Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 607–611, January 1988
Medical Sciences

Synergy between tumor necrosis factor and bacterial products causes hemorrhagic necrosis and lethal shock in normal mice

(Shwartzman phenomenon/endotoxin shock/lipopolysaccharide/mycoplasma/corynebacterium)

JAY L. ROTHSTEIN* AND HANS SCHREIBER

Division of Biological Sciences, The University of Chicago, 5841 Maryland Avenue, Chicago, IL 60637

Communicated by Janet D. Rowley, September 25, 1987 (received for review August 1, 1987)

ABSTRACT **We find a strong synergism between tumor necrosis factor (TNF) and bacteria or their products. Endotoxin-"free" recombinant TNF, even at very high doses (160 μg), did not alone cause hemorrhagic necrosis (HN) in the skin of normal mice. Similarly, TNF alone had a low systemic toxicity in tumor- and pathogen-free mice. However, TNF given intravenously with nanogram quantities of the endotoxin lipopolysaccharide caused lethal shock. Furthermore, subcutaneous injection of lipopolysaccharide made skin susceptible to subsequent induction of HN by TNF injected in the same site 24 hr later. Mycoplasma-infected cells or corynebacteria also synergized with TNF to cause HN or lethal shock. In addition, we find that lymphotoxin, a cytokine functionally and genetically related to TNF, also synergized with the bacteria to cause HN, whereas interleukin 1α or interferon γ did not. Together, the results indicate that a synergy between TNF and bacteria or their products causes HN and lethal shock in normal mice.**

Certain bacterial products, such as the endotoxin lipopolysaccharide (LPS), induce the release of a factor into serum that causes hemorrhagic necrosis (HN) of tumors and is called tumor necrosis factor (TNF; ref. 1). TNF has been cloned and the recombinant material, like the native product, causes HN of tumors (2). It is of major concern that, in addition, the recombinant TNF has been found to induce many of the deleterious effects of endotoxin, such as lethal shock and disseminated HN (3). It is not clear why this systemic toxicity of TNF *in vivo* was only found after recombinant preparations of TNF became available and why it had not been observed in previous studies using nonrecombinant sources of TNF (for review, see ref. 4). The higher systemic toxicity of recombinant TNF could be due to the use of higher doses, to structural differences between the recombinant and nonrecombinant TNF molecules, or to impurities in the recombinant preparations (4).

Interestingly, we find that recombinant TNF, which is virtually endotoxin-free, has a low toxicity in the absence of added endotoxin or other microbial agents so long as tumor-free and pathogen-free animals are tested. However, we observe a very strong synergism between TNF and LPS endotoxin or between TNF and other bacteria in causing lethal shock or HN in normal tissues.

MATERIALS AND METHODS

Mice. Six- to 10-week-old, pathogen-free, female mice of the C3H/HeN, C3H/HeJ, athymic NCR *nu/nu*, and BALB/cAn strains were purchased from a germ-free-derived, defined-flora colony at the Frederick Cancer Research Institute (Frederick, MD) and maintained in pathogen-free conditions in laminar flow hoods.

Cytokines and Bacteria. Recombinant human TNF (lot 3056-55) and recombinant human lymphotoxin (LT; lot 4260-1) had specific activities of 5.01×10^7 units (U)/mg and 1.02×10^8 U/mg, respectively, based on cytotoxicity of actinomycin D-treated L929 mouse fibroblast cells (5). Recombinant murine interferon γ (IFN-γ; lot 4407-47) had a specific activity of 1.97×10^7 U/mg. TNF, LT, and IFN-γ were obtained from Genentech. Recombinant human interleukin 1α (IL-1α; lot SM63) had a specific activity of 5×10^9 U/mg and was obtained from Hoffmann–La Roche. Endotoxin levels of cytokines, expressed as endotoxin units (EU), were TNF ≤ 0.125 EU/ml, IL-1α ≤ 15 EU/ml, IFN-γ ≤ 2 EU/ml, and LT ≤ 0.062 EU/ml, as measured in the *Limulus* amoebocyte lysate assay given that 1 EU is equal to 0.1 ng/ml of USP standard *Escherichia coli* endotoxin (lot EC5). The LPS endotoxin of *E. coli* 0111:B4 (lot 743397, Difco) was diluted in nonpyrogenic saline (Invenex Labs, Melrose Park, IL) to 10 mg/ml and sterilized by 0.22-μm filtration before use. Heat-killed *Corynebacterium parvum* (lot 608122, Behring Diagnostics, San Diego, CA) was diluted in nonpyrogenic saline to 10 mg/ml before use. *Mycoplasma orale* was passaged by intracellular growth *in vitro* and expanded with the cells for the generation of lysate as described below.

Cell Lines. The UV-induced fibrosarcomas 1591-RE and 1316-RE, normal heart-lung fibroblast strains, and 10T½ cells (an untransformed fibroblast line) were obtained and passaged as described (6).

Assay for HN and Lethal Shock. The lower backs of mice were shaven and then depilated with a chemical depilator (Nair, Carter-Wallace, New York). Test material was injected subcutaneously (s.c.) with or without TNF. Removing the hair before the start of the experiment did not influence the results and for convenience, therefore, we usually depilated the skin before the start of the experiment. The degree of HN was scored 24 hr later and in some cases skin sections were examined microscopically. Cell lysates used for induction of necrosis were generated by three cycles of freeze-thawing followed by a 30-min sonication. In some experiments, mice were primed for HN with LPS (100 μg) injected s.c. 24 hr before cytokine injection. To study the systemic toxicity of mixtures of TNF and LPS or TNF and *C. parvum*, the materials were coinjected i.v. by way of the retroorbital plexus.

RESULTS

Synergism Between LPS and TNF in Causing Lethal Shock. When normal pathogen-free C3H/HeN mice were injected i.v. with human TNF no lethality was observed except at the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: EU, endotoxin unit(s); HN, hemorrhagic necrosis; IFN-γ, interferon γ; IL-1α, interleukin 1α; LPS, lipopolysaccharide; LT, lymphotoxin; PML, polymorphonuclear leukocyte(s); TNF, tumor necrosis factor.

*To whom reprint requests should be addressed.

Table 1. LPS synergizes with TNF in causing lethal shock

Dose of TNF, μg†	Dose of LPS (μg) causing lethality*								
	0	0.023	0.093	0.38	1.5	6	25	100	400
0.0	0/5	0/2	0/2	0/2	0/5	0/8	0/3	**1/17**	**2/2**
0.6	0/5	0/2	0/2	0/2	0/7	0/7	0/5	**2/5**	**2/2**
2.5	0/5	0/2	0/2	0/2	**1/7**	**3/7**	**1/5**	**4/5**	**2/2**
10.0	0/11	**2/6**	**3/6**	**3/5**	**5/7**	**7/7**	**2/2**	**2/2**	**2/2**
40.0	0/18	**3/6**	**4/6**	**5/5**	**7/7**	**7/7**	**2/2**	ND	ND
160.0	**2/19**	**5/5**	**5/5**	**2/2**	**3/3**	**3/3**	**1/1**	ND	ND

ND, not done.

*Number of deaths/number of mice. Lethality occurred within 2–24 hr. Data are pooled from two to seven independent experiments, each also containing mice injected with LPS alone and TNF alone as controls. Numbers set in boldface type indicate lethality was observed.

†C3H/HeN mice received an i.v. injection of TNF in nonpyrogenic saline at the various doses alone or together with LPS.

highest dose (160 μg per mouse or 8 mg/kg); but even at this dose, only about 10% of the mice died after the TNF injection (Table 1, second column); death occurred in <24 hr with symptoms similar to those observed after injection of high doses of LPS (piloerection, lethargy, diarrhea, fluid exudation around eyes). i.v. injections of 100 μg of LPS alone per mouse caused <10% lethality (Table 1). In contrast, we observed in repeated experiments that combined doses (lower by a factor of 8 than individual doses of TNF or LPS that caused only 10% lethality) killed 100% of the mice. Furthermore, doses of LPS as low as 23 ng caused 50% lethality when applied with a nonlethal dose of TNF. An isobologram (Fig. 1 *Upper*) dramatizes that the combined doses of TNF and endotoxin were markedly synergistic and not additive. Similar synergism was also observed when another strain of LPS-sensitive pathogen-free mice (BALB/cAn) was treated with combinations of TNF and LPS.

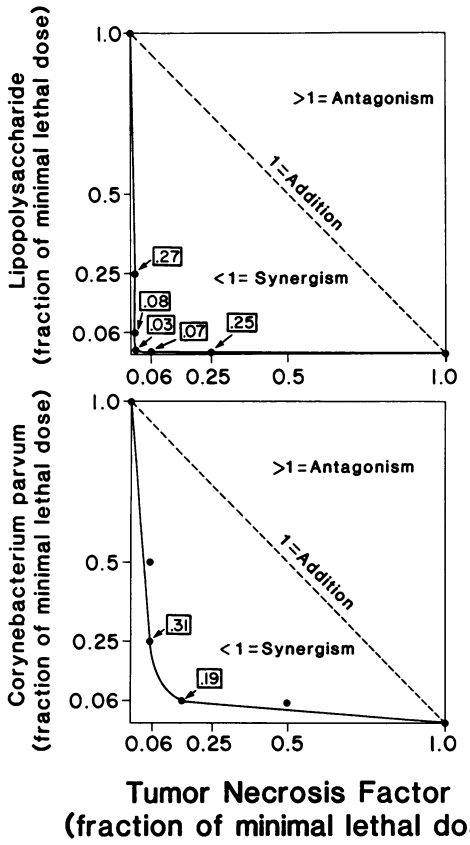

FIG. 1. LPS and TNF (*Upper*) or *C. parvum* and TNF (*Lower*) synergize in causing lethal shock in mice. The effects are expressed as isobols with solid circles representing doses at which lethality was observed. Doses are plotted as fractions of the lowest (minimal) doses of LPS (100 μg = 1), *C. parvum* (4.8 mg = 1), and TNF (160 μg = 1) that were found to cause any lethality on their own (corner points). At this minimal lethal dose, LPS (100 μg) caused 6% lethality, TNF (160 μg) caused 10%, and *C. parvum* (4.8 mg) caused 50%. The two agents show synergism in causing lethal shock when both agents were given together because the sums of the fractions (boxed values <1) of the minimal lethal doses of these two agents were sufficient to kill the mice. The extremely concave curve in *Upper* is typical for maximum synergism between two agents, in this case LPS and TNF. *C. parvum* (*Lower*) is also synergistic but to a lesser degree. Additive effects would have resulted in a straight line (dotted) with values of the sums = 1 and antagonistic effects would have resulted in a convex curve with values of the sums > 1. TNF and LPS toxicity data points are also shown in Table 1, TNF and *C. parvum* toxicity data are derived from two to seven independent experiments, each containing control doses of TNF or *C. parvum* alone. Data are calculated as described by Sande and Mandell (7).

Table 2. TNF causes HN in LPS-prepared skin: Independence from T-cell immunity but sensitivity to x-ray

Strain	First injection*	Interval, hr	Second injection	Interval, hr	Incidence of necrosis (%)†	
C3H/HeN	LPS + TNF	24	—	—	0/8	(0)
Normal	LPS	24	TNF	24	21/26	(81)‡
	TNF	48	—	—	0/5	(0)
	LPS	48	—	—	0/5	(0)
	TNF	24	LPS	24	0/5	(0)
650 rads§	LPS	24	TNF	24	0/10	(0)
Nude	LPS	24	TNF	24	6/7	(86)‡
C3H/HeJ	LPS + TNF	24	—	—	0/8	(0)
	LPS	24	TNF	24	0/5	(0)
BALB/cAn	LPS	24	TNF	24	3/4	(75)‡
	TNF	48	—	—	0/5	(0)

*Single or mixed reagents were injected s.c. in a final volume of 0.25 ml into the lower back skin of mice from which the hair had been removed by a chemical depilator. In all cases, the dose of LPS was 100 μg and the dose of TNF was 10 μg.

†Number of mice with HN/number of mice. Percentages of mice with HN are given in parentheses. HN was confirmed histologically in 4 of the 24 mice tested showing polymorphonuclear leukocyte (PML) infiltrates and extravasation of blood. In the other instances, the term HN was simply used to describe a macroscopically visible 0.5- to 2.5-cm (diameter) dark red to black flat discoloration of the skin. Lesions begin to develop 7 hr after the last injection as erythema with scattered petechiae, which become confluent hemorrhagic lesions by 18–24 hr, at which time the lesions are scored. The lesions disappeared after 5–7 days. TNF alone injected s.c. did not cause detectable histologic changes. For each mouse strain, data are pooled from two independent experiments.

‡The sizes of the HN induced by LPS followed 24 hr later by TNF in C3H/HeN, nude, and BALB/cAn mice were similar [1.3 ± 0.1, 1.4 ± 0.2, and 1.4 ± 0.2 cm (mean ± SEM), respectively].

§Mice were exposed to 650 rads (1 rad = 0.01 gray) of γ-radiation from a ^{137}Cs source (J. L. Shepard and Associates, San Fernando, CA) 24 hr before injections.

However, the synergism was not observed in a substrain of C3H mice (C3H/HeJ) known to be genetically resistant to the lethal effects of LPS (data not shown), suggesting that the observed "toxification" of TNF was indeed mediated by the LPS in the endotoxin preparation.

LPS Primes Normal Skin for the Induction of HN by TNF. s.c. injection of TNF into normal skin at doses of 160 μg did not cause HN (data not shown). Furthermore, coinjection of a mixture of 100 μg of LPS and 10 μg of TNF into C3H/HeN mice did not induce HN at the site of injection, though 1 of the 8 mice injected died of shock within 24 hr following injection (Table 2). However, sequential s.c. injection of LPS followed 24 hr later by TNF caused a massive HN in 21/26 of the C3H/HeN mice, of which 11/21 died of lethal shock. All mice that died developed HN of the skin before death. In contrast, injection of TNF followed 24 hr later by LPS failed to induce any HN or lethality, suggesting that LPS but not TNF sensitized the s.c. site for induction of necrosis.

The development of HN was independent of a functional T-cell immunity since athymic nude mice developed HN after sequential injections of LPS and TNF (Fig. 2*A*). Furthermore, γ-irradiation (650 rads), which causes leukopenia, prevented the HN produced by sequential LPS and TNF injections. As expected, C3H/HeJ mice, known to be genetically resistant to the endotoxin effects (8) of LPS, did not develop HN after sequential LPS/TNF treatments, whereas BALB/cAn did develop such necrosis similar to C3H/HeN mice. However, the LPS-resistant C3H/HeJ mice responded, as determined histologically, to s.c. LPS injection with an influx of PML greater in magnitude than the LPS-sensitive C3H/HeN mice, which confirmed an earlier observation (9).

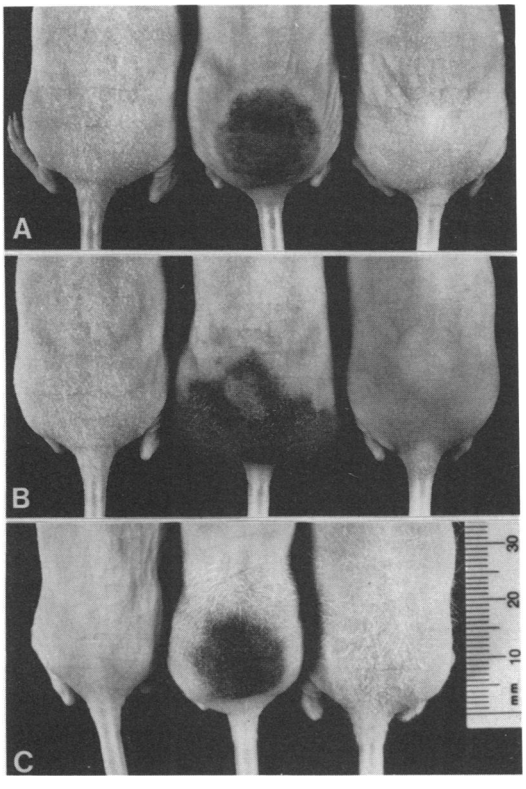

FIG. 2. (*A*) HN induced by TNF and LPS. Nude mice were primed with 100 μg of LPS 24 hr before the start of the experiments and either left unchallenged (right) or injected with 10 μg of TNF (center) where HN was detected 24 hr later. HN was not seen in mice injected with 10 μg of TNF alone (left). (*B*) The center panel shows HN induced by TNF and mycoplasma-positive lysates. Mice injected with 10 μg of TNF and lysates of 10^7 mycoplasma-positive cells developed HN 24 hr later, whereas no necrosis was seen with TNF alone (left) or TNF and lysates from 10^7 mycoplasma-negative cells (right). (*C*) The center panel shows HN induced by *C. parvum* and TNF. Nude mice developed HN 24 hr after injection of 300 μg of *C. parvum* and 10 μg of TNF but not with *C. parvum* alone (right) or TNF alone (left).

Certain Bacteria Can Synergize with TNF to Cause HN or Lethal Shock. The finding of synergism between TNF and LPS would have much broader implications if TNF also synergized with other bacteria or bacterial products. Mycoplasma organisms do not have a cell wall but produce lipoglycans, which are similar but not identical to the LPS in the cell wall of Gram-negative organisms (10). We, there-

Table 3. Certain bacteria synergize with TNF to cause HN

Host	Treatment: Bacterium	Treatment: TNF	Incidence of necrosis (%)*
Normal C3H/HeN	None	+	0/9 (0)
	MP-pos. tumor cells†	+	8/9 (89)‡
	MP-pos. tumor cells	−	0/5 (0)
	MP-neg. tumor cells	+	0/7 (0)
	MP-neg. tumor cells	−	0/5 (0)
	MP-pos. tumor cells (1 hr, 100°C)	+	2/2 (100)‡
	MP-pos. tumor cells (1 hr, 100°C)	−	0/2 (0)
	MP-pos. fibroblasts§	+	8/8 (100)‡
	MP-neg. fibroblasts	+	0/4 (0)
	C. parvum (killed)	+	13/14 (93)‡
	C. parvum (killed)	−	0/14 (0)
650 rads, C3H/HeN	None	+	0/3 (0)
	MP-pos. tumor cells	+	0/3 (0)
	C. parvum (killed)	+	1/10 (10)‡
Athymic C3H/HeN	None	+	0/3 (0)
	MP-pos. tumor cells	+	2/2 (100)‡
	MP-pos. tumor cells	−	0/4 (0)
	C. parvum (killed)	+	4/4 (100)‡
	C. parvum (killed)	−	0/5 (0)
Normal C3H/HeJ	None	+	0/7 (0)
	MP-pos. tumor cells	+	9/10 (90)‡
	C. parvum (killed)	+	7/7 (100)‡
	C. parvum (killed)	−	0/6 (0)
Normal BALB/cAn	None	+	0/5 (0)
	MP-pos. tumor cells	+	4/5 (75)‡
	C. parvum (killed)	+	10/10 (100)‡
	C. parvum (killed)	−	0/5 (0)
650 rads, BALB/cAn	None	+	0/5 (0)
	C. parvum (killed)	+	0/5 (0)
Athymic NCR nude	None	+	0/4 (0)
	MP-pos. tumor cells	+	3/5 (60)‡
	C. parvum (killed)	+	4/4 (100)‡

MP-pos., mycoplasma-positive; MP-neg., mycoplasma-negative.

*Experiments with more than three mice were pooled from two or more experiments, each containing a negative control (bacterium alone and TNF alone) and a positive control (bacterium and TNF). Percentages of mice with HN are given in parentheses. For other details, see footnote † of Table 2.

†1591-RE and 1316-RE were used as MP-pos. tumor cells, which induced necrosis in six of seven and two of two mice, respectively. Elsewhere in the table, MP-pos. tumor cells refer to 1591-RE only.

‡The sizes of the HN induced by MP-pos. lysates and TNF or *C. parvum* and TNF were similar [1.1 ± 0.2, 1.0 ± 0.1, 1.6 ± 0.1, 1.3 ± 0.1, 1.0, 1.3 ± 0.7, 1.9 ± 0.2, 1.6 ± 0.1, 1.4 ± 0.2, 1.6 ± 0.2, 1.5 ± 0.2, 1.1 ± 0.1, 1.3 ± 0.2 cm (mean ± SEM), respectively, in the sequence indicated].

§The normal fibroblast line 10T½ and normal heart-lung fibroblasts were used as MP-pos. fibroblasts, which induced necrosis in four of four and four of four mice, respectively. MP-neg. fibroblasts refer to 10T½ only.

fore, injected lysates of either mycoplasma-uninfected or mycoplasma-infected cells together with TNF s.c. (Table 3). Massive HN of the skin developed at the site of injection of lysates from mycoplasma-positive tumor cells or mycoplasma-positive normal cells and TNF but, unlike what was observed after s.c. injection of LPS and TNF, none of the mice died of the treatment. Mycoplasma-positive cell lysates and TNF (Fig. 2*B*) caused a similar HN in athymic and normal mice, but γ-irradiated mice did not respond, indicating that the induction of HN with mycoplasma-positive cell lysates and TNF was (like the HN induced by LPS and TNF) T-cell independent and radiosensitive. As expected, the LPS-sensitive and LPS-insensitive C3H strains both responded equally well to mycoplasma and TNF as did BALB/cAn mice. An unrelated (Gram-positive) organism, *C. parvum*, coinjected s.c. as a bacterial suspension mixed with TNF, also caused massive HN (Fig. 2*C*) at the skin site of injection without causing deaths in three strains of mice, including nude mice (Table 3). Boiling *C. parvum* or the mycoplasma-positive cell lysates for 1 hr did not destroy the activity of the materials to synergize with TNF. *C. parvum* and TNF failed to cause deaths when injected together s.c. However, *C. parvum* with TNF injected together i.v. synergized in causing lethal shock, though the degree of synergism in causing lethality was less than that caused by LPS and TNF (Fig. 1 *Upper* and *Lower*).

IL-1α or IFN-γ Fails to Synergize with Bacteria or TNF in the Induction of HN. IL-1α has a number of functional similarities with TNF (for review, see ref. 11) and a nonrecombinant material containing IL-1 has been implicated in the Shwartzman reaction (12). However, Table 4 shows that the recombinant cytokine, IL-1α, failed to substitute for TNF in synergizing with *C. parvum* to induce HN. Similarly, IFN-γ also failed to synergize with *C. parvum* to induce HN. In contrast, LT (13, 14), which is encoded by a closely linked gene (15) with significant sequence homology to TNF, can substitute for TNF in causing HN with *C. parvum*, although in repeated experiments LT was less effective. IFN-γ is known to synergize with TNF in killing tumor cells *in vitro* (16–18). However, neither IFN-γ nor IL-1α could replace the bacterial component and synergize with TNF to induce HN.

DISCUSSION

Determining the reasons for the toxicity of recombinant TNF was important not only because toxicity would limit the therapeutic usefulness of recombinant preparations but also because such studies may help us to understand the biologic effects of TNF. Since the endotoxin LPS is a potent stimulator for macrophages to produce TNF (6) and since antibodies to recombinant TNF can prevent the development of endotoxin shock (19), it is conceivable that TNF, as a single protein mediator, causes the deleterious effects without further participation of endotoxin. Although such a possibility appears plausible, especially since TNF alone can be directly cytotoxic to malignant cells (1) or inhibit the function of certain enzymes *in vitro* (20), such a notion is not supported by the results of the experiments presented here.

In this paper, we show that certain bacteria or bacterial substances can have a high degree of synergy with TNF to cause HN and/or lethal shock. At present, we cannot determine whether the marginal systemic toxicity of TNF observed at the highest doses was due to TNF alone, whether it was caused by contamination of the recombinant TNF preparation with very small amounts of endotoxin, or whether a low level of circulating endotoxin absorbed from the intestinal flora might account for the phenomenon. Similarly, there was no evidence for TNF alone being sufficient to induce HN since injecting even large doses of TNF into normal skin failed to induce any micro- or macroscopically detectable tissue injury. Thus, it appears that at least two synergistic signals (*i*, TNF; *ii*, a bacterial component) are required for lethal shock or HN to occur.

Heat killed *C. parvum* bacteria or mycoplasma-infected normal or malignant cell lysates, given together with TNF, had effects similar to LPS and TNF and even synergized in endotoxin-resistant mice. Together this suggests that the observed synergy may occur with infections that involve quite different bacteria or bacterial products and TNF. TNF could be given passively, be produced actively by host macrophages, or be replaced by LT, which is produced by T cells that commonly participate in the defense against infections. Possibly, different bacteria that synergize with TNF or LT share a common moiety or induce a common mediator. However, it does not appear to be IFN-γ or IL-1α since neither of these cytokines could substitute for the bacterial components and synergize with TNF to induce HN.

Our study suggests that the bacterial moiety and TNF are not sufficient to cause HN or lethality on their own but require a third component that comes from the host (Fig. 3). Evidence comes from the fact that LPS and TNF induced neither lethality nor HN in mice genetically resistant to the toxic effects of endotoxin. Furthermore, leukopenia, which

Table 4. Neither IL-1α nor IFN-γ can substitute for TNF, LT, or *C. parvum* in the induction of HN

s.c. treatment*	Incidence of HN							Total (%)†	
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7		
TNF	0/2	0/2	0/2	—	—	0/2	—	0/8	(0)
LT	0/2	0/2	—	—	—	—	—	0/4	(0)
IFN-γ	0/2	0/2	0/2	—	—	0/2	—	0/8	(0)
IL-1α	—	—	—	—	—	0/2	0/2	0/4	(0)
C. parvum	0/2	0/2	0/2	—	—	—	—	0/6	(0)
+ TNF	5/5	5/5	2/2	2/2	2/2	—	—	16/16	(100)‡
+ LT	3/5	1/2	—	—	—	—	—	4/7	(57)†
+ IFN-γ	0/5	0/5	0/2	—	—	—	—	0/12	(0)
+ IL-1α	—	—	—	0/2	0/2	—	—	0/4	(0)
IFN-γ + TNF	—	—	—	—	—	0/2	0/2	0/4	(0)
IL-1α + TNF§	—	—	—	—	—	0/2	0/2	0/4	(0)

*C3H/HeN mice were injected s.c. with *C. parvum* (300 μg) and/or the appropriate recombinant cytokine (each cytokine was used at a dose of 10 μg per mouse). The incidence of HN was determined 24 hr later.

†Number of mice showing HN/number of mice tested. The percentages are given in parentheses.

‡The sizes of the HN induced by *C. parvum* and TNF or *C. parvum* and LT were similar [1.7 ± 0.1 and 1.9 ± 0.2 cm (mean ± SEM), respectively].

§IL-1α injected s.c. (10 μg per mouse) followed 24 hr later by TNF (10 μg per mouse) also failed to induce HN (experiment 1, 0/2; experiment 2, 0/2).

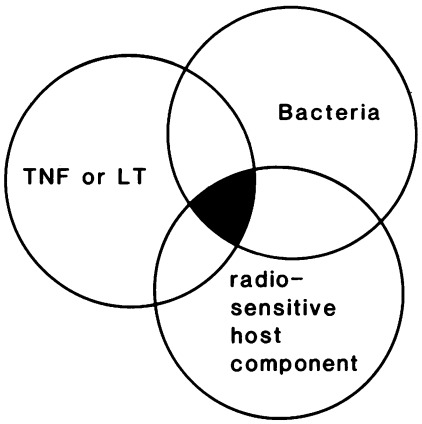

FIG. 3. Factors implicated in the induction of HN. Bacteria and TNF or LT act together synergistically to activate a radiosensitive, T-cell-independent host component, resulting in localized HN.

has been shown to prevent the Shwartzman reaction (21–23), induced in this study by γ-irradiation (650 rads), prevented the induction of HN in sensitive mice, whereas functional T cells were not required. This suggests that the host component was radiosensitive and T-cell independent. The third component might be derived from PML since these cells are implicated in the systemic and local Shwartzman reaction (22). Possibly, the Shwartzman reaction (24) may depend on endogenously produced TNF that synergizes with sensitizing endotoxin.

The time when PML invade LPS-, mycoplasma-, or *C. parvum*-injected skin correlated with the time when TNF had to be injected to induce HN at the prepared skin site. The mere attraction of PML to the site of LPS injection must not be sufficient to prepare skin for the induction of HN by TNF, however, since LPS injection into the skin of LPS-resistant C3H/HeJ mice caused an equal or even more effective PML infiltrate than observed in the LPS-sensitive C3H strain (even though the LPS-resistant strain failed to respond to TNF injection into the infiltrated skin with HN). Cellular responsiveness to TNF and the bacterial agent for the induction of HN appears to be required at a step subsequent to PML infiltration. Thus, a second LPS-mediated event must be required that is defective in LPS-resistant mice.

Experimentally, TNF treatment has been shown to cause necrosis and regression of certain murine or human tumors in mice, although the sensitivity of these tumors to TNF *in vivo* does not always correlate with a sensitivity of the tumor cells to TNF *in vitro*. Since TNF alone did not cause necrosis of normal tissues, one would expect that a tumor sensitive to TNF *in vivo* provides a second signal analogous to the one provided by bacteria in the HN of normal skin by TNF. The circulation of this second factor may be responsible for the increased susceptibility of tumor-bearing individuals to the toxic effects of TNF (4, 25). In cases in which tumors are resistant to TNF *in vivo*, this proposed second signal is not produced but could be replaced by simultaneous application of bacterial agents and TNF. On the basis of the described synergism between TNF and bacterial agents, patients bearing a tumor that has a predisposition for TNF toxicity or who are exposed advertently to bacterial agents (26) or inadvertently in the form of intercurrent infections would be more susceptible to systemic complications of TNF treatment such as lethal shock.

Although the observed synergy might appear to have adverse effects on the normal host, it is more likely that the synergy is part of an important local defense mechanism of the host against infections. For example, HN, by causing local ischemia, may prevent the rapid spread or diffusion of bacteria and of toxic products. The breakdown of this local containment reaction may be detrimental to the host because circulation of bacterial toxins inducing systemic release of cytokines may cause lethal shock.

We thank Dr. Donald Rowley for critical review of the manuscript, Dr. H. Michael Shepard (Genentech) for providing very generously TNF, LT, and IFN-γ, Dr. Peter Lomedico (Hoffman-LaRoche) for IL-1α, and Dr. Joseph G. Tully (National Institute of Allergy and Infectious Diseases, Mycoplasma Section) for analysis and culturing of mycoplasma. This work was supported by National Institutes of Health Grants CA-19266, CA-22677, and CA-37156. J.L.R. was supported by National Institutes of Health Grant T-32 AI-07090.

1. Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N. & Williamson, B. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3666–3670.
2. Pennica, D., Nedwin, G. E., Hayflick, J. S., Seeburg, P. H., Derynck, R., Palladino, M. A., Kohr, W. J., Aggarwal, B. B. & Goeddel, D. V. (1984) *Nature (London)* **312,** 724–729.
3. Tracey, K. J., Beutler, B., Lowry, S. F., Merryweather, J., Wolpe, S., Milsark, I. W., Hariri, R. J., Fahey, T. J., Zentella, A., Albert, J. D., Shires, G. T. & Cerami, A. (1986) *Science* **234,** 470–474.
4. Old, L. J. (1985) *Science* **230,** 630–632.
5. Kramer, S. M. & Carver, M. E. (1986) *J. Immunol. Methods* **93,** 201–206.
6. Urban, J. L., Shepard, H. M., Rothstein, J. L., Sugarman, B. J. & Schreiber, H. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5233–5237.
7. Sande, M. A. & Mandell, G. L. (1985) in *The Pharmacological Basis of Therapeutics*, eds. Gilman, A. G., Goodman, L. S., Rall, T. W. & Murad, F. (Macmillan, New York), 7th Ed., pp. 1080–1105.
8. Heppner, G. & Weiss, D. W. (1965) *J. Bacteriol.* **90,** 696–703.
9. Moeller, G. R., Terry, L. & Snyderman, R. (1978) *J. Immunol.* **120,** 116–123.
10. Gabridge, M. G., Chandler, D. K. F. & Daniels, M. J. (1985) *Mycoplasmas* **4,** 313–351.
11. Beutler, B. & Cerami, A. (1986) *Nature (London)* **320,** 584–588.
12. Beck, G., Habicht, G. S., Benach, J. L. & Miller, F. (1986) *J. Immunol.* **136,** 3025–3031.
13. Ruddle, N. H. & Waksman, B. H. (1968) *J. Exp. Med.* **128,** 1267–1279.
14. Gray, P. W., Aggarwal, B. B., Benton, C. V., Bringman, T. S., Henzel, W. J., Jarrett, J. A., Leung, D. W., Moffat, B., Ng, P., Svedersky, L. P., Palladino, M. A. & Nedwin, G. E. (1984) *Nature (London)* **312,** 721–724.
15. Muller, U., Jongeneel, C. V., Nedospasov, S. A., Fischer-Lindahl, K. & Steinmetz, M. (1987) *Nature (London)* **325,** 265–267.
16. Williams, T. W. & Bellanti, J. A. (1983) *J. Immunol.* **130,** 518–520.
17. Williamson, B. D., Carswell, E. A., Rubin, B. Y., Prendergast, J. S. & Old, L. J. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5397–5401.
18. Sugarman, B. J., Aggarwal, B. B., Hass, P. E., Figari, I. S., Palladino, M. A., Jr., & Shepard, H. M. (1985) *Science* **230,** 943–945.
19. Beutler, B. A., Milsark, I. W. & Cerami, A. C. (1985) *Science* **229,** 869–871.
20. Kawakami, M., Pekala, P. H., Lane, M. D. & Cerami, A. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 912–916.
21. Becker, R. M. (1948) *Proc. Soc. Exp. Biol. Med.* **69,** 247–250.
22. Stetson, C. A. & Good, R. A. (1951) *J. Exp. Med.* **93,** 49–64.
23. Thomas, L. & Good, R. A. (1952) *J. Exp. Med.* **96,** 605–624.
24. Shwartzman, G. (1928) *J. Exp. Med.* **48,** 247–268.
25. Asher, A., Mule, J. J., Reichert, C. M., Shiloni, E. & Rosenberg, S. A. (1987) *J. Immunol.* **138,** 963–974.
26. Coley, W. B. (1891) *Ann. Surg.* **14,** 199–220.