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CLASSIFICATION	SYSTEM NUMBER 506699
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System Number:

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CLINICAL IMMUNOLOGY AND IMMUNOPATHOLOGY
Vol. 83, No. 2, May, pp. 185–189, 1997
Article No. II974350

RAPID COMMUNICATION

Effect of Melarsoprol Treatment on Circulating IL-10 and TNF-α Levels in Human African Trypanosomiasis

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DCIEM Pub. No. 96-P-55

The pathogenesis of human African trypanosomiasis (HAT) has been the object of considerable research interest but has remained incompletely understood. The importance of cytokines in the pathophysiology of this protozoan infection is now widely recognized, but the full spectrum of cytokines involved has yet to be determined. In the present investigation we compared the plasma concentrations of TNF-α and IL-10 in normal African controls and patients suffering from advanced meningocephalic (late-stage) *Trypanosomiasis brucei (T. b.) gambiense* infections, before and after treatment with the arsenical trypanocide melarsoprol. We found that patients with late-stage *T. b. gambiense* exhibit chronically elevated circulating levels of both of these cytokines, and that these levels quickly decline following melarsoprol treatment. These findings confirm that TNF-α is involved in the immunopathogenesis of late-stage African trypanosomiasis and suggest that IL-10 may also play an important regulatory role in this disease. © 1997 Academic Press

INTRODUCTION

African trypanosomes of the subspecies *Trypanosomiasis brucei gambiense* are notoriously potent modulators of the host's immune system (20). The ability of these extracellular protozoa to evade the immune destruction produces a chronic debilitating disease that is frequently fatal if unmedicated (1). Melarsoprol remains the drug of choice for late-stage Gambian human African trypanosomiasis (HAT) with central nervous system (CNS) involvement; it is effective in killing trypanosomes in the bloodstream and lymph nodes and also crosses the blood–brain barrier. Clinically, even patients with advanced meningoencephalitis feel better within 1 week of initiation of treatment (38). Despite its efficacy, melarsoprol causes severe side effects associated with propylene glycol-induced cellulitis and the unpredictable risk of potentially fatal reactive arsenical encephalopathy (27). Up to 10% of melarsoprol-treated patients abruptly experience grossly elevated cerebrospinal fluid (CSF) and plasma TNF-α levels (18), followed by fever, a worsening of preexisting symptoms, and death within 10 days (38). In such cases massive trypanolysis is thought to provoke a violent inflammatory host response which aggravates existing CNS lesions (26).

Activated tissue macrophages and circulating monocytes are responsible for the bulk of the production of proinflammatory monokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 in response to various infectious and noninfectious stimuli (35). Overproduction of proinflammatory cytokines can be highly deleterious to the host, and is an essential element in the pathological sequelae of many diseases, including septic shock (37), cerebral malaria (13), as well as American (15) and African trypanosomiasis (18).

IL-10 plays a major role in dampening cell-mediated immune responses and inflammatory reactions. Although IL-10 has pleiotrophic biological actions, its major function appears to be inhibition and antagonism of proinflammatory monokine synthesis (22). IL-10 specifically inhibits TNF-α production (11) and down-regulates its own production via an autoregulatory feedback mechanism (9). Elevated IL-10 levels have been detected in biological fluids under various pathological circumstances. In particular, very high levels of IL-10 have been measured in the serum of patients suffering from cerebral malaria (28) and septic shock (21).

A better understanding of the complex cytokine interactions regulating HAT infections is essential to elucidate the mechanism(s) of generalized immunosuppression and to develop novel therapeutic interventions aimed at controlling this disease. The objective of the present study was to investigate the potential contribution of IL-10 and TNF-α to the pathophysiology of late-stage Gambian trypanosomiasis.

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PATIENTS AND METHODS

Study site and patient population. The study was conducted on 8 Congolese patients (female:male ratio = 1:1; 18–37 years of age, mean ± SD = 26.7 ± 6.9) suffering from HAT at the stage of meningoencephalitis. On the basis of clinical symptoms, the patients were selected by a medical surveillance team from villages located in the Ngabé, the Bouenza, and the Cuvette endemic foci and were subsequently examined at the neurology ward of the University Hospital of Brazzaville. Informed consent to participate in the study was obtained from the patients, as well as by agreement with the Ministry of Health of the Republic of Congo. Trypanosomes were present in the blood or a lymph gland puncture, and/or in the CSF of all patients diagnosed with *T. b. gambiense* using a serologic immunofluorescence test. The clinical and laboratory criteria used to evaluate disease severity have been previously described (29).

Healthy subjects. Six healthy Ivorian males (20–25 years of age, 23.3 ± 1.9) volunteered and gave their informed consent to serve as controls and were tested with a similar experimental design at the University Hospital of Yopougon (Abidjan, Côte d'Ivoire). The protocol was approved by Ivorian health authorities.

Patients and controls tested negative for malaria and human immunodeficiency virus (HIV)-1. Due to logistical and cultural constraints, ages could not be matched between the two groups. In order not to disturb the subjects sleep during data collection, they were housed in an air-conditioned room (ambient temperature: 24°C) adjacent to the recording room to which equipment leads and catheters were passed through an opening in the adjoining wall. Participants remained in bed during the 24-hr experimental period and all blood samples were taken with patients in a supine position so as to minimize variations in plasma volume resulting from postural shifts.

Blood sampling and drug treatment schedule. Peripheral blood samples were collected in EDTA-coated tubes. The patients were examined before and after 6 days of combination drug treatment. Chemotherapy consisted of 2 days of adjunctive corticosteroid therapy (30 mg/day to help minimize the side effects associated with melarsoprol), followed by 1 day of intravenously administered melarsoprol (one injection of 3.6 mg/kg Mel B Arsobal with a maximum of 200 mg/day (14); Rhône-Poulenc, France) along with promethazine, and 2 days of treatment with one injection per day of melarsoprol alone.

Determination of IL-10 and TNF-α levels. Plasma concentrations of IL-10 and TNF-α were measured according to the manufacturer's instructions using commercially available solid-phase enzyme immunoassay (EIA) kits (Quantikine, R&D Systems Inc., Minneapolis, MN) with sensitivities of 2.0 and 0.2 pg/mL, respectively. The absorbancies were read at 450 nm, using an automated microplate reader (EL340, Bio-Tek Instruments, Winooski, VT). All determinations were performed in duplicate with the same lot of EIA kits to minimize interassay variations.

Statistical analysis. Cytokine concentrations are expressed as the median and the 90% confidence intervals (CIs). Intergroup comparisons were made using a nonparametric Mann–Whitney U test for unpaired data. All analyses were two-tailed and an α level of $P \leq 0.05$ was considered to indicate statistical significance. The ages of patients and control subjects are given as the mean ± SEM.

RESULTS

Plasma cytokine concentrations. Figure 1 displays the median plasma concentrations of IL-10 (A) and TNF-α (B) in patients and controls. Before drug treatment (BT) was initiated, both IL-10 (median = 31.64 pg/mL; CI = 13.59–48.02) and TNF-α (median = 33.93 pg/mL; CI = 20.68–60.70) levels in trypanosomiasis patients were significantly ($P < 0.001$) elevated relative to normal African controls (CT). After treatment (AT), IL-10 levels dropped below the detection threshold (2.0 pg/mL) of the assay. Similarly, TNF-α values were significantly ($P < 0.001$) diminished (median = 6.45 pg/mL; CI = 0.40–11.72), but not completely abrogated, following chemotherapy relative to BT values.

DISCUSSION

In this study, we investigated the circulating levels of the pro-inflammatory cytokine, TNF-α, and the prototypic immunosuppressive cytokine, IL-10 in HAT, before and after treatment with melarsoprol. Patients with advanced meningocephalic (late-stage) *T. b. gambiense* infection were found to exhibit chronically elevated levels of both of these soluble mediators, and these levels quickly declined following melarsoprol treatment.

For some time trypanosome-induced, host-derived TNF-α has been recognized as an important pathological and protective mediator of both animal and human trypanosomiasis (16, 18). The consequences of TNF-α production during protozoan infections are largely dependent upon its time, rate, and site of production (4). Secretion of TNF-α during the early stages of trypanosomal infection induces fever, elicits the hepatic acute-phase response, and activates the mononuclear phagocytic system (10, 23). These nonspecific immunoinflammatory responses initiated by TNF-α provide host protection by inhibiting parasite development,

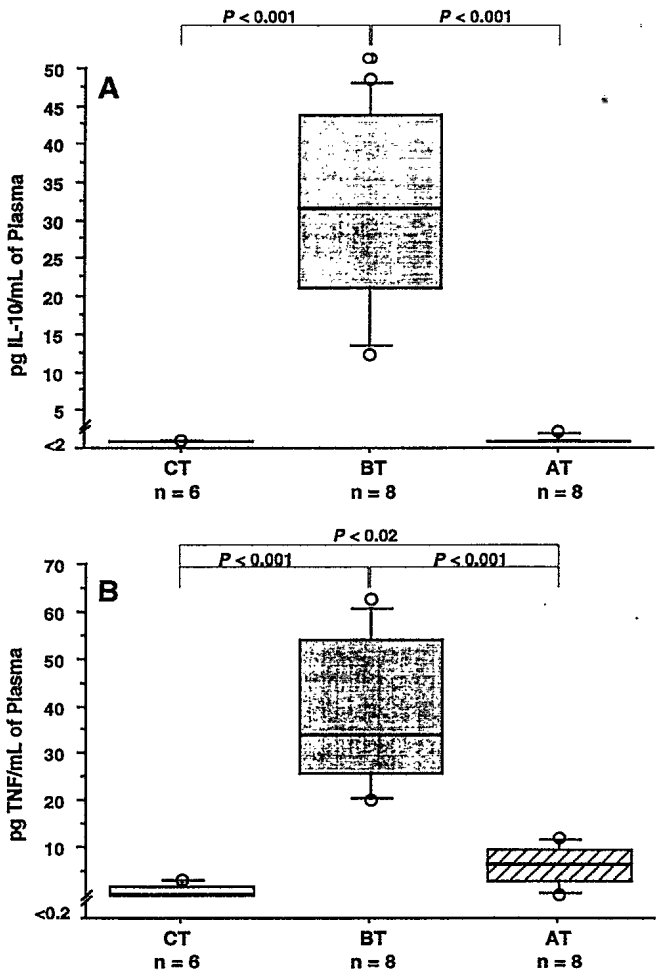

FIG. 1. Box-and-wisker plots of circulating plasma cytokine concentrations in healthy African controls (CT), unmedicated late-stage trypanosomiasis patients (BT), and patients after 6 days (AT) of combination chemotherapy. The median is indicated by a horizontal bar across each box. Boxes encompass 50% of the data and delineate the lower (25th) and upper (75th) percentiles. Wisker caps on each box represent the 10th and 90th percentile points of data. Individual data points (O) show the outliers.

limiting parasite spread, and preparing the host for a prolonged specific immune defense (34).

While a regulated release of TNF-α can stimulate beneficial antiparasitic host responses, its overproduction can be detrimental by promoting excessive inflammatory cascades, eventually leading to multiple organ failure and death if unchecked (37). This dual role of TNF-α has been shown in several protozoan infections, but is most evident in cerebral malaria (13, 35). In the case of experimental trypanosomiasis infections, administration of soluble trypanosomal lysates induces murine TNF-α secretion resulting in direct trypanolysis or inhibition of parasite growth (16, 17). In contrast, cotreatment with neutralizing anti-TNF-α antibodies abolishes these antiparasitic effect (19). Severe anemia and cachexia are major causes of death in bovine trypanosomiasis and have been linked to excessive TNF-α production (33). Indeed, many of the clinical and pathophysiologic features of trypanosomiasis including anemia, fever, and cachexia resemble those induced by *in vivo* administration of recombinant TNF-α (4). Several recent investigations of HAT confirm that circulating TNF-α concentrations are significantly increased in patients relative to healthy African controls (24, 29, 30). This work also implies that chemotherapy can quickly reduce circulating TNF-α levels (24).

Soluble parasite-derived factors are implicated as possible triggers of TNF-α production during trypanosomal infection (2). Pentreath *et al.* (25) reported markedly elevated endotoxin (LPS) levels ($\geq$100 pg/mL) in the blood and CSF of late-stage trypanosomiasis. Clinically, these levels are comparable to severe endotoxemia. The source of the LPS is not clear, but could be a direct by-product of trypanolysis, a consequence of intestinal or hepatic damage, and/or secondary bacterial infection (25).

In addition to mononuclear phagocytes, CNS cells such as astrocytes/microglias produce several inflammatory cytokines, including TNF-α and IL-10 following clinical injury or experimentally induced neuropathy (7, 31). The brains of late-stage *T. b. brucei*-infected mice commonly exhibit upregulation of TNF-α (12). Our finding that successfully treated patients exhibit substantially reduced, although still above normal, TNF-α levels 6 days after chemotherapy suggests that TNF-α levels of cured patients may stabilize quickly following therapy. Conceivably then, prophylactic blockade of TNF-α activity could be used to mitigate morbidity and mortality in patients undergoing chemotherapy. On the other hand, it also implies an advantageous role for moderate TNF-α levels in the recovery from trypanosomiasis. In support of this view, recent work with TNF-α receptor knock-out mice shows that TNF-α is largely protective in the CNS where it prolongs neuronal and microglial cell survival following excitotoxic or ischemic insults (5). Likewise, IL-10 expression has been detected in murine brain tissue during inflammatory or infectious diseases, where it is thought to have a neuroprotective role by inhibiting excessive microglial production of inflammatory monokines (7).

Although to the best of our knowledge, this is the first report of IL-10 levels in HAT, evidence from other protozoan illnesses suggests multiple possible regulatory roles in this disease (3, 36). Chronic exposure to LPS, in late-stage HAT, most likely triggers IL-10 release from activated macrophages. IL-10 is produced relatively late in the cytokine cascade as part of an endogenous protective mechanism which down-regulates early synthesis of proinflammatory monokines as

well as its own production (8, 9). Excessive IL-10 production, however, may contribute to the generalized immunosuppression characteristic of chronic African trypanosomiasis infections (32) through its inhibition of nitric oxide (NO) synthesis (6) and/or via down-regulation of T cell-mediated immune responses (21, 22).

CONCLUSION

Results of this study have demonstrated that both TNF-α and IL-10 are significantly elevated in late-stage HAT, and that elevated levels can be reversed after successful treatment with the antiparasitic drug, melarsoprol. The normal physiologic equilibrium between TNF-α and IL-10 is disrupted by chronic exposure to parasites. It appears that the initial release of TNF-α during acute trypanosomal infection is a protective response to limit disease severity. However, overproduction of TNF-α can become detrimental to the host and promotes the progression of immunopathologic symptoms of advanced disease. In this context, it is conceivable that the observed elevation of IL-10 production is an attempt of the host to counteract the inflammatory cascade precipitated primarily by TNF-α. Melarsoprol therapy appears to be able to restore the metabolism of proinflammatory TNF-α and the anti-inflammatory IL-10 to a normal or near-normal homeostatic state.

ACKNOWLEDGMENTS

The authors are indebted to Dr. J. Sarda for the organization of the medical field surveillance, to Drs. S. Bisser, A. Montmayeur, and K. Scott for invaluable assistance in the care of the patients and collection of the blood samples, and to B. Abadie, T. Brown, S. Chalbou, A. Charpenet, N. Fidier, and A. Roux for valuable technical assistance. This work was supported by Grant TDR/ID 910048 from the World Health Organization, and by grants from the French Ministry of Cooperation and the Defence and Civil Institute of Environmental Medicine, Toronto, Canada.

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Received August 28, 1996; accepted with revision February 17, 1997

#506699