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Pharmacologic Inhibitors of Tumor Necrosis Factor Production Exert Differential Effects in Lethal Endotoxemia and in Infection with Live Microorganisms in Mice

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Tumor necrosis factor- α (TNF- α) and interleukin- 1β (IL- 1β) are principal mediators of septic shock; inhibition of TNF- α production may ameliorate outcome in severe infections. Pentoxifylline, chlorpromazine, and thalidomide inhibit TNF- α production. Their effects were tested in lethal endotoxemia in sensitized mice. Only chlorpromazine significantly improved survival. Chlorpromazine and pentoxifylline significantly reduced postendotoxin circulating TNF- α , by 89% and 76%, respectively. Chlorpromazine also significantly reduced IL- 1β and soluble TNF receptor-P75. No drug improved survival in *Klebsiella pneumoniae*-infected mice despite significantly lower circulating TNF- α concentrations in chlorpromazine- or pentoxifylline-treated animals. The three compounds decreased circulating TNF- α in *Candida albicans*-infected mice, but survival was not influenced. In neutropenic mice, chlorpromazine had no influence on candidae in organs, but in normal mice, *Candida* counts in kidneys were higher in chlorpromazine-treated mice. Thus, inhibition of TNF- α production was of no benefit in *K. pneumoniae* infection and worsened outcome in *C. albicans* infection.

The proinflammatory cytokines interleukin- 1β (IL- 1β) and tumor necrosis factor- α (TNF- α) play an important role in the pathophysiology of sepsis [1]. TNF- α and IL- 1β concentrations are increased early in sepsis, and clinical studies demonstrate that high concentrations of TNF- α in the circulation correlate with the severity of the disease and with a poor prognosis [2, 3]. Treatment with anti-TNF- α antibodies attenuates the lethal effects of endotoxin in mice and protects against shock after infusion of gram-negative bacteria in primates [4, 5]. Similarly, treatment with either of the naturally occurring antagonists of IL-1 and TNF, IL-1 receptor antagonist and soluble TNF receptor (sTNFR), has been shown effective in lethal endotoxemia and in various models of experimental infection [6–11]. TNF- α also appears to have an important role in experimental *Candida albicans* infection. The mannoprotein constituents of the *Candida* cell wall induce the in vitro production of TNF- α by macrophages [12], and in vivo infusion of *C. albicans* in mice induces a rise in TNF- α plasma concentrations, which peak at

24 h [13]. Therefore, TNF- α synthesis may be an important target for pharmacologic intervention in severe infections.

Pentoxifylline, a methylxanthine derivative and phosphodiesterase inhibitor, inhibits the generation of mRNA for TNF- α in vitro [14] and decreases the in vivo production of TNF- α in humans and in experimental animals [15–17]. Pentoxifylline has been reported to increase survival of mice in endotoxic shock [16]. Chlorpromazine is a phenothiazine derivative with a broad spectrum of actions that protects against the toxicity of endotoxin in various experimental models. At least part of this protection is achieved through inhibition of TNF synthesis [18]. Thalidomide has been used in the past as a sedative and antiemetic drug. Because of its teratogenic effects it has limited use as an antiinflammatory and immunosuppressive agent in the treatment of erythema nodosum leprosum, rheumatoid arthritis, and severe graft-versus-host disease [19–21]. Thalidomide has been shown to inhibit TNF- α production by monocytes when these cells are triggered with endotoxin [22].

In the present study, we evaluated to what extent treatment with pentoxifylline, chlorpromazine, or thalidomide could increase survival after endotoxin challenge in sensitized mice. We measured circulating concentrations of TNF- α , IL- 1β , and the two soluble TNF receptors, sTNFR-P55 and sTNFR-P75, after endotoxin challenge. We also evaluated the effects of pentoxifylline, chlorpromazine and thalidomide in lethal infections with *Klebsiella pneumoniae* or *C. albicans*. Because of the major importance of polymorphonuclear leukocytes in the defense against *C. albicans* [23] and their involvement in the actions of the cytokines during in-

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fection, we studied the effects of the drugs in normal and neutropenic mice infected with *C. albicans*.

Materials and Methods

Animals. Female Swiss mice, 6–8 weeks old, weight 20–25 g, were obtained from a local colony. The animals were fed standard laboratory chow (Hope Farms, Woerden, Netherlands) and housed under specific pathogen-free conditions.

Drugs. Pentoxifylline was obtained from Hoechst Pharma (Frankfurt am Main, Germany). Chlorpromazine was obtained from Rhone-Poulenc Rorer (Amstelveen, Netherlands), and thalidomide was provided by K. Zwingenberger (Grunenthal, Stolberg, Germany). Pentoxifylline (40 or 80 mg/kg) and chlorpromazine (4 mg/kg) were given in pyrogen-free saline by intraperitoneal (ip) injection, since this is a convenient and effective route for administration of these compounds [16, 18]. Because no water-soluble form of thalidomide is available, this drug was given by gastric instillation in 1% carboxymethylcellulose at a dose of 400 mg/kg. Control mice received vehicle by the same route. Pentoxifylline and chlorpromazine were given 30 min and thalidomide 1 h before endotoxin injection. In the experiments with live microorganisms, all treatments were started 1 day before infection and continued daily thereafter. Pentoxifylline was given at 80 mg/kg/day twice daily, chlorpromazine 4 mg/kg/day once daily, and thalidomide 400 mg/kg/day once daily.

Lethal endotoxemia in sensitized mice. Lipopolysaccharide (LPS; *Escherichia coli* serotype O55:B5) was obtained from Sigma (St. Louis). Mice were sensitized with actinomycin D (Sigma) or D-galactosamine (Merck, Darmstadt, Germany). Animals were injected ip with actinomycin D (0.6 or 0.8 mg/kg) 10 min before the ip injection of LPS (30 µg/kg) as described [18]. In the experiments in which the mice were sensitized with D-galactosamine, the animals were given D-galactosamine (560 mg/kg) and LPS (40 µg/kg) simultaneously ip [24]. After injection of LPS, 6 mice from each treatment group were anesthetized with ether and bled from the retroorbital plexus at 90 min for measurement of circulating TNF-α concentrations and another 6 mice per treatment group at 3 h for measurement of circulating concentrations of IL-1β and sTNFR-P55 and -P75. In the remaining mice, survival was assessed daily for 7 days.

***K. pneumoniae* infection.** *K. pneumoniae* (ATCC 43816), a strain that produces a lethal infection in normal mice, was inoculated in the left thigh muscle of the animals as described [25]. Inocula ranged from 2×10^2 to 2×10^5 cfu. Twenty-four hours after infection, subgroups of mice were sacrificed and blood was collected for measurement of TNF-α plasma concentrations. To quantitate the recovery of the microorganisms from the livers and spleens, the organs were removed aseptically, weighed, and homogenized in sterile saline in a tissue grinder. To bring the bacterial counts after culture into the optimal range for reading, samples of liver and spleen were diluted in sterile saline. The suspensions were plated on sheep blood agar, and colony-forming units were counted after overnight incubation. Survival of the animals was observed for 5 days after infection. In a separate experiment, the animals were infected with an inoculum of 10^4

cfu, and subgroups of 6 mice were sacrificed to measure TNF-α plasma concentrations at various time points after infection. In these animals, we measured the recovery of the microorganism from blood, liver, and spleen.

***C. albicans* infection.** *C. albicans* (10^5 cfu) was given intravenously (iv) in the lateral tail vein. At 24 h after injection, subgroups of mice were sacrificed, blood was taken for determination of plasma TNF-α concentrations, and the recovery of the microorganisms from livers, kidneys, and spleens was quantitated in a manner similar to the quantitation of *K. pneumoniae*. Organ suspensions were plated on Sabouraud dextrose agar, and colonies were counted after overnight incubation. Survival of the animals was monitored for 8 days. In addition, mice rendered granulocytopenic by subcutaneous injections of cyclophosphamide, 150 mg/kg 4 days before and 100 mg/kg 1 day before infection, were injected with 10^4 cfu of *C. albicans*.

TNF-α measurement. Blood was collected in 1-mL tubes containing 7.5 µg of EDTA (Becton Dickinson, Rutherford, NJ) and 250 µL of aprotinin (Bayer, Leverkusen, Germany) and centrifuged for 5 min at 13,000 g. After separation, the plasma was stored at -20°C until assayed for TNF-α by ELISA. Briefly, 96-well immunoassay plates were coated overnight at 4°C with VIQ, a rat monoclonal antibody specific for mouse TNF-α (provided by Peter Krammer, Institute for Immunology and Genetics, German Cancer Research Center, Heidelberg) [26]. Nonoccupied binding sites were blocked with 1% bovine serum albumin. The samples were added onto the plate for 1 h at room temperature. A standard titration curve was obtained by serial dilutions of a known sample of recombinant mouse TNF-α in medium identical to the samples. Plates were then washed four times with wash buffer and incubated with a rabbit anti-mouse polyclonal antibody and peroxidase-conjugated swine anti-rabbit IgG. After substrate was added for 18 min, the reaction was stopped with 4 M H₂SO₄, and photospectrometry (450 nm) was done. The lower detection limit was 300 pg/mL. In some of the experiments, ELISA was done with TN3 as the monoclonal antibody directed against mouse TNF-α as described [27]. TN3 was provided by Celltech (Slough, UK) [28].

IL-1β measurement. Plasma was collected in the same manner as for TNF-α measurement. IL-1β was measured on plasma samples obtained 3 h after LPS injection by specific RIA as described [29].

sTNFR measurement. Plasma was collected in the same manner as for TNF-α measurement. sTNFR-P55 and sTNFR-P75 were measured in plasma samples obtained 3 h after LPS injection by specific ELISA as described [30].

Statistical analysis. Survival curves were analyzed using the Kaplan-Meier logarithm of rank test [31]. Differences in concentrations of circulating cytokines and differences in organ counts of the microorganisms were analyzed using the Kruskal-Wallis test with χ^2 approximation. Results were considered statistically significant at $P < .05$.

Results

Lethal endotoxemia in sensitized mice. Mortality occurred in the first 48 h after endotoxin injection without

further mortality during the next 5 days of follow-up. In the two models of endotoxin-induced mortality studied, actinomycin D- and D-galactosamine-sensitized mice, treatment with chlorpromazine significantly improved survival ($P < .05$). Treatment with pentoxifylline and thalidomide did not result in a better survival than control treatment (figure 1). In a separate experiment in which treatment with pentoxifylline was started 48 h before endotoxin challenge, survival of treated mice was not better than that of controls. Chlorpromazine and pentoxifylline had a strong inhibitory effect on immunoreactive TNF- α concentrations in plasma 90 min after endotoxin, whereas thalidomide did not influence circulating TNF- α (figure 2A). In actinomycin D-sensitized mice, TNF- α concentrations did not differ significantly between the groups treated with pentoxifylline and chlorpromazine, but in mice sensitized with D-galactosamine, TNF- α concentrations in chlorpromazine-treated mice were significantly lower than in pentoxifylline-treated mice ($P < .01$; results not shown).

The effects of the drugs on circulating IL-1 β concentrations 3 h after endotoxin injection is shown in figure 2B. Plasma IL-1 β concentrations in chlorpromazine-treated mice were significantly lower than in control animals, but pentoxifylline and thalidomide did not influence circulating IL-1 β concentrations.

None of the compounds influenced the concentrations of sTNFR-P55 3 h after endotoxin (not shown). However, chlorpromazine significantly decreased the concentration of sTNFR-P75 compared with controls (figure 2C).

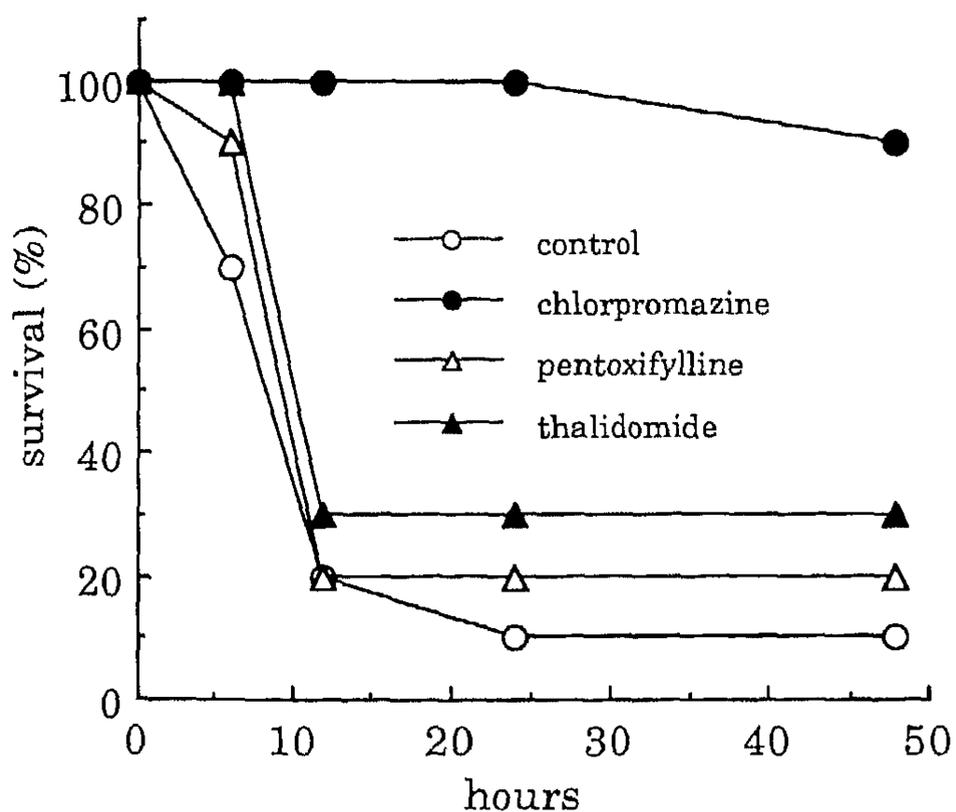


Figure 1. Percentage survival of D-galactosamine-sensitized Swiss mice after injection with lipopolysaccharide ($n = 10$, results of 1 representative experiment). $P < .05$ for chlorpromazine treatment. In mice sensitized with actinomycin D, same trends were seen.

K. pneumoniae infection. At 30 min after intramuscular (im) injection of 10^4 cfu of *K. pneumoniae*, blood cultures were still sterile, and TNF- α was not detectable in the circulation. However, at 90 min, TNF- α could be detected and remained relatively constant during the observation period (concentration range, 1.1–3.3 ng/mL); from this time point on, the microorganisms were present in the blood, liver, and spleen, indicating that there was an ongoing sepsis in these animals.

Plasma TNF- α concentrations at 24 h after infection were significantly decreased in the groups treated with pentoxifylline and chlorpromazine compared with controls. Pentoxifylline reduced TNF- α by 22% (from 0.9 ± 0.1 to 0.7 ± 0.2 ng/mL, $n = 10$, $P < .05$), and chlorpromazine reduced TNF- α by 30% (0.5 ± 0.2 to 0.3 ± 0.07 ng/mL, $n = 10$, $P < .05$). Although thalidomide reduced circulating TNF- α concen-

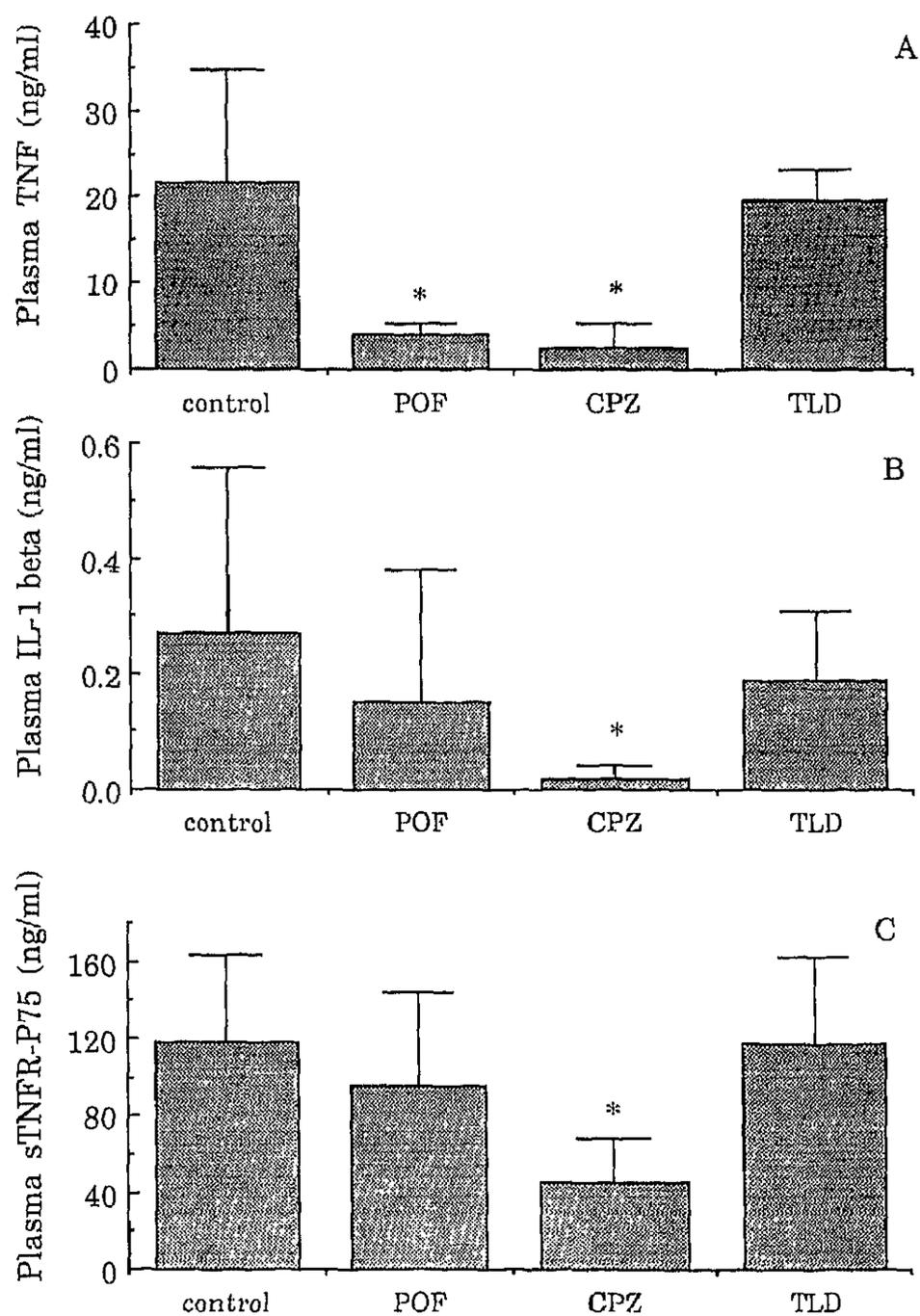


Figure 2. Circulating concentrations of cytokines after lipopolysaccharide (LPS) injection. **A**, TNF after 90 min ($*P < .01$ and $P < .005$ for pentoxifylline [POF] and chlorpromazine [CPZ], respectively). **B**, Interleukin-1 β (IL-1) after 3 h ($*P < .001$ for CPZ). **C**, Soluble TNF receptor-P75 (sTNFR-P75) after 3 h ($*P < .01$ for CPZ). Results are mean \pm SD ($n = 6$). TLD, thalidomide.

trations by 69%, this difference was not statistically significant because of the large variation in the control group (controls, 1.3 ± 1.5 ; thalidomide treatment, 0.4 ± 0.1 ng/mL; $n = 10$).

Survival was not significantly improved by either drug. Bacterial counts tended to be lower in the livers and spleens of the treated animals, regardless of the drug used, but these differences did not consistently reach statistical significance. Because chlorpromazine had the strongest effect on bacterial counts, we investigated the antimicrobial effect of chlorpromazine in vitro. Chlorpromazine did not inhibit the growth of *K. pneumoniae* in vitro (data not shown).

C. albicans infection in nonneutropenic mice. Plasma TNF- α concentrations were lower in chlorpromazine- and thalidomide-treated animals than in their respective controls, while pentoxifylline was not able to inhibit TNF- α production significantly during this infection (figure 3A). Survival was not influenced by any of the drugs used. Candidal outgrowth tended to be higher in pentoxifylline- and thalidomide-treated animals than in controls, but these differences were not statistically significant. However, chlorpromazine-treated animals showed significantly higher *Candida* counts in kidneys than did controls (figure 4).

C. albicans infection in neutropenic mice. Pentoxifylline significantly lowered plasma TNF- α concentrations (figure 3B). Chlorpromazine and thalidomide tended to inhibit TNF- α synthesis ($P = .065$ and $.07$, respectively). Circulat-

ing TNF- α concentrations were substantially higher in neutropenic mice than in nonneutropenic animals. Survival and recovery of *C. albicans* from the organs were not influenced by any of the drugs tested (figure 4).

Discussion

In this study we investigated the influence of three pharmacologic inhibitors of TNF- α synthesis on outcome of lethal endotoxemia and infection. Endotoxin-induced mortality was studied in mice sensitized with D-galactosamine or actinomycin D. Normal mice exhibit a low and varying sensitivity to endotoxin, thus hampering reproducibility of experiments. Endotoxin-induced mortality in sensitized mice has been shown to be mediated by cytokine production [10, 18, 24]. To rule out specific effects of either D-galactosamine or actinomycin D, both compounds were used in experiments with sensitized mice. We found that chlorpromazine was the most powerful inhibitor of postendotoxin TNF- α production and the only one that increased survival in lethal endotoxemia. Despite substantial reduction in TNF- α production by pentoxifylline, this drug did not improve survival after endotoxin challenge in our experiments. Our findings with chlorpromazine are in accordance with those of Gadina et al. [18], but despite equal and even higher dosage schedules of pentoxifylline used in our experiments, we could not confirm the data of Schade et al. [16], who showed a beneficial effect of pentoxifylline on survival. These differences cannot readily be explained. Thalidomide appeared not to be an inhibitor of TNF- α production after in vivo administration of endotoxin. This finding underscores the differences in influence on cytokine production that may be observed in in vivo versus in vitro experiments [22]. However, thalidomide did decrease plasma TNF- α concentrations after *Candida* infection in nonneutropenic mice. Differences in pharmacokinetics between the orally administered thalidomide and ip chlor-

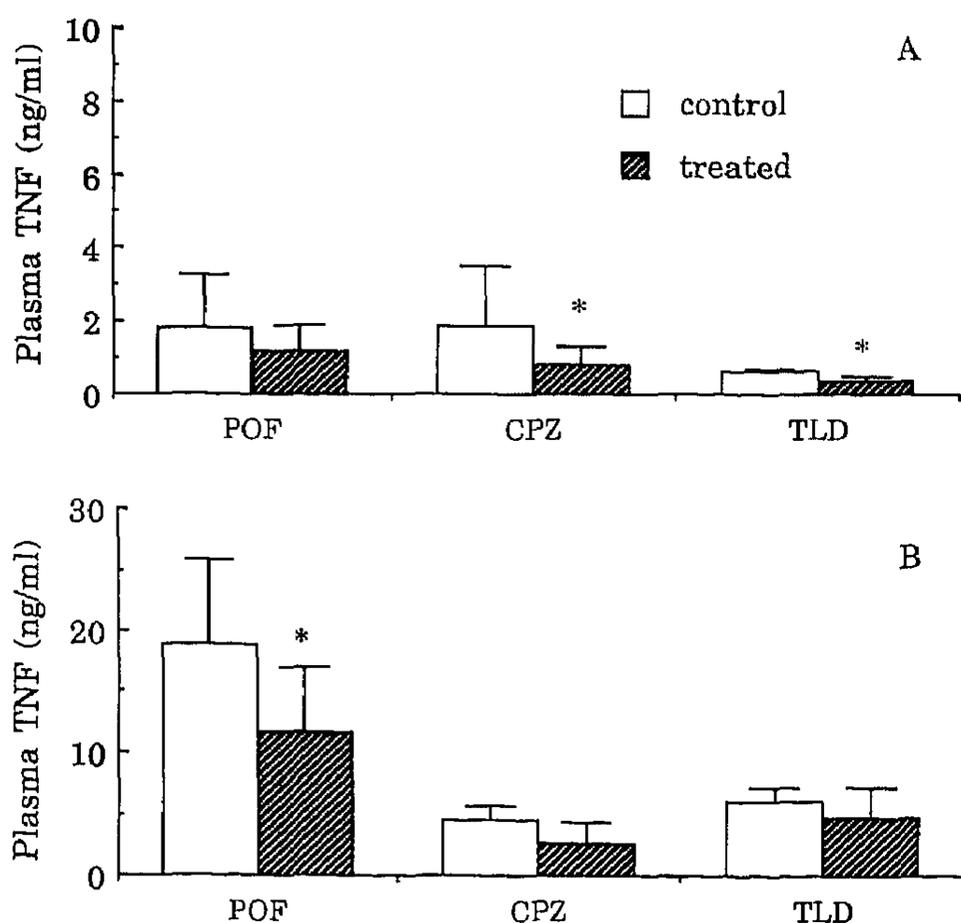


Figure 3. TNF- α plasma concentrations 24 h after injection of *Candida albicans* into nonneutropenic mice (A; $*P < .05$) or neutropenic mice (B; $*P < .05$). POF, pentoxifylline; CPZ, chlorpromazine; TLD, thalidomide. Results are mean \pm SD ($n \geq 10$ mice).

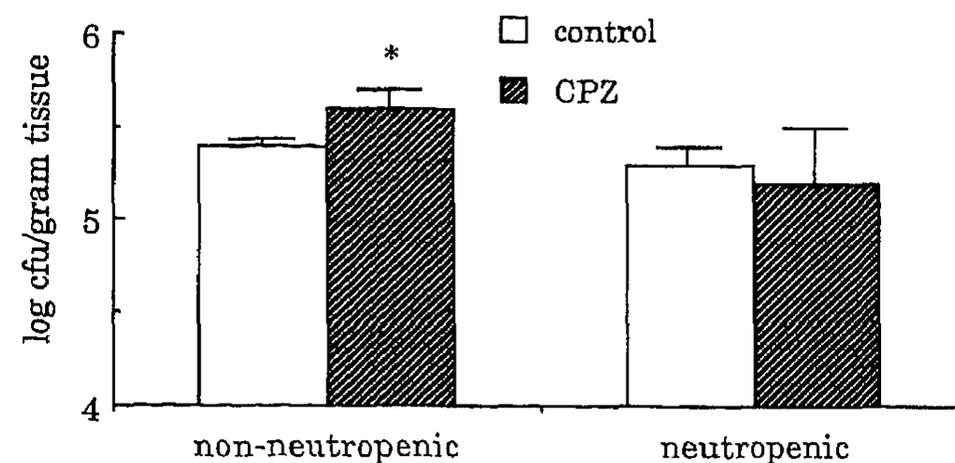


Figure 4. *Candida albicans* outgrowth from kidneys of nonneutropenic and neutropenic animals after treatment with chlorpromazine (CPZ) compared with controls. Results are mean \pm SD; $n \geq 10$ mice. $*P < .05$.

promazine and pentoxifylline may account for these divergent effects.

Since pentoxifylline also substantially reduced postendotoxin circulating TNF- α concentrations, it is clear that reduction of TNF- α production cannot solely account for the beneficial effect of chlorpromazine in lethal endotoxemia. In addition to substantially reducing TNF- α production, chlorpromazine profoundly decreased postendotoxin IL-1 β , and as far as we know this has not been reported before. As antagonism of IL-1 increases survival after lethal endotoxemia in mice [7], interference with the production of this cytokine may contribute to the effects observed. Chlorpromazine has a wide range of pharmacologic activities, from antipsychotic and antihistaminic actions [32] to inhibition of phospholipase A₂ [33]. To what extent these activities contribute to the effects in lethal endotoxemia is not clear. Pentoxifylline did not influence circulating IL-1 β concentrations, a finding in accordance with reports that phosphodiesterase inhibitors, such as pentoxifylline, selectively decrease TNF- α production without affecting IL-1 production capacity [34].

It is of interest that chlorpromazine strongly reduced the concentration of circulating sTNFR-P75 at 3 h after endotoxin, without affecting the concentration of circulating sTNFR-P55. It was recently reported that injection of endotoxin in mice results in an increase in both sTNFR-P55 and sTNFR-P75 as early as 30 min after the injection. sTNFR-P55 slowly diminished after a peak 30 min after LPS, whereas sTNFR-P75 increased to a plateau concentration 4–8 h after LPS administration [30]. Since both sTNFR-P55 and sTNFR-P75 are well detectable in plasma 3 h after LPS injection, it is unlikely that we missed a difference in sTNFR-p55 concentrations induced by one of the compounds. Recently, evidence was gained that in mice, sTNFR-P75 and not sTNFR-p55 is responsible for the inactivation of TNF and clearance of the cytokine from the circulation [35]. It is unlikely that the strong decrease in sTNFR-P75 induced by chlorpromazine is brought about by its inhibiting effect on the production of TNF- α or IL-1 β , since neither anti-TNF antibody nor IL-1 receptor antagonist decreased the concentration of circulating sTNFR after endotoxin injection [30]. Thus, the mechanism by which chlorpromazine reduces sTNFR-P75 concentration is probably more direct but remains to be elucidated.

From a clinical standpoint, it is somewhat worrisome that despite a marked inhibition of circulating TNF- α concentrations, neither of the drugs we tested improved outcome in *K. pneumoniae* infection. An infection with live microorganisms gives rise to a much more complicated response of the host than an injection of LPS. After LPS injection, circulating TNF- α concentrations peak at 90 min and decrease rapidly thereafter [2]. As shown here, there is an ongoing release of TNF after im injection of *K. pneumoniae*. These data are in accordance with the observations of others regarding the

efficacy of anti-TNF antibodies in experimental *E. coli* infection: They may be protective after iv but not after ip injection of *E. coli* [36]. Thus, the lack of an effect of pharmacologic TNF inhibition is reminiscent of the inability of anti-TNF antibodies to improve outcome in animal models of infection that resemble the gram-negative infection we have studied [4, 26, 37].

In *C. albicans* infection in mice, we found no influence of the three drugs on the survival of the animals. A recent study also failed to show an effect of pentoxifylline on outcome of infection with *C. albicans* in rats [17]. In our pentoxifylline-treated nonneutropenic mice, the concentrations of TNF- α tended to be reduced, but they were significantly inhibited only in the chlorpromazine- and thalidomide-treated groups. Only pentoxifylline significantly affected TNF- α concentrations in neutropenic mice. Since the same doses of pentoxifylline and chlorpromazine did decrease TNF- α synthesis in *K. pneumoniae* infection, these differences may be due to differences in the regulation of TNF- α production in the two infection models. In this respect it should be mentioned that endotoxin does not induce TNF- α synthesis in endotoxin-resistant C3H/HeJ mice, while *C. albicans* does [13].

In nonneutropenic mice, chlorpromazine treatment was associated with an increased outgrowth of candidae from the kidneys. However, in neutropenic mice, none of the drugs tested influenced the recovery of the microorganisms from the organs. This observation is in accordance with the report by Steinshamn and Waage [38], who found that treatment with a monoclonal antibody against TNF- α increased outgrowth of *C. albicans* from the kidneys of normal but not of neutropenic mice. Taken together, these observations in infection models are in agreement with the observations that TNF- α increases the intracellular killing of *C. albicans* by neutrophils in vitro [39, 40]. Apart from its influence on TNF production, direct interference with killing function of neutrophils may also play a role in the increased candidal outgrowth in chlorpromazine-treated mice [41–43].

We conclude that chlorpromazine protects sensitized mice against death due to lethal endotoxemia and that this protection is accompanied by a strong inhibition of in vivo TNF- α and IL-1 β synthesis. The anticytokine effect of chlorpromazine may be counteracted by decreased concentrations of circulating sTNFR-P75. Pentoxifylline, chlorpromazine, and thalidomide do not improve survival in the models of bacterial and fungal infections studied. In experimental infections, which are probably more relevant for infections in humans than are LPS challenges, the capacity of the drugs tested to inhibit TNF- α production appeared limited. In the case of chlorpromazine, the potential benefit of reducing circulating concentrations of TNF- α in lethal infections seems to be counteracted by a reduced phagocytosing capacity of neutrophils. In these experimental infections, the effects of

pharmacologic inhibition of TNF- α production are reminiscent of the effects observed with antibodies to TNF- α .

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References

- Dinarello CA. Role of interleukin-1 and tumor necrosis factor in systemic responses to infection and inflammation. In: Gallin JI, Goldstein IM, Snyderman R, eds. *Inflammation: basic principles and clinical correlates*. New York: Raven Press, 1992:211-31.
- Cannon JG, Tompkins RG, Gelfand JA, et al. Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. *J Infect Dis* 1990;161:79-84.
- Marks JD, Marks CB, Luce JM, et al. Plasma tumor necrosis factor in patients with septic shock. Mortality rate, incidence of adult respiratory distress syndrome, and effects of methylprednisolone administration. *Am Rev Respir Dis* 1990;141:94-7.
- Silva AT, Bayston KF, Cohen J. Prophylactic and therapeutic effects of a monoclonal antibody to tumor necrosis factor- α in experimental gram-negative shock. *J Infect Dis* 1990;162:421-7.
- Tracey KJ, Fong Y, Hesse DG, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature* 1987;330:662-4.
- Wakabayashi G, Gelfand JA, Burke JF, Thompson RC, Dinarello CA. A specific receptor antagonist for interleukin 1 prevents *Escherichia coli*-induced shock in rabbits. *FASEB J* 1991;5:338-43.
- Alexander HR, Doherty GM, Buresh CM, Venzon DJ, Norton JA. A recombinant human receptor antagonist to interleukin 1 improves survival after lethal endotoxemia in mice. *J Exp Med* 1991;173:1029-32.
- Alexander HR, Doherty GM, Venzon DJ, Merino MJ, Fraker DL, Norton JA. Recombinant interleukin-1 receptor antagonist (IL-1ra): effective therapy against gram-negative sepsis in rats. *Surgery* 1992;112:188-93.
- Mancilla J, Garcia P, Dinarello CA. The interleukin-1 receptor antagonist can either reduce or enhance the lethality of *Klebsiella pneumoniae* sepsis in newborn rats. *Infect Immun* 1993;61:926-32.
- Lesslauer W, Tabuchi H, Gentz R, et al. Recombinant soluble tumor necrosis factor receptor proteins protect mice from lipopolysaccharide-induced lethality. *Eur J Immunol* 1991;21:2883-6.
- Ashkenazi A, Marsters SA, Capon DJ, et al. Protection against endotoxic shock by a tumor necrosis factor receptor immunoadhesin. *Proc Natl Acad Sci USA* 1991;88:10535-9.
- Vecchiarelli A, Puliti M, Torosantucci A, Cassone A, Bistoni F. In vitro production of tumor necrosis factor by murine splenic macrophages stimulated with mannoprotein constituents of *Candida albicans* cell wall. *Cell Immunol* 1991;134:65-76.
- Riipi L, Carlson E. Tumor necrosis factor (TNF) is induced in mice by *Candida albicans*: role of TNF in fibrinogen increase. *Infect Immun* 1990;58:2750-4.
- Strieter RM, Remick DG, Ward PA, et al. Cellular and molecular regulation of tumor necrosis factor- α production by pentoxifylline. *Biochem Biophys Res Commun* 1988;155:1230-6.
- Zabel P, Wolter DT, Schonharting MM, Schade UF. Oxpentifylline in endotoxaemia. *Lancet* 1989;2:1474-7.
- Schade UF, von der Bosch J, Schonharting M. Pentoxifylline increases survival of mice in endotoxic shock. *Prog Clin Biol Res* 1989;301:223-7.
- Lechner AJ, Rouben LR, Potthoff LH, Tredway TL, Matuschak GM. Effects of pentoxifylline on tumor necrosis factor production and survival during lethal *E. coli* sepsis vs. disseminated candidiasis with fungal septic shock. *Circ Shock* 1993;39:306-15.
- Gadina M, Bertini R, Mengozzi M, Zandalasini M, Mantovani A, Ghezzi P. Protective effect of chlorpromazine on endotoxin toxicity and TNF production in glucocorticoid-sensitive and glucocorticoid-resistant models of endotoxic shock. *J Exp Med* 1991;173:1305-10.
- Sampaio EP, Kaplan G, Miranda A, et al. The influence of thalidomide on the clinical and immunologic manifestation of erythema nodosum leprosum. *J Infect Dis* 1993;168:408-14.
- Gutierrez-Rodriguez O, Starusta-Bacal P, Gutierrez-Montes O. Treatment of refractory rheumatoid arthritis—the thalidomide experience. *J Rheumatol* 1989;16:158-63.
- Wood PM, Proctor SJ. The potential use of thalidomide in the therapy of graft-versus-host disease—a review of clinical and laboratory information. *Leuk Res* 1990;14:395-9.
- Sampaio EP, Sarno EN, Galilly R, Cohn ZA, Kaplan G. Thalidomide selectively inhibits tumor necrosis factor alpha production by stimulated human monocytes. *J Exp Med* 1991;173:699-703.
- Kullberg BJ, van't Wout JW, van Furth R. Role of granulocytes in increased host resistance to *Candida albicans* induced by recombinant interleukin-1. *Infect Immun* 1990;58:3319-24.
- Wallach D, Holtmann H, Engelmann H, Nophar Y. Sensitization and desensitization to lethal effects of tumor necrosis factor and IL-1. *J Immunol* 1988;140:2994-9.
- van der Meer JWM, Barza M, Wolff SM, Dinarello CA. A low dose of recombinant interleukin 1 protects granulocytopenic mice from lethal gram-negative infection. *Proc Natl Acad Sci USA* 1988;85:1620-3.
- Echtenacher B, Falk W, Mannel DN, Krammer PH. Requirement of endogenous tumor necrosis factor/cachectin for recovery from experimental peritonitis. *J Immunol* 1990;145:3762-6.
- von Asmuth EJ, Maessen JG, van der Linden CJ, Buurman WA. Tumor necrosis factor alpha (TNF-alpha) and interleukin 6 in a zymosan-induced shock model. *Scand J Immunol* 1990;32:313-9.
- Sheehan KC, Ruddle NH, Schreiber RD. Generation and characterization of hamster monoclonal antibodies that neutralize murine tumor necrosis factors. *J Immunol* 1989;142:3884-93.
- Blok WL, Vogels MTE, Curfs JHAJ, Eling WMC, Buurman WA, van der Meer JWM. Dietary fish-oil supplementation in experimental gram-negative infection and in cerebral malaria in mice. *J Infect Dis* 1992;165:898-903.
- Bemelmans MH, Gouma DJ, Buurman WA. LPS-induced sTNF-receptor release in vivo in a murine model. Investigation of the role of tumor necrosis factor, IL-1, leukemia inhibiting factor, and IFN-gamma. *J Immunol* 1993;151:5554-62.
- Peto R, Pike MC, Armitage P. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. *Br J Cancer* 1977;35:1-39.
- Baldessarini RJ. Drugs and the treatment of psychiatric disorders. In: Goodman Gilman A, Rall TW, Nies AS, Taylor P, eds. *Goodman Gilman's the pharmacological basis of therapeutics*. 8th ed. New York: Pergamon Press, 1990:383-435.
- Vadas P, Stefanski E, Pruzanski W. Potential therapeutic efficacy of inhibitors of human phospholipase A2 in septic shock. *Agents Actions* 1986;19:194-202.
- Endres S, Fulle HJ, Sinha B, et al. Cyclic nucleotides differentially

regulate the synthesis of tumour necrosis factor- α and interleukin- 1β by human mononuclear cells. *Immunology* 1991;72:56-60.

35. Bemelmans MH, Gouma DJ, Buurman WA. Influence of nephrectomy on tumor necrosis factor clearance in a murine model. *J Immunol* 1993;150:2007-17.
36. Zanetti G, Heumann D, Gerain J, et al. Cytokine production after intravenous or peritoneal gram-negative bacterial challenge in mice. Comparative protective efficacy of antibodies to tumor necrosis factor- α and to lipopolysaccharide. *J Immunol* 1992;148:1890-7.
37. Bagby GJ, Plessala KJ, Wilson LA, Thompson JJ, Nelson S. Divergent efficacy of antibody to tumor necrosis factor- α in intravascular and peritonitis models of sepsis. *J Infect Dis* 1991;163:83-8.
38. Steinshamn S, Waage A. Tumor necrosis factor and interleukin-6 in *Candida albicans* infection in normal and granulocytopenic mice. *Infect Immun* 1992;60:4003-8.
39. Djeu JY, Blanchard DK, Halkias D, Friedman H. Growth inhibition of *Candida albicans* by human polymorphonuclear neutrophils: activation by interferon- γ and tumor necrosis factor. *J Immunol* 1986;137:2980-4.
40. Ferrante A. Tumor necrosis factor alpha potentiates neutrophil antimicrobial activity: increased fungicidal activity against *Torulopsis glabrata* and *Candida albicans* and associated increases in oxygen radical production and lysosomal enzyme release. *Infect Immun* 1989;57:2115-22.
41. Takanaka K, Taniguchi K, Masuda Y, O'Brien PJ. Reversible drug effects on the metabolic activation of polymorphonuclear leukocytes. *Chem Biol Interact* 1990;73:309-21.
42. Bertini R, Wang JM, Mengozzi M, et al. Effects of chlorpromazine on PMN-mediated activities in vivo and in vitro. *Immunology* 1991;72:138-43.
43. Kelder PP, de Mol NJ, 'T Hart BA, Janssen LH. Metabolic activation of chlorpromazine by stimulated human polymorphonuclear leukocytes. Induction of covalent binding of chlorpromazine to nucleic acids and proteins. *Chem Biol Interact* 1991;79:15-30.