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Bacterial Lipopolysaccharide Potentiates Gamma Interferon-Induced Cytotoxicity for Normal Mouse and Rat Fibroblasts

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Gamma interferon (IFN-γ) can be cytolytic for normal mouse fibroblasts isolated from embryonic or adult tissue (R. Dijkmans, B. Decock, H. Heremans, J. Van Damme, and A. Billiau, Lymphokine Res. 8:25–34, 1989). This cytotoxicity has been shown to be transcription and translation dependent, thereby suggesting involvement of a suicidelike mechanism. The dose of IFN-γ required for cytotoxicity is higher than that needed for antiviral and macrophage activation but can be reduced 10- to 100-fold by cotreatment of the cells with tumor necrosis factor or interleukin-1 (IL-1) or both, two cytokines that by themselves are not toxic for these cells. Here, we show that bacterial lipopolysaccharide (LPS), which alone has no effect on the viability of mouse fibroblasts, stimulates cell suicide induced by IFN-γ. The effect was observed in cultures that were virtually free of nonfibroblastoid cells. LPS showed its toxicity-enhancing effect only if applied on the cells simultaneously with or immediately after treatment with IFN-γ. Pretreatment of the cells with LPS was ineffective. Inclusion of antibodies directed against tumor necrosis factor α or IL-1α in the culture medium did not block the cytotoxic effect of combined IFN-γ plus LPS treatment. The time courses of cell toxicity appearance in fibroblasts treated with combined IFN-γ plus LPS or IFN-γ plus IL-1 were similar. In addition to LPS, heat-killed gram-negative (*Escherichia coli*) but also gram-positive (*Staphylococcus aureus, Listeria monocytogenes*) bacteria were found to enhance IFN-γ-induced cell death. These findings suggest that IFN-γ formed in vivo during infectious processes directly aggravates tissue destruction.

The pathogenicity of bacteria is largely determined by their secretion of exotoxins or release of endotoxins. The term endotoxin generally refers to the phospholipid-polysaccharide-protein macromolecules associated with the cell wall of gram-negative bacteria. Lipopolysaccharide, the most active component of this complex, consists of a lipid and a polysaccharide component. In contrast to many exotoxins, bacterial endotoxins have few direct toxic actions and most of the pathological phenomena observed after infection with gram-negative bacteria are accounted for by overstimulation of the immune and the blood clotting systems (12). For example, lipopolysaccharide is one of the factors that, upon massive release in the circulation, may lead to a shock syndrome characterized by a drastic fall of blood pressure and sometimes death. Lipopolysaccharide also activates the complement system via both the classical and the alternate pathways. Lipopolysaccharide is a polyclonal stimulator for B cells, thereby giving rise to the production of large amounts of nonspecific immunoglobulins. Lipopolysaccharide can trigger the production of a variety of biologically active mediators (tumor necrosis factor, interleukin-1, etc.) by mononuclear phagocytes. Through its interaction with macrophages it may also induce the production of gamma interferon by T lymphocytes and natural killer cells (10, 11, 14).

In a previous study (4), we showed that gamma interferon is cytotoxic for normal mouse fibroblast cultures. We also reported that this gamma interferon-induced lethal effect is enhanced by tumor necrosis factor and interleukin-1, which are not cytotoxic for these cells without gamma interferon. The gamma interferon-induced cytotoxic effect was shown to depend on active RNA and protein synthesis of the target cells, suggesting a suicidelike reaction. In this paper, we report that lipopolysaccharide, which by itself has no effect on the viability of mouse and rat fibroblasts, can strongly potentiate the gamma interferon-induced cytotoxicity for these cells. This finding suggests a novel possible mechanism of bacterial pathogenicity: gamma interferon may synergize with bacterial endotoxin to destroy cells at the inflammatory site.

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MATERIALS AND METHODS

Abbreviations. EMEM, Eagle minimal essential medium; FCS, fetal calf serum; IFN-γ, gamma interferon; IL-1, interleukin-1; LPS, lipopolysaccharide; MEF, mouse embryonic fibroblasts; Mu, murine; REF, rat embryonic fibroblasts; TNF, tumor necrosis factor.

Products. Crude supernatant from Chinese hamster ovary (CHO) cells that had been transformed with an expression plasmid containing MuIFN-γ cDNA was used in most experiments as a source of MuIFN-γ (5). Culture supernatant from CHO cells transformed with the expression plasmid with the MuIFN-γ cDNA in the opposite orientation toward the promoter was used as a control. Crude IFN-γ-containing supernatant gave results similar to those of monoclonal antibody affinity-purified MuIFN-γ (4). IFN concentrations were determined by a viral cytopathic effect reduction assay (8) and were expressed in international units (National Institutes of Health international reference standard Gg02-901-533). The endotoxin concentration of IFN-γ preparations was less than 100 pg/ml as determined by a *Limulus* amoebocyte chromogenic assay (performed by P. Van Limbergen, Blood Transfusion Service, Leuven). Recombinant mouse TNF-α and a polyclonal antiserum against recombinant mouse TNF-α were obtained from A. Bosman and H. Van Heuverswijn (Innogenetics, Ghent, Belgium). Natural human IL-1β was prepared in our laboratory (17). A purified goat anti-mouse IL-1α antiserum was provided by R. Chiz-

zonite (Hoffmann-La Roche Inc., Nutley, N.J.). Cycloheximide and actinomycin D were from Serva (Heidelberg, Federal Republic of Germany). Bacterial endotoxins (phenol extracts from *Serratia marcescens*, *Salmonella enteritidis*, and *Escherichia coli*), phorbol 12-myristate-13-acetate, and all-*trans* retinoic acid were from Sigma Chemical Co. (St. Louis, Mo.). Concanavalin A was from Calbiochem-Behring (San Diego, Calif.). Toxic shock syndrome toxin-1 was from Toxin Technology (Madison, Wis.), and poly(rI)-poly(rC) was from P-L Biochemicals, Inc. (Milwaukee, Wis.).

Methods. Cytotoxicity assays were performed as described before (4). Briefly, mouse fibroblast cultures were established from NMRI mouse embryo (MEF) or Wistar rat embryo (REF) tissue. Secondary MEF were seeded in 96-well microdilution plates (40,000 cells per well) and cultured for 24 h in EMEM supplemented with 10% FCS. MEF were then refed with EMEM (100 μl per well) supplemented with 2% FCS and serial threshold dilutions of IFN-γ (time zero). The cells were cultured at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. After 24 h, the medium was replaced by the same volume of EMEM supplemented with 2% FCS and the cells were cultured at 37°C for an additional 24 h. The cells were then stained with neutral red, and the viability of the culture was expressed in absorption units (A_{542}).

Percent toxicity was expressed as follows: $100 - \{100 \times [(A_{542}\text{ sample} - A_{542}\text{ positive control})/(A_{542}\text{ negative control} - A_{542}\text{ positive control})]\}$ were positive control is a cell culture that was totally destroyed by mengovirus and negative control is a culture treated identically as the sample except that IFN-γ was omitted. The 50% toxic dose is the concentration of IFN-γ required to obtain 50% toxicity.

Alterations to this experimental protocol were as follows. LPS or heat-killed bacteria were added to the culture medium before, simultaneously with, or after contact with IFN-γ. Heat-killed bacteria were prepared as follows. *E. coli* (NCIB 8743), *Staphylococcus aureus* (13N2904), *Listeria monocytogenes* (ATCC 984), and *Candida albicans* (C011) cells were grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) for 48 h at 27°C. The culture broth was separated from the cells by centrifugation, and cells were washed in phosphate-buffered saline and autoclaved (10 min, 120°C) in phosphate-buffered saline. Heat-killed cells were then diluted and applied on IFN-γ-treated MEF. In some experiments, the heat-killed cells were further purified. Heat-killed cells were centrifuged (1 min, 16,000 × *g* in an Eppendorf 5415 centrifuge), and the precipitate was washed twice in 10 volumes of phosphate-buffered saline and reconstituted in 1 volume of phosphate-buffered saline. In some instances, as indicated, actinomycin D (800 ng/ml) or cycloheximide (400 ng/ml) was added to the culture medium (0 to 16 h or 16 to 48 h).

Seroneutralization experiments were performed as follows. IFN-γ dilutions (with or without other lymphokines or LPS) were supplemented with antiserum and incubated for 3 h at 37°C before application on MEF. Anti-MuIL-1α antiserum was used at a final concentration of 60 μg/ml (neutralizes 30 U/ml in D10 bioassay; R. Chizzonite, personal communication). Anti-TNF-α antiserum was used at 1,000 neutralizing units per ml. After 24 h, the MEF culture medium was replaced by the same volume of EMEM supplemented with 2% FCS and antiserum. The concentration of TNF was determined by using its capacity to induce toxicity in L929 cells.

Mouse peritoneal cells were isolated from NMRI mice. Cells were incubated in EMEM supplemented with 10% FCS in plastic tissue culture bottles. After 1 h, nonadherent cells were withdrawn; adherent cells were isolated by trypsinization. Mouse muscle cells were isolated from adult NMRI mice as described earlier (4).

TABLE 1. Enhancement of IFN-γ-induced cell death of MEF by LPS

IFN-γ (IU/ml)	Viability (A_{542}) after the following LPS (ng/ml) treatment[a]:			
	0	10	100	1,000
0	1.08 (0)	1.01 (0)	1.08 (0)	1.04 (0)
0.16	1.09 (−1)	1.03 (2)	1.09 (−1)	1.02 (2)
1.6	1.01 (8)	0.65 (42)	0.19 (96)	0.18 (97)
16	1.01 (8)	0.16 (99)	0.17 (98)	0.17 (98)
160	0.92 (17)	0.16 (99)	0.18 (97)	0.17 (98)
1,600	0.19 (96)	0.15 (100)	0.16 (99)	0.18 (97)

[a] Viability (mean of duplicate samples) of MEF treated with IFN-γ (0 to 16 h) and LPS (0 to 48 h). Values in parentheses show percent toxicity.

RESULTS

Effect of LPS on IFN-γ-induced cytotoxicity. Serial threefold dilutions of CHO cell-derived recombinant MuIFN-γ were added to monolayers of secondary MEF (time zero). After 24 h, the cells were refed with fresh medium and further incubated at 37°C. At 48 h, cultures treated with MuIFN-γ at doses of approximately 1,000 U of MuIFN-γ per ml showed cytotoxicity. The addition of as much as 10 μg of *S. marcescens* LPS (phenol extract) per ml without IFN-γ to MEF failed to affect the vital staining or the microscopically observed morphology of the cells. However, significant cytotoxicity was induced by combined treatment with IFN-γ and *S. marcescens* endotoxin at concentrations at which neither of the two individual compounds had an effect on the viability of the cells (Table 1). The enhancing effect was also observed when *E. coli*- and *S. enteritidis*-derived endotoxins were used. No differences in effectivity among these endotoxins were observed. LPS showed this activity if present from 0 to 24 h, from 24 to 48 h, or from 0 to 48 h, the latter time schedule being the most effective (Fig. 1). In contrast, the addition of LPS before stimulation with IFN-γ (from −24 to 0 h) had no effect on the IFN-γ-induced cytotoxicity. Enhancement of cytotoxicity was also evident on secondary cultures of adult mouse muscle cells; the 50% toxic dose for IFN-γ alone was 500 U/ml, and that of IFN-γ plus 100 ng of LPS per ml was less than 20 U/ml. (The 50% toxic dose is the concentration of IFN-γ that kills 50% of the cells, as measured by neutral red staining of the cells). Treatment with actinomycin D or cycloheximide blocked the cytotoxic effect of combined IFN-γ plus LPS. Whereas 8 U of IFN-γ per ml plus 100 ng of LPS per ml was sufficient to cause 50% toxicity in control MEF, no toxicity was observed in the presence of actinomycin D (800 ng/ml, 0 to 16 h) or cycloheximide (400 ng/ml, 0 to 16 h), even at 500 U of IFN-γ per ml. LPS had no influence on the antiviral effect of IFN-γ on MEF. Concanavalin A (as much as 30 μg/ml), poly(rI)-poly(rC) (100 μg/ml), phorbol 12-myristate-13-acetate (100 ng/ml), all-*trans* retinoic acid (150 μM), and toxic shock syndrome toxin-1 (1 μg/ml) were ineffective as enhancers of IFN-γ-induced cytotoxicity (data not shown). Mouse L929 cells which were earlier shown not to be sensitive to the cytotoxic effect of IFN-γ alone were also insensitive to IFN-γ plus LPS (Table 2).

To exclude the possibility that the effect of LPS was due to its interaction with a contaminating nonfibroblastoid cell

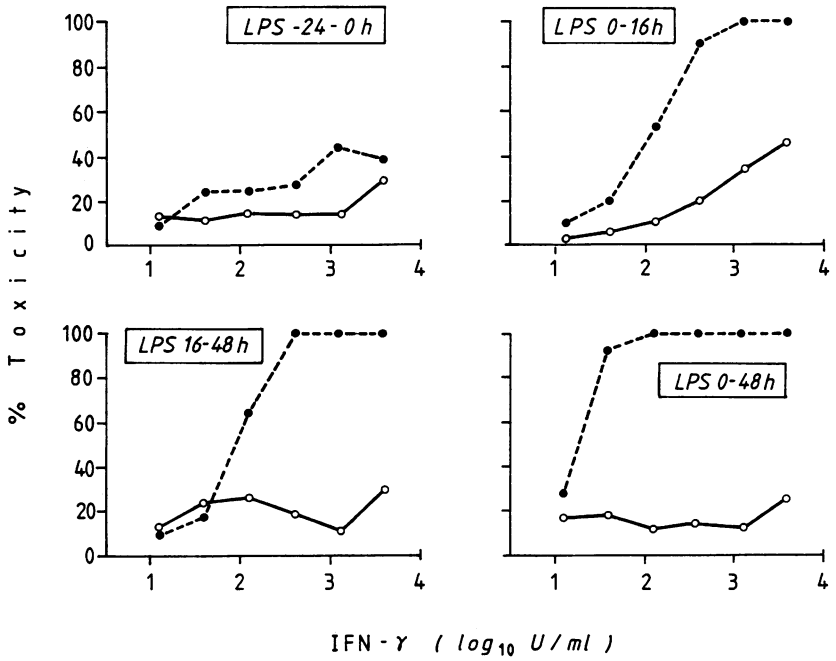

FIG. 1. Effect of time of LPS addition. MEF were treated with IFN-γ from 0 to 16 h, refed with fresh medium, and stained at 48 h. LPS (100 ng/ml) was present in the culture medium at the indicated time intervals. ——, without LPS; - - - -, with LPS.

(macrophage, lymphocyte, endothelial cell, etc.), we wanted to test the effect of combined IFN-γ plus LPS treatment on fibroblast cultures that were less likely than mouse embryo cells to contain such cells. Since mouse embryonic and adult tissue fibroblast cultures undergo a growth crisis after approximately four passages in vitro and cultures expanded from cells surviving this crisis may have a transformed phenotype, we analyzed the effect of MuIFN-γ on fibroblast cultures derived from rat embryos (REF), which have a longer lifespan in culture. MuIFN-γ alone (as much as 3.8 $\log_{10}$ U/ml) was not toxic for REF. However in combination with LPS, cytotoxicity was induced in REF at passage 2 up to passage 8 (Table 2). Another argument against the involvement of macrophages in the IFN-γ-induced cytotoxicity for MEF was the finding that artificial contamination of MEF with mouse peritoneal cells (total, nonadherent, or adherent) did not cause a further increase in cytotoxicity, or rather counteracted it (Table 2).

TABLE 2. Susceptibility of mouse and rat cells for MuIFN-γ-induced cell death

Cells[a]	50% toxic dose ($\log_{10}$ IU/ml)		
	IFN-γ	IFN-γ + LPS[b]	IFN-γ + IL-1[c]
Mouse			
MEF 1	2.7	0.9	1.3
MEF 1 + PC	ND[d]	1.3	1.4
MEF 1 + NAPC	>2.7	1.1	1.4
MEF 1 + APC	>2.7	2.7	>2.7
MEF 4	ND	0.6	1.1
MEF 4 + APC	ND	0.4	ND
L929	>3.8	>3.8	>3.8
Rat			
REF 2	>3.8	2.3	1.7
REF 3	>3.8	3.0	ND
REF 5	>3.8	2.8	ND
REF 7	>3.8	2.0	1.7
REF 8	>3.8	2.2	1.5

[a] Number shows passage number. MEF were mixed with 20% mouse peritoneal cells (PC), adherent peritoneal cells (APC), or nonadherent peritoneal cells (NAPC).
[b] LPS at 0.1 μg/ml (MEF), 1 μg/ml (REF), or 10 μg/ml (L929 cells).
[c] IL-1 at 10 U/ml.
[d] ND, Not determined.

Induction of TNF and IL-1 is not a major mechanism responsible for the effect of LPS. In a previous study (4), we showed that TNF and IL-1 are capable of enhancing the IFN-γ-induced cytotoxicity. The possibility had to be considered that induction of these cytokines in MEF might be a major mechanism responsible for the effect of LPS. This hypothesis was supported by the lack of synergism observed in cultures treated with IFN-γ in combination with suboptimal doses of LPS plus suboptimal doses of TNF or IL-1. For example, 200 U of IFN-γ per ml plus 3 U of IL-1 per ml induced 70% toxicity; IFN-γ plus 1 ng of LPS per ml induced 50% toxicity, and the combination of IFN-γ plus IL-1 plus LPS yielded 90% toxicity, which is not more than an addition of the individual toxicity values. For comparison, IFN-γ plus a threefold-higher dose of IL-1 (10 U/ml) was 100% toxic. In another experiment, it was shown that the 50% toxic dose of IFN-γ in combination with LPS was identical to that required in the combination IFN-γ plus LPS plus IL-1 or IFN-γ plus LPS plus TNF (Table 3). A further indication for involvement of IL-1 in mediating LPS-induced cytotoxicity enhancement was the observation of induction of MuIL-1α RNA and, to a lesser extent, MuIL-1β RNA in IFN-γ-treated MEF (data not shown). In case induction of IL-1 would be required, the time course of appearance of cytotoxicity induced by IFN-γ plus IL-1 was expected to be different from that induced by IFN-γ plus LPS. The kinetics of the IFN-γ-induced cytotoxicity was examined as follows. MEF were cultured at 37°C for 24 h in the presence of IFN-γ and LPS or IL-1, refed, and further cultured in presence of LPS or IL-1. MEF were stained with neutral red at 18, 22,

TABLE 3. Effect of LPS on cytokine-induced cell death of MEF

Treatment[a]	50% toxic dose (log_{10} IU/ml)[a]	
	−LPS	+LPS
IFN-γ	3.0	1.0
IFN-γ + IL-1	1.9	1.0
IFN-γ + TNF	2.5	1.0
IFN-γ + IL-1 + TNF	1.2	ND[b]

[a] IL-1, 10 U/ml; TNF, 5 ng/ml; LPS, 100 ng/ml.
[b] ND, Not determined.

24, 26, 31, 38.25, 42, 46, 48.5, 53.5, 66, and 72 h after first contact with IFN-γ (Fig. 2). Cytotoxicity was first apparent at 42 h and was maximal at 48 h. No differences in kinetics were observed between either treatment. A treatment with higher or lower doses of IFN-γ than those employed in the experiment shown in Fig. 2 or a combined treatment with IFN-γ and TNF had a similar time course (data not shown). Although these results showed that the surmised induction of IL-1 and TNF is not rate limiting in the establishment of the toxicity, they did not disprove that IL-1 and TNF are required for the effect since induction of these cytokines by LPS may be very rapid.

Therefore, we tested whether addition of antibodies against IL-1α and TNF-α inhibited the IFN-γ-induced cytotoxicity. Solutions containing dilutions of MuIFN-γ in combination with LPS, MuTNF-α, or human IL-1β were supplemented with polyclonal antisera against MuIL-1α or MuTNF-α, preincubated for 3 h at 37°C, and applied on MEF monolayers. After 24 h, the culture medium was removed and replaced by fresh EMEM supplemented with 2% FCS and the antibody preparations. Antibodies against MuIL-1α had hardly any effect on the outcome of either treatment (Table 4). The concentration of anti-IL-1 antibodies was not a limiting factor in the seroneutralization since a 10-fold-higher dose of it was not more effective (data not shown). Although anti-TNF-α antibodies caused a slight but significant ($P < 0.05$) neutralization of the IFN-γ–LPS-induced toxicity, they were likewise unable to block the effect (Table 4). Here again, it is unlikely that the dose of anti-TNF was too low for complete seroneutralization since this dose neutralized >99% of the activity of 50 ng of TNF per ml in the L929 cytotoxicity assay, and no detectable TNF (<1 ng/ml) was present in the culture medium of IFN-γ–LPS-treated MEF. As could be expected, cytotoxicity on MEF caused by IFN-γ plus TNF was severely reduced. A combined treatment with anti-IL-1 and anti-TNF was not more effective in neutralizing IFN-γ–LPS-induced toxicity than treatment with the individual antibodies (data not shown). Thus, secretion of IL-1α and TNF-α by LPS–IFN-γ-treated MEF may not be a major mechanism responsible for the LPS-induced enhancement of cytotoxicity.

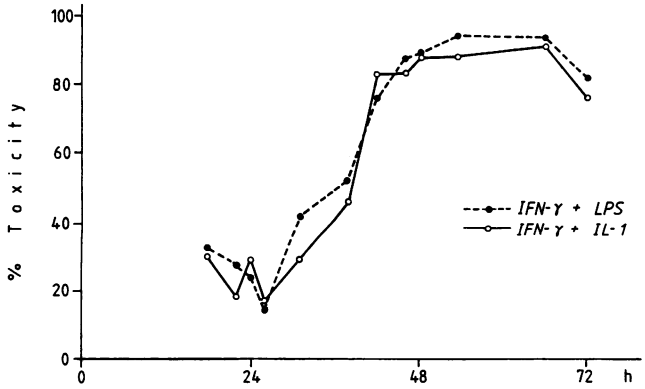

FIG. 2. Kinetics of cytotoxicity. IFN-γ (500 U/ml) was added at 0 h and withdrawn at 18 h. LPS (100 ng/ml) or IL-1 (10 U/ml) was present from 0 h until staining.

TABLE 4. Effect of anti-TNF-α and anti-IL-1 on IFN-induced cell death in MEF

Treatment[a]	Increase in 50% toxic dose of IFN-γ in presence of[b]:	
	Anti-IL-1	Anti-TNF
IFN-γ	0.0 (1)	0.23 (0.13; 3)
IFN-γ + LPS	0.09 (0.03; 7)	0.28 (0.10; 6)
IFN-γ + IL-1	0.2 (1)	0.10 (0.06; 3)
IFN-γ + TNF	−0.1 (1)	1.13 (0.08; 3)

[a] LPS, 100 ng/ml; mouse TNF-α, 5 ng/ml; human IL-1β, 10 U/ml.
[b] 50% toxic dose (log_{10} international units per milliliter of IFN-γ) with antiserum − 50% toxic dose without antiserum. Values are means (standard error; *n*) over *n* independent experiments, each assayed in duplicate. The 50% toxic doses for IFN-γ alone, IFN-γ plus LPS, IFN-γ plus IL-1, and IFN-γ plus TNF were 3.0 to 3.5, 0.9 to 1.3, 1.5 to 2.0, and 2.6 to 3.0 log_{10} U/ml, respectively.

Heat-killed bacteria are also capable of enhancing IFN-γ-induced cell death. To examine whether the effect of LPS could be generalized to other microbial products, we examined the effect of heat-killed bacteria and yeast cells on IFN-γ-induced cell death. The enhancing effect was not restricted to LPS; heat-killed *E. coli* cells were also effective (Table 5). This effect was observed at endotoxin concentrations that were much lower than those required for enhancement in the absence of *E. coli* cells. Moreover, enhancement was observed with virtually endotoxin-free preparations of gram-positive bacteria. The enhancing bacterial compound could not be removed from the cells by washing and was shown to be present in both heat-killed and untreated bacterial suspensions. Cytotoxicity enhancement was not seen with preparations of heat-killed yeast and animal cells (Table 5). These data suggest that bacterial compounds other than LPS increase the sensitivity of MEF for IFN-γ-induced cell death.

DISCUSSION

The most significant conclusion of the experiments presented here is that LPS strongly enhances the IFN-γ-

TABLE 5. Enhancement of IFN-γ-induced cell death in MEF by microbial products

Product or bacteria	Toxic concn	Lowest effective concn[a]	Endotoxin[b] (pg/ml)
LPS	>100 ng/ml	10 ng/ml	
E. coli[c]	>50 × 10^8 CFU/ml	15 × 10^5 CFU/ml	19
S. aureus[c]	>32 × 10^6 CFU/ml	32 × 10^4 CFU/ml	<10
L. monocytogenes[c]	>35 × 10^8 CFU/ml	10 × 10^7 CFU/ml	<30
C. albicans[c]	>65 × 10^5 CFU/ml	>65 × 10^5 CFU/ml	
L929 cells[c]	>10^5 cells/ml	>10^5 cells/ml	

[a] Lowest concentration giving at least 50% toxicity in combination with 600 IU of MuIFN-γ per ml. MEF were in contact with IFN-γ from 0 to 24 h and with products from 24 to 48 h. Concentration of heat-killed microbes is expressed in CFU per milliliter as measured before heat killing.
[b] Endotoxin concentration of the product at its lowest effective concentration. Limulus amoebocyte chromogenic assay.
[c] Heat killed.

induced cytotoxicity for normal mouse fibroblasts. This finding sheds new light on the role of IFN-γ in defense against and recovery from microbial diseases. A large body of in vivo studies has clearly demonstrated that IFN-γ is an essential component in resistance against microbes and parasites including *Plasmodium berghei*, *Toxoplasma gondii*, *L. monocytogenes*, and *Histoplasma capsulatum* (3, 13). This antimicrobial effect of IFN-γ is generally thought to occur through its activation of macrophage antimicrobial capacities. However, other mechanisms such as inhibition of parasite growth by depletion of essential growth elements (15) may not be excluded. Besides being a protective factor, IFN-γ also has been implicated as an agent that aggravates the harmful effects of microbial infections (9).

We showed here that mouse fibroblasts become much more susceptible to the cytolytic action of IFN-γ in the presence of the gram-negative bacterial product LPS but also in the presence of heat-killed gram-negative and gram-positive bacteria. A recent study (2) shows that mouse L929 cells infected with *Chlamydia psittaci* are killed by IFN-γ, whereas noninfected cells are resistant. The finding that microbial infection of fibroblastoid cells (2) or contact of such cells with certain microbial products (this report) may enhance their susceptibility to IFN-γ-induced suicide suggests that IFN-γ-induced cell death has relevance to host cell toxicity induced by microbial attack. Local destruction of connective tissue may elicit a stronger immune response against the invading microorganism and/or may facilitate migration of macrophages to the inflammatory site. Local cell death may thus constitute an advantage for the host. However, in case of massive infection, IFN-γ-induced and LPS-amplified cell suicide may contribute considerably to the pathology of the disease, and in this case therapeutic depression of IFN-γ production or inhibition of IFN-γ action may diminish the severity of the disease.

We do not know which mechanism is responsible for the increased cytotoxicity elaborated by LPS treatment of mouse and rat fibroblastoid cell cultures. A hypothesis was that contaminating adherent nonfibroblastoid cells would become stimulated by LPS and consequently would produce TNF or IL-1, two cytokines that have already been shown to amplify IFN-γ-induced cell suicide. This hypothesis was mainly based on the consideration that LPS is a potent stimulator of macrophages (13) and that only few activities of LPS on fibroblasts are demonstrated (7). The presence of nonfibroblastoid cells in MEF cultures would also explain why cytotoxicity was more pronounced for some MEF isolates (e.g., Table 1) than for others (e.g., Table 2). However, differences in susceptibility may also be caused by slight variations in culture conditions (cell density [16], pH [1], batch of serum [6]). In addition, contaminating macrophages seem not to be involved in the process since (i) cytotoxicity was observed in high-passage REF which certainly do not contain macrophages, (ii) no class II-positive cells could be detected in MEF cultures by fluorescence-activated cell sorter analysis (data not shown), and (iii) mixed cultures of mouse embryonic cells and mouse adherent peritoneal cells were not more susceptible than mouse embryo cells alone. Finally, secretion of IL-1α or TNF-α or both by LPS-stimulated cells cannot, at least completely, explain the effect of LPS since inclusion in the culture medium of antibodies against these cytokines failed to block cytotoxicity.

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