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FOUR DIFFERENT INTERLEUKIN-1 SPECIES SENSITIZE TO THE LETHAL ACTION OF TUMOUR NECROSIS FACTOR

Bart Everaerdt, Peter Brouckaert, Alan Shaw*, and Walter Fiers

Laboratory of Molecular Biology, State University, Ledeganckstraat 35, 9000 Gent, Belgium

*Glaxo Institute for Molecular Biology, Route des Acacias 46, 1211 Genève 24, Switzerland

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We studied the induction of lethal shock by Tumour Necrosis Factor (TNF) in mice and observed a remarkable difference between the effect of human and murine TNF, which could be eliminated by co-administration of sensitizing agents. We identified interleukin-1 (IL1) as a natural sensitizer, rendering mice as susceptible to human TNF as to murine TNF. This IL1 activity was found to be exerted to the same extent both by human and murine IL1-α or IL1-β, and was also different from the sensitization obtained with galactosamine, since these agents had an additive effect. Pretreatment of the animals with indomethacin, a cyclooxygenase inhibitor, provided partial protection against TNF lethality in IL1-sensitized but not in galactosamine-sensitized mice.

Tumour Necrosis Factor (TNF) is a pleiotropic cytokine which is the principal mediator of both the antitumour and the shock-inducing properties of endotoxin (reviewed in 1). Cloning and expression in E. coli provided both human and murine TNF in sufficient quantity and purity to allow in vitro as well as in vivo investigation of their pleiotropic activities (2).

Interleukin-1 (IL1) is another macrophage-derived, pleiotropic cytokine having an overlapping, but distinct pattern of activities (reviewed in 3). It has been recently reported that IL1 can act synergistically with TNF to cause hypotension (4), hypothermia and lethality (5).

The presence of IL1 activity in human serum was shown to correlate with the fatal outcome of meningococcal sepsis (6). However, real proof for its involvement in the development of septic

Abbreviations: GalN, galactosamine; h, human; IL, interleukin; indo, indomethacin; i.p., intraperitoneally; i.v., intravenously; LPS, lipopolysaccharide; m, murine; PBS, phosphate-buffered saline; TNF, tumour necrosis factor.

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shock has not yet been presented. On the other hand, the observation that antibodies against TNF could provide protection against a lethal injection of LPS in mice, is indirect evidence for the importance of this mediator in sepsis (7).

In studies on the lethal shock induced by TNF, we observed a marked species specificity, which can be abolished by the action of sensitizing agents: human TNF (hTNF) at doses up to 250 µg is not lethal in mice (unless sensitization occurs), whereas mTNF does not need extraneous sensitizers to cause lethal toxicity (the LD_{50} is about 10 µg/mouse; Brouckaert et al., in preparation). We here present results of a more detailed investigation into the synergism between TNF and IL1, a combination which is likely to be present in natural, pathological situations.

MATERIALS AND METHODS

Laboratory animals: Female C57BL/cnb mice (SCK, Mol, Belgium) were used at the age of 8 to 10 weeks. The animals were kept in 12 h light/dark cycles in a temperature-controlled, air-conditioned room and received pelleted food and water ad libitum.

Cytokines and reagents: hTNF and mTNF, produced in E. coli, were purified to apparent homogeneity (2) and were a gift from Dr. J. Tavernier (formerly Biogent, Gent, Belgium). hTNF had a specific activity of 2.5×10^7 U/mg and contained less than 0.11 ng endotoxin/mg protein; mTNF had a specific activity of 7.5×10^7 U/mg and contained less than 0.96 ng endotoxin/mg protein. The four recombinant IL1 preparations (mIL1-α, mIL1-β, hIL1-α and hIL1-β) were prepared in the Glaxo Institute for Molecular Biology, Genève, Switzerland); their endotoxin contamination did not exceed 3.85, 0.35, 1.4 and 0.30 ng/mg protein, respectively. The endotoxin contamination was determined by a chromogenic Limulus amoebocyte lysate assay (Coatest; KabiVitrum, Stockholm, Sweden).

E. coli 0111:B4 endotoxin was purchased from Difco Laboratories (Detroit, MI, USA), D-galactosamine (GalN) from Janssen Chimica (Beerse, Belgium) and indomethacin from Sigma Chemical Co. (St Louis, MO, USA).

Cytokines were stored at -80 °C and diluted in endotoxin-free, phosphate-buffered saline immediately before injection. Intravenous (lateral tail vein) and intraperitoneal injections had a total volume of 0.2 ml and 0.5 ml, respectively. Indomethacin was dissolved in 1.4 % $NaHCO_3$.

Statistical analysis: Mortality was recorded every 30 min up to 24 h, thereafter regularly twice a day up to 72 h. No deaths occurred after this period. Significance of the observed differences in lethality was analysed using the X^2 test, with Yates' correction for small samples. Significance of the differences in survival time was analysed using the Mantel modification of Gehan's Generalized Wilcoxon Test (8).

RESULTS

Co-administration of IL1 leads to increased toxicity and elimination of species specificity of TNF in vivo: IL1 increased the

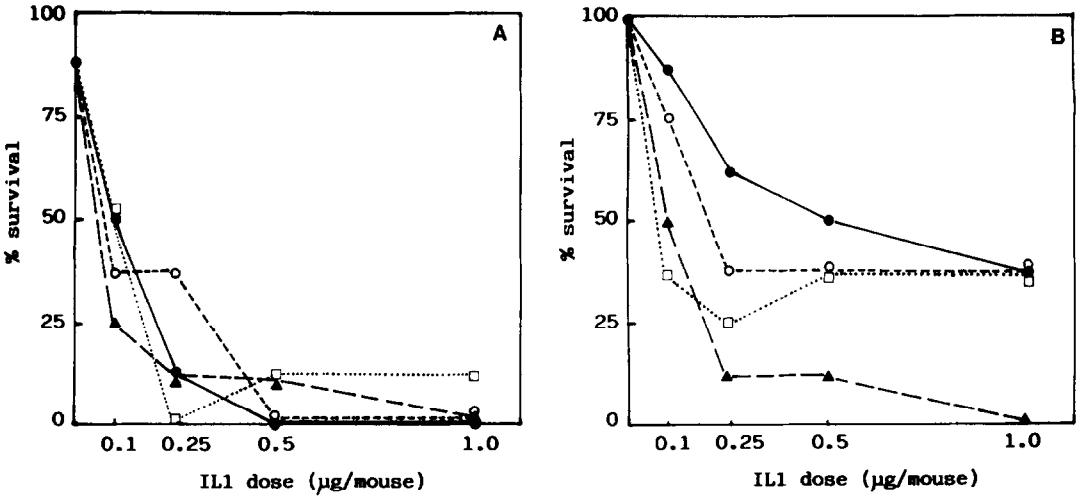

Fig. 1. Synergism of IL1 with mTNF and hTNF in the induction of lethality.
The indicated dose of hIL1-α (●——●); hIL1-β (□···□), mIL1-α (▲– – –▲) or mIL1-β (○---○) was i.v. injected simultaneously with 2.5 µg mTNF (A) or 7.5 µg hTNF (B) in 0.2 ml endotoxin-free PBS. Groups consisted of 8 mice. Lethality was assessed after 72 h.

toxicity of both mTNF and hTNF in a dose-dependent manner (Fig. 1). Also, there were no significant differences between either murine or human IL1-α or IL1-β for this activity. The enhanced toxicity observed by co-administration of IL1 was not due to endotoxin contamination of the IL1 preparations, since a dose of 100 pg LPS, which is more than ten times the largest contaminating amount of LPS per µg IL1, did not result in increased lethality (Table 1).

We have reported previously that in vitro there is little species specificity when comparing human to murine TNF (9); the specific activity, as assayed on murine L929 cells, is only threefold higher for mTNF as compared with hTNF. But in vivo, mTNF is approx. fiftyfold more toxic to mice than hTNF (Brouckaert et al., in preparation). Interestingly, this species specificity of mTNF- and hTNF-induced lethality disappears in the presence of IL1. hTNF, which up to 250 µg does not induce lethality in mice (Table 1, and

TABLE 1. Lethality observed in control groups

2.5 µg mTNF	1/8*	1 µg hIL1-α	0/8
2.5 µg mTNF + 100 pg LPS	0/8	1 µg hIL1-β	0/8
20 µg mTNF	5/5	1 µg mIL1-α	0/8
100 µg hTNF	0/5	1 µg mIL1-β	0/8

*Number of dead mice / Total number of mice.
The cytokines were injected i.v. in 0.2 ml PBS. Lethality was assessed after 72 h.

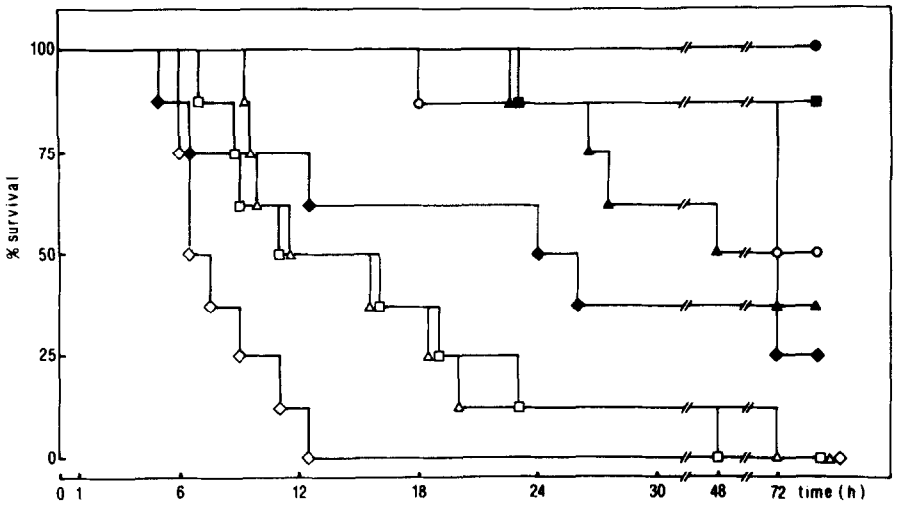

Fig. 2. Effect of indomethacin on the lethality induced by a combination of TNF and IL1.
mIL1-α (●, 0.1 μg; ■, 0.25 μg; ▲, 0.5 μg; ◆, 1.0 μg) was i.v. injected simultaneously with 2.5 μg mTNF in 0.2 ml PBS. Indomethacin (125 μg/mouse; full symbols) or buffer (open symbols) was i.p. injected in 0.5 ml of 1.4 % $NaHCO_3$ 2 h before the injection of the cytokines. Groups consisted of eight mice. Significance (p) of the differences in survival time between mice pretreated with buffer and indomethacin was < 0.025, < 0.005, < 0.01 and < 0.05, respectively, for the IL1 doses mentioned above.

unpublished results), became lethal in combination with IL1, more particularly in doses comparable with those of mTNF (Fig. 1).

The cyclooxygenase inhibitor indomethacin reduces the toxicity of the combination of TNF and IL1: We have previously reported that pretreatment with indomethacin can reduce TNF-induced side-effects and leads to improved survival (10). The effect of pretreatment with indomethacin was studied in mice receiving mTNF and mIL1-β. This pretreatment completely prevented the diarrhoea occurring soon after the combined administration of mTNF and IL1, and also partly reduced the lethality observed after an injection with this combination. In all groups, indomethacin pretreatment clearly prolonged survival of the mice (Fig. 2). The difference in final mortality was, however, only statistically significant ($p < 0.05$) at a dose of 0.25 μg IL1. However, indomethacin did not protect mice against the lethal combination of TNF and GalN (Table 2).

Additive sensitization by IL1 and GalN: We examined whether the sensitizing action of IL1 was still exerted when the animals were also treated with GalN, which specifically blocks hepatic RNA synthesis by depletion of free UTP and renders mice very sensitive to TNF (11); we found that the species difference between hTNF and mTNF was abolished. Furthermore, we observed increased lethality by

TABLE 2. Effect of GalN (20 mg/mouse) on the lethality induced by TNF and/or IL1, and lack of protection by indomethacin

GalN + 0.05 µg mTNF	3/10*	GalN + 0.015 µg mTNF + buffer	0/6
GalN + 0.05 µg mTNF + 0.25 µg mIL1-β	6/10	GalN + 0.15 µg mTNF + buffer	6/6
		GalN + 0.45 µg mTNF + buffer	5/6
GalN + 1.00 µg mIL1-β	0/8	GalN + 0.015 µg mTNF + indo	0/6
GalN + 5.00 µg mIL1-β	0/8	GalN + 0.15 µg mTNF + indo	5/6
		GalN + 0.45 µg mTNF + indo	6/6

*Number of dead mice / Total number of mice.
Mixtures of GalN and cytokines were injected i.p. in 0.5 ml PBS. Indomethacin (75 µg/mouse) in 1.4 % $NaHCO_3$ buffer was given i.p. 2 h before the GalN/TNF treatment. Lethality was assessed after 72 h.

administering IL1 together with a combination of TNF and GalN (Table 2). IL1 alone or combined with GalN did not cause lethality at the doses tested (Table 2), although diarrhoea, hypothermia and lethargy were observed. Remarkably, after treatment with TNF (or LPS) together with GalN, disease symptoms were not observed until shortly before death.

Kinetics of the synergism of IL1 and TNF: We further assessed the lethality when IL1 was administered at different times before or after TNF injection. The synergism appears to be most pronounced when both cytokines are administered simultaneously (Table 3). The fact that the observed lethality is lower than could be expected from the results shown in Fig. 1, is most likely due to the i.p. administration of IL1 in this experiment.

DISCUSSION

The involvement of TNF in septic shock has been well documented. TNF was detected in the serum of patients with meningococcal infection (12) or in healthy volunteers after endotoxin administration (13). In experimental animals, injection with anti-

TABLE 3. Time dependency of TNF + IL1 synergistic toxicity

Time of IL1 administration relative to TNF	lethality
- 5 h	1/6*
- 2 h	0/6
0 h	4/6
+ 2 h	1/6
+ 4 h	1/6
+ 6 h	0/6

*Number of dead mice / Total number of mice.
10 µg hTNF was injected i.v. at t = 0 h in 0.2 ml PBS, whereas 5 µg hIL1-β was injected i.p. in 0.5 ml PBS at the times indicated.

TNF antibodies has provided protection against lethal bacteraemia (14) or an otherwise lethal dose of endotoxin (7). However, the occurrence of septic shock most likely involves the interplay of several cytokines and hormones, with additive, synergistic and possibly antagonistic effects.

One of the cytokines that act in concert with TNF to induce lethal shock, could be IL1, which is induced not only by endotoxin, but also by TNF (15). As previously observed (5), we confirmed that IL1 greatly potentiates the lethal shock action of TNF. Moreover, IL1 leads to the disappearance of the species-specific difference between the effects of hTNF and mTNF in mice.

In a previous publication, we have already described a model that can provide an explanation for the phenomena we see at work here (16). We concluded that two distinct processes, viz. sensitization and a challenging effect, are both required for lethality to occur after TNF injection. The sensitizing effect can be exerted by mTNF, but not by hTNF, so that only mTNF, and not hTNF, can induce lethal toxicity in normal, healthy mice. Sensitization of the animals can also be exerted by GalN, ethanol, tumours, infections, endotoxin and some cytokines other than mTNF, such as interferon-γ and IL1 (but not IL6). This renders them equally susceptible to mTNF as to hTNF. Our results do not indicate any difference in the sensitizing potential of either murine or human IL1-α or IL1-β. The four species of IL1 tested, in agreement with our model and as previously found for GalN (11, 16), sensitize the mice to the lethal effect of TNF, and at the same time abolish the species difference seen when the toxicity of hTNF and mTNF is compared in naive animals. However, the actions of GalN and IL1 are not identical. Typically, in the GalN model, mice injected with a lethal dose of TNF appear completely healthy until one to half an hour before death, which occurs between 5 and 8 h after the injection. In this short time period, the body temperature of the animals drops from normal values to only a little above room temperature just before death. This fall in body temperature does not occur when a sublethal dose of TNF plus GalN is administered. On the other hand, when a lethal or even sublethal combination of IL1 and TNF is given, disease symptoms, such as hypothermia and diarrhoea, are already apparent half an hour after the injection. These symptoms and lethality induced by TNF plus IL1 can be diminished by pretreatment with indomethacin, which does not protect against lethality occurring after a TNF/GalN injection. The latter observation is in agreement with results for LPS/GalN

described previously (17). Finally, when both IL1 and GalN are injected together with TNF, their effects appear to be additive.

Contrary to results reported by others (18), we did not observe lethality when IL1 and GalN were co-administered. We do not know whether this discrepancy is due to the strain of mice used or to the presence of contaminating LPS in the IL1 preparations.

In conclusion, we found that IL1 can act as a natural sensitizer which renders mice much more susceptible to the toxic effects of TNF and which causes the loss of the species-specific difference in lethality between hTNF and mTNF in vivo. Since tumour-bearing animals were found to be more sensitive to the toxic effects of both LPS and TNF than tumour-free controls (19), such sensitivity might be due to the presence, either locally or systemically, of elevated IL1 levels in response to the inflammatory stimulus from the tumour. Consequently, inhibition of such IL1 effects would be very desirable, not only for anti-tumour therapy with TNF, but also for controlling the toxicity in septic shock.

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