The cytokine response to critical illness

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Abstract
The aims of this review are to provide a basic introduction to the biology of cytokines and to summarise the results of studies, both laboratory and clinical, relating to the cytokine response to critical illness. Elucidation of the cytokine response to conditions such as sepsis, trauma, and burns may be important for several reasons. It may improve understanding of the pathophysiological processes triggered by these insults. It may allow the severity of the insult to be gauged, and maybe even provide prognostic information about individual patients. Similarly it may allow the effectiveness of resuscitation and further treatment to be monitored. Finally, knowing about the cytokine response may provide the key to developing new treatments.


Key terms: cytokines; sepsis; trauma; burns; prognosis

Cytokines are a superfamily of low molecular weight glycoproteins which act as intercellular messengers. They include the interleukins, interferons, tumour necrosis factors, growth factors, transforming growth factors, colony stimulating factors, and the chemokines (table 1). They control and regulate a large number of essential physiological processes (table 2). The considerable structural homology of these proteins within the animal kingdom reflects their importance to the proper functioning of multicellular organisms. As intercellular messengers they may act in a number of different ways:

(1) Autocrine communication – that is, the cytokine interacts with the cell that produced it and modifies that cell’s function. This is not necessarily a feedback process as it may regulate processes other than its own production.

(2) Paracrine communication – that is, the cytokine interacts with cells adjacent to the producer cell and thus exerts very local control of cell functions.

(3) Endocrine communication – that is the cytokine passes into the systemic circulation and interacts with cells very distant to the producer cell.

Cytokines are produced by a wide range of different cell types: for example, T cells, macrophages, monocytes, fibroblasts, and endothelial cells. These cells do not produce one cytokine. Different trigger stimuli acting on the same cell type may result in the production of different cytokines. Indeed T helper cells may produce several different cytokines simultaneously in response to an activating stimulus. Cytokines, unlike polypeptide hormones are synthesised de novo in response to specific stimuli (table 3). There appears to be no evidence that they are ever stored intracellularly and then released. Triggers for cytokine production include processed antigens and specific antigens, viruses, double stranded DNA, and bacterial lipopolysaccharides. All these triggers could be considered as potential threats to the wellbeing of the organism: hence the importance of cytokines to the body’s defence mechanisms. The processes by which cytokine production is switched on are not entirely clear. However their production can be controlled at various stages: (1) transcription of genetic material; (2) translation; (3) during processing in endoplasmic reticulum and in the Golgi body; (4) secretion.

Processing in intracellular organelles may result in glycosylation, the addition of fatty acid chains, or cleavage of the protein from a precursor form to an active form. Cytokines may be secreted as either an active form or as a precursor, which is later cleaved to become active.

Table 1 Cytokine families

| Interleukins | IL-1 to IL-15 |
| Interferons | IFNα, IFNβ, IFNγ |
| Tumour necrosis factors | TNFα, TNFβ |
| Growth factors |
| Transforming growth factors |
| Colony stimulating factors |
| Chemokines |

Table 2 Physiological roles of cytokines

| Cellular control: | Proliferation, differentiation, metabolism |
| Immune control: | Cytotoxic cells, host defences against viruses and parasites, Regulation of inflammatory response and fever, Regulation of haematopoiesis, Wound healing and tissue remodelling |

Table 3 Cytokines versus polypeptide hormones

| Produced by multiple cell types | One hormone: one producer cell type |
| Multiple actions and duplication of cytokine effects | One hormone: one action |
| Single target cell | Specific target cells/ organs |
| Principally local action (can act systemically) | Systemic/endocrine actions |
| De novo production | Storage and release |
| Response to a challenge | Homeostasis |
active. Most cytokines are released as soluble proteins but some remain membrane bound. In this form they may act in a paracrine fashion, interacting with receptors on adjoining cells. As proteins, cytokines are unable to penetrate cell membranes and so in order to have any bioactivity they must bind to receptors. Cytokine receptors are themselves proteins made up of one or more polypeptide chains. They have three domains: (1) an extracellular domain with a three dimensional structure that determines both the affinity and the specificity of that receptor; (2) a transmembrane domain that fixes the receptor to the cell membrane; and (3) an intracellular domain which interacts with second messenger systems to provide an effector mechanism.

The sensitivity of a cell to a cytokine will depend on the number of receptors on its surface and on the affinity and specificity of those receptors. Receptor numbers are determined by the rate at which receptors are inserted into the membrane and the rate at which they are lost by internalisation. Insertion will depend on the rate of de novo synthesis and the rate of recycling of internalised receptors. Internalisation of receptors, once ligand binding has taken place, is an important regulatory process. By reducing the number of available receptors it may downregulate the effect of a cytokine and render the cell temporarily insensitive to the continued presence of the cytokine. Internalisation may be an important effector mechanism in the case of interferon (IFN), allowing it to reach the cell nucleus and so have a direct effect on gene activation.

Not all receptors are membrane bound. There are several soluble receptors. These may be quite specific for an individual cytokine (for example, the p55 and p75 soluble tumour necrosis factor (TNF) receptors), or they may have a more general protein binding function, for example, α2 macroglobulin. These soluble receptors may act as passive carriers of cytokines, or as a circulating reservoir of the cytokine, as is the case for insulin-like growth factor-1 (IGF-1). They may also be produced to damp down the effects of a cytokine: interleukin (IL)-1 receptor antagonist (IL-Ira) is a specific antagonist of IL-1.

Second messengers are essential for the transduction of the cytokine signal. There are a number of different messenger systems (table 4), which set off sequences of protein and enzyme activations resulting in the biological effects of the cytokine. At the intracellular level the main functions of cytokines are to alter gene expression and to control surface protein synthesis. Gene expression can be altered in four main ways: (1) a silent gene may be activated; (2) a gene already being expressed can be upregulated; (3) a gene already being expressed can be downregulated; and (4) an active gene may be shut down.

### The cytokine response to sepsis

There has been considerable interest in recent years in the cytokine response in the critically ill, both to improve our understanding of the pathophysiology and to try to devise new treatments. In this brief review we will concentrate on presenting some of the findings of laboratory studies as well as clinical investigations into sepsis, trauma, and burns. Given the readership of this journal, the emphasis will be on the cytokine response to trauma. For the sake of clarity we will focus on the triad of pro-inflammatory cytokines: IL-1, IL-6, and TNFα, although a few others will need to be mentioned. The principal functions of these cytokines are summarised in tables 5–9.

#### MODELS OF SEPSIS

Many different models of sepsis have been used to study the cytokine response. The model used by Tracey et al serves as a good example.1 This used anaesthetised baboons given a lethal dose of E.coli such that all animals died within eight hours from an accelerated form of septic shock: a fall in blood pressure, increase in heart rate, an initial rise in cardiac output followed by a catastrophic fall associated with anuric renal failure and death

### Table 4 Second messengers

<table>
<thead>
<tr>
<th>Tyrosine kinases</th>
<th>G proteins</th>
<th>Adenylyl cyclase and CAMP</th>
<th>Phospholipases</th>
<th>Inositol phosphates and calcium ions</th>
<th>Diacylglycerols and protein kinase C</th>
<th>Arachidonic acid and prostaglandins</th>
</tr>
</thead>
</table>

### Table 5 Interleukin-1 (previously known as endogenous pyrogen)

<table>
<thead>
<tr>
<th>Function</th>
<th>Receptor</th>
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<tbody>
<tr>
<td>Mediator of host inflammatory response</td>
<td>T cell activation; B cell proliferation; Collagen production; Tissue repair</td>
</tr>
<tr>
<td>Adhesion molecule expression</td>
<td>Acute phase protein synthesis; Pyrogen</td>
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### Table 6 Interleukin-2 (T cell growth factor)

<table>
<thead>
<tr>
<th>Function</th>
<th>T cell activation; B cell proliferation; Collagen production; Tissue repair</th>
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</thead>
<tbody>
<tr>
<td>Autocrine growth factor for T cells</td>
<td>Promotes antibody synthesis by B cells</td>
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### Table 7 Interleukin-6 (hepatocyte stimulating factor)

<table>
<thead>
<tr>
<th>Function</th>
<th>Acute phase protein synthesis; B cell differentiation; Cytotoxic T cell differentiation</th>
<th>Cell proliferation</th>
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### Table 8 Interleukin-8 (a secondary mediator of inflammation)

<table>
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<th>Function</th>
<th>Neutrophil chemotaxis; Neutrophil activation</th>
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### Table 9 Tumour necrosis factor (mediator of septic shock)

| Function | Induces IL-1 and IL-8 (IL-6); Cytokine synergism; Pyrogen; Acute phase protein synthesis; Myocardial depression; Intravascular thrombosis; Vascular smooth muscle relaxation (via nitric oxide) |
|----------|---------------------------------|-------------------|

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from pulmonary oedema. It was found that in these animals there was a rise in TNFα, which reached a peak about 1–5–2–5 hours after the injection of E coli. TNFα became undetectable by 4–6 hours. If the animals were given a monoclonal anti-TNFα antibody one hour before the E coli injection there was a temporary improvement in their haemodynamic status, but the 100% mortality was unchanged. Anti-TNFα given two hours before the E coli injection resulted in 100% survival at 24 hours, which strongly implicated TNFα in the pathogenesis of shock and death following a severe bacteraemia. The same model was used by Fong et al to investigate the production of IL-1 and IL-6. They found that there was a rise in IL-1 within two hours of the E coli injection, which reached a peak at about three hours. The rise in IL-6 was somewhat delayed in comparison and had yet to reach a peak at eight hours, which was the final blood sampling time in the study. The production of these cytokines could be attenuated by the prior administration of anti-TNFα, suggesting that TNFα is instrumental in triggering the cytokine response to severe bacteraemia.

The importance of endotoxin as a trigger for the production of cytokines has been shown in studies on human volunteers. A bolus of endotoxin (4 ng/kg) was injected into volunteers and resulted in a rise in TNFα starting 90 minutes after the injection. The rise persisted for 180 minutes. In contrast to the baboon studies, there was no increase in IL-1. Van Deventer et al found that a 2 ng/kg bolus of E coli endotoxin resulted in a rise in TNFα starting at 30–45 minutes and reaching a peak at 60–90 minutes. IL-1 was undetectable, but there was a rise in IL-6. This was seen about 15 minutes after the rise in TNFα and the peak level was reached at 120–150 minutes. Both studies found that the appearance of TNFα in the circulation coincided with the onset of influenza-like symptoms, an increase in temperature and heart rate, and a neutropenia. A neutrophilia was only seen after TNFα had been cleared from the circulation. The failure of both of these studies to detect the production of IL-1 is in contrast to the study by Dinarello et al, investigating the pyrogenicity of TNFα. In this study rabbits were injected with a bolus of recombinant TNFα at a dose of 1 μg/kg, which resulted in a monophasic fever peaking at about 50 minutes. When a much higher dose was used (10 μg/kg) the fever was found to be biphasic, with a second peak at 3–5 hours. This was found to be the result of the production of IL-1, suggesting that at high doses TNFα triggered the production of IL-1. It may be that in the volunteer and sepsis the production of TNFα produced in response to the endotoxin was insufficient to trigger IL-1 synthesis.

Caeal ligation and puncture is a widely used model of sepsis, mimicking a visceral perforation, and so may be a more clinically valid model than direct intravascular injections of monocultured bacteria or pure endotoxin. Ertel et al used this model in rats and found increases in circulating endotoxin, TNFα, IL-1, and IL-6. In contrast to studies using a pure endotoxin or E coli challenge, which cause a transient rise in TNFα, Ertel et al found that TNFα levels remained raised for several hours and then only decreased gradually, despite increasing plasma endotoxin levels. This suggested that although the stimulus for production, namely endotoxin, was still present some other mediator was down-regulating production.

CLINICAL SEPSIS

There have been many studies recording the cytokine response to sepsis of various origins. Waaage et al in Norway have been particularly interested in the response in meningococcal disease. In 1987 they found that TNFα was raised in these patients and that a fatal outcome was associated with a TNFα level exceeding 140 pg/ml. In patients with meningococcal septic shock they found raised levels of IL-6, and a 50% mortality rate when IL-6 levels exceeded 3 ng/ml. IL-1 was only increased in the most severely ill patients and these already had raised concentrations of TNFα, IL-6, and endotoxin. By looking at the timing of cytokine production they found that IL-6 was produced later than TNFα (see also 3).

In septic shock of various causes there is also an increase in TNFα, which correlates with the severity of the shock and the eventual outcome. The presence of circulating TNFα in septic patients was associated with a higher mortality in the study by Debeuts et al. They found that 25% of their patients showed detectable circulating TNFα. The mortality from this group was 73% compared to only 34% for the group showing no detectable TNFα. In terms of patient characteristics, sepsis score, and multiple organ failure, there was no difference between the two groups.

IL-6 has been studied specifically in septic patients being admitted to an intensive care unit. Those patients already in shock were found to have higher IL-6 concentrations than those who were still normotensive. A correlation was found between IL-6 and heart rate, lactate level, and platelets as well as Ca and C4a (products of complement activation). Patients who subsequently died had higher IL-6 levels on admission than survival survivors. However, monitoring the course of IL-6 after admission did not provide additional information: levels tended to decline irrespective of outcome. In a series of 15 patients with intra-abdominal sepsis there was a strong correlation between the serum concentration of IL-6 and the sepsis score of Elebute and Stoner. In contrast to the studies noted above, no correlation was found between serum TNFα concentration and sepsis score. Overall it appears that in sepsis, and particularly in septic shock, there is activation of cytokine production, especially TNFα and IL-6. The production of TNFα seems to precede that of IL-6. IL-1 is detectable in a minority of patients: usually the most severely ill, probably because they have sufficiently raised levels of TNFα to trigger IL-1 production. Within a given population of
patients, those with the higher levels of TNFα or IL-6 seem to have a higher mortality, even if other indicators of disease severity are the same.

The cytokine response to trauma
There is now good evidence that the inflammatory cytokines are involved in the response to tissue injury. This evidence comes from studies into the effects of elective surgery — a controlled form of trauma — and from studies using animal models of haemorrhagic and traumatic shock.

ELECTIVE SURGERY
One of the earliest clinical studies looked at six patients undergoing elective cholecystectomy. An IL-6 response was detectable as early as 30 minutes after the initial incision. All the patients showed a response within 1-5 hours and peak IL-6 levels were reached at 1-5-4 hours after incision. Peak IL-6 levels were found to correlate with the duration of surgery. There were no increases in either endotoxin, IL-1, or TNFα and it was suggested that IL-6 might be an early marker of tissue damage. Since then several studies have confirmed the IL-6 response to elective surgery. The fact that peak IL-6 levels in the 1989 study by Shenkin et al correlated with duration of surgery suggested that there might be a link between the severity of surgery and the cytokine response. In a further study this group looked at the response to different types of elective surgery. Patients undergoing minor surgery (mainly breast lumpectomy, stripping of varicose veins, and partial thyroidectomy), cholecystectomy, hip replacement, colorectal, and major vascular surgery were compared. The latter two groups of patients showed the highest peaks in IL-6 and there was a significant correlation between the integrated IL-6 response over 48 hours and the duration of surgery, suggesting that the overall IL-6 response was related to the magnitude of tissue damage. A comparison of open and laparoscopic cholecystectomy also found that the IL-6 response was greater and lasted longer in those patients having open surgery and so greater degrees of tissue trauma. TNFα is not typically raised in elective surgery. In most studies there is no detectable increase in IL-1. However Baigrie et al did find a rise in IL-1β which preceded that of IL-6 by several hours in patients undergoing elective repair of abdominal aortic aneurysms. This IL-1β response was much smaller than that of IL-6 and of a much shorter duration. The failure to detect IL-1β in other studies may be due to sampling schedules simply missing an early very transient rise. Alternatively it may be due to the production of IL-1 inhibitors as found by Di Padova et al. Overall these studies of elective surgery strongly suggest that IL-6 is a sensitive marker of tissue damage.

ANIMAL MODELS
Animal studies have yielded further insights into the cytokine response to trauma by allowing the response to haemorrhage and hypotension to be studied in a controlled and reproducible manner. Ayala et al have used a mouse model of haemorrhagic shock in which the animals are bled to a mean blood pressure of 35 mm Hg (pre-bleed blood pressure around 95 mm Hg) and maintained at that level for 60 minutes before being resuscitated with their shed blood and an additional infusion of Ringer’s lactate (twice the shed blood volume). In this model it was found that TNFα increased at 30 minutes during the shock phase and remained increased for two hours after resuscitation, but had disappeared by four hours. Levels of endotoxin were checked to assess whether the stimulus for this increase might be an endotoxaemia. However, these were found to be no different to those of the surgical control animals. They also noted that IL-6 levels were elevated two hours after resuscitation but that the control animals showed a similar IL-6 response. This suggested that the surgical preparation used on the control animals had itself elicited a cytokine response. To investigate this, the model was modified to include a laparotomy before the haemorrhage to try to isolate the two stimuli (soft tissue trauma and haemorrhage). In this later rat study they found that IL-6 levels rose after the laparotomy, but before the start of haemorrhage. TNFα was undetectable before haemorrhage and reached a peak four hours after the onset of haemorrhage. Interestingly IL-6 concentrations continued to increase during haemorrhage and were significantly higher than in the control animals at the same time. This suggested that soft tissue trauma might be the trigger for IL-6 production and that haemorrhage might augment this response. TNFα production seemed to be determined by haemorrhage rather than trauma. A more recent study by Schmand et al, using the Ayala mouse model, showed that the increases in IL-6 seen in simple haemorrhage, haemorrhage and trauma (laparotomy), and haemorrhage and trauma with a longer hypotensive period (90 minutes) were not significantly different but were 8-10 times higher than in the control animals. They did not look at TNFα. In contrast to the previous studies, the work of Schmand et al suggested that the IL-6 response had been fully triggered by the simple haemorrhage model and that any further insult caused no further cytokine response. The results of the two studies by Ayala et al suggested that soft tissue trauma and haemorrhage/hypotension provided distinct stimuli triggering the production of quite different cytokines.

CLINICAL STUDIES
These conclusions prompted a study to see whether cytokine levels could be related to the degree of trauma or to the degree of haemorrhage in multiply injured patients (Foex B A et al unpublished). Ten patients were studied. The injury severity score (ISS) was used to assess the degree of trauma. The degree of haemorrhage was assessed by the blood transfusion requirement during the first 48 hours after injury. Blood was sampled as
soon as possible after injury and as close to every two hours as possible for the next 48 hours. Levels of IL-1, IL-6, and TNFα were measured. The patients showed a range of ISS from 9–50 and a range of transfusion requirements from 0–14 units over the 48 hour study period. Overall, five of the patients died after the end of the study period either from the sequelae of head injuries or from multiorgan failure (MOF). IL-1 levels were raised in five out of eight patients from whom enough plasma was obtained. These increases occurred in the patients with lower ISS. IL-6 was raised in nine of the 10 patients and there was no correlation between peak IL-6 levels and either the ISS, the degree of haemorrhage, or the eventual outcome. TNFα was raised briefly in two patients. These two patients were the most severely injured and also received the largest blood transfusions – 11 and 14 units of blood respectively. There was no characteristic pattern of cytokine production in this group of patients but it was noticeable that cytokine levels could fluctuate widely over a short period of time. This was particularly apparent in the case of IL-1 and TNFα – which showed very transient peaks – compared to IL-6, which showed much more sustained increases.

Svoboda et al studied a series of 42 patients with ISS > 16.24 Serial measurements of IL-1β, IL-2, IL-6, and TNFα were made on entry to the ICU then daily for six days and at weekly intervals until death or discharge. A slight increase in IL-1β lasting 2–10 days was noted in seven of the 42 patients, but IL-2 was raised in four patients on day 1 only. IL-6 was raised in 27 patients and there was a good correlation between IL-6 level on day 1 and the ISS (more than 90% of patients with an ISS of >25 had raised IL-6). No correlations were found between IL-6 and the revised trauma score (RTS) or TRISS scores. In contrast to the ISS, these scores are determined by physiological derangements rather than by the degree of tissue injury. If IL-6 is a marker of tissue damage it is maybe not surprising that its levels are not related to physiological changes. IL-6 levels rapidly decreased over 48 hours in patients making a successful recovery. However, those who developed multiple organ failure and died showed huge increases in IL-6. All patients with an IL-6 concentration of >400 pg/ml subsequently died, but early IL-6 concentrations were not predictive of the development of multiple organ failure or eventual outcome. Only four patients showed an early increase in TNFα and there was no correlation with ISS or outcome. As with IL-6, those patients who developed multiple organ failure showed increases in TNFα. The highest TNFα concentrations were seen in those patients who later died. Increases in IL-6 were also noted by Hoch et al in 30 trauma patients.25 These were divided into three groups according to ISS (mild injury, ISS <10; moderate injury, ISS 11–24; severe injury, ISS >25). IL-1, IL-6, IL-8, TNFα, and endotoxin concentrations were measured within two hours of injury, then four hourly for the first 24 hours and then daily until day 5. From the outset the severely injured group had a significantly higher concentrations of both IL-6 and IL-8 than the other two groups. These levels increased over the first 24 hours and then gradually declined. TNFα concentrations were only just raised above the detection threshold of their assay. Neither IL-1 nor endotoxin were detectable in any group. They subsequently found a correlation between further increases in IL-6 in those patients with an ISS of >25 and the extent of anatomical injury and degree of shock.26 Initial IL-6 concentrations were not predictive of the development of MOF.

The role of TNFα in the cytokine response to trauma remains very unclear. One possible trigger for the production of cytokines is the translocation of bacteria or endotoxin from the gastrointestinal tract. Endotoxin is a potent stimulator of TNFα production27 and a potent stimulator of other cytokines, notably IL-6. It has been suggested that the TNFα response is too transient to be detected in most studies. To overcome this possibility Ferguson et al obtained samples from the site of the accident or scene of the accident.27 In this way they were able to study TNFα, IL-6, and IL-8 concentrations in 42 patients (mean ISS 21.7). Mean time to sampling was 81 minutes. They found raised concentrations of TNFα in 91% of patients, raised IL-6 in 80%, and raised IL-8 in 49% of their patients. Looking at patients whose blood samples were taken earlier than the mean time, the figures were: TNFα raised in 80%, IL-6 raised in 67%, and IL-8 raised in 32%. They concluded that TNFα was active in the inflammatory response to trauma and that it might be activated early and may have modulated subsequent cytokine activity. Rabinovici et al, however, found that in 100 less severely injured patients (mean ISS 12.8), TNFα concentrations were only increased in 35%.28 Interestingly 44% of their control population of healthy volunteers showed an increased concentration of TNFα. They did, however, find that in those patients developing septic complications there were more frequent increases in TNFα concentrations. TNFα levels were undetectable in the 25 patients studied by Meade et al.29 In contrast Endo et al found raised concentrations of TNFα in 28 out of 29 patients in haemorrhagic shock (blood pressure <80 mm Hg on admission to hospital).30 These TNFα values were higher than those in healthy controls. They found no correlation between TNFα concentrations and the degree of haemorrhage (as assessed by the volume of blood transfusion). This suggested that TNFα production might be triggered by haemorrhage and hypotension, but as an all or none phenomenon rather than a graded response. Surprisingly they found no difference in TNFα values between those patients developing multiple organ failure and those who did not. Far fewer patients showed a rise in IL-6 (12 of 29) and IL-8 (23 of 29). Again there was no difference according to blood transfusion requirement and there was no difference between those going into multiple organ failure and those who did not.
Cytokine response to critical illness

The results of all these studies reveal considerable variations in the cytokine response to trauma. One of the reasons for this variability is the very heterogeneous nature of trauma patients with regard to the mechanism of injury and the types of injury sustained. This variability is not really addressed by the various scoring systems commonly used. Studying the response to elective surgery is one way of attempting to circumvent this problem.

The cytokine response to burns

Another approach has been to study the response to burns, a very specific form of trauma, and to try to correlate the magnitude of the response to the extent of the burn injury. This approach was used in 12 burn patients and revealed a good correlation between the IL-6 response and the extent of the burn. IL-6 concentrations reached a peak on day 4 after injury, which is later than for surgery or multiple trauma. Higher IL-6 concentrations were reached in those patients who became septic than in those who did not. TNFa was raised in a smaller percentage of patients and showed no particular pattern. TNFa concentration was not related to the extent of the burn but was greater in septic patients. In contrast, Cannon et al found no correlation between cytokine levels and burn size when they made serial measurements of IL-1b and TNFα in 31 patients suffering 10–95% body surface area burns. There was no correlation between cytokine levels and APACHE II score, but increased levels of IL-1β and TNFα on day 1 did correlate with increases in body temperature. A correlation has also been found between increased plasma IL-6 concentrations and increased rectal temperature during the first 24 hours following moderate burns and scalds in children. Although IL-1 was originally known as endogenous pyrogen it was generally not detectable in the plasma of these pyrexial children, although it was present in blister fluid.

The question remains as to the exact trigger mechanism for the cytokine production seen in trauma and burns patients. The macrophage hypothesis of activated macrophages producing cytokines and other mediators which then result in systemic inflammation leaves unanswered the question of what activates the macrophages. Bacterial or endotoxin translocation from the gastrointestinal tract has been advanced as one possible mechanism for the activation of macrophages. This has been supported by various animal studies which have provided evidence for bacterial translocation. The situation remains very controversial in the setting of human trauma. Some groups have found little evidence for it, while others have found good evidence for it. Deitch et al have suggested that even in the absence of live translocating organisms or endotoxin in the portal and systemic circulations the gut can still act as a cytokine generator through the activation of the gut associated lymphoid tissue (GALT). And apparently it could be that cytokine production is triggered directly at the wound site. Evidence for this comes from both a rat model and a clinical situation. Raised concentrations of IL-6 were found in both wound fluid and serum of rats subjected to polyvinyl alcohol sponge implantation and in fluid draining from mastectomy scars. This suggested that cytokines found in the systemic circulation might have originated at the wound site. Both IL-1 and IL-6 were found in the blisters of burned children, although IL-1 was generally absent from the circulation, again suggesting local production. It may be that two mechanisms act together or act independently on different cytokines, IL-6 being triggered to a greater extent at the wound site by tissue trauma while TNFα and IL-1 might be more sensitive to changes in gut barrier function and activation of the GALT.

Clinical experience with cytokine antagonists

In order to apply the findings of laboratory and animal experiments to clinical practice the model of cytokine release in critical illness must be simplified. Such a model is illustrated in the figure. This model can be used to illustrate the progressive investigation of cytokine antagonists as therapeutic tools in the fight to reduce mortality from sepsis.

ENDOTOXIN

Endotoxin triggers cytokine release in Gram negative sepsis. For this reason, blocking endotoxin was the obvious starting point for therapeutic interventions. The earliest trial of an antiserum to endotoxin was reported by Ziegler et al in 1982. This study showed that in patients with proven Gram negative sepsis, a human antiserum to the lipopolysaccharide moiety of endotoxin significantly reduced mortality. Mortality decreased from 39% to 22% in bacteraemic patients (P = 0.011) and from 77% to 44% in those with bacteraemia and shock (P = 0.003). These beneficial results were later confirmed.

The antiserum used in these studies was prepared by vaccinating healthy individuals with a mutant. Serum collected from vaccinated individuals was then used to treat critically ill patients. This polyclonal antiserum was impractical to produce and administer in the long term. A human monoclonal IgM antibody to the lipid A domain of endotoxin was therefore developed.

A double blind, placebo controlled study showed that this antibody, HA-1A, was effective in patients with Gram negative bacteraemia. Of the 543 patients enrolled in the study, 200 (37%) had documented Gram negative bacteraemia. In these patients, the 28 day mortality decreased from 49% to 30% in HA-1A treated patients (P = 0.014). In patients with Gram negative bacteraemia and shock at study entry, mortality decreased from 59% to 33% (P = 0.017). The study results were taken as illustrating the potential of human monoclonal antibody medicine and based on this study HA-1A
A simplified model of mediator release in sepsis. This illustrates the stages in the investigation of cytokine antagonists in the management of patients with severe sepsis. TNF, tumour necrosis factor; IL, interleukin; PAF, platelet activating factor; NO, nitric oxide.

(Centoxin) was licensed in several European countries but not in the USA. A second study of HA-1A was undertaken—the CHESS (Centocor HA-1A efficacy in septic shock) study. Interim analysis of this study showed an increase in mortality among HA-1A treated patients without Gram negative bacteraemia relative to the placebo group (P < 0.1). For safety reasons, the study was stopped but the results have not been published. HA-1A is still a licensed drug available in some European countries. However, information on its efficacy is lacking and no rapid test for circulating endotoxin levels has been developed to enable accurate patient selection.

TUMOUR NECROSIS FACTOR
TNF is released in response to various insults. Because its release is not confined to Gram negative sepsis, immunotherapy directed at circulating TNF in septic patients appeared rational. An early study used a murine monoclonal antibody directed against human TNF (CB0006) in an open, escalating dose evaluation of the safety, pharmacokinetics, and clinical efficacy in patients with severe sepsis. CB0006 was well tolerated in spite of the development of anti-mouse antibodies in 49 of 50 patients. The anti-TNF antibody did not, however, offer a survival benefit. The 28 day mortality was 41% overall and there was no significant difference between the four treatment groups. A retrospectively defined subgroup analysis of 35 patients with increased TNF levels at study entry indicated that patients in this group who received the highest dose of CB0006 (10 mg/kg) had an increased survival rate, 86% v <50%. TNF cannot be measured easily and because of its rapid clearance concentrations are variable. As a result, measurement of TNF to identify patients who may benefit from an anti-TNF antibody is not a realistic option.

A study of a different anti-TNF, a soluble receptor for tumour necrosis factor, was performed. Preliminary results showed an increased mortality in the treated group. The results of this study have not been published and, since TNF has some beneficial effects, the clinical use of anti-TNF agents is being re-evaluated.

INTERLEUKIN-1
IL-1 occupies an important place in the cytokine cascade and so was a target for blockade. IL-1 is antagonised in the body by IL-1ra, a naturally occurring protein which binds to IL-1 receptors, blocking IL-1 mediated responses without agonistic activity. A human recombinant IL-1ra has been produced. Initial results indicated a dose dependent reduction in mortality; the placebo mortality was 44% compared to groups treated with 17, 67, and 133 mg/h of IL-1ra, where mortality was 32%, 25%, and 16% respectively. There followed a multicentre, randomised, double blind, placebo controlled trial on 893 patients with sepsis syndrome. This study showed no survival benefit between the placebo group and patients receiving 1 or 2 mg/kg/h of IL-1ra. The mortality rates were 34%, 31%, and 29% respectively. Retrospective analysis showed that treated patients with a risk of mortality greater than 24%, calculated by the APACHE III score, had a decreased mortality and increased survival time. In this subgroup of 580 patients, the mortality rates were 45% (for placebo), 38%, and 35% (for 1 and 2 mg/kg/h respectively). IL-1ra also significantly reduced mortality in patients with organ failure. Further multicentre, randomised, double blind, placebo controlled study was undertaken to confirm these findings prospectively. Interim analysis of this study, performed after enrolment of some 700 patients, showed no significant advantage of IL-1ra over placebo. Because of this the study was halted and the results have not been published.

OTHER CYTOKINES
Blockade of other cytokines implicated in the pathogenesis of sepsis has been investigated. A multicentre, randomised, double blind, placebo controlled trial to investigate the role of a platelet activating factor (PAF) antagonist in sepsis showed no survival benefit for this agent. The 28 day mortality for the placebo group was 51%, and that for treated patients, 42% (P = 0.17). A retrospective analysis in patients with Gram negative sepsis showed a decreased mortality in treated patients: 57% v 33% (P = 0.1). In patients with Gram negative sepsis and shock at study entry, mortality was also decreased: 65% v 37%. Because of the problem of prospectively identifying patients likely to benefit from the treatment, further studies of PAF antagonists have been postponed.

Inhibition of nitric oxide synthetase has also been investigated, but there is no convincing evidence of a decreased mortality. Other agents such as prostaglandins and leukotrienes are under investigation. However, trials are smaller and tend to be in subgroups of septic patients. Bradykinin, although not a cytokine, is an inflammatory mediator generated in trauma, burns, and sepsis, causing cytokine activation as well as hypotension, vascular leak, endothelial cell retraction, and pain. A bradykinin antagonist, CP-0127, has recently been studied in a placebo controlled trial in patients with the systemic inflammatory response syndrome (SIRS) and sepsis in the USA. Preliminary results show an overall...
Cytokine response to critical illness

non-significant improvement in both risk adjusted seven day survival (P = 0.09) and 28 day survival (P = 0.51). For Gram negative infections 28 day survival was significantly improved (P < 0.005), such that mortality fell from 50% in the placebo group to 22% in the group receiving the highest dose of CP-0127. Further studies are under way.

LESSONS
Many lessons have been learned from these studies. It is now realised that the pathophysiology of sepsis is more complex than previously imagined and the hope for a "magic bullet" cure has faded. Disappointingly, several large studies have not been reported and data from these studies have not been analysed. The epidemiology and pathophysiology of sepsis, particularly the role of underlying illnesses, may be elucidated further by analysis of these data.

Ethical lessons have also been learned. The cost of anti-cytokines is considerable and this has raised the issues of the cost-effectiveness of care, particularly if survival time is increased but mortality rate unaltered, and the ethical dilemmas of rationing. Academic issues have been raised. These include whether changing clinical practice on the basis of limited information is justifiable, optimising design of clinical trials, and resolving the conflicting interests of academia, patient interest, and a drug industry financed by venture capital.

The fear of sepsis from organisms resistant to antibiotics is becoming widespread. This fear, however, should not encourage reliance on agents without proven efficacy.

At the moment antagonists to cytokines are not fulfilling their promise or our hopes. Cytokines are released in critical illnesses such as sepsis, burns, and trauma but antagonising these cytokines does not improve mortality. A combination of different antagonists may be more effective than each alone, and this is the present hope. In the meantime, the impact of cytokine release and the importance of cytokine antagonism can be reduced by early resuscitation so that the initial insult to the patient is minimised.

Conclusions
The original premise in this review was that knowledge about cytokines is important on a number of different levels in the seriously ill patient. Has this been borne out? Understanding of the biochemical and immunological consequences of sepsis, trauma, and burns has certainly improved as a result of cytokine studies. There remains much controversy about whether measurement of cytokine concentrations can provide useful information about the severity of an insult or the patient's progress. In the case of sepsis it appears that serial measurements can be used to monitor deterioration or recovery on the intensive care unit. In the case of trauma this really only applies if the patient subsequently develops multiple organ failure. Finally, have any therapeutic breakthroughs been made as a result of this increased knowledge? At the moment the answer appears to be no, although several other agents are still being investigated.

This review is based on lectures given by Prof A Shenkin, Dr B A Foxx, and Dr M P Shelly at the Annual Scientific Meeting of the Emergency Medicine Research Society, held in Liverpool in November 1994.

Recommended reading
The following books give a fuller introduction to the biology of cytokines:

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Foix, Shelly


The cytokine response to critical illness.

B A Foëx and M P Shelly

doi: 10.1136/emj.13.3.154

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