

Detection of Plasma Tumor Necrosis Factor, Interleukins 6, and 8 during the Jarisch-Herxheimer Reaction of Relapsing Fever

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Summary

The Jarisch-Herxheimer Reaction (J-HR) is a clinical syndrome occurring soon after the first adequate dose of an antimicrobial drug to treat infectious diseases such as Lyme disease, syphilis, and relapsing fever. Previous attempts to identify factors mediating this reaction, that may cause death, have been unsuccessful. We conducted a prospective trial in Addis Ababa, Ethiopia on 17 patients treated with penicillin for proven louse-borne relapsing fever due to *Borrelia recurrentis* to evaluate the association of symptoms with plasma levels of tumor necrosis factor (TNF), interleukins 6, and 8 (IL-6 and -8). 14 of the 17 (82%) patients experienced a typical J-HR consisting of rigors, a rise in body temperature ($1.06 \pm 0.2^\circ\text{C}$) peaking at 2 h, leukopenia ($7.4 \pm 0.6 \times 10^{-3}$ cells/mm³) at 4 h, a slight decrease, and then rise of mean arterial blood pressure. Spirochetes were cleared from blood in 5 ± 1 h after penicillin. There were no fatalities, but constitutional symptoms were severe during J-HR. Plasma TNF, IL-6, and -8 were raised in several patients on admission, but a seven-, six-, and fourfold elevation of these plasma cytokine concentrations over admission levels was detected, respectively, occurring in transient form coincidental with observed pathophysiological changes of J-HR. Elevated plasma cytokine levels were not detected in the three patients who did not suffer J-HR. We conclude that the severe pathophysiological changes characterizing the J-HR occurring on penicillin treatment of louse-borne relapsing fever are closely associated with transient elevation of plasma TNF, IL-6, and -8 concentrations.

The Jarisch-Herxheimer Reaction (J-HR)¹ is an eponymous title for a clinical phenomenon, first described at the turn of the century, resulting from the treatment of early syphilis with mercury (1, 2). The original description was that the spots of roseola syphilis became more defined and numerous on treatment, and that the reaction was accompanied by fever, sweating, and anorexia which occurred with 24 h of mercury inunction. It is now recognized that similar transient reactions occur soon after the first adequate dose of a drug, usually an antibiotic, in the treatment of a wide spectrum of infectious diseases (3). Antibiotic treatment of spirochetal infections, for example syphilis, relapsing fever, yaws, leptospirosis, Lyme disease, and Vincent's angina, has typically been identified with J-HR, but the phenomenon has been described in many other bacterial infections. In ad-

dition, J-HR has been described in protozoal infection. For example, in African trypanosomiasis, it is a serious complication of treatment (4).

Pathophysiological changes occurring on penicillin treatment of early syphilis have been previously documented (5), and consist of an increase in body temperature of more than 0.8°C peaking between 6 and 8 h, often with rigors, associated with a fall in systemic arterial blood pressure and an increase in metabolic rate. The clinical worsening of symptoms associated with antibiotic treatment of louse-borne relapsing fever, caused by *Borrelia recurrentis*, is the most severe form of J-HR documented. Patients with this infection present with high fever, severe constitutional disturbance, hepatalgia, and splenomegaly (3). The diagnosis of relapsing fever is made by microscopic observation of spirochetes, typical of *Borrelia recurrentis*, on Wright's stained blood smears. The level of bacteremia may be intense with densities of up to 10^5 organisms per mm⁻³. The name relapsing fever describes one of the

¹ Abbreviations used in this paper: J-HR, Jarisch-Herxheimer Reaction.

principal clinical features of this infection in which there is spontaneous resolution of high pyrexia, usually by crisis, within 10 d, followed by transient remission lasting 5 or 6 d, followed by relapse of symptoms. Each remission is accompanied by the production of IgG and IgM specific for the responsible bacterial strains causing relapse, and up to four relapses may occur during the course of the infection.

The J-HR of relapsing fever may be fatal with a mortality approaching 40% (6). The J-HR of louse-borne relapsing fever (7-9) is very predictable in its form, and is similar to that of syphilis, but it occurs sooner after the administration of antibiotics, at ~90 min, and is associated with considerably more severe constitutional disturbances. It is hardly surprising that the severe pathophysiological changes of the J-HR of relapsing fever have provided a paradigm for studies on the pathogenesis of inflammation and shock. However, previous studies aimed at defining the nature of mediators involved in the etiology of this syndrome have failed. Since many of the features of J-HR resemble those now known to be caused by cytokines in man or animals, we have carried out experiments to test the hypothesis that the J-HR of louse-born relapsing fever is associated with the appearance of cytokines, TNF, IL-6, and -8 in the circulation.

Materials and Methods

Selection of Patients. Patients attending the emergency outpatient clinic of the Black Lion Hospital, Addis Ababa, with a proven diagnosis of relapsing fever, were included in the study. The diagnosis of relapsing fever was made on clinical suspicion, and proven by visual identification of *Borrelia recurrentis* organisms on blood smears stained with Wright's stain which were examined using light microscopy. Patients were excluded from the study if evidence of any other infection was found and in particular, if malarial parasites were seen on the blood smear in addition to *Borrelia*. Only patients experiencing the first episode of fever in the course of *B. recurrentis* infection were included. In all, 17 patients were enrolled into the study. The age range was 14-40 yr old, and all patients were male.

Protocol and Clinical Parameters. Patients were nursed throughout the study reclining on a bed. Clinical measurements of rectal temperature (using a mercury thermometer) and blood pressure were made on admission until steady values had been obtained, then immediately before intramuscular injection of 600,000 U procaine penicillin, and at 0.5, 1, 2, 4, 8, 12, and 24 h after injection. In addition, 10 ml of blood were drawn from a peripheral vein at the same time points, and placed into endotoxin-free tubes containing EDTA as anticoagulant. Thin blood smears were made on glass microscope slides and stained using Wright's stain. In addition, an aliquot of blood was taken for dilution and estimation of total white cell count using a hemocytometer. The mean of three estimations of white cell count was taken for each blood sample. Each blood sample was centrifuged at 1,000 g for 5 min, and plasma was immediately frozen in aliquots and stored at -20°C before cytokine assay. Microscopy of the stained blood films was carried out by an independent observer who examined them by eye for the presence of *B. recurrentis*. A minimum of 25 microscopic fields ($\times 100$) were examined for the presence of the organism. A negative score was not given until all microscopic fields examined were free of the organism. Patients were discharged from the hospital at 24 h, but some discharged themselves after 12-24 h.

Cytokine Assays. All cytokine assays were performed in the Department of Pathology, the University of Michigan. Samples were assayed within 3 mo of aspiration, and were continuously maintained in -20°C until the time of assay. Measurements were made in a blinded fashion so that those investigators handling coded samples were unaware of patients' clinical details or of the timing of samples.

TNF Analysis. TNF bioactivity was assayed using the highly sensitive cell line, WEHI 164, subclone 13 (the generous gift of Anders Waage, University of Trondheim, Norway), which is able to detect TNF at a concentration of 2 pg/ml (10). We have previously shown that this assay is unaffected by IL-1, -2, or -6, and that there is no synergy between interferon- γ and TNF in this assay (11). Briefly, samples were serially diluted in 96-well microtiter plates. The WEHI cells were resuspended at 5×10^4 cells/ml in RPMI 1640 with 10% FCS, 2 mM L-glutamine, and 0.5 μ g/ml actinomycin D (Calbiochem Corp., La Jolla, CA). The next day, cell lysis was detected by adding MTT-tetrazolium (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazol blue) and incubating the plates an additional 4 h. The dark purple tetrazolium salts were then dissolved in acidified isopropanol. Units of TNF were calculated based on a recombinant human standard run in the same assay.

IL-6 Assay. The IL-6 assay was performed using the B9 cell line, which is very sensitive to IL-6. Briefly, serial dilutions of the samples were placed into 96-well microtiter plates, to which were added 5,000 B9 cells in IMDM with 2 mM L-glutamine, 25 mM Hepes, 1% penicillin/streptomycin, and 10% FCS (12). The cells were incubated for 68 h at 37°C, and pulsed for the final 6 h with MTT-tetrazolium. The crystals were dissolved with acidified isopropanol, and the units calculated based on a standard curve run in the same assay. We have previously established the specificity of the B9 assay for IL-6, and have shown that it is unaffected by multiple other cytokines (13).

IL-8 ELISA. IL-8 was measured with an ELISA procedure after a previously described protocol (14). A polyclonal rabbit anti-IL-8 antibody was prepared by repeated intradermal injections of purified rIL-8. The IgG from high-titer antisera was purified on protein-A agarose columns (Pierce Chemical Co., Rockford, IL). ELISA plates (Nunc-Immuno Plate Maxisorb, Neptune, NJ) were coated with 50 μ l/well of anti-IL-8 diluted to 1 μ g/ml in borate-buffered saline (50 mM H₃BO₃, 120 mM NaCl, pH 8.6) and incubated overnight at 4°C. The plates were washed 3 times with wash buffer (PBS with 0.05% vol/vol Tween 20), and blocked for 1-2 h with PBS containing 2% BSA at 37°C. 50 μ l of sample was placed in each well, incubated for 1 h at 37°C, and the plates were then washed. Next, biotinylated anti-IL-8 was added (3.5 μ g/ml, 50 μ l/well, prepared in wash buffer with 2% FCS), the plates incubated at 37°C for 30 min, and then washed. Avidin-horseradish peroxidase (Dako Corp., Carpinteria, CA) was diluted 1:5,000, 100 μ l was added to each well, and the plates were incubated for 30 min at 37°C. After washing, 100 μ l of substrate solution was added (0.67 mg/ml orthopenylenediamine dichloride [Dako Corp.], 0.0125% H₂O₂ in 25 mM citrate phosphate buffer, pH 5.0), the color was developed for 4-5 min, and then stopped by adding 50 μ l of 3 M H₂SO₄. All samples were run in duplicate, and the concentration of IL-8 was calculated from a standard curve prepared with rIL-8.

Statistical Analysis. The changes in each cytokine level were analyzed by repeated measures of analysis of variance, to determine if there was a difference between the time points. All cytokines demonstrated a difference. Since the data was not normally distributed, the values at the various time points were compared with the admission levels by the Wilcoxon rank sum test.

Ethical Approval. Ethical approval for the study was obtained from the Ethical Committee, Department of Medicine, Black Lion Hospital, The University of Addis Ababa. Antibiotic treatment of any patient was not delayed because of inclusion in the study. Consent was given by each patient before the studies commenced.

Results

Patients

17 patients were enrolled in the study. Patients were all febrile on admission with rectal temperature of $39.5 \pm 0.9^\circ\text{C}$ (SEM), and had been ill with symptoms consistent with a first episode of *B. recurrentis* infection for 4.1 ± 1.0 d (SEM) before inclusion in the study. 14 patients (82%) experienced a severe J-HR with rigors and profound constitutional symptoms. Three patients experienced no J-HR reaction. There were no deaths in the study period, and all patients were discharged from the hospital. All patients completed 12 h of study and 11 patients completed 24 h of study. Six discharged themselves from the hospital early, between 12 and 24 h.

Clinical Parameters

Fig. 1 shows results of clinical parameters measured during the study as a function of time. Rigors were experienced by all 14 patients experiencing J-HR and occurred between 60 and 120 min after penicillin administration, each episode lasting between 20 and 30 min. Even though all patients were febrile on admission to the study, all 14 patients suffering J-HR experienced a rise in body temperature which peaked at 120 min of $1.06 \pm 0.2^\circ\text{C}$ (SEM) and coincided with rigors. In addition, a leukopenia ($7.4 \pm 0.6 \times 10^{-3}$ cells mm^{-3}) beginning with the onset of rigor was documented. Furthermore, the typical small decrease then rise in mean arterial blood pressure was observed. Spirochetemia, while not being quantitatively recorded, had disappeared in all patients within a mean of 4.5 h after antibiotic treatment was commenced.

Plasma Cytokine Concentrations

Admission to Study. Plasma TNF levels were elevated (> 3 pg/ml) on admission to the study in nine patients. The range of plasma levels at this time was 3 pg/ml $^{-1}$ to 55 pg/ml. Admission plasma IL-6 levels were elevated in 12 patients with a range of 90–6,673 pg/ml. Similarly elevated IL-8 levels in plasma were detected in eight of the patients with a range of 76–846 pg/ml.

During J-HR. Results of plasma cytokine concentrations during J-HR are shown in Table 1. A transient release of all three cytokines was measured with peak values occurring 2–4 h after penicillin administration (126 ± 38 pg/ml for TNF; $9,578 \pm 1,808$ pg/ml for IL-6; $8,102 \pm 4,491$ pg/ml for IL-8). By 12 h, when the three main clinical parameters measured had returned to normal levels, the circulating concentrations of all three cytokines had fallen dramatically from the peak levels recorded. At 24 h after antibiotic administration, plasma cytokine concentrations had returned to admission levels for TNF, and IL-6 and were not detectable for IL-8.

The pattern of cytokine appearance in plasma was interesting

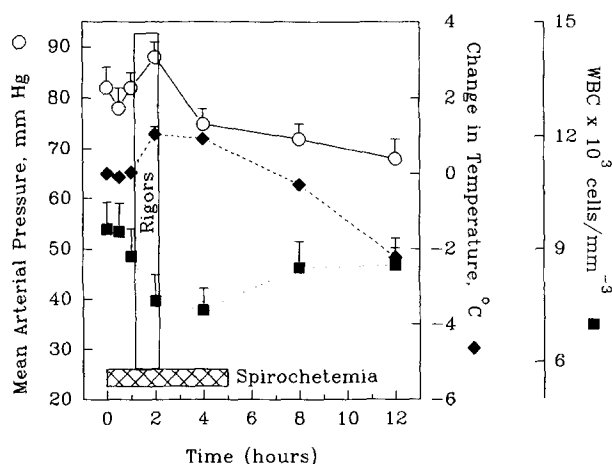


Figure 1. Kinetics of symptomatology of J-HR. Penicillin was given intramuscularly at time 0. The symptoms consisted of the rapid elevation of temperature (shown as increase over admission temperature), decrease in white blood cells, and initial slight rise in blood pressure followed by hypotension. These values are all displayed as the mean for 10–14 patients. (XXXX) Mean time of clearance of spirochetemia. (□) Range for time of onset of rigors.

Table 1. Peripheral Blood Cytokine Levels during the Jarisch-Herxheimer Reaction

Time	TNF	IL-6	IL-8
h	pg/ml	pg/ml	pg/ml
0	15 ± 5	1,478 ± 527	173 ± 65
	0–55	0–6,673	0–848
0.5	37 ± 14	1,020 ± 369	171 ± 67
	0–142	0–5,358	0–1,001
1	38 ± 13	1,375 ± 435	168 ± 56
	0–137	0–4,940	0–871
2	86 ± 23	3,836 ± 651	503 ± 330
	0–232	1,261–9,975	0–4945
4	126 ± 38	9,578 ± 1,808	8,102 ± 4,991
	0–469	2,262–28,447	109–70,000
8	8 ± 4	2,003 ± 696	216 ± 111
	0–40	66–9,403	0–1,567
12	12 ± 7	289 ± 159	178 ± 69
	0–51	0–1,841	0–639
24	14 ± 10	40 ± 21	ND
	0–47	0–245	

At the indicated time points after administration of penicillin, blood samples were drawn and cytokine levels were measured in plasma. Each value is the mean ± SEM for 6–14 patients at each time point. Values on the second line represent the range observed at each time point.

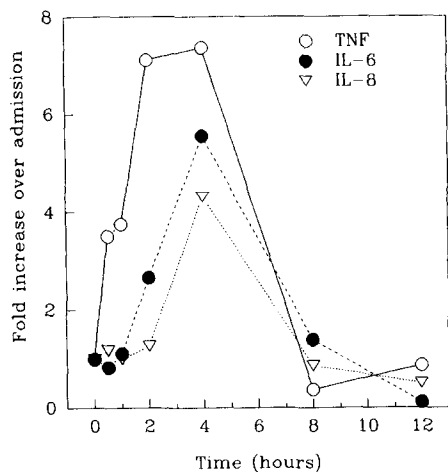


Figure 2. Kinetics of cytokine release. Data are expressed as fold increase over admission levels. Compared with baseline levels, TNF showed the most rapid induction, and attained the greatest relative increase. TNF is significantly increased over baseline by 1 h, IL-6 by 2 h, and IL-8 by 4 h.

(Fig. 2). Since the patients had been symptomatic for an average of 4 d before diagnosis, it was not surprising that several patients had circulating cytokines detectable before the initiation of antibiotic treatment. To provide a clearer understanding of the relative changes in cytokine levels, the data in Fig. 2 is expressed as a fold increase over admission levels. These data show that TNF was the most rapidly induced, and showed the greatest increase relative to admission levels.

A detailed description of the rise in plasma cytokine concentrations and body temperature in the early phase of the J-HR is shown in Fig. 3 in conjunction with the time of onset of rigor. This figure shows that TNF is elevated over admission levels within 30 min. These TNF levels were significantly greater than pretreatment controls at the 1 h time point (Wilcoxon rank sum test), whereas IL-6 and -8 levels were not elevated above baseline at this time point. Plasma IL-6 was elevated above admission at the 2 h timepoint, and IL-8 at the 4 h time point (all comparisons made using the Wilcoxon rank sum test). Thus, the evolution of plasma cytokine levels in this form of J-HR occurred in a distinct temporal sequence with the early, rapid rise in TNF being sequentially followed by IL-6 and then by -8. This figure clearly shows the relationship to the appearance of plasma cytokines with the development of rigors and pyrexia. TNF appeared before the onset of symptoms, IL-6 was detected as the symptoms developed, and IL-8 was detected well after the onset of rigors and pyrexia.

Discussion

The J-HR is a well-known, but infrequently diagnosed complication of antimicrobial therapy. The reaction has been documented in the treatment of many bacterial infections (3), but particular attention has been focused on the phenomenon in spirochetal infections, namely syphilis (5), relapsing fever

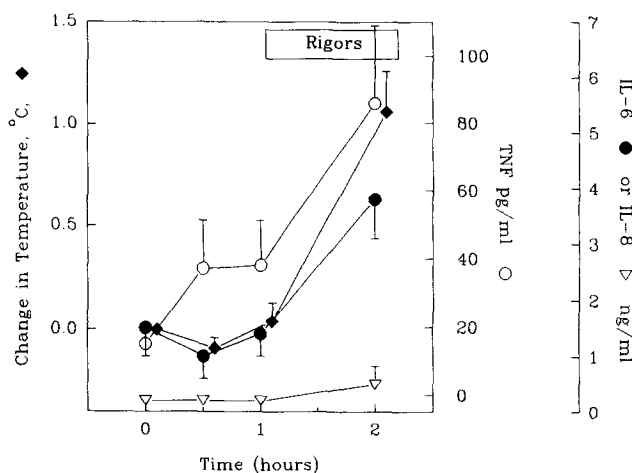


Figure 3. Early kinetics of cytokine release and development of symptoms. The change in temperature, and development of rigors is shown relative to the elevation of plasma cytokine concentration. The rise of plasma TNF concentrations preceded the elevation in temperature and development of rigors, whereas elevation of plasma IL-6 coincided with these symptoms. Elevation of plasma IL-8 concentration lagged behind the development of symptoms. All values are mean \pm SEM for 10–14 patients.

(6–9), and leptospirosis (15). There has been considerable debate in the past concerning the nature and the identity of the mediators involved in the severe clinical manifestations of the J-HR, particularly for syphilis and relapsing fever, but no definitive etiological explanation for the phenomenon has emerged. The present study sheds light on this question for the first time, and suggests that a cytokine cascade consisting, at the least, of TNF, IL-6 and 8, may be responsible. We have demonstrated that these cytokines appear in the circulation in transient fashion related to the appearance of severe symptoms, and pathophysiological changes associated with the most severe form of the J-HR reaction, namely those associated with relapsing fever caused by *B. recurrentis* infection.

Analysis of the plasma cytokine profiles demonstrates that the appearance of elevated TNF in the plasma precedes that of IL-6, which in turn precedes that of IL-8. In the current study 82% of patients suffered a J-HR, a percentage consistent with published data of previous studies in this condition (6, 7). The three patients who did not experience a J-HR in the current study were cured of *B. recurrentis* bacteremia, and became afebrile after penicillin treatment, but demonstrated no release of any of the three measured cytokines.

The peak plasma levels of TNF measured in this study of 126 ± 38 pg/ml (range 0–469) are close to levels detected upon hospital admission of patients who subsequently died of meningococcal bacteria (16), and slightly lower than peak levels of TNF detected in plasma of human volunteers receiving bolus infusion of endotoxin (17). In addition, the peak detected plasma levels of IL-6, namely $9,578 \pm 1,808$ pg ml⁻¹ (range 2,262–28,447), are comparable with those measured previously in septic shock and bacteremia (18), but considerably higher than human volunteers receiving bolus infusion of endotoxin (19) or rTNF (20). Elevated levels of plasma IL-8

have not previously been reported in any human infection, but elevated levels have been detected in a primate model of bacteremia (21). Bolus infusion of endotoxin into human volunteers has been reported to cause a rise in plasma IL-8 concentration (19), but peak levels reported in that study were only ~10% of the peak values detected in the study reported here on J-HR.

Thus, the peak values of the cytokine concentrations recorded in this J-HR study are of the same order of magnitude as those recorded in previous studies of lethal bacteremia. In this study of the J-HR of relapsing fever no fatalities occurred, however severe pathophysiological changes were documented. The transient nature of elevated plasma TNF, IL-6, and -8 is similar to that recorded in humans in response to a bolus infusion of endotoxin (17, 19). Furthermore, a similar profile of cytokine release has been recorded in vitro (22), reflecting control of cytokine production at the level of mRNA concentration within stimulated cells. A reaction resembling J-HR has been demonstrated in renal transplant patients about 1 h after receiving antithymocyte globulin (23) or OKT3 mAb (24) to treat rejection, and transient elevation of plasma TNF concentration was associated with fever and constitutional symptoms. Treatment of cancer patients with high-dose rTNF induces symptomatology very similar to that observed during the J-H reaction (25). In addition, experiments extending these studies showed that incubation of peripheral blood mononuclear cells in vitro with either antithymocyte globulin or OKT 3 mAb induced secretion of TNF.

The stimulus for cytokine generation and release into the circulation during the J-HR clearly deserves more detailed investigation. The presence of soluble pyrogenic agents has been documented in plasma of patients experiencing J-HR by bioassay employing intravenous infusion of such plasma into rabbits (26). However, the identity of the factor(s) responsible was not determined. It was suggested that endotoxin per se may be the mediator, since the pathophysiological events of the J-HR closely resemble those of endotoxin administration (27). However although a further study suggested that endotoxin might be responsible, it also demonstrated that endotoxin fractions prepared from *B. recurrentis* and injected intravenously into rabbits caused none of the pathophysiological changes associated with J-HR (38).

The correlation of disappearance of the spirochetes from the circulation we have demonstrated in the present study, with the observed pathophysiological changes and pulsatile

release of cytokines, suggests that removal of bacteria, presumably by phagocytosis, may represent the stimulus for cytokine release. Spirochetes are not removed en masse from the circulation until penicillin is administered, and it is likely that abnormal forms of the organism, produced in response to the antibiotic, are rendered susceptible to phagocytosis by macrophages, for example Kupffer cells in the liver. In our laboratory, we have recently shown that phagocytosis of pathogens by human macrophage cell lines (THP-1) or peripheral blood monocyte/macrophages caused a rapid increase in mRNA for TNF, IL-6 and -8, and that these cytokines are released into the incubating medium (G. E. Griffin, unpublished observations). It is therefore possible that phagocytosis of spirochetes made susceptible by the action of penicillin is an important stimulus for the production and release of cytokines. This hypothesis is strengthened by the study that a nonendotoxin heat stable particulate pyrogen has been isolated from *B. recurrentis*, that was proposed as a possible stimulus for the J-HR (26). This hypothesis is testable using a murine model of Borreliosis (29) in which the administration of ampicillin has been shown to produce a J-HR type reaction. In addition, a previous study of the interaction of Lyme disease spirochetes with adherent human monocytes or murine macrophage cell lines demonstrated the production of IL-1 as determined by bioassay (30). This bioassay in this study was probably not specific for IL-1 and may well have detected IL-6. Lyme disease spirochetes were phagocytosed in vitro by macrophages in the absence of opsonins, serums, or antibiotics. In addition, experiments were carried out that eliminated endotoxin as a stimulus for cytokine release in this system. Furthermore, in another series of experiments, it was shown that *Borrelia* spirochetes induced the production of leukocyte pyrogen and thromboplastin from human blood leukocytes (31).

Attempts have been made to ameliorate the severity of the J-HR using pharmacological agents. The use of intravenous hydrocortisone was ineffective (7), and the use of an opiate-partial agonist, Meptazinol, was only partially successful (32) in relapsing fever. The use of a more potent glucocorticoid, prednisone, in the J-HR of syphilis was also unsuccessful in reducing symptoms (33). The demonstration in the present study that cytokines may have a role to play in the etiology of J-HR may lead to more rational therapy aimed at blocking the action of cytokines using specific mAbs that have already been shown to be efficacious in animal models of severe infection (34, 35).

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