis: The statistical significance for comparison of IFN-γ titers between groups was determined using Student’s *t*-test. The chi-square test was used to determine the significance of survival rate. *P* value of less than 0.05 was considered to be significant.

RESULTS

Enhancement by *P. acnes* of host resistance to *Toxoplasma* infection: Survival of mice injected with *P. acnes* (10 mg/mouse) on various days before *Toxoplasma* challenge is shown in Fig. 1. All of the mice injected with *P. acnes* on day −1 and noninjected control mice died by day 15 postinfection, while 100% of mice challenged on days 3, 5 and 7 after the injection with *P. acnes* survived. Fifty per cent of mice challenged with *Toxoplasma* at 10 days after injection with *P. acnes* died by day 15 (*P<0.05). Although fewer mice injected with *Toxoplasma* on day 14 or day 21 after the treatment with *P. acnes* died than controls, the survival rates were not statistically significant. To investigate the dose-dependent effect on protection, mice injected with varying doses of *P. acnes* 7 days earlier were challenged with *Toxoplasma* (Fig. 2). All mice treated with either 10 mg or 20 mg of *P. acnes* survived, while all controls died by day 12 of infection. A significantly increased protection was also noted in the group given 5 mg of *P. acnes* (*P<0.01), while no significant protection was conferred by injection with 1 mg of the bacterium. From the results, mice were injected with 10 mg of *P. acnes* on day −7 in the following experiments.

Effect of *P. acnes* on IFN-γ production during *Toxoplasma* infection: The production of IFN-γ by *P. acnes*-injected mice was evaluated in comparison with that by noninjected controls upon challenge with *T. gondii* (Table 1). The kinetics and levels of IFN-γ produced in the bloodstream were observed to be similar from day 2 onward in both *P. acnes*-injected and noninjected mice. However, the levels of IFN-γ produced in mice injected
Table 1. Production of IFN-γ in the bloodstream of *P. acnes*-injected and noninjected mice following *T. gondii* infection

<table>
<thead>
<tr>
<th>Hours after infection</th>
<th>IFN-γ titer (U/ml)±sD</th>
<th>Injected</th>
<th>Noninjected</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5.0±2.2**</td>
<td>5.0±2.2**</td>
<td>&lt;1</td>
</tr>
<tr>
<td>12</td>
<td>15.0±5.2**</td>
<td>15.0±5.2**</td>
<td>&lt;1</td>
</tr>
<tr>
<td>24</td>
<td>26.6±4.4**</td>
<td>26.6±4.4**</td>
<td>1.4±0.6</td>
</tr>
<tr>
<td>48</td>
<td>38.2±9.8</td>
<td>38.2±9.8</td>
<td>28.8±9.6</td>
</tr>
<tr>
<td>72</td>
<td>19.8±5.8</td>
<td>19.8±5.8</td>
<td>16.6±3.6</td>
</tr>
<tr>
<td>96</td>
<td>40.8±11.1</td>
<td>40.8±11.1</td>
<td>37.6±6.2</td>
</tr>
<tr>
<td>144</td>
<td>123.4±19.0</td>
<td>123.4±19.0</td>
<td>117.6±18.9</td>
</tr>
<tr>
<td>192</td>
<td>74.8±17.0</td>
<td>74.8±17.0</td>
<td>66.6±12.8</td>
</tr>
</tbody>
</table>

a) Titers are mean±S.D. from 5 mice.
Symbol: **, Significantly different from noninjected mice at *P<0.01*.

with *P. acnes* were significantly higher than those produced in noninjected mice in the early stage of infection. In mice injected with the bacterium, considerable amounts of IFN-γ were detected at 6 hr, and the titers increased gradually as infection progressed. In contrast, a trace amount or an undetectable level of IFN-γ was produced by 24 hr in noninjected control mice, then the titer began to increase at 48 hr postinfection. No IFN-γ was detectable in any serum samples collected from mice at different times (3 hr to 3 days) after injection with the bacterium (Data not shown).

**Effect of anti-IFN-γ MAb on anti-toxoplasma resistance in *P. Acnes*-injected mice:** A single injection of anti-IFN-γ MAb on day 0 resulted in a total abrogation of anti-toxoplasma resistance in *P. acnes*-injected mice (Fig. 3). All of these mice died from 7 to 13 days postchallenge. A significantly increased mortality was also noted when the mice were injected with anti-IFN-γ MAb on day −7 (*P<0.05*). In contrast, 80% of *P. acnes*-treated mice survived when injected with the MAb on day 3 or day 4. All of the *P. acnes*-injected mice receiving normal rat globulin survived. Thus, the anti-IFN-γ MAb was found to be highly effective in abrogating the resistance when administered at around *Toxoplasma* challenge.

**Effects of immunosuppressive drugs and antibody on anti-toxoplasma resistance in *P. Acnes*-injected mice:** As depicted in Fig. 4, daily administration of Cs-A from day 0 to day 2 enhanced dramatically the susceptibility to *Toxoplasma* infection in *P. acnes*-injected mice. By contrast, only 20% of mice receiving Cs-A from day 4 to day 6 died of toxoplasmosis. Thus, Cs-A showed a highly suppressive effect on *P. acnes*-induced resistance when given during the early stage of *Toxoplasma* infection. The treatment of *P. acnes*-injected mice with carrageenan also resulted in a severe exacerbation of infection. By contrast, no significant increase in mortality was noted in mice receiving anti-asialo GM1 antibody. All *P. acnes*-injected control mice receiving normal rabbit globulin survived.

**Effects of immunosuppressive drugs and antibodies on IFN-γ production:** The IFN-γ titers in sera and spleen extracts from *P. acnes*-injected mice on days 1 and 6 were determined in comparison with those from mice treated with immunosuppressive drugs or antibodies (Table 2). A single injection of anti-IFN-γ MAb on day 0 or day 4 resulted in a marked inhibition of IFN-γ production in the early (day 1) or later (day 6) stage of infection, respectively. Daily injection of Cs-A for 3 days starting on day −1 or
Table 2. Effect of immunosuppressive drugs and antibodies on production of endogenous IFN-γ in P. acnes-injected mice following T. gondii infection

| Treatment                     | IFN-γ titer (U/ml)²⁻²⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻⁻

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum</th>
<th>Spleen</th>
<th>Serum</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control mice³ᵇ</td>
<td>20.2±3.8</td>
<td>13.2±3.4</td>
<td>102.8±17.5</td>
<td>114.6±21.6</td>
</tr>
<tr>
<td>Anti-IFN-γ MAb³ᵇ</td>
<td>&lt;1**</td>
<td>&lt;1**</td>
<td>&lt;1**</td>
<td>&lt;1**</td>
</tr>
<tr>
<td>Anti-asiato GM1 Ab</td>
<td>16.6±5.2</td>
<td>11.9±2.6</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Cyclosporin A³ᵈ</td>
<td>&lt;1***</td>
<td>2.2±0.6**</td>
<td>34.7±7.1**</td>
<td>28.8±10.2***</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>15.8±3.7</td>
<td>10.4±3.2</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

a) IFN-γ titers were determined from 5 mice at indicated days after infection.
b) Mice received normal rat globulin.
c) Mice received the MAb on day 0 or day 4.
d) Mice were injected with Cs-A for 3 days starting at day −1 or day 4 of infection, and samples were collected at 6 hr after the final injection.
Symbol: **, Significantly different from control mice at P<0.01.

The results of this study showed that the treatment of mice with heat-killed P. acnes enhanced host resistance against a lethal challenge with T. gondii. The protection against Toxoplasma was demonstrated after 3 days of P. acnes injection and persisted for about 1 week. Thus, the resistance conferred by P. acnes appeared to be a transient in nature. This finding is consistent with that of the previous report on P. acnes-induced resistance against Listeria (L.) monocytogenes or T. gondii infection [19].

A major goal of the present study was to explore a possible mechanism of P. acnes-induced resistance to T. gondii. Since IFN-γ is known to be a major mediator that influences host defence against Toxoplasma [11, 17, 18], we expected that this lymphokine may also play a role for such nonspecific resistance. Indeed, a single injection of anti-IFN-γ MAb just before Toxoplasma challenge resulted in a severe aggravation of the infection. Likewise, treatment of mice with Cs-A for 3 consecutive days starting at day 0 converted subclinical infection into clinical disease with high mortality. Despite the marked suppressive effect on IFN-γ production, both Cs-A and anti-IFN-γ MAb had no significant effect on the mortality when injected later than day 3 of infection (Figs. 3, 4 and Table 2). Taken together, these results suggest that the early IFN-γ production by T cells is an essential event for the generation and expression of anti-toxoplasma resistance in P. acnes-injected mice. More direct evidence for the participation of early produced IFN-γ in P. acnes-induced anti-toxoplasma resistance was provided by the results showing that mice treated with P. acnes produced significantly more IFN-γ than those produced by nontreated control mice up to 24 hr of infection (Table 1).

In this study, the contribution of asiato GM1-bearing cells, which mainly correspond to NK cells, to P. acnes-induced resistance to Toxoplasma was also examined, since NK cells have been recently shown to participate in the early host defence against another microorganism by secreting IFN-γ [4]. In our study, however, the administration of anti-asiato GM1 antibody 1 day before infection had no significant consequence on the host defence and production of endogenous IFN-γ, suggesting that NK cells may not be required for resistance to Toxoplasma infection in P. acnes-injected mice. This failure to prevent the anti-toxoplasma resistance is unlikely due to the use of an inadequate dosage of anti-asiato GM1 antibody, because the dose that we used is comparable to 2.5-fold higher than the effective dose used by others to exacerbate infection with L. monocytogenes in murine models [4, 12]. Thus, the cell populations responsible for the production of endogenous IFN-γ and anti-toxoplasma resistance in P. acnes-injected mice were found to be similar to those observed in specific mediation of protective immunity in which Toxoplasma infection was severely exacerbated by host treatment with either anti-IFN-γ or anti-CD4 and/or anti-CD8 MAbs [5, 6, 18].

The mechanism causing such early production of endogenous IFN-γ in P. acnes-injected mice remains to be clarified. However, one possibility may be that the inoculum used for challenging infection contained lipopolysaccharide (LPS)-like material, as it is well known that P. acnes alters the responsiveness of mice to various kinds of IFN inducers such as LPS and staphylococcal enterotoxin A (SEA) with the markedly enhanced production of IFN-γ [10, 13, 14]. One common feature of these IFN inducers is the transient nature of the resulting IFN production by which a maximum level of IFN-γ can be obtained in P. acnes-treated mice at about 3 hr after stimulation and then rapidly disappears [13, 14]. In our study, however, the IFN-γ activities induced in P. acnes-injected mice upon Toxoplasma challenge were first
detectable at 6 hr and continued for at least 18 hr with the relatively low titers. These findings appear to rule out the possibility that the induction of early IFN-γ production may be due to contamination with LPS- or SEA-like materials in the inoculum.

Although the present study has revealed the importance of endogenous IFN-γ in P. acnes-induced protection against Toxoplasma, our results still raise the question how the early produced IFN-γ mediates such nonspecific resistance. A direct cytotoxic effect of IFN-γ on extracellular parasites is unlikely [9, 16, 17]. The ability of IFN-γ to activate the microbicidal function of macrophages has been well documented with various pathogens. In our study, the treatment of mice with carrageenan resulted in a total abrogation of anti-toxoplasma resistance conferred by P. acnes (Fig. 4). Also, IFN-γ has been reported to be a potent mediator of the specific immune responses by causing increased IgG1 antigen expression on various antigen presenting cells, including macrophages [8]. Based on these findings, it can be postulated that IFN-γ produced during the early stage of Toxoplasma infection controls the first step of the parasite multiplication by activating macrophages, thereby allowing hosts to survive and then to mount the specific immune responses to kill or to inhibit the remaining parasites. Studies are now in progress to define the T cell subsets responsible for the production of endogenous IFN-γ and anti-toxoplasma resistance in P. acnes-injected mice.

REFERENCES


