

Cytokines involved in human septic shock—the model of the Jarisch–Herxheimer reaction

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Studies of the cytokine cascade in animal models of infection and human experiments involving endotoxin infusion have contributed fundamentally to understanding the role of cytokines in human sepsis. The complexity of this cytokine cascade has been difficult to unravel in clinical sepsis. However, the Jarisch–Herxheimer reaction has been identified as a model of the cytokine cascade in human sepsis and has provided an excellent model for experiments involving blocking agents. TNF blocking has been shown to be important for protection in animal models of sepsis, but has been somewhat disappointing in humans because adverse events have generally outweighed benefits.

Introduction

The discovery of the role of cytokines in local and systemic inflammation has been one of the major advances in our understanding of the pathophysiology of infectious diseases. For example, it is now possible to understand the mechanisms of fever, alterations in vascular permeability and synthesis of acute phase proteins, which are all consequences of the action of cytokines. Initial euphoria following some of the early discoveries of cytokine biology has now been tempered by the appreciation that clinical disease is mediated by the activity of many cytokines, some which are pro-inflammatory and some down-regulatory.

The purpose of this review is to summarize cytokine cascades in animal and human models of infection and to relate these to clinical sepsis. In addition, the Jarisch–Herxheimer reaction (JHR), a complication of antibiotic treatment of louse-borne relapsing fever, will be described as a model of the cytokine cascade in a clinical situation resembling sepsis. The cytokine cascade involved in this reaction will be compared with that seen in human septicaemia and recent clinical experiments employing agents designed to block pro-inflammatory cytokines in this condition will be considered.

Animal models of sepsis syndrome¹

Several animal models of the sepsis syndrome have been studied with many different types of microorganisms or molecules such as endotoxin (LPS) administered by various routes. The immune status has been manipulated

in various ways in some of these models in order to elucidate mechanisms of host response. The simplest model has involved the administration of a sub-lethal dose of LPS to rabbits, rodents or human volunteers.¹ The pattern of pro-inflammatory cytokine release into plasma was similar in each of these models, with pulsatile, dose-dependent release of tumour necrosis factor- α (TNF) and tachyphylaxis in response to repeated LPS administration. Many studies have demonstrated that pathophysiological changes seen in LPS-induced shock strongly resemble the response to intravenous TNF in rats and crucial experiments have shown that monoclonal antibodies against TNF are protective against lethal doses of LPS. Such data from animal models emphasize the important role of this cytokine in sepsis, but discussion of most of the early studies failed to specify that suppression of TNF bioactivity in plasma does not prevent morbidity, or that circulating concentrations of other pro-inflammatory cytokines are reduced but not abolished. For example, interleukin-6 (IL-6) plasma concentrations are approximately halved in rats treated with monoclonal anti-TNF antibodies and receiving LPS and, although bioactive TNF is detectable in plasma, the animals still develop fever, lose weight and synthesize acute phase proteins as a result of other pro-inflammatory cytokines.² More complex models of sepsis involve infusion of live bacteria into animals: the baboon–*Escherichia coli* model has been particularly useful in elucidating the complex cytokine profile of sepsis in primates. In this model, TNF has been shown to be pivotal in the pathophysiology of bacteraemia and pre-treatment with monoclonal anti-TNF antibodies reduced mortality

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but did not prevent disease. The role played by TNF in paracrine release of other pro-inflammatory cytokines was also demonstrated in this model, particularly for IL-1 and IL-6.

Primate and rodent models, although extremely important in elucidating the cytokine cascade, represent more aggressive disease than naturally acquired infection. Infection acquired through mucosal routes has received little attention and clearly is an important area for study since it relates more closely to the immune and metabolic responses to natural infection.

Cytokines in human bacterial infection

The cytokine response detected in human infections is complex compared with defined animal models or endotoxin infusion in human volunteers. For example, TNF is often not detected in the plasma of patients presenting with acute sepsis, although high concentrations of TNF generally indicate a poor prognosis.³ Serum of patients with meningococcal sepsis has shown a highly complex pattern of cytokines,⁴ and high concentrations of IL-6 and IL-1 as well as TNF are related to poor prognosis. Other studies have not demonstrated any relationship between plasma IL-1 concentration and mortality in septic shock. For example, in acute melioidosis, caused by *Pseudomonas pseudomallei*, plasma TNF concentration on clinical presentation to hospital was generally a very poor marker of prognosis (probably because elevations of TNF are very short-lived and the peak concentration may have passed), as were, to a lesser extent, the concentrations of IL-6 and IL-8.⁵ More traditional indicators of disease severity, such as age, documented bacteraemia, reduced urine output and arterial pH, proved better prognostic indices than cytokine measurements. In a separate study involving profoundly ill patients on an intensive care unit, plasma cytokine concentrations were relatively poor prognostic markers, even when measured longitudinally (J. Friedland, personal communication).

The precisely controlled pulsatile release of TNF determines its transient and unpredictable presence in plasma. In addition, stimuli promoting cytokine synthesis and release may differ significantly. There is also compelling evidence that TNF release is likely to be related to host genotype in terms of TNF gene promoter polymorphism.⁶ Thus, potentially many host and microbial factors determine the pattern and magnitude of the human cytokine response involved in infection.

This complex situation and the limitations in our understanding mean that the treatment of sepsis with anti-cytokine agents remains somewhat empirical. However, it is clear that TNF is pivotal in the processes of sepsis and, not surprisingly, strategies directed against this molecule, including monoclonal antibodies and soluble receptors, have received considerable attention.

The Jarisch–Herxheimer reaction in louse-borne relapsing fever

Louse-borne relapsing fever

Louse-borne relapsing fever is an infection caused by the spirochaete *Borrelia recurrentis*, a microorganism that has caused many epidemics in North Africa, the Middle East and Europe. The infection is currently endemic in some countries of poor socio-economic status, in particular Ethiopia and Bolivia. *B. recurrentis* lives in the haemolymph of the human body louse and gains access to the human circulation through the skin after the louse is crushed. Patients with this infection present with high fever, severe constitutional illness and hepatosplenomegaly.⁷ Diagnosis is made by identifying spirochaetes in Wright's stained blood smears. Bacteraemia may be heavy with typical densities of up to 10^5 organisms/mm³. The name relapsing fever relates to the remitting nature of the fever in which episodes are relieved by crisis followed by relapse of symptoms about a week later. These relapses are caused by antigenic variation due to reassortment of the gene cassettes for the surface antigen so that the organism can escape from the specific IgG and IgM in the serum, which is directed at the previously exposed surface antigens.

The Jarisch–Herxheimer reaction

The JHR is a classic clinical syndrome in which there is a profound worsening of symptoms immediately following antimicrobial treatment of infection. JHR was first described at the turn of the century in two independent papers by Jarisch (in Vienna) and Herxheimer (in Berlin), in syphilitic patients following treatment with mercury. It is now recognized that similar transient phenomena occur soon after the first dose of an appropriate antibiotic in the treatment of a wide spectrum of infectious diseases. The most severe form of the reaction is that associated with antibiotic treatment of spirochaetal infection. The severity of JHR associated with such infections varies considerably; the two infections most precisely studied in this respect are syphilis and louse-borne relapsing fever. The JHR in these conditions is classically associated with an increase in body temperature of approximately 0.8–1.5°C within 1–2 h of antibiotic administration, rigors, a fall in systemic arterial blood pressure and a fall in peripheral blood white cell count. The JHR associated with louse-borne relapsing fever is associated with a mortality of approximately 5%.

Clinical characteristics of Jarisch–Herxheimer reaction

Patients presenting with relapsing fever are febrile and extremely ill on presentation to hospital with body temperatures of approximately 39.5°C. In a series of studies in

Model of Jarisch–Herxheimer reaction

Addis Ababa, Ethiopia, patients with relapsing fever who experienced JHR had a rise of body temperature of $1.06 \pm 0.2^\circ\text{C}$ (S.E.M.) peaking 120 min after the administration of penicillin.⁸ In addition, rigors were experienced between 60 and 120 min and leucopenia and a fall in arterial blood pressure were demonstrated (Figure 1).

Cytokine cascade associated with Jarisch–Herxheimer reaction

The symptoms and clinical features of JHR are similar to those of both endotoxin administration and the sepsis syndrome in humans and it is therefore not surprising that the mediators involved in JHR have been sought and investigated. Early studies detected bioactive substances in the plasma of patients with JHR but their chemical nature was not determined. More recently, massive release of cytokines into the circulation in JHR after penicillin treatment of relapsing fever was shown.⁸ In these patients, in whom TNF, IL-6 and IL-8 concentrations were measured, a highly reproducible cascade of cytokine release was demonstrated (Figure 2a). Detailed analysis of cytokine release in the early phase of JHR showed that TNF concentration increases within 30 min following penicillin administration and that this increase preceded pyrexia and the rises in plasma IL-6 and IL-8 concentrations (Figure 2b). In summary, TNF appeared in the plasma before the onset of symptoms, IL-6 was detected as symptoms developed and IL-8 was detected in plasma well after the onset of rigors and pyrexia. A similar pattern of cytokine release has been described following tetracycline treatment of louse-borne relapsing fever.

Peak plasma concentrations of TNF (126 ± 38 pg/mL) were similar to those in patients admitted to hospital with fatal meningococcal septicaemia³ but lower than those seen following bolus endotoxin administered to

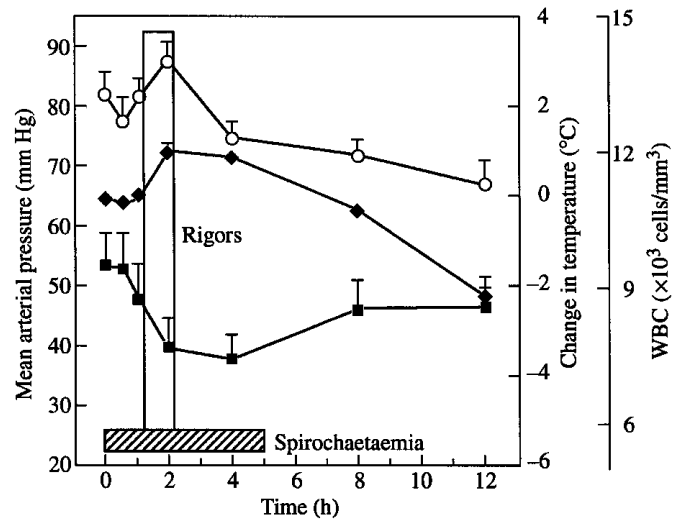


Figure 1. Clinical changes in Jarisch–Herxheimer reaction associated with penicillin treatment of louse-borne relapsing fever by intramuscular injection. ○, mean arterial pressure; ◆, change in temperature; ■, WBC.

volunteers.⁹ Peak plasma concentrations of IL-6 resembled those measured in patients with sepsis syndrome but were considerably higher than those measured in volunteers given endotoxin. Finally, the plasma concentrations of IL-8 recorded in JHR were ten times higher than those recorded following bolus endotoxin administration.¹⁰

The stimulus for the massive cytokine release in the JHR is extremely potent and is not yet defined. Penicillin rapidly alters the morphology of the dividing spirochaetes and makes them susceptible to phagocytosis—it is possible that sudden removal of such organisms (Figure 1) from the circulation, presumably by phagocytosis, is a potent stimulus for cytokine release. Indeed phagocytosis of

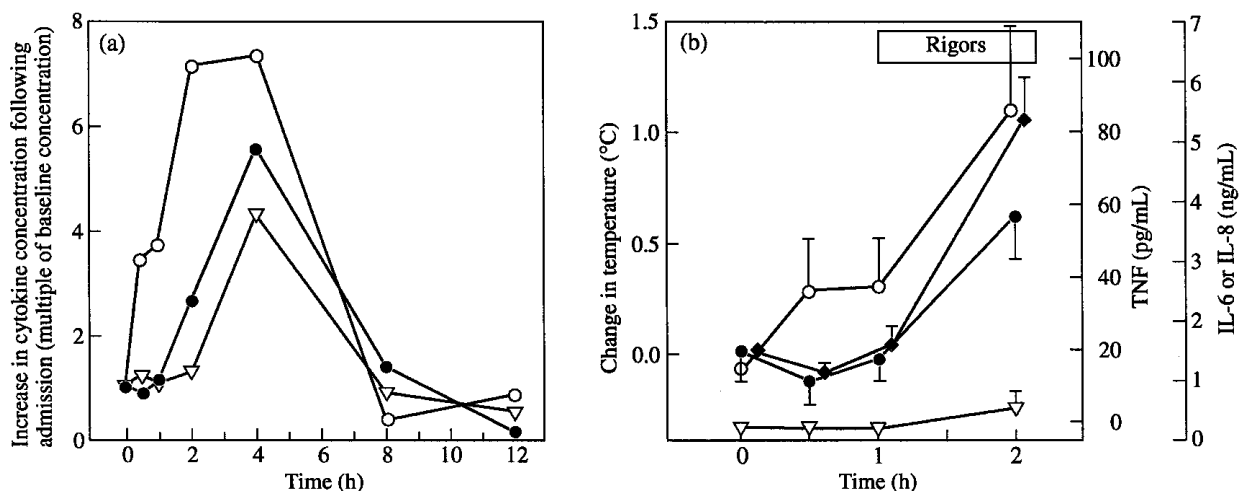


Figure 2. (a) Evolution of the cytokine profile in plasma of patient experiencing Jarisch–Herxheimer reaction of relapsing fever (see Figure 1) following antibiotic treatment with a single intramuscular dose of penicillin. (b) An expansion of the first 2 h of the response with serum concentrations of TNF, IL-6 and IL-8 and the rise in temperature. ○, TNF; ●, IL-6; ▽, IL-8; ◆, temperature.

micro-organisms has been shown to be a potent signal for cytokine release.¹¹ However, phagocytosis of inert particles by macrophages is not a stimulus for TNF release and there are likely to be additional components of micro-organisms which trigger cytokine production and release. Indeed a non-endotoxin heat-stable particulate pyrogen has been isolated from *B. recurrentis*.

Pharmacological modulation of Jarisch–Herxheimer reaction

The cytokine release during JHR in louse-borne relapsing fever provides a model for investigating pathophysiological mechanisms of human sepsis. Several agents have been used in attempts to control clinical symptoms of JHR in louse-borne relapsing fever. Corticosteroids and an antipyrogen (paracetamol) have little effect on JHR, but an opioid partial agonist, meptazinol, reduced the severity of symptoms. The discovery of the cytokine cascade and its relationship to the evolution of JHR prompted a study employing an ovine anti-TNF Fab fragment.¹² In this study, increases in temperature, pulse rate and systolic blood pressure were all substantially reduced. In addition there was profound inhibition of IL-6 and IL-8 release into plasma, as a result of preventing paracrine TNF activity. An important conclusion of this study was that the clearance of spirochaetes from the circulation was not influenced by the anti-TNF treatment. This is extremely important in terms of the potential therapeutic use of anti-TNF in human infection. Previous experiments using a weak blocker of TNF release, oxpentifylline, demonstrated that this agent had no effect on the severity of JHR or plasma concentrations of TNF, indicating that release of TNF in this clinical situation can be mediated by a pathway other than that antagonized by oxpentifylline.

Anti-cytokine strategies in infection

It is not surprising that therapeutic trials in human sepsis have focused on anti-TNF strategies. Such trials have contributed to our understanding of the pathophysiology of sepsis and to the design of clinical trials involving a complex and diverse group of patients. However, trials of antibodies directed against TNF have not been entirely successful. Evidence of increased survival has been weak and increased side effects, such as opportunistic infection, have been responsible for a major reassessment of anti-cytokine strategies in sepsis. The concept that appropriate release of TNF is crucial in the immune response to bacteria is strongly supported by experiments using sub-lethal doses of *Listeria monocytogenes* and *Salmonella typhimurium*.^{13,14} In these mouse models there was increased mortality and decreased immune response to the pathogen.¹⁵ In addition, the crucial role of this cytokine in the immune response to mycobacteria was demonstrated

by pre-treatment of mice with monoclonal antibody directed against TNF, which abolished granuloma formation induced by Bacille Calmette Guérin.¹⁶ This effect may be related to the autocrine effects of TNF released from macrophages interacting with lymphocytes.

Our knowledge of the autocrine effects of TNF release when cells of the immune system link through specific adhesion molecules is evolving rapidly.¹⁷ Such events, which may take place during antigen presentation, are crucial for protective immune responses. It is likely that strategies using powerful TNF antagonists in human sepsis need to be more sophisticated, perhaps containing panels of blocking agents against a range of cytokines that preserve protective immune responses, whilst preventing the injury associated with sepsis.

Conclusions

Great advances have been made in our understanding of the pathophysiology of the sepsis syndrome, particularly in terms of the role played by pro-inflammatory cytokines. In human and animal models, TNF clearly has a major role in clinical events. The JHR provides an elegant model of the human cytokine cascade in events resembling sepsis and TNF has been shown to play a dominant role in the pathophysiology of this condition. However, optimism that anti-TNF strategies may control the sepsis syndrome has been tempered by adverse events in animal models of infection¹⁵ and clinical trials.^{13,14}

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