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Induction of Murine Gamma Interferon Production by Lipopolysaccharide and Interleukin-2 in *Propionibacterium acnes*-Induced Peritoneal Exudate Cells

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Lipopolysaccharide (LPS) induces high levels of gamma interferon (IFN-γ) in the circulation of mice pretreated with heat-killed *Propionibacterium acnes*. The following results were obtained in the present study. (i) LPS, as well as interleukin-2 (IL-2), was also able to induce IFN-γ in vitro in peritoneal exudate cells (PEC) from such mice. Splenocytes and lymph node cells from these mice or resident peritoneal cells from control mice produced trace or undetectable amount of IFN-γ upon exposure to LPS. (ii) A synergistic effect on IFN-γ induction was observed when LPS was added to a culture of PEC together with IL-2. (iii) Indomethacin augmented the induction of IFN-γ by LPS or IL-2, and prostaglandin E_2 reversed its effect. (iv) Deprivation of plastic-adherent or nylon wool-adherent cells abolished the induction by LPS or IL-2, whereas it did not affect that by concanavalin A. (v) Culture supernatant of plastic-adherent cells incubated with LPS stimulated the nylon wool-nonadherent cells to produce IFN-γ in the presence of IL-2, but interleukin-1 or phorbol myristic acetate did not replace the LPS-stimulated supernatant. (vi) The ability of PEC to produce IFN-γ measured as a function of time after *P. acnes* injection increased in proportion to their natural killer (NK)-like activity against YAC-1 cells. Moreover, treatment of PEC with monoclonal anti-Thy-1 antibody or with anti-asialo GM1 antiserum plus complement eliminated the production of IFN-γ and the NK-like activity simultaneously, whereas treatment with monoclonal anti-Lyt-2 antibody plus complement did not. These results suggest that IL-2 and some unidentified factor released from plastic-adherent cells by LPS stimulation cooperatively induce IFN-γ production in activated, Thy-1- and asialo GM1-positive NK-like cells appearing in inflammatory reactions and that prostaglandin E_2 regulates IFN-γ production in these cells.

It is well known that gamma interferon (IFN-γ) plays important roles in immune responses by modulating the expression of Ia antigen (2, 16, 27) or cell surface receptors (8, 33), by promoting the maturation of antibody-secreting cells (17, 25), or by activating macrophages (1). However, the mechanism of IFN-γ production in relation to its roles has not yet been fully elucidated. No definite conclusion has yet been reached as to how antigens or mitogenes can stimulate the ultimate producer cells or regarding the detailed characteristics of the cells participating in its induction. The difficulty involved in analyzing these details may be ascribed to the complexity of the cells involved and to the long lag period required for the release of IFN-γ into the culture.

Recently, interleukin-2 (IL-2), instead of antigens or mitogens, has been shown to induce IFN-γ in both murine and human natural killer (NK) cells (9, 15, 18, 32). This is significant because it suggests that IFN-γ is finally induced by endogenous substances produced under physiological conditions. However, the question of how macrophages are involved in the induction by IL-2 is still unanswered. Kawase et al. (15) claimed the existence of a macrophage-derived secondary factor which stimulates IFN-γ production. The identification of such factors would contribute to the elucidation of the induction mechanism of IFN-γ.

On the other hand, a large amount of literature has been written about the effects of bacterial preparations on the capacity of animals to produce IFN. *Mycobacterium bovis* BCG alters the responsiveness of mice to lipopolysaccharide (LPS) with enhanced production of circulating IFN (37). *Propionibacterium acnes* (5, 30) and *Listeria monocytogenes* (10, 19) have also been reported to have similar effects, especially on the enhancement of local IFN production by *P. acnes*. Such experiments with bacterial organisms may yield useful information concerning the induction mechanism of IFN-γ. We have also found that mice pretreated with *P. acnes* or BCG are capable of secreting high levels of IFN-γ into the blood when challenged with various T-cell mitogens or LPS (21, 34). On the basis of these observations, studies were conducted to evaluate the capacity of LPS to induce IFN-γ in vitro and to clarify the cells and factors participating in IFN-γ induction by LPS in comparison with other inducers.

Here, we demonstrate that NK-like cells induced in the peritoneal cavity of mice treated with *P. acnes* produce IFN-γ upon stimulation with LPS dependent on plastic-adherent cells. The cooperation of IL-2 and an unidentified soluble factor released from plastic-adherent cells is suggested.

MATERIALS AND METHODS

Animals. Inbred C57BL/6 and outbred ICR female mice, 8 to 12 weeks of age, were obtained from Shizuoka Laboratory Animal Center (Hamamatsu, Japan).

Reagents. The following reagents were used in this study: LPS (*Escherichia coli* 0127:B8; Difco Laboratories, Detroit, Mich.); concanavalin A (ConA; Pharmacia, Uppsala, Sweden); indomethacin and phorbol myristic acetate (Sigma Chemical Co., St. Louis, Mo.); recombinant human alpha interleukin-1 (rIL-1; Genzyme Co., Boston, Mass.); partially

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purified murine IL-2 (Litton Bionetics, Inc., Charleston, S.C.), partially purified human IL-2 (Electro-Nucleonics Inc., Silver Spring, Md.); recombinant human IL-2 (rIL-2; kindly donated by Takeda Chemical Industries, Osaka, Japan); and prostaglandin E_2 (PGE_2; kindly donated by Ono Pharmaceutical Co., Osaka, Japan).

For most of the studies, rIL-2 was used, but no significant differences in the results were observed among the three IL-2 preparations.

Antibodies. Monoclonal anti-Thy-1.2 and anti-Lyt-2.2 antibodies and rabbit complement were purchased from Cedarlane Laboratory (Hornby, Ontario, Canada). Rabbit anti-asialo GM1 antiserum was purchased from Wako Pure Chemical Industries (Osaka, Japan). Specific antiserum to murine IFN-α/β (14) and anti-IFN-γ antiserum produced by J. A. Georgiades (23) or by E. Falcoff (28) were kindly provided by Y. Kawade of the Institute for Virus Research, Kyoto University, Kyoto, Japan.

Bacteria. *P. acnes* ATCC 11827 (also known as "*Corynebacterium parvum*") was grown in brain heart infusion medium supplemented with L-cysteine (0.03%) and Tween 80 (0.03%). Cells were harvested, washed with phosphate-buffered saline (PBS) by centrifugation, resuspended in PBS, and exposed to a temperature of 60°C for 1 h.

Treatment of animals and cell preparation. Mice were injected intraperitoneally (i.p.) with 0.2 ml of PBS containing 1 mg of heat-killed *P. acnes*, and after 5 days peritoneal exudate cells (PEC) were gathered from the peritoneal cavity by washing out with minimal essential medium supplemented with 10% fetal bovine serum (Flow Laboratories, North Ryde, New South Wales, Australia) and heparin (5 U/ml). Cells were washed by centrifugation and suspended in RPMI 1640 medium supplemented with fetal bovine serum (10%), L-glutamine (2 mM), and gentamicin (40 μg/ml). Control (resident) peritoneal cells were obtained from mice injected with PBS. Spleens and mesenteric lymph nodes were excised, minced, and filtered through wire mesh. Bone marrow cells were flushed out of femurs with minimal essential medium and 26-gauge needles.

Induction of IFN-γ. All the cells were suspended at a concentration of 5×10^6 cells per ml, and 0.18-ml portions of this suspension were placed onto a 96-well microtest culture plate (Becton Dickinson Labware, Oxnard, Calif.). Inducers dissolved in 0.02 ml of PBS were added, and incubation was carried out in an atmosphere containing 5% CO_2 at 37°C. At appropriate incubation times (unless otherwise mentioned, usually overnight), culture fluid was harvested for IFN assay.

IFN assay and characterization of IFNs. Determination of IFN titers and characterization of IFNs produced were performed as described previously (21), using vesicular stomatitis virus, mouse L cells, and specific antisera to IFNs. All IFN titers are expressed in international reference units.

Estimation of cells participating in production of IFN-γ. Cells participating in IFN-γ production were estimated by susceptibility to complement-mediated lysis after treatment with specific antibodies directed to the antigens Thy-1, Lyt-2, or asialo GM1. PEC (10^7 cells per ml) were incubated with appropriately diluted antibodies for 20 min at 4°C and for a further 20 min at 37°C before being washed by centrifugation. At 45 min after the addition of complement, the cells were washed three times with minimal essential medium and suspended in RPMI 1640 medium (M.A. Bioproducts, Walkersville, Md.). Viable cells were counted by the trypan blue dye exclusion test, and cell concentrations were adjusted to 5×10^6/ml and used for IFN-γ induction. Control experiments were done by treating the cells with complement alone. Cells enriched for T lymphocytes and NK-like cells were prepared by applying the cells to a nylon wool column after removing the plastic-adherent cells twice. Plastic-nonadherent cells (5×10^7) were applied to the nylon wool column (10 ml), and after incubating for 60 min at 37°C, nylon wool-nonadherent cells were eluted with warmed RPMI 1640 medium containing 10% fetal bovine serum.

Cells enriched for macrophages were prepared by treating the PEC (10^7/ml) with appropriately diluted anti-Thy-1 antibody plus complement and by removing the nonadherent cells after incubating on plastic microtest plates (5×10^6 cells per ml). These cells were used for the experiments involving reconstitution of the systems for IFN-γ production.

Preparation of LPS-stimulated culture supernatant from plastic-adherent cells. *P. acnes*-induced PEC suspended in indomethacin-containing medium (10 μg/ml) were transferred to plastic dishes (5×10^6 cells per ml), and after incubating at 37°C for 1 h, nonadherent cells were vigorously removed. LPS was added to the culture (10 μg/ml), and after another incubation for 4 h, the culture supernatant was harvested and used for the induction experiments with or without IL-2. The culture supernatant usually contained a small amount of IFN, which was completely neutralized by anti-IFN-α/β antiserum.

Assay of NK-like activity. YAC-1 cells maintained in RPMI 1640 medium were used for the assay of NK-like activity. Cells were adjusted to 5×10^6/ml, and a 0.1-ml sample of the suspension was added to 0.1 ml of $Na_2{}^{51}Cr_2O$ (1 mCi/ml; New England Nuclear Corp., Boston, Mass.). The mixture was incubated for 1 h at 37°C in a humidified atmosphere of 5% CO_2. The ^{51}Cr-labeled YAC-1 cells were washed three times, resuspended, and overlaid (10^4 cells in 0.1 ml of RPMI 1640 medium) on the PEC in round-bottomed 96-well microplates at an effector/target cell number ratio of 50:1. The plates were centrifuged at $300 \times g$ for 5 min, and after a 4-h incubation at 37°C, 0.1 ml of the supernatant from each well was harvested for assay of radioactivity in an automatic gamma scintillation spectrophotometer (Packard 5320). Cytotoxicity was calculated and expressed as percent lysis according to the method described by Brunner et al. (4). The maximum release was obtained by exposing the labeled cells to 0.1 ml of 10% H_2SO_4, and the spontaneous release was counted from the supernatant of the target cells alone.

The characteristics of the cells cytotoxic to YAC-1 cells were estimated in parallel with the estimation of cells participating in IFN-γ production, using the vigorously treated PEC.

RESULTS

Induction kinetics. The induction kinetics of IFN-γ production by LPS, rIL-2, and ConA are shown in Fig. 1. The release of IFN-γ into the culture fluid began approximately 4 h after the addition of inducers and reached maximum levels after about 7 h. The time needed for maximum induction was much shorter than that usually observed in conventional inductions in spleen cells with ConA, which takes a few days (29). Peritoneal cells from control mice did not produce significant levels of IFN-γ upon exposure to LPS or rIL-2. The ability of PEC to produce IFN-γ in response to these inducers was maximal about 5 days after *P. acnes* treatment, when hepatosplenomegaly became notable. The effective doses of LPS and rIL-2 could be lowered to as little as 0.1 μg/ml and 1 U/ml, respectively.

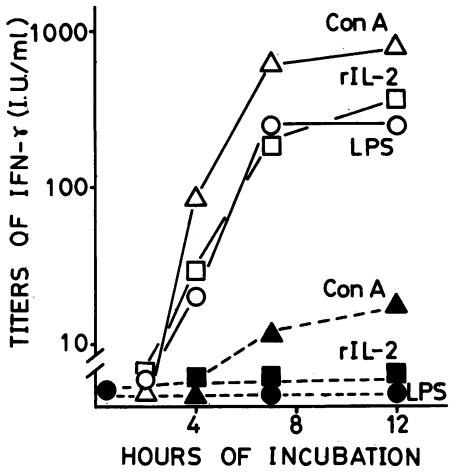

FIG. 1. Induction kinetics of IFN-γ production by LPS, rIL-2, and ConA in *P. acnes*-induced PEC. PEC harvested from C57BL/6 mice injected i.p. with 1 mg of heat-killed *P. acnes* 5 days previously (——) or resident peritoneal cells from control mice (----) were washed by centrifugation, suspended in RPMI 1640 medium (5×10^6 cells per ml), and transferred to 96-well, flat-bottomed microtest culture plates. LPS (10 μg/ml), rIL-2 (10 U/ml), or ConA (5 μg/ml) was added to the culture, and after appropriate time intervals, culture supernatants were taken out for IFN assay. Average values of IFN-γ titers obtained from three experiments were all determined after neutralizing the samples with anti-murine IFN-α/β antiserum. Symbols: ○, ●, LPS; □, ■, rIL-2; △, ▲, ConA.

Characterization of IFNs produced in PEC by LPS stimulation. The IFNs produced by PEC in response to LPS were carefully characterized in the same manner as that reported previously (21). The results indicated that the early appearing IFN released at low levels around 2 h after induction was α/β type, while the IFN appearing at about 7 h was γ type (Table 1). These findings were similar to those of in vivo experiments in the respect that IFN-α/β appeared first in the circulation of *P. acnes*-treated mice and then IFN-γ appeared after challenge with LPS (21). Because IFN activities were completely neutralized by a combination of anti-IFN-α/β and anti-IFN-γ antisera, the residual IFN activity after neutralization with anti-IFN-α/β antiserum could be regarded as γ type. Thus, titers of IFN-γ were usually expressed by the values obtained after neutralizing the samples with anti-IFN-α/β antiserum.

Induction of IFN-γ by LPS in cells from various sources. Cells were prepared from various tissues and compared with regard to their capacity to produce IFN-γ. *P. acnes*-induced PEC were the most competent in production of IFN-γ (Table 2). Spleen cells, lymph node cells, and bone marrow cells secreted much less IFN-γ when stimulated by various inducers.

TABLE 1. Characterization of IFNs induced by LPS in *P. acnes*-induced PEC

Treatment	IFN titer at time (h) after induction[a]			
	2	4	7	16
None	107 ± 27	213 ± 53	213 ± 53	266 ± 53
pH 2.0	67 ± 13	120 ± 40	133 ± 27	107 ± 27
Anti-IFN-α/β	<20	27 ± 6	160 ± 0	187 ± 70
Anti-IFN-α/β + anti-IFN-γ	<20	<20	<20	<20

[a] PEC prepared from three *P. acnes*-treated ICR mice were used. After the addition of LPS (10 μg/ml) to the individual cultures, samples were taken at the times indicated and tested for antigenicity and acid stability. Mean values ± standard error of three samples are shown.

TABLE 2. Comparison of capacity of various tissues from *P. acnes*-treated mouse to produce IFN-γ

Treatment	Tissue[a]	IFN-γ titer induced by[b]:				
		PBS	LPS	rIL-2	LPS + rIL-2	ConA
P. acnes treated	PEC	20	160	160	640	640
	Spleen	20	20	40	80	80
	Lymph node	<10	<10	20	20	160
	Bone marrow	<10	<10	20	20	160
Control	PEC	<10	<10	<10	40	20
	Spleen	<10	<10	10	20	80
	Lymph node	<10	<10	<10	20	40
	Bone marrow	<10	<10	<10	10	20

[a] Various tissues were taken from one ICR mouse treated with *P. acnes* 5 days previously or from one control mouse and used for IFN-γ production.
[b] IFN-γ synthesis was induced by incubating the cultures with LPS (10 μg/ml), rIL-2 (10 U/ml), ConA (5 μg/ml), or LPS (10 μg/ml) plus rIL-2 (10 U/ml).

It was notable that a synergistic effect on IFN-γ production was observed when LPS was added to the culture of PEC together with rIL-2. The IFN-γ titers induced by the combination were usually four times higher than those individually induced.

Effect of indomethacin on IFN-γ production. Because it has been reported that arachidonic acid metabolites regulated IFN-γ production either positively (12) or negatively (35), the effect of indomethacin was examined in the present experimental system (Table 3). The administration of indomethacin (10 μg per mouse i.p.) to *P. acnes*-treated mice 1 h before cell preparation and further addition of the agent (10 μg/ml) to the culture resulted in an enhancement of IFN-γ production when induced by either LPS or rIL-2. However, the drug did not have such a pronounced effect on the induction by ConA. In vivo administration alone or in vitro addition alone was also effective, but the combined use was a little more efficient. The enhanced IFN-γ induction by indomethacin was also observed in spleen cells when stimulated by rIL-2. Yet the induction by LPS in spleen cells was negligible even in the presence of indomethacin.

Effect of PGE_2 on IFN-γ production. To confirm that the effect of indomethacin was due to the inhibition of prostaglandin synthesis, we added PGE_2 to the culture (10 μg/ml) to see whether it would reverse the effect of indomethacin. The addition of PGE_2 reversed the effect of indomethacin, and it also suppressed the induction by LPS or by rIL-2 in the absence of indomethacin (Table 4). However, the induction by ConA was only slightly influenced by PGE_2.

Effect of deprivation of plastic-adherent cells on IFN-γ induction by LPS. Since it is widely accepted that macrophages are involved in IFN-γ production, the effect of removal of plastic-adherent and nylon wool-adherent cells was examined (Table 5). Because plastic-adherent cells accounted for a large portion of *P. acnes*-induced PEC (usually 70 to 80%), the yield of nonadherent cells was low. The results indicate that LPS alone, as well as rIL-2 alone, was unable to induce IFN-γ in nylon wool-nonadherent cells. However, ConA was able to bring about considerably augmented induction in these cells, perhaps owing to the enrichment of the producer cells. LPS or rIL-2 was able to induce IFN-γ in cells reconstituted with plastic-adherent

TABLE 3. Effect of indomethacin on IFN-γ production in PEC and spleen cells from mice treated with *P. acnes*

Cells	Indomethacin	IFN-γ titer induced by[a]:				
		PBS	LPS	rIL-2	LPS + rIL-2	ConA
PEC	−	13 ± 3	120 ± 40	133 ± 27	427 ± 106	427 ± 106
PEC	+	67 ± 13	267 ± 53	373 ± 53	533 ± 106	480 ± 160
Spleen	−	<10	<10	40 ± 20	67 ± 13	53 ± 13
Spleen	+	<10	17 ± 3	213 ± 53	267 ± 53	80 ± 0

[a] Mean ± standard error of three experiments with ICR mice. See Table 2, footnote *b*, for inducer concentrations.

cells and nylon wool-nonadherent cells. Thus, induction of IFN-γ by LPS or rIL-2 was dependent on the plastic-adherent cells, perhaps macrophages, whereas induction by ConA was independent or much less dependent on macrophages in the present experimental system.

Induction of IFN-γ by the cooperation of IL-2 and the LPS-stimulated culture supernatant of plastic-adherent cells. For the purpose of clarifying the way plastic-adherent cells are involved in IFN-γ production, we examined the culture supernatant of plastic-adherent cells incubated with LPS for its ability to induce IFN-γ in nylon wool-nonadherent cells. rIL-2 alone or the LPS-stimulated culture supernatant alone did not induce IFN-γ in nylon wool-nonadherent cells (Table 6). However, these cells produced IFN-γ as a result of combined stimulation by them. The LPS-stimulated culture supernatant was still effective when it was diluted by 32 times. The control culture supernatant of the plastic-adherent cells incubated with PBS instead of LPS did not exhibit the inducibility even in the presence of rIL-2. Phorbol myristic acetate, which has been reported to enhance IFN-γ production in spleen cells (11), or human rIL-1 was unable to replace the active LPS-stimulated culture supernatant. LPS itself together with rIL-2 induced a trace amount of IFN-γ in nylon wool-nonadherent cells, but the amount was not significant compared with that produced by the culture supernatant. Thus, the existence of a secondary factor cooperating with IL-2 in IFN-γ induction was strongly suggested.

Estimation of cells participating in IFN-γ production in *P. acnes*-induced PEC. To analyze the IFN-γ induction by LPS and IL-2 in *P. acnes*-induced PEC, we did another experiment for estimating the number of cells producing IFN-γ. The NK-like activity of resident peritoneal cells, as well as the capacity to produce IFN-γ in these cells, was almost negligible. However, the capacity to produce IFN-γ in the PEC increased in the same way as the cytotoxicity of the PEC to YAC-1 cells after the injection of *P. acnes* (Fig. 2), that is, both the IFN-γ producibility and the NK-like activity proceeded correspondingly. Furthermore, an elimination experiment was also undertaken. Table 7 shows one of the typical results of experiments on the effect of treatment of the PEC with specific antibodies followed by complement-mediated lysis on both NK-like activity and IFN-γ production by them. Treatment of the PEC with monoclonal anti-Thy-1 antibody or with anti-asialo GM1 antiserum plus complement abolished the NK-like activity almost completely, and moreover, most of the IFN-γ production by any inducer was eliminated at the same time. However, treatment of the PEC with monoclonal anti-Lyt-2 antibody plus complement did not affect either of the activities.

TABLE 4. Inhibition of IFN-γ production by PGE_2 in *P. acnes*-induced PEC in the absence or presence of indomethacin[a]

Group	Indomethacin	PGE_2	IFN-γ titer induced by:			
			PBS	LPS	rIL-2	ConA
1	−	−	10	160	160	640
2	+	−	20	320	640	640
3	−	+	<10	40	80	160
4	+	+	<10	40	80	320

[a] Single ICR mouse treated with *P. acnes* 5 days before was used. See Table 2, footnote *b*, for inducer concentrations.

DISCUSSION

In our previous reports (21, 34), we demonstrated that mice treated with *P. acnes* or BCG readily released IFN-γ as well as IFN-α/β into the circulation upon challenge with LPS. In addition, this induction by LPS was suggested to be dependent on macrophages and on Thy-1- or asialo GM1-bearing cells. In the present study, we provided evidence from in vitro experiments that LPS can stimulate the production of IFN-γ as well as IFN-α/β in PEC from such mice (Fig. 1; Table 2). The enhanced production of IFN-γ in these cells was also observed when other inducers, IL-2 or ConA, were used. The induction of IFN-γ production by LPS was not observed in resident peritoneal cells, and the lag period for the induction was as short as 4 h (Fig. 1), and much shorter than that for primary induction with spleen cells and lectin (usually more than 24 h). These findings suggest that IFN-γ-producing cells in *P. acnes*-induced PEC have gone through several processes required for IFN-γ production.

Recently, while this manuscript was in preparation, Blanchard et al. (3) reported the IL-2 and macrophage-dependent induction of IFN-γ by LPS in "aged" splenocytes. They also demonstrated that the addition of LPS to freshly prepared splenocyte cultures which had been treated with IL-2 before exposure to LPS resulted in the induction of a large amount of IFN-γ. Our present results are similar to theirs in some respects, for example, the synergism of LPS

TABLE 5. Effect of deprivation of plastic-adherent cells on IFN-γ production in *P. acnes*-induced PEC

Responder population	IFN-γ titer induced by[a]:				
	PBS	LPS	rIL-2	LPS + rIL-2	ConA
Unfractionated PEC	20	160	320	640	640
Nylon wool nonadherent[b]	<10	<10	<10	80	2,560
Plastic adherent[c]	<10	<10	<10	<10	<10
Nylon wool nonadherent + plastic adherent[d]	20	320	320	1,280	2,560

[a] See Table 2, footnote *b*, for inducer concentrations.

[b] PEC prepared from C57BL/6 mice injected i.p. with killed *P. acnes* 5 days previously were combined, and after the plastic-adherent cells were removed twice, cells were applied to a nylon wool column.

[c] PEC were treated with anti-Thy-1 antibody plus complement, transferred to a microtest plate, incubated at 37°C for 1 h, and washed vigorously to remove the nonadherent cells.

[d] After the plastic-adherent cells were prepared, equal numbers of nylon wool-nonadherent cells were placed on them and incubated with inducers.

TABLE 6. IFN-γ induction by LPS-stimulated culture supernatant in nylon wool-nonadherent cells of *P. acnes*-induced PEC

Induction with:	IFN-γ titer[a]
PBS	<10
rIL-2 (10 U/ml)	<10
LPS (10 μg/ml) + rIL-2	43 ± 20
Phorbol myristic acetate (0.01–0.1 μg/ml) + rIL-2	10
rIL-1 (1–100 U/ml) + rIL-2	10
Control supernatant (PBS)[b]	33 ± 6
IL-2-stimulated supernatant[b] + rIL-2	27 ± 7
LPS-stimulated supernatant[b]	13 ± 4
LPS-stimulated supernatant[b] + rIL-2	533 ± 106
Control supernatant (resident peritoneal cells)[c] + rIL-2	13 ± 4

[a] Mean ± standard error of three experiments with C57BL/6 mice.
[b] Plastic-adherent cells prepared as described in the text were incubated with PBS, rIL-2 (10 U/ml), or LPS (10 μg/ml) for 4 h at 37°C, and the culture supernatant was added to the induction media at a dilution of 1/5.
[c] Culture supernatant was prepared by incubating resident peritoneal adherent cells with LPS (10 μg/ml) in the same manner as that for *P. acnes*-induced PEC.

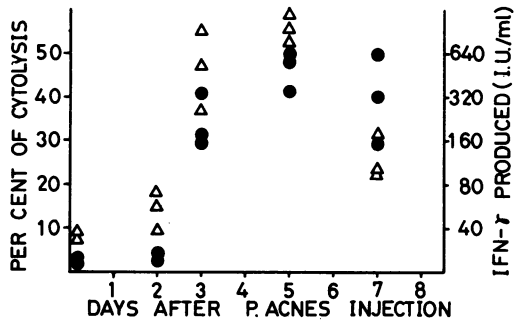

FIG. 2. Time course of increase in NK-like activity and capacity of IFN-γ production in *P. acnes*-induced PEC. Both cytolytic activity against YAC-1 cells (△) and ability to produce IFN-γ (●) of the *P. acnes*-induced PEC from individual mice (C57BL/6) were measured with the passage of time after *P. acnes* injection. The assay for the cytolytic activity was performed with ^{51}Cr-labeled YAC-1 cells at an effector-to-target cell ratio of 50:1. The induction of IFN-γ production was carried out by LPS (10 μg/ml) in the presence of indomethacin (10 μg/ml).

and IL-2 (Table 2), simultaneous induction of IFN-α/β and IFN-γ, or macrophage dependency. Thus, their aged splenocytes may correspond to our bacteria-activated PEC, and it would be of interest to compare the detailed induction mechanisms of both systems.

Cells from spleen, lymph node, or bone marrow of *P. acnes*-treated mice produced only trace amounts of IFN-γ by stimulation with LPS or IL-2 (Table 2). However, this low level of induction may have been due to the low proportion of macrophages in these tissues or to endogenous inhibitors of IFN-γ production such as arachidonic acid metabolites, since the induction by LPS in these cells in the presence of plastic-adherent cells of *P. acnes*-induced PEC (data not shown) and the induction by IL-2 in spleen cells in the presence of indomethacin (Table 3) were considerably augmented.

There have been several papers on the factors influencing IFN-γ production (12, 35). Of these factors, prostaglandin, which is known to be intimately involved in regulating the responses of macrophages to LPS, was examined in the present study (Table 4). In our experiments with *P. acnes*-induced PEC, indomethacin enhanced IFN-γ production by LPS or by IL-2, and the addition of PGE_2 reversed its effect. Because PGE_2 is known to be released from macrophages (7), it is likely that macrophages are concerned with the regulation of IFN-γ production in inflammatory reactions through PGE_2 secretion. On the other hand, the induction of IFN-γ by ConA was little influenced by indomethacin or PGE_2. No explanation for this result is given in the present study, but there is a possibility that ConA is able to stimulate the producer cells directly, whereas the induction by LPS or IL-2 requires other functional cells which are sensitive to PGE_2. Since leukotriene, another type of arachidonic acid metabolite, has been shown to upregulate IL-2-induced IFN-γ production (12), it would be interesting to elucidate the opposing roles of both types of arachidonic acid metabolite in IFN-γ induction.

Many pieces of evidence have confirmed that IL-2 can induce IFN-γ in human peripheral blood lymphocytes (13, 18, 24, 32) or murine spleen cells (6, 15, 36). In our present experiments, IL-2 stimulated IFN-γ production in *P. acnes*-induced PEC but not in resident peritoneal cells (Table 2). The induction by IL-2 together with LPS exhibited a synergistic effect, and the deprivation of plastic-adherent cells abrogated the induction by IL-2 despite enrichment of the possible producer cells (Table 5). Thus, IL-2 alone failed to stimulate the nonadherent cells to secrete IFN-γ, while ConA induced considerable augmentation. Moreover, the addition of LPS-stimulated culture suppernatant of plastic-adherent cells restored the induction by IL-2 (Table 6). It thus seems probable that in the case of unseparated PEC, an endogenous, unidentified factor released from plastic-adherent cells cooperates with added IL-2, while to the contrary, small amounts of endogenous IL-2 may work together when LPS is used as an inducer.

Although there have been numerous reports indicating the indispensability of macrophages in IFN-γ production (29), there is no definite agreement about the way in which they function. Classically, they may work as the source of IL-1, which augments the production of IL-2. However, in our experiments, rIL-1 plus rIL-2 failed to induce IFN-γ in nylon

TABLE 7. Effect of elimination of cells bearing asialo GM1, Thy-1, or Lyt-2 antigen on IFN-γ production and on NK-like activity in *P. acnes*-induced PEC[a]

Treatment	IFN-γ titer induced by:					NK-like activity (% lysis)
	PBS	LPS	rIL-2	LPS + rIL-2	ConA	
Complement alone	40	320	320	640	640	37
Anti-asialo GM1 + complement	<10	20	40	40	160	5
Anti-Thy-1.2 + complement	<10	<10	<10	<10	<10	8
Anti-Lyt-2.2 + complement	20	320	160	640	640	41

[a] PEC from C57BL/6 mice injected with *P. acnes* 5 days previously were used. See Table 2, footnote *b*, for inducer concentrations.

wool-nonadherent cells of *P. acnes*-induced PEC, while rIL-2 plus LPS-stimulated culture supernatant of plastic-adherent cells did so (Table 6). Blanchard et al. (3) also indicated that rIL-1 was unable to replace the adherent cells in their experiments with aged splenocytes. However, while they insisted on the necessity for direct contact between the macrophages and producer cells, our results indicated that LPS-stimulated culture supernatant of plastic-adherent cells could act as substitute for the adherent cells (Table 6). Kawase et al. (15) also claimed that a secondary factor was needed for IFN-γ induction by IL-2 in NK cells. This discrepancy may have been due to the differences in the types of producer cells used, the former being T lymphocytes and the latter being NK-like cells.

Although it has been said that the cellular source of IFN-γ is T lymphocytes bearing Lyt-2 antigen (11, 26), there are recent pieces of evidence to suggest that NK cells can produce IFN-γ (9, 15), while on the other hand, it has been shown that i.p. administration of *P. acnes* or BCG dramatically raises the NK activity in the PEC (20, 31). Accordingly, in the present study, we examined the relationship between the *P. acnes*-induced NK-like cells and IFN-γ production (Fig. 2; Table 7). Both the capacity to produce IFN-γ and the NK-like activity against YAC-1 cells increased correspondingly after *P. acnes* injection (Fig. 2). The results of our experiments on cell elimination with specific antibodies (Table 7) also indicated that nylon wool-nonadherent, Thy-1$^+$, asialo GM1$^+$, Lyt-2$^-$ NK-like cells were responsible for IFN-γ production in *P. acnes*-induced PEC. It was unexpected that treatment of the cells with monoclonal anti-Thy-1 antibody plus complement abolished both IFN-γ production and the cytotoxicity against YAC-1 cells. Yet, considering that NK cells are composed of cells bearing heterogeneous surface markers including Thy-1 antigen (22), it is quite probable that Thy-1-bearing NK cells are concerned with IFN-γ production in local inflammatory sites. It would be of interest to examine the roles and the detailed nature of such NK-like cells in inflammatory and immune reactions.

Because IFN-γ is known to enhance the expression of Ia antigen on macrophages (16) and Ia-positive macrophages are known to have important roles as antigen-presenting cells in immune responses, the clarification of the induction mechanism of IFN-γ production in inflammation-induced cells would be of significance. Studies on IFN-γ induction by LPS in inflammatory cells would also yield information about the roles which LPS may play against bacterial infections.

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LITERATURE CITED

1. **Adams, D. O., and T. A. Hamilton.** 1984. The cell biology of macrophage activation. Annu. Rev. Immunol. **2**:283–318.
2. **Basham, T. Y., and T. C. Merigan.** 1983. Recombinant interferon-γ increases HLA-DR synthesis and expression. J. Immunol. **130**:1492–1494.
3. **Blanchard, D. K., J. Y. Djeu, T. W. Klein, H. Friedman, and W. E. Stewart II.** 1986. Interferon-γ induction by lipopolysaccharide: dependence on interleukin 2 and macrophages. J. Immunol. **136**:963–970.
4. **Brunner, K. T., J. Manuel, J.-C. Cerottini, and B. Chapuis.** 1968. Quantitative assay of the lytic action of immune lymphoid cells on ^{51}Cr-labelled allogeneic target cells in vitro; inhibition by isoantibody and by drugs. Immunology **14**:181–196.
5. **Farber, P. A., and L. A. Glasgow.** 1972. Effect of *Corynebacterium acnes* on interferon production in mice. Infect. Immun. **6**:272–276.
6. **Farrar, W. L., H. M. Johnson, and J. J. Farrar.** 1981. Regulation of the production of immune interferon and cytotoxic T lymphocytes by interleukin 2. J. Immunol. **126**:1120–1125.
7. **Goodwin, J. S., and D. R. Webb.** 1980. Regulation of the immune response by prostaglandins. Clin. Immunol. Immunopathol. **15**:106–122.
8. **Guyre, P. M., P. M. Morganelli, and R. Miller.** 1983. Recombinant immune interferon increases immunoglobulin G Fc receptors on cultured human mononuclear phagocytes. J. Clin. Invest. **72**:393–397.
9. **Handa, K., R. Suzuki, H. Matsui, Y. Shimizu, and K. Kumagai.** 1983. Natural killer (NK) cells as a responder to interleukin 2 (IL 2). II. IL 2-induced interferon γ production. J. Immunol. **130**:988–992.
10. **Havell, E. A.** 1986. Augmented induction of interferons during *Listeria monocytogenes* infection. J. Infect. Dis. **153**:960–969.
11. **Johnson, H. M., and B. A. Torres.** 1982. Phorbol ester replacement of helper cell and interleukin 2 requirements in gamma interferon production. Infect. Immun. **36**:911–914.
12. **Johnson, H. M., and B. A. Torres.** 1984. Leukotrienes: positive signals for regulation of γ-interferon production. J. Immunol. **132**:413–416.
13. **Kasahara, T., J. J. Hooks, S. F. Dougherty, and J. J. Oppenheim.** 1983. Interleukin 2-mediated immune interferon (IFN-γ) production by human T cells and T cell subsets. J. Immunol. **130**:1784–1789.
14. **Kawade, Y.** 1980. An analysis of neutralization reaction of interferon by antibody: a proposal on the expression of neutralization titer. J. Interferon Res. **1**:61–70.
15. **Kawase, I., C. G. Brooks, K. Kuribayashi, S. Olabuenaga, W. Newman, S. Gillis, and C. S. Henney.** 1983. Interleukin 2 induces γ-interferon production: participation of macrophages and NK-like cells. J. Immunol. **131**:288–292.
16. **King, D. P., and P. P. Jones.** 1983. Induction of Ia and H-2 antigens on a macrophage cell line by immune interferon. J. Immunol. **131**:315–318.
17. **Leibson, H. J., M. Gefter, A. Zlotnik, P. Marrack, and J. W. Kappler.** 1984. Role of γ-interferon in antibody-producing responses. Nature (London) **309**:799–801.
18. **Mukaida, N., T. Kasahara, J. Hosoi, K. Shioiri-Nakano, and T. Kawai.** 1986. Effects of anti-Tac antibody on the response of large granular lymphocytes to interleukin-2. Immunology **57**:137–143.
19. **Nakane, A., and T. Minagawa.** 1985. Sequential production of alpha and beta interferons and gamma interferon in the circulation of *Listeria monocytogenes* infected mice after stimulation with bacterial lipopolysaccharide. Microbiol. Immunol. **29**: 659–669.
20. **Ojo, E., O. Haller, and H. Wigzell.** 1978. *Corynebacterium parvum*-induced peritoneal exudate cells with rapid cytolytic activity against tumor cells are nonphagocytic cells with characteristics of natural killer cells. Scand. J. Immunol. **8**:215–222.
21. **Okamura, H., K. Kawaguchi, K. Shoji, and Y. Kawade.** 1982. High-level induction of gamma interferon with various mitogens in mice pretreated with *Propionibacterium acnes*. Infect. Immun. **38**:440–443.
22. **Ortaldo, J. R., and R. B. Herberman.** 1984. Heterogeneity of natural killer cells. Annu. Rev. Immunol. **2**:359–394.
23. **Osborne, L. C., J. A. Georgiades, and H. M. Johnson.** 1980. Classification of interferons with antibody to immune interferon. Cell. Immunol. **53**:65–70.
24. **Reem, G. H., and N.-H. Yeh.** 1984. Interleukin 2 regulates expression of its receptor and synthesis of gamma interferon by human T lymphocytes. Science **225**:429–430.
25. **Sidman, C. L., J. D. Marshall, L. D. Shultz, P. W. Gray, and H. M. Johnson.** 1984. γ-Interferon is one of several direct B cell-maturing lymphokines. Nature (London) **309**:801–804.
26. **Sonnenfeld, G., A. D. Mandel, and T. C. Merigan.** 1979. *In vitro*

production and cellular origin of murine type II interferon. Immunology **36**:883–889.

27. **Steeg, P. S., R. N. Moore, H. M. Johnson, and J. J. Oppenheim.** 1982. Regulation of murine macrophage Ia antigen expression by a lymphokine with immune interferon activity. J. Exp. Med. **156**:1780–1793.
28. **Stefanos, S., L. Catinot, J. Wietzerbin, and E. Falcoff.** 1980. Production of antibodies against mouse immune T (type II) interferon and their neutralizing properties. J. Gen. Virol. **50**:225–229.
29. **Stewart, W. E., II.** 1981. Mitogens and immune recognition induction, p. 44–46. *In* The interferon system. Springer-Verlag, New York.
30. **Storch, E., and H. Kirchner.** 1985. Interferon inducibility in mice treated with *Corynebacterium parvum*. Antiviral Res. **5**:117–123.
31. **Tracey, D. E., S. A. Wolfe, J. M. Durdik, and C. S. Henney.** 1977. BCG-induced murine effector cells. I. Cytolytic activity in peritoneal exudates: an early response to BCG. J. Immunol. **119**:1145–1151.
32. **Trinchieri, G., M. Matsumoto-Kobayashi, S. C. Clark, J. Seehra, L. London, and B. Perussia.** 1984. Response of resting human peripheral blood natural killer cells to interleukin 2. J. Exp. Med. **160**:1147–1169.
33. **Vogel, S. N., L. L. Weedon, R. N. Moore, and D. L. Rosenstreich.** 1982. Correction of defective macrophage differentiation in C3H/HeJ mice by an interferon-like molecule. J. Immunol. **128**:380–387.
34. **Wada, M., H. Okamura, K. Nagata, T. Shimoyama, and Y. Kawade.** 1985. Cellular mechanisms in *in vivo* production of gamma interferon induced by lipopolysaccharide in mice infected with *Mycobacterium bovis* BCG. J. Interferon Res. **5**:431–443.
35. **Wakasugi, N., J.-L. Virelizier, F. Arenzana-Seisdedos, B. Rothhut, J.-M. M. Huerta, F. Russo-Marie, and W. Fiers.** 1985. Defective interferon-γ production in the human neonate. II. Role of increased sensitivity to the suppressive effects of prostaglandin E. J. Immunol. **134**:172–176.
36. **Yamamoto, J. K., W. L. Farrar, and H. M. Johnson.** 1982. Interleukin-2 regulation of mitogen induction of immune interferon (IFNγ) in spleen cells and thymocytes. Cell. Immunol. **66**:333–341.
37. **Youngner, J. S., and W. R. Stinebring.** 1965. Interferon appearance stimulated by endotoxin, bacteria, or viruses in mice pretreated with *Escherichia coli* endotoxin or infected with *Mycobacterium tuberculosis*. Nature (London) **208**:456–458.