INFECTION AND IMMUNITY, Mar. 1999, p. 1455–1460 Vol. 67, No. 3
0019-9567/99/$04.00+0

Involvement of Tumor Necrosis Factor Alpha and Interleukin-1β in Enhancement of Pentylenetetrazole-Induced Seizures Caused by *Shigella dysenteriae*

YAEL YUHAS,[1] LESTER SHULMAN,[2] ABRAHAM WEIZMAN,[1] ELIZABETH KAMINSKY,[1] ALEXEY VANICHKIN,[1] AND SHAI ASHKENAZI[1,3]*

The Basil and Gerald Felsenstein Medical Research Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv,[1] Central Virology Laboratory, Sheba Medical Center, Tel Hashomer,[2] and Schneider Children's Medical Center of Israel, Petah Tikva,[3] Israel

Received 5 August 1998/Accepted 3 December 1998

Neurologic manifestations, mainly convulsions, are the most frequent extraintestinal complications of shigellosis. We used an animal model to study the roles of tumor necrosis factor alpha (TNF-α) and interleukin-1 β (IL-1β) in *Shigella*-related seizures. Administration of *Shigella dysenteriae* 60R sonicate enhanced the sensitivity of mice to the proconvulsant pentylenetetrazole (PTZ) within 7 h. This was indicated by a significantly higher mean convulsion score and an increased number of mice responding with clonic-tonic seizures in the *Shigella*-pretreated group. Preinjection of mice with anti-murine TNF-α (anti-mTNF-α) or anti-murine IL-1β (anti-mIL-1β) 30 min prior to administration of *Shigella* sonicate abolished their enhanced response to PTZ at 7 h. Mean convulsion scores were reduced by anti-mTNF-α from 1.2 to 0.8 ($P = 0.017$) and by anti-mIL-1β from 1.3 to 0.7 ($P = 0.008$). Preinjection of anti-mTNF-α also reduced the percentage of mice responding with clonic-tonic seizures, from 48 to 29% ($P = 0.002$), and preinjection of anti-mIL-1β reduced it from 53 to 21% ($P = 0.012$). Neutralization of TNF-α or IL-1β did not protect the mice from death due to *S. dysenteriae* 60R. These findings indicate that TNF-α and IL-1β play a role in the very early sensitization of the central nervous system to convulsive activity after *S. dysenteriae* administration. Similar mechanisms may trigger neurologic disturbances in other infectious diseases.

Neurologic disturbances are the most frequent complications of acute gastroenteritis caused by bacteria of the genus *Shigella*. They include convulsions, severe headaches, hallucinations, and encephalopathy, which can be fulminant, leading rapidly to unconsciousness and death (1, 2, 5, 10).

The pathogenesis of *Shigella*-associated neurologic symptoms is unclear. Shiga toxin (ST), the main toxic product of *Shigella dysenteriae*, has been implicated in neurotoxicity, as its administration caused paralysis and death in mice and rabbits (13). Two other toxins of the same family, Shiga-like toxins I and II (SLT I and SLT II), which are produced by enterohemorrhagic *Escherichia coli* strains and are similar to ST in structure, cell binding receptor, and biological activity (for a review see reference 20), were also associated with neurotoxicity. Administration of SLTs to laboratory animals or inoculation with SLT-producing *E. coli* induced neurologic symptoms (8, 9, 15, 29), and human infections with SLT-producing strains are often accompanied by neurologic complications (9, 12). However, the primary damage after toxin administration is found in the vascular endothelium of the central nervous system (CNS) rather than in the neuronal cells (13, 29), and the histopathological findings in human autopsy studies do not always correlate with the severe manifestations (12).

Lipopolysaccharide (LPS) and the proinflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin 1β (IL-1β) may also be involved in diseases associated with ST and SLT toxicity. Barrett et al. have shown that LPS either increased or inhibited SLT II lethality in mice and rabbits, depending on the timing of its application, and that the toxicity of SLT II in mice was macrophage-dependent (3, 4). TNF-α and IL-1β, the major mediators of LPS toxicity, are also induced by SLTs (25). Both TNF-α and IL-1β upregulate toxin receptor expression on endothelial cells and increase SLT cytotoxicity (33). In the early stages of disease, phagocytosis of *Shigella* triggers TNF release (21), and macrophages infected with invasive *Shigella* secrete large quantities of IL-1β (36). The increased production of these cytokines has been implicated in the extensive inflammatory reaction and the severe tissue damage of the colon (26). High concentrations of TNF-α and IL-1β may also account for systemic manifestations. Recently, we found elevated plasma levels of TNF-α in children with shigellosis, with significantly higher concentrations in those exhibiting neurologic complications (18).

To investigate the underlying mechanisms of the neurologic disturbances of shigellosis, we recently developed an animal model to study the roles of host mediators and bacterial products in the induction of seizures (35). Administration of the proconvulsant pentylenetetrazole (PTZ) to mice induces clonic-tonic seizures within minutes of its application, owing to its antagonistic activity at the benzodiazepine/γ-aminobutyric acid (GABA) receptor complex. The ability of bacterial products to modulate the sensitivity of mice to PTZ-induced seizures was used to study their involvement in the neural processes that lead to convulsions.

Employing this model, we showed that crude preparations of *S. dysenteriae* 60R (a producer of ST) and of *E. coli* H-30 (a producer of SLT I) enhanced the response to PTZ-induced seizures and that ST and LPS acted in concert in this respect (35). In the present study, we demonstrate that the enhancement of PTZ-induced seizures caused by *S. dysenteriae* is mediated by TNF-α and IL-1β.

* Corresponding author. Mailing address: P.O.B. 8145, Petah Tikva 49181, Israel. Phone: 972-3-9253680/1. Fax: 972-3-9253056/3899. E-mail: shkenazi@post.tau.ac.il.

MATERIALS AND METHODS

Mice. ICR outbred male mice 22 to 28 days of age (weight range, 17 to 25 g) were maintained under standard conditions. The animal experimentation guidelines followed in the animal studies were approved by the Animal Experimentation Committee of the Rabin Medical Center.

Materials. PTZ (Sigma Chemical Co., St. Louis, Mo.) was dissolved in pyrogen-free saline. Evans blue (Sigma) was dissolved in sterile pyrogen-free saline before use.

Antisera. Rabbit polyclonal anti-murine TNF-α (anti-mTNF-α) and rabbit polyclonal anti-murine IL-1β (anti-mIL-1β) antibodies were purchased from Genzyme Corp. (Cambridge, Mass.).

Preparation of bacterial sonicate. Strain 60R of *S. dysenteriae* serotype 1 was grown in syncase broth for 48 h with shaking, lysed by sonication, and filter sterilized as described (22). The bacterial sonicate was analyzed for protein content, cytotoxic activity, and lethality in mice. Cytotoxic activity was quantitatively determined on HeLa cells as described previously (35). Protein content was measured with the Bio-Rad protein assay (Bio-Rad Laboratories GmbH, Munich, Germany). Lethality studies were performed in groups of six mice injected intraperitoneally (i.p.) with a fourfold dilution of bacterial sonicate, as described previously (35). Death was recorded daily, and the 50% lethal dose (LD_{50}) was calculated according to the method of Reed and Muench (28).

PTZ-induced convulsions. Induction and scoring of seizures was performed as described (35). Groups of six mice were inoculated i.p. with 50 mg of PTZ per kg of body weight (a dose which causes clonic-tonic seizures in 50% of animals), and their reactions were observed for 10 min. Reactions included several phases, as follows: unresponsiveness, myoclonic jerks, clonic seizures, and tonic seizures (forelegs and then hind legs rigidly extended to the rear), occasionally followed by death. Not all mice went through all the phases. For statistical analysis, each phase was given a numerical score, as detailed previously (19, 32): the score was 0 for unresponsiveness, 1 for myoclonic contractions, 2 for clonic seizures, 3 for tonic seizures, and 4 for death. The response of each mouse was scored as the highest phase reached, and a mean seizure severity score was calculated for each group. The percentages of mice that had clonic-tonic seizures in the treatment groups were also statistically compared.

Enhancement of PTZ-induced seizures. Groups of six to eight mice were inoculated i.p. with 1,000 50% cytotoxic doses (CD_{50}) (~4 LD_{50}) of *S. dysenteriae* 60R sonicate. This dose caused death in 80 to 100% of the mice within 36 to 96 h. Saline-treated mice were used as controls. At 7 and 24 h after injection of *S. dysenteriae* sonicate or saline, mice were injected with PTZ (50 mg/kg) and scored for their response compared with that of controls.

Pretreatment with antibodies. Rabbit anti-mTNF-α (30 μl/mouse), rabbit anti-mIL-1β (30 μg/mouse), or normal rabbit serum (NRS) was injected intravenously (i.v.) in a 200-μl volume 30 min before injection of bacterial sonicate.

Cytokine assay. Plasma for TNF-α and IL-1β assessment was collected at 0, 1, 3, 5, and 24 h after *Shigella* sonicate administration and stored at −70°C until assay. TNF-α levels were determined by a cytotoxicity bioassay on mouse L-929 cells (18). Each test included a standard curve of recombinant human TNF-α (specific activity, 2.5×10^7 U/mg of protein), kindly provided by T. Amarant (Reprogen Ltd., Rehovot, Israel). IL-1β was measured by an enzyme-linked immunosorbent assay kit (R&D Systems Inc., Minneapolis, Minn.).

Determination of BBB integrity. Blood-brain barrier (BBB) permeability was tested as described previously (7). Mice were injected i.v. with 100 μl of 1% Evans blue, an albumin binding dye, in saline. One hour later mice were anesthetized with pentobarbital sodium (Nembutal) (60 mg/kg) and flushed with saline introduced into the cardiac left ventricle. The brains were then removed for photography.

Statistical analysis. The difference in the incidence of seizures among the various groups in each experiment was compared by chi-square test for multiple comparisons or by Fisher's exact test, as appropriate. Convulsion scores were compared by two-tailed unpaired *t* test (for two groups) or by one-way analysis of variance (for three or more groups).

RESULTS

Induction of TNF-α and IL-1β production by *S. dysenteriae* 60R sonicate. Injection of *S. dysenteriae* 60R sonicate rapidly induced circulating TNF-α and IL-1β. High levels of TNF-α were present 1 h after injection, and the levels declined rapidly to undetectable concentrations at 5 h (Fig. 1); IL-1β appeared after TNF-α, the levels peaking at 3 h and returning to baseline by 24 h (Fig. 1).

Effects of anti-mTNF-α and anti-mIL-1β on enhancement of PTZ-induced seizures by *S. dysenteriae* sonicate. Administration of *S. dysenteriae* 60R sonicate 7 and 24 h before PTZ administration increased the susceptibility of the mice to PTZ. This was reflected in the significantly higher mean convulsion score (Fig. 2) and the greater number of mice responding with clonic-tonic seizures as compared to control, saline-treated mice (Table 1).

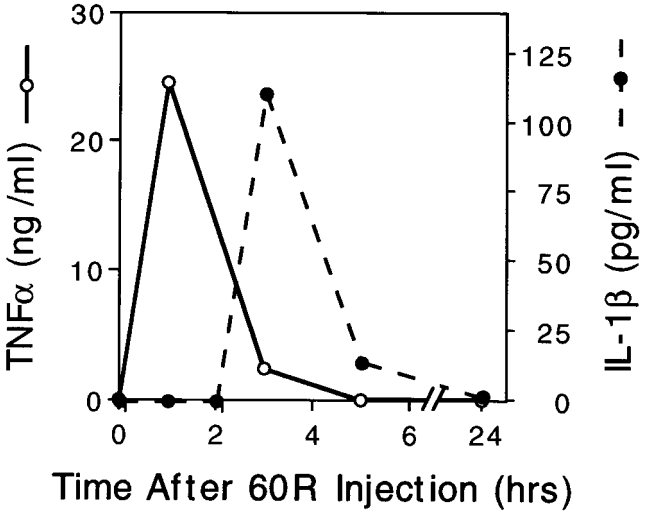

FIG. 1. Plasma TNF-α and IL-1β production in response to *S. dysenteriae* 60R sonicate (60R). Mice were injected with *S. dysenteriae* 60R sonicate (4 LD_{50}, i.p.). TNF-α production was determined by bioassay and IL-1β production was determined by enzyme-linked immunosorbent assay kit, as described in Materials and Methods. The values are the means of at least five determinations.

To determine whether TNF-α and IL-1β participate in the sensitization of mice to PTZ-induced seizures by *S. dysenteriae*, we pretreated the mice with anti-mTNF-α or anti-mIL-1β. Pretreatment with anti-mTNF-α before inoculation with *S. dysenteriae* 60R sonicate abolished the greater response to PTZ at 7 h. The mean convulsion scores for control mice, mice given *S. dysenteriae* 60R sonicate, and mice pretreated with anti-mTNF-α prior to inoculation with *S. dysenteriae* 60R sonicate were 0.7, 1.2, and 0.8, respectively ($P < 0.001$ for saline versus *Shigella* and $P = 0.017$ for *Shigella* versus anti-mTNF-α followed by *Shigella*) (Fig. 2A). The incidences of seizure for the three groups (Table 1) were 27% (17 of 67 saline-treated mice), 48% (32 of 67 *Shigella* sonicate-treated mice), and 29% (20 of 68 mice treated with anti-mTNF-α plus *Shigella*) ($P = 0.002$ for saline versus *Shigella* and $P = 0.021$ for *Shigella* versus anti-mTNF-α plus *Shigella*).

A reduced response after anti-mTNF-α pretreatment was also noticed when PTZ was administered 24 h after bacterial sonicate inoculation, but the reduction was milder and did not reach statistical significance (Fig. 2A and Table 1).

Pretreatment with anti-mIL-1β yielded results similar to those for anti-mTNF-α. The effect of anti-mIL-1β was also most evident when PTZ was applied 7 h after bacterial sonicate inoculation (Fig. 2B). Mean convulsion scores were 0.5, 1.3, and 0.7 for saline-treated, *S. dysenteriae* 60R sonicate-treated, and anti-mIL-1β-plus-*S. dysenteriae* 60R sonicate-treated groups, respectively ($P = 0.001$ for saline versus *Shigella* and $P = 0.010$ for *Shigella* versus anti-mIL-1β plus *Shigella*). Preinjection of anti-mIL-1β also completely abolished the increase in the number of mice responding to PTZ with clonic-tonic seizures; the seizure incidences were 23% (7 of 30 mice) in the saline-treated group, 53% (16 of 30 mice) in the *Shigella* sonicate-treated group, and 21% (6 of 28 mice) in the group treated with anti-mIL-1β plus *Shigella*) ($P = 0.016$ for saline versus *Shigella* and $P = 0.012$ for *Shigella* versus anti-mIL-1β plus *Shigella*) (Table 1).

The effect of anti-mIL-1β on the convulsion score remained significant when PTZ was administered 24 h after bacterial

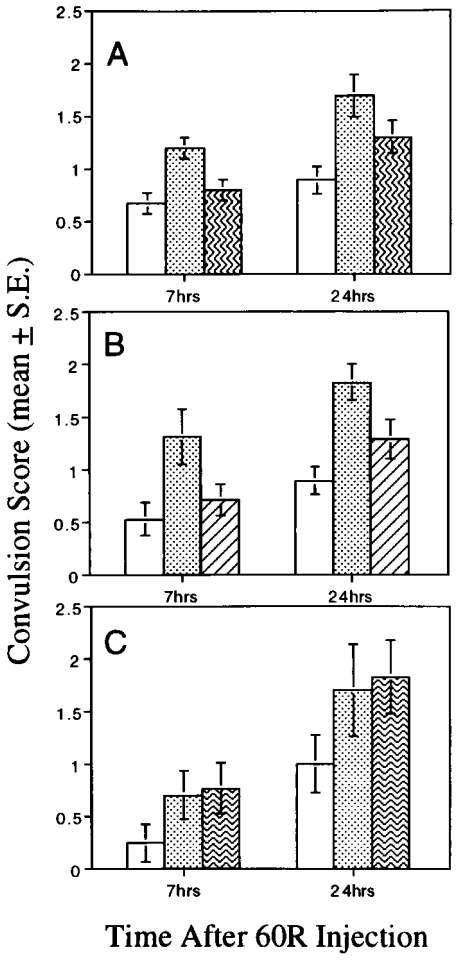

FIG. 2. Effect of pretreatment with anti-mTNF-α or anti-mIL-1β on the enhancement of PTZ-induced seizures by *S. dysenteriae* sonicate (60R). Mice were injected i.p. with saline alone (□) or with 60R alone (▨) or were pretreated with the indicated antibodies i.v. 30 min before administration of 60R. PTZ was applied i.p. at 7 and 24 h. Each panel shows the findings for a different pretreatment. (A) For mice pretreated with anti-mTNF-α (▨), at 7 h the *P* value was <0.001 for the convulsion scores in the groups treated with saline ($n = 63$) and with 60R ($n = 67$), and it was 0.017 for the scores for the groups treated with 60R and with anti-mTNF-α plus 60R ($n = 68$). At 24 h, the *P* value was 0.001 for saline- ($n = 49$) and 60R-treated ($n = 56$) groups, and the *P* value was 0.170 for 60R- and anti-mTNF-α-plus-60R-treated ($n = 48$) groups. (B) For mice pretreated with anti-mIL-1β (▨), at 7 h, *P* was 0.001 for the scores in the saline-treated ($n = 30$) and 60R-treated ($n = 30$) groups, and it was 0.008 for those in the 60R- and anti-mIL-1β-plus-60R-treated ($n = 28$) groups. At 24 h, *P* was <0.001 for saline-treated ($n = 48$) and 60R-treated ($n = 48$) groups, and *P* was 0.036 for 60R- and anti-mIL-1β-plus-60R-treated ($n = 47$) groups. (C) For mice pretreated with NRS (▨), the *P* values were 0.017 for saline-treated ($n = 11$) and 60R-treated ($n = 12$) groups and 0.1 for 60R- and NRS-plus-60R-treated groups at 7 h and <0.05 for saline-treated ($n = 18$) and 60R-treated ($n = 18$) groups and 0.7 for saline- and NRS-plus-60R-treated groups at 24 h.

sonicate inoculation; the scores were 0.9, 1.8, and 1.3 for the groups treated with saline, *Shigella*, and *Shigella* plus anti-mIL-1β, respectively ($P < 0.001$ for saline versus *Shigella* and $P = 0.036$ for *Shigella* versus anti-mIL-1β plus *Shigella*) (Fig. 2B). However, there was no significant difference between the total numbers of mice with clonic-tonic seizures in the *Shigella* and anti-mIL-1β-plus-*Shigella* groups (Table 1).

Pretreatment of mice with NRS prior to *S. dysenteriae* 60R sonicate administration did not reduce the enhanced response to PTZ (Fig. 2C), nor did administration of anti-mTNF-α or anti-mIL-1β affect the response to PTZ in mice pretreated with saline (data not shown).

BBB permeability. Elevated levels of IL-1β and TNF-α have been associated with disruption of BBB integrity (24). To investigate the possibility that severe BBB injury contributes to the enhanced response to PTZ, we examined the permeability of the BBB at various time points after *S. dysenteriae* 60R sonicate injection (Fig. 3). At 7 h, only 1 of 15 brains examined was slightly stained with Evans blue, whereas at 24 and 48 h 60% ($n = 10$) and 100% ($n = 5$), respectively, were permeable.

To further exclude the possibility that enhanced response to PTZ results from extensive disruption of the BBB, we examined the brains of the mice exhibiting an increased response to PTZ after treatment for 7 h with *S. dysenteriae* 60R sonicate. Six of the 11 mice in this group had clonic-tonic seizures, whereas none of 6 control (saline-pretreated) mice did. None of the brains of the mice that responded with seizures were stained with Evans blue (Fig. 3).

Effects of anti-mTNF-α and anti-mIL-1β on clinical manifestations. The mice that received *S. dysenteriae* 60R sonicate died within 5 days. Clinical symptoms, which usually appeared after 24 h, included ruffled fur, weakness, weight loss, and shortly before death, flaccid paralysis of the hind legs. Pretreatment with anti-mTNF-α or anti-mIL-1β neither attenuated the clinical symptoms nor protected the mice from death. A slightly increased mortality rate (Table 2) and shorter survival time were observed in the mice receiving anti-mTNF-α (Fig. 4). However, both anti-mTNF-α and anti-mIL-1β prevented the massive weight loss observed at 24 h after bacterial sonicate administration (Table 2).

DISCUSSION

Mice show an increased susceptibility to PTZ as early as 6 to 7 h after administration of *S. dysenteriae* 60R sonicate. This resembles the situation in human shigellosis where, strikingly, neurologic disturbances appear very early in the course of the disease, sometimes preceding the onset of diarrhea (2). Therefore, our model is useful for studying the early neural processes that lead to convulsions in human infections.

The rapid sensitization of the mice to PTZ by *Shigella* sonicate is a result of the mutual action of ST and LPS, as we have previously shown (35). TNF-α and IL-1β are quickly induced after *Shigella* administration by LPS and possibly by ST itself. Both TNF-α and IL-1β have been implicated in the neurologic manifestations of infectious diseases, such as bacterial meningitis, cerebral malaria, and human immunodeficiency viral encephalitis (7, 16, 30), owing mainly to the correlation between cerebrospinal fluid TNF-α and IL-1β levels and the severity of the neurologic damage. Neurologic disorders, including seizures, have also been reported to occur during cancer therapy with TNF-α or IL-1β (27, 31) and in transgenic mice showing CNS-specific expression of TNF-α (23).

In all these pathological conditions, however, TNF-α and IL-1β are present for a prolonged time. Here, we demonstrate that the administration of antibodies to TNF-α or IL-1β abolishes the enhanced *S. dysenteriae*-induced sensitivity to PTZ which occurs rapidly (within 7 h) after bacterial administration. Yet, since by the time mice exhibit the increased sensitivity TNF-α and IL-1β are no longer detectable in the circulation, it is possible that other factors induced by TNF-α and IL-1β may also contribute to the sensitization of the CNS. To the best of our knowledge, this is the first time that a causal relationship between TNF-α or IL-1β and the induction of seizures has been demonstrated in an animal model of an infectious disease.

TABLE 1. Effects of pretreatment with anti-mTNF-α and anti-mIL-1β on *S. dysenteriae* 60R enhancement of PTZ-induced seizures

Treatment at:		No. of mice responding with convulsion score of ≥2[a]/no. tested (%) [*P* value] at:	
−30 min	0 h	7 h	24 h
Saline	Saline	17/63 (27)	17/49 (35)
Saline	*S. dysenteriae* 60R	32/67 (48) [0.002][b]	29/45 (56) [0.058][b]
Anti-mTNF-α	*S. dysenteriae* 60R	20/68 (29) [0.021][c]	23/48 (48) [0.119][c]
Saline	Saline	7/30 (23)	14/48 (29)
Saline	*S. dysenteriae* 60R	16/30 (53) [0.016][b]	31/48 (65) [<0.005][b]
Anti-mIL-1β	*S. dysenteriae* 60R	6/28 (21) [0.012][d]	24/47 (51) [0.129][d]

[a] Tonic-clonic seizures.
[b] *S. dysenteriae* 60R- versus saline-pretreated mice.
[c] Anti-mTNF-α-plus-*S. dysenteriae* 60R- versus *S. dysenteriae* 60R-pretreated mice.
[d] Anti-mIL-1β-plus-*S. dysenteriae* 60R- versus *S. dysenteriae* 60R-pretreated mice.

There are several possible pathways by which TNF-α and IL-1β can affect brain function. TNF-α and IL-1β can modulate the CNS from the periphery. There is a line of evidence showing that a variety of illness responses, such as fever, headache, slow-wave sleep, and behavioral changes, which are governed by the brain, are mediated by cytokines produced in the periphery, which stimulate the CNS through afferent nerves (34). Alternatively, as some studies have demonstrated, TNF-α and IL-1β can cross the BBB (11). They are also produced within the brain by glial cells. Elevated brain mRNA levels for TNF-α and IL-1β were shown after systemic administration of LPS (34). It is possible, therefore, that TNF-α and IL-1β produced locally in the brain are also involved in the sensitization to PTZ-induced seizures by *S. dysenteriae*. These cytokines affect many functions in the CNS, including neurotransmission and neurotoxicity (reviewed in reference 30). Some of their activities are modulated by the induction of secondary messengers, such as nitric oxide (NO). It has been reported that TNF-α and IL-1β synergistically mediate neurotoxicity through NO induction (6). NO acts also as a neurotransmitter, and its overproduction has been linked to induction of seizures (17). Another gene upregulated in the brain after systemic administration of either LPS or IL-1β is the immediate-early gene *c-fos*, which is activated during seizures (14, 34). TNF-α and IL-1β may also act indirectly by increasing ST toxicity. ST is transported very rapidly to the CNS, where it binds to specific receptors on endothelial cells (29). It can be postulated that both TNF-α and IL-1β upregulate ST receptors and increase ST cytotoxicity, as they do in vitro.

Which one of the diverse activities of TNF-α and IL-1β in the CNS is relevant to the enhancement of seizures by *S. dysenteriae* remains an enigma. In any case, since the increased

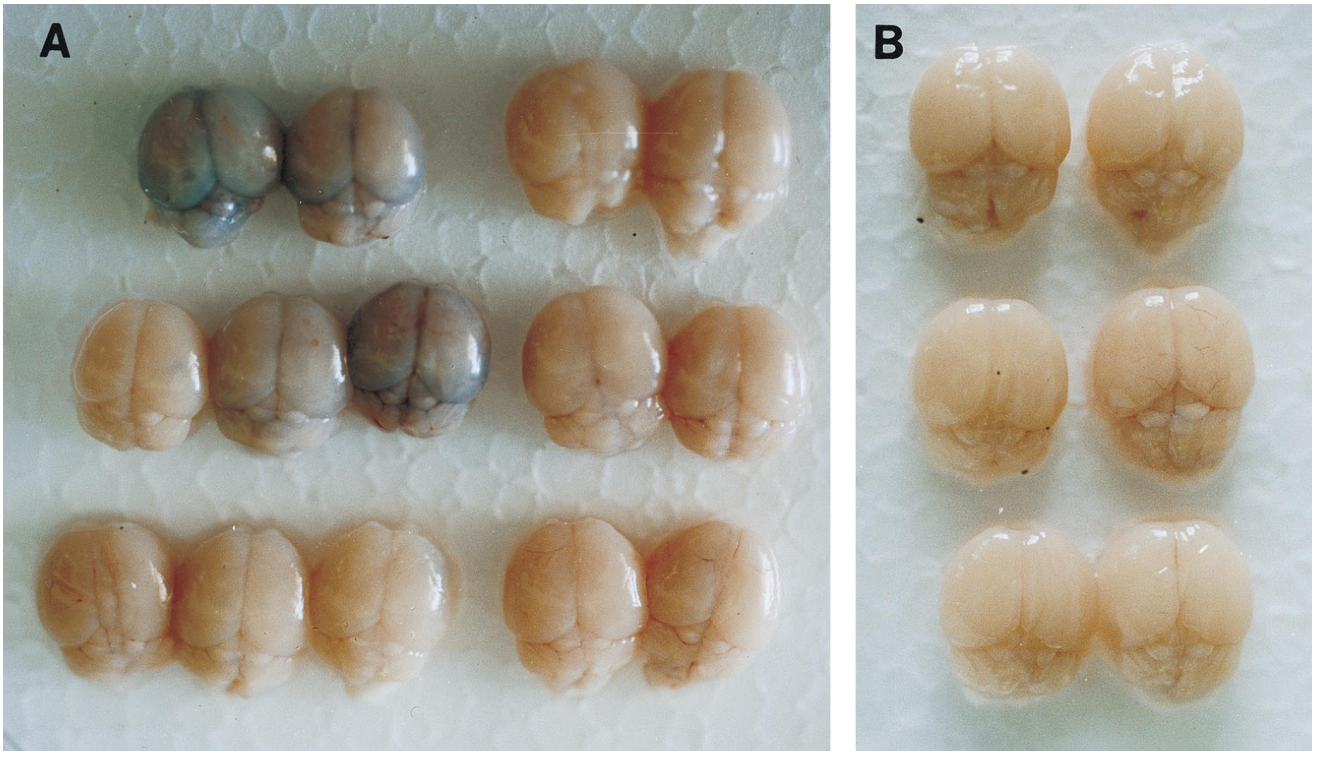

FIG. 3. Evans blue penetration of BBB in mice injected with *S. dysenteriae* 60R sonicate. Evans blue was injected i.v. 1 h before brain examination, as described in Materials and Methods. (A) Brains of mice injected with *Shigella* 60R sonicate (left) or saline (right) 7, 24, and 48 h (bottom to top) before injection of Evans blue. (B) Brains of mice injected with *Shigella* 60R sonicate and responding with seizures to PTZ at 7 h.

TABLE 2. Effects of pretreatment with anti-mTNF-α and anti-mIL-1β on mouse survival and weight loss after *S. dysenteriae* 60R sonicate inoculation[a]

Treatment at:		Total no. of deaths/no. of mice injected (%)	Mean change in body wt[b] (no. of mice)
−30 min	0 h		
Saline	Saline	0/46	+1.26 (30)
Saline	*S. dysenteriae* 60R	34/46 (74)	−0.94 (29)
Anti-mTNF-α	*S. dysenteriae* 60R	41/46 (89)	−0.3 (30)
Saline	Saline	0/28	+1.56 (36)
Saline	*S. dysenteriae* 60R	26/28 (93)	−0.66 (36)
Anti-mIL-1β	*S. dysenteriae* 60R	26/27 (96)	−0.02 (36)

[a] Mice were injected i.p. with *S. dysenteriae* 60R sonicate (4 LD_{50}). Deaths were recorded daily for 7 days.

[b] In grams per mouse. Mice were weighed individually. Values are mean changes in body weight 24 h after *S. dysenteriae* 60R sonicate injection.

sensitivity to the proconvulsant PTZ requires the presence of both LPS and ST, as we have previously shown (35), some kind of cooperative mechanism must exist. The fact that neutralization of either TNF-α or IL-1β was sufficient to reduce seizure incidence indicates that both cytokines play an essential role in the processes that lead to the increased susceptibility to proconvulsants.

The inhibition of TNF-α or IL-1β was much less effective in reducing the incidence of enhanced seizures when PTZ was applied 24 h after *Shigella* sonicate. This implies that at this later stage, the enhancement of seizures is mediated by additional mechanisms. Moreover, the neutralization of TNF-α or IL-1β did not protect the mice from death, which indicates that *S. dysenteriae* is capable of inducing neuronal damage and death by mechanisms that do not involve induction of circulatory TNF-α and IL-1β. In fact, administration of TNF-α slightly increased the mortality rate and shortened the life span. This points to the complex effects, both protective and deleterious, of TNF-α during the course of the disease.

In conclusion, using the model of PTZ-induced seizures, we showed that both TNF-α and IL-1β sensitize the CNS to convulsive activity very shortly after *S. dysenteriae* administration. Similar mechanisms may trigger the early neurologic disturbances observed in human shigellosis and enterohemorrhagic *E. coli* infections. This model may be used to elucidate the pathogenesis of neurologic symptoms associated with other infectious diseases.

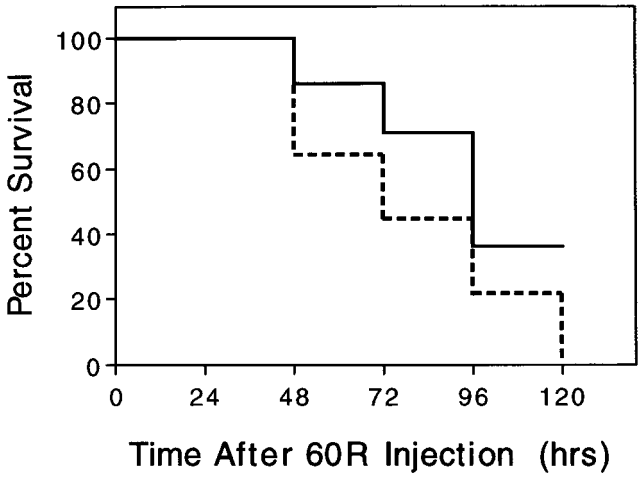

FIG. 4. Effect of pretreatment with anti-mTNF-α on *S. dysenteriae* 60R-induced mortality in mice. Mice were injected i.v. with saline (——) or anti-mTNF-α (– – –) 30 min prior to *S. dysenteriae* 60R sonicate inoculation (4 LD_{50}) (n = 14 for each group). Deaths were recorded daily.

ACKNOWLEDGMENTS

We thank Amiram Ravid, The Felsenstein Medical Research Center, for fruitful discussions of the results.

This study was supported by Tel Aviv University Research Foundation grant 572/96.

REFERENCES

1. **Ashkenazi, S., G. Bellah, and T. G. Clearly.** 1989. Hallucinations as an initial manifestation of childhood shigellosis. J. Pediatr. **114:**95–96.
2. **Ashkenazi, S., G. Dinari, A. Zevulunov, and M. Nitzan.** 1987. Convulsions in childhood shigellosis. Clinical and laboratory features in 153 children. Am. J. Dis. Child. **141:**208–210.
3. **Barrett, T. J., M. E. Potter, and I. K. Wachsmuth.** 1989. Bacterial endotoxin both enhances and inhibits the toxicity of Shiga-like toxin II in rabbits and mice. Infect. Immun. **57:**3434–3437.
4. **Barrett, T. J., M. E. Potter, and N. A. Strockline.** 1990. Evidence for participation of the macrophage in Shiga-like toxin II-induced lethality in mice. Microb. Pathog. **9:**95–103.
5. **Barrett-Connor, E., and J. D. Connor.** 1970. Extraintestinal manifestations of shigellosis. Am. J. Gastroenterol. **53:**234–245.
6. **Chao, C. C., S. Hu, L. Ehrlich, and P. K. Peterson.** 1995. Interleukin-1 and tumor necrosis factor-α synergistically mediate neurotoxicity: involvement of nitric oxide and of *N*-methyl-D-aspartate receptors. Brain Behav. Immun. **9:**355–365.
7. **Clark, I. A., J. D. MacMicking, K. M. Gray, K. A. Rockett, and W. B. Cowden.** 1992. Malaria mimicry with tumor necrosis factor: contrasts between species of murine malaria and Plasmodium falciparum. Am. J. Pathol. **140:**159–162.
8. **Fujii, J., T. Kita, S.-I. Yoshida, T. Takeda, H., Kobayashi, N. Tanaka, K. Ohsato, and Y. Mizuguchi** 1994. Direct evidence of neuron impairment by oral infection with verotoxin-producing *Escherichia coli* O157:H− in mitomycin-treated mice. Infect. Immun. **62:**3447–3453.
9. **Fujii, J., Y. Kinoshita, T. Kita, A. Higure, T. Takeda, N. Tunaka, and S.-I. Yoshida.** 1996. Magnetic resonance imaging and histopathological study of brain lesions in rabbits given intravenous verotoxin 2. Infect. Immun. **64:**5053–5060.
10. **Goren, A., S. Freier, and J. H. Passwell.** 1992. Lethal toxic encephalopathy due to childhood shigellosis in a developed country. Pediatrics **89:**1189–1193.
11. **Gutierrez, E. G., W. A. Banks, and A. J. Kastin.** 1993. Murine tumor necrosis factor alpha is transported from blood to brain in the mouse. J. Neuroimmunol. **47:**169–176.
12. **Hamano, S. I., Y. Nakanishi, T. Nara, et al.** 1993. Neurological manifestations of hemorrhagic colitis in the outbreak of *Escherichia coli* O157:H7 infection in Japan. Acta Paediatr. **82:**454–458.
13. **Howard, J. G.** 1955. Observations on the intoxication produced in mice and rabbits by the neurotoxin of Shigella shiga. Br. J. Exp. Pathol. **36:**439–446.
14. **Johansson, B., V. Georgiev, T. Kuosmanen, and B. B. Fredholm.** 1996. Long-term treatment with some methylxanthines decreases the susceptibility to bicucullin- and pentylenetetrazol-induced seizures in mice. Relationship to c-fos expression and receptor binding. Eur. J. Neurosci. **8:**2447–2458.
15. **Karpman, D., H. Connell, M. Svensson, F. Scheutz, P. Alm, and C. Svanborg.** 1997. The role of lipopolysaccharide and Shiga-like toxin in a mouse model of *Escherichia coli* O157:H7 infection. J. Infect. Dis. **175:**611–620.
16. **McCracken, G. H., Jr., M. M. Mustafa, O. Ramilo, K. D. Olsen, and R. C. Risser.** 1989. Cerebrospinal fluid interleukin 1β and tumor necrosis factor concentrations and outcome from neonatal Gram negative enteric bacillary meningitis. Pediatr. Infect. Dis. J. **8:**155–159.
17. **Moncada, S., and A. Higgs.** 1993. The L-arginine-nitric oxide pathway. N. Engl. J. Med. **329:**2002–2012.
18. **Mor, M., Y. Yuhas, E. Kaminsky, G. Dinari, and S. Ashkenazi.** 1996. Induction of tumor necrosis factor and nitric oxide by Shigella strains isolated from patients with or without neurologic manifestations. Isr. J. Med. Sci. **32:**1271–1275.
19. **Neumann, P. E., and R. L. Collins.** 1991. Genetic dissection of susceptibility to audiogenic seizures in inbred mice. Proc. Natl. Acad. Sci. USA **99:**5408–5412.
20. **O'Brien, A. D., V. L. Tesh, A. Donohue-Rolfe, M. P. Jackson, S. Olsnes, K. Sandvig, A. A. Lindberg, and G. T. Keusch.** 1992. Shiga toxin: biochemistry, genetics, mode of action and role in the pathogenesis. Curr. Top. Microbiol. Immunol. **180:**67–94.
21. **Perdomo, O. J. J., J. M. Cavaillon, M. Huerre, H. Ohayon, P. Gounon, and P. J. Sansonetti.** 1994. Acute inflammation causes epithelial invasion and mucosal destruction in experimental shigellosis. J. Exp. Med. **180:**1307–1319.
22. **Prado, D., T. G. Cleary, L. K. Pickering, et al.** 1986. The relation between production of cytotoxin and clinical features in shigellosis. J. Infect. Dis. **54:**149–155.

23. **Probert, L., K. Akassoglou, M. Pasparakis, G. Kontogeorgos, and G. Kollias.** 1995. Spontaneous inflammatory demyelinating disease in transgenic mice showing central nervous system-specific expression of tumor necrosis factor alpha. Proc. Natl. Acad. Sci. USA **92:**11294–11298.
24. **Quagliarello, V. J., B. Wispelwey, W. J. Long, Jr., and W. M. Scheld.** 1991. Recombinant human interleukin-1 induces meningitis and blood-brain barrier injury in the rat. J. Clin. Investig. **87:**1360–1366.
25. **Ramegowda, B., and V. L. Tesh.** 1996. Differentiation-associated toxin receptor modulation, cytokine production, and sensitivity to Shiga-like toxins in human monocytes and monocytic cell lines. Infect. Immun. **64:**1173–1180.
26. **Raqib, R., B. Wretlind, J. Andersson, and A. A. Lindberg.** 1995. Cytokine secretion in acute shigellosis is correlated to disease activity and directed more to stool than to plasma. J. Infect. Dis. **171:**376–384.
27. **Redman, B. G., Y. Abubakr, T. Chou, P. Esper, and L. E. Flaherty.** 1994. Phase II trial of recombinant interleukin-1 beta in patients with metastatic renal cell carcinoma. J. Immunother. Emphas. Tumor Immunol. **16:**211–215.
28. **Reed, L. Y., and H. Muench.** 1938. A simple method of estimating fifty percent end-points. Am. J. Hyg. **27:**493–497.
29. **Richardson, S. E., T. A. Rotman, V. Jay, C. R. Smith, L. E. Becker, M. Petric, N. F. Olivieri, and M. A. Karmali.** 1992. Experimental verocytotoxemia in rabbits. Infect. Immun. **60:**4154–4167.
30. **Rothwell, N. J., and S. J. Hopkins.** 1995. Cytokines and the nervous system. II. Actions and mechanisms of action. Trends Neurosci. **18:**130–135.
31. **Saks, S., and M. Rosenblum.** 1992. Recombinant human TNF-alpha: preclinical studies and results from early clinical trials. Immunol. Ser. **56:**567–587.
32. **Seyfried, T. N., IV.** 1982. Convulsive disorders, p. 97–124. *In* H. L. Foster, J. D. Small, and J. G. Fox (ed.), The mouse in biomedical research. Academic Press, London, United Kingdom.
33. **Van de Kar, N. C. A. J., L. A. H. Monnens, M. A. Karmali, and V. W. M. Van Hinsbergh.** 1992. Tumor necrosis factor and interleukin-1 induced expression of the verocytotoxin receptor globotriaosylceramide on human endothelial cells: implications for the pathogenesis of hemolytic uremic syndrome. Blood **80:**2755–2764.
34. **Watkins, L. R., S. F. Maier, and L. E. Goehler.** 1995. Immune activation: the role of pro-inflammatory cytokines in inflammations, illness responses and pathological pain states. Pain **6:**289–302.
35. **Yuhas, Y., A. Weizman, G. Dinari, and S. Ashkenazi.** 1995. An animal model to study the neurotoxicity of bacterial toxins and the application of the model to demonstrate that Shiga toxin and lipopolysaccharide cooperate in inducing neurologic disorders. J. Infect. Dis. **171:**1244–1249.
36. **Zychlinsky, A., C. Fitting, J. M. Cavaillon, and P. J. Sansonetti.** 1994. Interleukin 1 is released by murine macrophages during apoptosis induced by *Shigella flexneri*. J. Clin. Investig. **94:**1328–1332.

Editor: E. I. Tuomanen