REVIEW ARTICLE

Cytokines in the systemic inflammatory response syndrome: a review

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ABSTRACT

Introduction: Patients subject to major surgery, suffering sepsis, major trauma, or following cardiopulmonary bypass exhibit a systemic inflammatory response.
This inflammatory response involves a complex array of inflammatory polypeptide molecules known as cytokines. It is well accepted that the loss of local control of the release of these cytokines leads to systemic inflammation and potentially deleterious consequences including the Systemic Inflammatory Response Syndrome, Multi-Organ Dysfunction Syndrome, shock and death.
Methods: The Medline database was searched for literature on mechanisms involved in the development of SIRS and potential targets for modifying the inflammatory response. We focus on the novel therapy of cytokine adsorption as a promising removal technology.
Results: Accumulating data from human studies and experimental animal models suggests that both pro- and anti- inflammatory cytokines are released following a variety of initiating stimuli including endotoxin release, complement activation, ischaemia reperfusion injury and others.
Discussion: Pro-and anti-inflammatory cytokines interact in a complex and unpredictable manner to influence the immune system and eventually cause multiple end organ effects. Cytokine adsorption therapy provides a potential solution to improving outcomes following Systemic Inflammatory Response Syndrome.

Keywords: *cytokine, systemic, inflammatory response, syndrome, SIRS.*

INTRODUCTION

Patients subject to major surgery, suffering sepsis, major trauma, or following cardiopulmonary bypass exhibit an 'acute phase' inflammatory response. This is characterised clinically by fever, drowsiness, and anorexia. Biochemical features are the synthesis of hepatic acute phase proteins, complement activation, leucocytosis and lymphopenia in the peripheral blood and disturbances in metabolism.
When the inflammatory response becomes uncontrolled, a Systemic Inflammatory Response Syndrome (SIRS) ensues. In some individuals this severe inflammatory response is down-regulated; in others it escapes control. In 1992 a consensus conference of the American College of Chest Physicians and the Society of Critical Care Medicine (ACCP/ SCCM) proposed a constellation of clinical signs by which SIRS would be recognised (1). These include tachypnoea, fever or hypothermia, tachycardia and leucocytosis or leukopaenia with

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a 'left shifted' differential white cell count (increased immature polymorphonucleocytes). Bone et al proposed that every severe insult to the body produces a response with pro- and anti-inflammatory components which together dictates the course of the illness (2).
The ACCP/ SCCM criteria have been shown to have clinical relevance – the Italian SEPSIS study showed an inverse correlation between the identification of SIRS (based on clinical criteria) and the development of sepsis, and subsequent mortality (3).
It is well established that major operative intervention, the systemic inflammatory response from sepsis and major trauma is associated with immunosuppression, both cell-mediated and humoral systems (3-8). Interestingly, the extent of the individual's inflammatory response is variable and unpredictable, this variability may be due to genetic variation. Although the full picture has yet to be completely elucidated, much advancement has been made in the last decade to better understand and target treatment towards this complex process. We review this topic and discuss current and potential future therapy.

METHODS

A search of the Medline database from 1950 to November 2008 using the OVID interface, combined with manual cross-referencing was performed using the following strategy: (Systemic Inflammatory Response Syndrome/ or SIRS.mp. or Surgery/) AND cytokines.mp. or Cytokines/ limit to (English language). Abstracts were reviewed for relevance to the topic.

Cytokines and inflammation

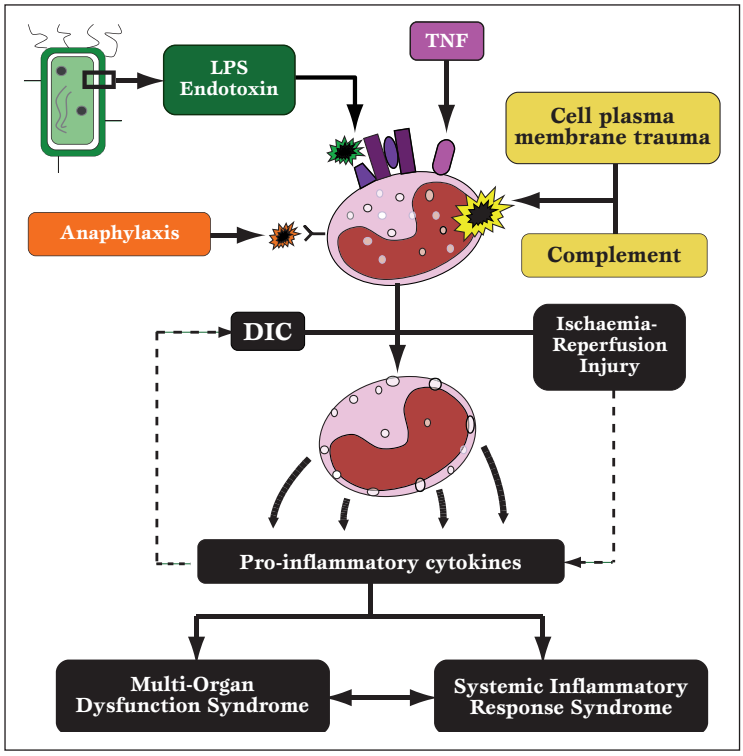
Cytokines belong to a large family of polypeptide signalling molecules that are released by various cells in response to an activating stimulus. They are small proteins of approximately 25kDa (range: 6-51) in size and bind to specific receptors in an autocrine, paracrine and/or endocrine manner.
The number of cytokine molecules identified is large and ever increasing. Precise effector pathways and detailed knowledge of inter-relationships between them are far from clear. There is however a growing body of evidence that a number of these cytokines are related to the establishment of SIRS and their persistent elevation is related to poor prognosis.
The inflammatory process involves the release of a pro- and anti-inflammatory cytokines. Anti-inflammatory cytokines act to localise and prevent over exuberant inflammation; it is the loss of this local control that leads to systemic inflammation and potential deleterious consequences, including SIRS, MODS (Multi-Organ Dysfunction Syndrome), shock and death (4) (*Figure 1*).

Potential triggers of cytokine release

Cytokines are released following a variety of initial stimuli which are summarised in *Figure 2* and include:

- Endotoxin/Lipopolysaccharide (LPS) – a protein fragment of the gram-negative bacteria cell wall (*Figure 3*). It was initially thought to be *the* instigating factor in the development of SIRS, however, a study of 100 patients with sepsis demonstrated gram-negative bacteraemia in only 12% (5); this argues against endotoxin as being essential in the development of SIRS. Furthermore, a study of 20 paediatric patients undergoing cardiopulmonary bypass (CPB; a process known to predispose to SIRS) demonstrated that although endotoxin, TNFα and IL-8 were elevated following bypass, levels of endotoxin did not correlate with duration of CPB, cytokine levels, or the development of SIRS/MODS.

Figure 1 - *Initiators of SIRS*



- **LPS "Endotoxin"** is a component of gram-negative bacterial cell walls and is continuously sheared off into surrounding interstitial fluid and serum. LPS degrades into the O-antigen and Core protein, which have little immunogenic effect and **Lipid-A** which is highly pro-inflammatory. Lipid-A binds the **CD14/TLR4/MD2** receptor on monocytes and tissue macrophages to trigger the **NF-κβ** protein family. This messenger translocates to the cell nucleus and initiates the production of pro-inflammatory cytokines via primer binding.
- **TNF** binds the TNF-R (types 1 and 2) to trigger three main intracellular pathways; the FADD, the TRAF-ASK1 and TRAF2-RIP pathways. These proteins activate intracellular caspase enzymes which degrade DNA by proteolysis and induce changes in DNA expression, thus cellular function and cause apoptosis. Dyscytokinaemia can results in uncontrolled cell death and organ dysfunction
- **Direct cell plasma membrane trauma** results in the production of ecosanoids by PLA_2 and the COX family (as well as other mediators). Prostaglandins affect tissue perfusion by controlling vasoconstriction/dilation and platelet aggregation. Leukotrines control vessel permeability as well as stimulating inflammatory cell chemotaxis. Lipoxins control cell adhesion and migration. Dysregulation of these mediators results in ishaemia/hyperaemia and tissue damage
- **Complement cascade activation** (via the classic or alternative pathway) results in the production of the membrane attack complex (C5b6789) which perforates the plasma membrane of the call on which it is formed. Formation can occur on any cell, but most commonly on bacteria, resulting in lysis and dissemination of bacterial antigens (eg. LPS). Normal cells express protectin on their surface, which prevent lysis by the membrane attack complex, however, uncontrolled activation of complement and production of this enzyme can overcome this protection and result in autolysis. Deficiences can also result in severe disseminated infection
- **Anaphylaxis** is an acute systemic type 1 hypersentivity reaction to an innocuous antigen. The resulting massive histamine release causes profound vasodilation, recruitment of inflammatory cells and the subsequent production of pro-inflammatory cytokines. If this process continues, it can result in tissue hypoxia and organ dysfunction.
- **DIC** results from uncontrolled activation of the clotting cascade by pro-inflammatory mediators. As a result, haemorrhage occurs throughout the body (micro/macro) and results in further tissue damage, hypoxia and pro-inflammatory cytokine results. Organ dysfunction can develop rapidly
- **Ischaemia-Reperfusion injury** occurs when a tissue has been hypoxic for a prolonged period and produces large quantities of pro-inflammatory & vasodilating mediators. When the tissue is reperfused, the effect is local hyperaemia and restuling tissue damage and the release of potent concentrations of cytokines into the systemic circulation. Reactive hyperaemia can induce rapid production of vasoconsricting mediators, leading to capillary level dysfunction. This cycle of ischaemia and hyperaemia leads onto tissue damage and further pro-inflammatory cytokine production

CD = cluster of differentiation, TLR = Toll-like receptor (TLR4 aka. CD284), MD2 = lymphocyte antigen 96 (aka. LY96), MAP = mitogen activated phosphokinases, ERK = extracellular signal-regulated kinases, JNK = c-Jun N-terminal kinases, DIC = Disseminated Intravascular Coagulation

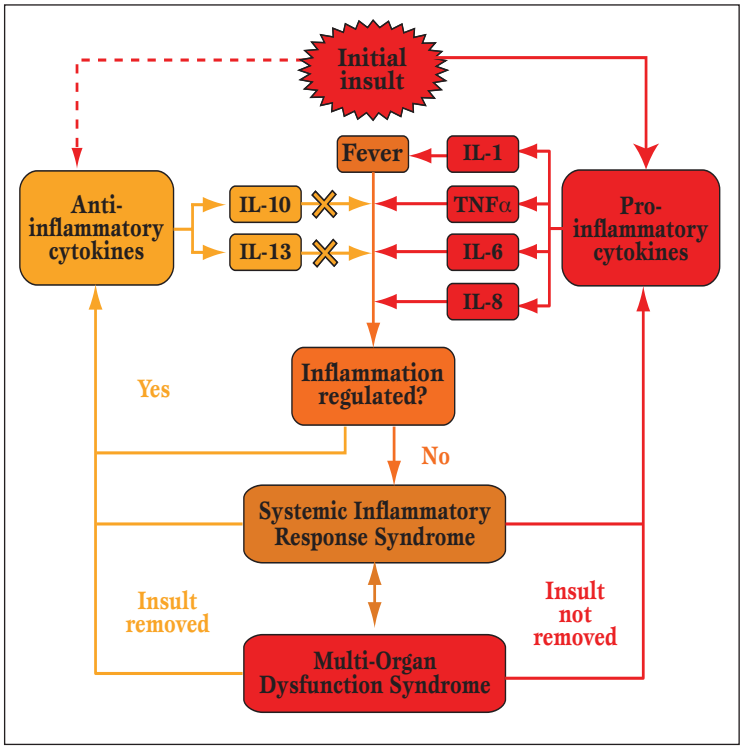


Figure 2 - *Balance between pro- and anti-inflammatory cytokines*

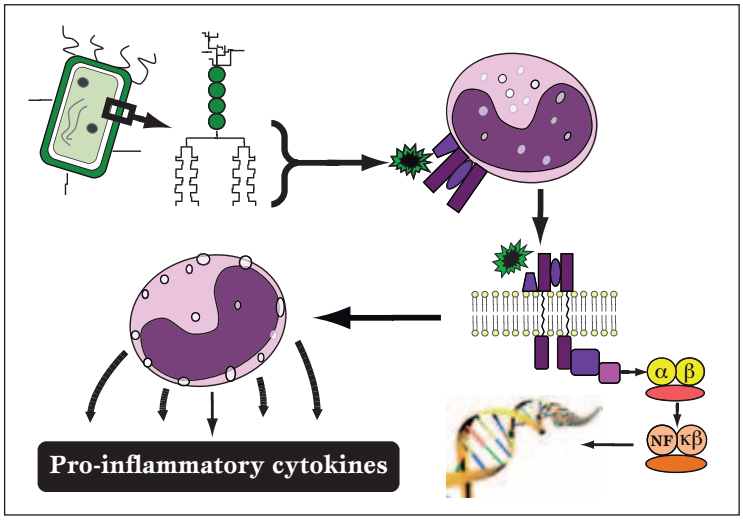


Figure 3 - *Mechanism of Lipopolysaccharide (LPS) "Endotoxin" pathogenesis*

Gram negative bacteria have a lipopolysaccharide (LPS) membrane outside the peptidoglycan layer.

1. LPS ("endotoxin") is sheared from the bacterial membrane continuously into surrounding interstitial fluid and serum
2. LPS degrades into the O-antigen and Core protein which have little immunogenic effect and **Lipid-A** which is highly pro-inflammatory
3. Lipid-A binds the **CD14/TLR4/MD2** receptor of tissue macrophages and serum monocytes to trigger intracellular pathways
4. The **NF-κβ** protein family is activated via a complex multiple step intracellular process, which translocates to the nucleus and initiates production of pro-inflammatory cytokines
5. This results in a mass release of pro-inflammatory cytokines including TNFα and IL-12

CD = cluster of differentiation, TLR = Toll-like receptor (TLR4 aka. CD284), MD2 = lymphocyte antigen 96 (aka. LY96), MAP = mitogen activated phosphokinases, ERK = extracellular signal-regulated kinases, JNK = c-Jun N-terminal kinases

In contrast, TNFα and IL-8 correlated well with duration of bypass and were associated with SIRS/MODS (10). The conclusions therefore are that endotoxaemia does occur in humans during various disease processes, however, a causative association with SIRS cannot be discerned (6).

- Complement – a family of plasma proteases belonging to the innate immune system, which when activated are capable of cleaving many proteins and activating cytokines. It is known that the complement proteins C3a and C3d elevate with sepsis (12), correlate with PAI-1 (plasminogen activator inhibitor-1) levels and inversely correlate with AT-III (anti-thrombin III). C3a levels are associated with poor APACHE II scores and fatal outcome. However, C3a and C3d levels do not correlate with TNFα or IL-6 levels nor do their levels decrease following treatment of sepsis.
  Lissauer et al (2007) showed that the levels of *classic* vs *alternative* complement proteins where elevated in different ratios is septic versus uninfected patients with SIRS. Furthermore, elevations were detectable up to 3 days prior to the clinical diagnosis of SIRS, although no temporal association could be established. The authors concluded that many of the complement proteins may be used as co-dependent-biomarkers for early diagnosis and targets for future treatment (7).
- Ischaemia-reperfusion injury. In rats, plasma TNFα levels were found to be elevated following 3 hours of bilateral lower limb ischaemia with further increases following 1 hour of reperfusion. Also, IL-6 levels progressively increased following reperfusion (8). A human cohort study of patients undergoing infrainguinal arterial reconstruction demonstrated that serum TNFα and gut mucosal permeability were higher in those operated on for critical limb ischaemia as opposed to intermittent claudication. In addition, gut permeability was found to correlate with period of arterial clamping (9). These studies implicate ischaemia-reperfusion injury as a potential trigger for SIRS.
- Oxidative stress – Measurement of plasma sulfhydryl groups (e.g., glutathione; GSH) and α-tocopherol in 26 trauma patients in the ICU showed progressive worsening in redox status (10) with a significant increase in plasma *oxidised* glutathione (thus a worsened redox status) and higher MODS scores on day 10. Additionally, a total loss of *reduced* plasma glutathione was seen in some of these patients indicating the collapse of the GSH-dependent anti-oxidative system. From this data, we can conclude a possible role of oxidative stress in the development of SIRS.

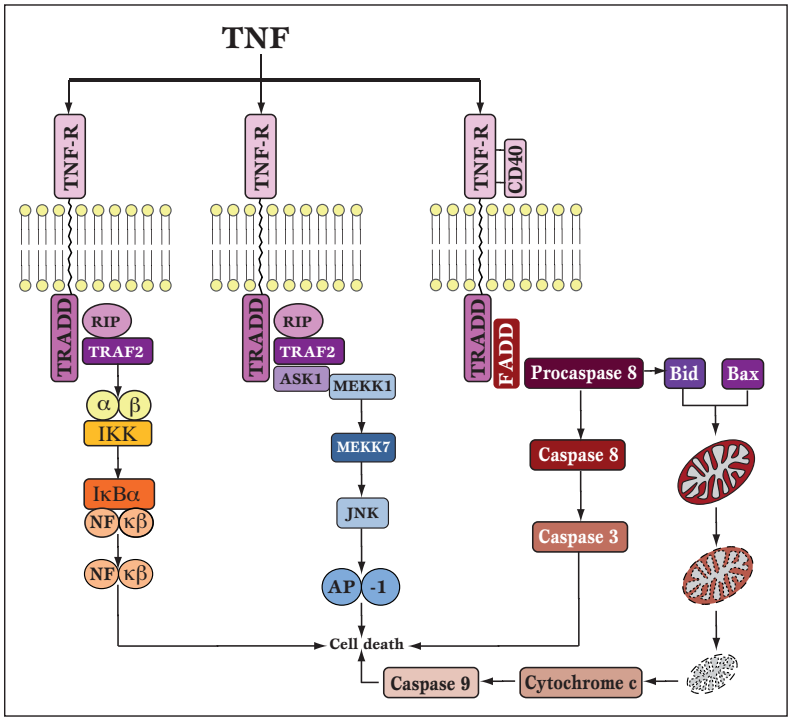
Pro-inflammatory cytokines

Cytokines are released in a cascade. Initial cytokines released include TNFα and IL-1ß; these stimulate further production of other proteins. The main pro-inflammatory cytokines are TNFα, IL-1, IL-6, IL-8 and macrophage inflammatory protein-1α (MIP-1α); these have consistently been shown to correlate with the mortality following severe injury (16,17), with TNFα and IL-6 levels also correlating with poor outcome from sepsis (11).

Tumour Necrosis Factor α (TNFα)

TNFα is a 17-kDa protein produced primarily by monocytes. Infusion of recombinant TNFα in humans results in SIRS with fever, haemodynamic abnormality, leukopaenia, elevated liver enzymes and coagulopathy (12).
TNF mediates its effects through the TNF receptor and multiple cell signalling path-

Figure 4
The cellular affects of TNFα



Tumour Necrosis Factor alpha (TNFα) is produced by macrophages, lymphocytes, fibroblasts and keratinocyte. TNFα binds the TNF receptors (TNF-R/CD120a/CD120b). There are 2 types of TNF-R;

- TNF-R type 1 (CD120a) – present on all cells and binds TNFα only.
- TNF-R type 2 (CD120b) – present on immune cells and binds both TNFα and TNFβ.

Stimulation of the TNF receptors results in receptor trimerisation and activation of downstream proteins. The effect of TNFα on any cell depends on myriad co-stimuli.

- CD40 co-stimulus results in FADD pathway activation.
- The caspase family of enzymes cleave cellular DNA.
- Bid binds to inserts pores into the mitochondrial membrane, causing leakage of cytochrome c. Cytochrome c binds APAF-1 to activate the caspase family and cause cellular DNA degradation.
- TRAF2-ASK1 pathway activation results in AP-1 production, which binds DNA to regulate the production of many proteins.
- TRAF2-RIP pathway activation results in NF- κβ production, which binds DNA primer sequences and stimulates mRNA, therefore protein production and a change in cell function.

APAF-1 = Activating Protease Apoptotic Factor-1, AP-1 = Activator Protein 1

ways (*Figure 4*); the propensity for each pathway depends on multiple other co-stimulatory and intra/extracellular factors:

- Stimulation of the NF-kβ pathway results in nuclear transcription of anti-apoptotic factors including Bcl-2, cFLIP and cIAP.
- Activation of the MAPK pathway results in the production of pro-apoptotic, proliferative and cell maturing factors.
- Activation of the FADD pathway results in cell apoptosis via autolysis by the caspase family cascade and Bid production. Co-stimulation by Fas-FasL and other CD complexes favours this pathway.

TNFα has a significant regulatory role in the development and propagation of the SIRS.

A number of studies involving infused endotoxin have shown that levels of TNFα peak in 60-90 mins (20, 21).

Therefore, TNFα appears rapidly in the plasma and is in prime position to facilitate the activation of other mediators of inflammation.

Interleukin-1 (IL-1)

IL-1 encompasses two related proteins, IL-1α and IL-1β both of which act on the same IL-1 receptors. IL-1 is synthesised by mono-

cytes, neutrophils and other cell types. Infusion of IL-1 into humans causes fever, haemodynamic abnormalities, anorexia, malaise, arthralgia, headache and neutrophilia. Infusion of endotoxin has been shown to causes an elevation of its levels (13). Although pro-inflammatory in nature, abnormally low levels may also have a role in the development of the SIRS (14). Data on IL-1 in SIRS is sparse and no conclusions can yet be drawn.

Interleukin 6 (IL-6)

IL-6 is a 21 kDa glycoprotein produced by many cell types. It is a potent mediator of fever, it increase release of acute phase proteins and it encourages chemotaxis via stimulation of the Toll-like receptor family. Studies of patients with severe sepsis for < 48hrs have shown a tight correlation between the elevation of IL-6, the severity of the SIRS and subsequent mortality (15,16).

In elective cardiopulmonary bypass cardiac surgery, high post pump IL-6 levels were found to predict subsequent worsening of lung function (17).

In mice subjected to a contact burn followed by intra-peritoneal LPS to simulate SIRS, treatment with a combination of anti-IL-6 and anti-IL-6R antibodies caused a significant reduction in inflammation and mortality. Anti-IL-6R antibody monotherapy had no effect (27). Also, IL-6 infused into anaesthetised dogs did not cause any significant haemodynamic abnormality (18).

This data suggests that the independent inflammatory potential of IL-6 is negligible; however its role may be in synergy with other cytokines.

Other interleukins

There is much equivocal or inconclusive data on a number of other pro-inflammatory cytokines including IL-8, IL-17 which are not been reported up on here.

Anti-inflammatory cytokines

To balance and control inflammation, co-existent anti-inflammatory cytokines are produced in synchrony with pro-inflammatory ones.

The main anti-inflammatory cytokines are IL-10 and IL-13. Rodriguez-Gaspar et al. showed anti-inflammatory cytokines also have a role in the pathogenesis of SIRS in sepsis; serum levels of pro-inflammatory cytokines (TNFα, IL-6, IL-8) were shown to be raised along with the anti-inflammatory cytokine IL-10.

Interestingly, the association between IL-10 and TNFα was stronger in patients with fatal outcomes (19).

Interleukin-10 (IL-10)

IL-10 is an 18 kDa anti-inflammatory cytokine, produced by monocytes and lymphocytes. It exhibits pleitropic effects in immunomodulation including: down regulation of Th1 cytokines (TNFα, IL-2, IL-3, INF-γ and GM-CSF), reduction of MHC class II antigen expression, increased B-cell survival time and blockade of the NF-kβ JAK-STAT pathway.

A comparative study of 12 SIRS patients and 12 healthy volunteers showed that levels of TNFα and IL-10 were higher in patients with SIRS and MODS, as compared to the healthy volunteers. In the murine model, intra-peritoneal loading of IL-10 attenuates the serum TNFα response to inflammatory stimulus (30,31). This suggests that IL-10 is antagonistic to TNFα in the pathogenesis of SIRS.

Interleukin 13 (IL-13)

IL-13 is a 17 kDa cytokine secreted mainly by T-helper cells. IL-13 induces the secretion of IgE from B-cells, up-regulates matrix metalloproteinases (MMPs) to reduce inflammation and stimulates lymphocyte proliferation. A study of ICU patients with SIRS demonstrated that IL-13 was elevated

and that the increase was greater in septic vs. non-septic patients. In SIRS, the elevation of IL-13 is proportional to TNFα and the degree of leukopaenia (20). This supports IL-13 likely importance in the pathogenesis of SIRS and as a regulator of TNFα and the leukocyte response.

Molecular mechanisms of cytokine release in SIRS

There seem to be multiple molecular mechanisms involved in the initiation, propagation and termination of SIRS. The following substances may be significant to the molecular basis of SIRS:

- Haemoxygenase-1 (HO-1) – implicated in the defensive response to oxidative stress; it has been found to attenuate TNFα mRNA synthesis and plasma TNFα levels, reduce lung injury and improves organ perfusion (33).
- Hypoxia inducible factor-1 (HIF-1) – a nuclear transcription factor influenced by IFNγ, TNF and IL-1β; important in the cellular response to ischaemia (34,35). HIF-1 activation results in increased expression of iNOS mRNA, iNOS proteins and thus the endogenous vasodilator molecule nitric oxide (NO). HIF-1 production has also been shown to increase NFκB activity, propagating inflammation. These effects can be blocked by a selective MAP (mitogen activated protein)-kinase inhibitors (21). These findings suggest that amplified NFκB activity and upregulation of NO production may be important pathways in the cytokine response in SIRS.
- NFκB – a DNA binding protein which plays a pivotal role in activation of inflammatory pathways (22). In septic patients, NFκB activity correlates well with mortality (38).
- Toll-Like Receptors (TLRs) – members of the pathogen associated molecular pattern (PAMP) recognition proteins; to-date there are 15 known subtypes, most with uncertain functions but some which have well elucidated locations and defined roles in regulating the innate immune system, particularly by triggering inflammation and cell death by apoptosis (23). One such receptor, TLR4, the so-called 'endotoxin receptor' recognises endotoxin. Microbial products and some endogenous molecules activate TLRs to cause widespread pro-inflammatory cytokine production, in turn causing organ failure, shock and death. TLR4 has been shown to be constitutively suppressed – it has been suggested that the initiating step in the pathogenesis of sepsis could be the release of TLR4 from suppression (24).

Treatment Strategies for SIRS

The treatment of SIRS can thought of in terms of pharmacological therapies and cytokine removal therapies. This second group are further sub-divided accordingly to the strategy used for cytokine removal. The rational and utility of various therapeutic strategies will be discussed based on their therapeutic mechanisms.

Pharmacological therapies

- Monoclonal antibodies

HA-1A is a human monoclonal IgM antibody that binds specifically to the lipid-A domain of endotoxin (LPS). A large RCT of patients with sepsis showed that HA-1A has no significant effect on mortality rate (Placebo: 43 % vs HA-1A: 39 %). However, patients specifically with gram-negative bacteraemia showed a significant reduction in 28 day mortality (from 49 % to 30 %) and death (from 57 % to 33 %) (41).

There have been many anti-TNFα antibodies used in clinical medicine. A meta-analysis of RCTs using TNFα antibodies in sepsis showed a small but significant benefit in anti-TNFα therapy. Furthermore, a recent

study of 2634 septic patients using a murine anti-TNFα antibody shows a significant reduction of 3.6 % in mortality (25). More recently a large double blinded placebo RCT published mortality rates of 35.9 % in the treatment (Afelimomab; anti-TNFα Fab2 monoclonal antibody fragment) group and 32.2 % in the placebo group. Afelimomab also resulted in a significant reduction in serum TNFα and IL-6 levels and a more rapid improvement in organ failure scores compared with placebo (43). These monoclonal therapies have yet to be proven specifically in SIRS patients. Results to date have been largely disappointing. This may be because the complexity of the dyscytokinaemia in SIRS is not amenable to single agent therapy alone.

- Polyclonal anti-LPS antibodies

The polyclonal anti-LPS antibodies HA-1A, Edobacomab (E5) did not show 14 or 28 day mortality benefit in a large RCT of patients with gram negative sepsis (26), although a similar second large RCT reported improved in incidence of Adult Respiratory Distress Syndrome (ARDS), CNS sequelae and resolution of MODS (27). Therefore, there are clinical trials currently underway assessing the efficacy of HA-1A and E5 in specific subsets of patients with SIRS (severe burns and bacterial overgrowth) with the hope of reducing incidence of sepsis.

Corticosteroids

Corticosteroids reduce IL-6, TNFα, and E-selectin levels and increase IL-10 secretion but have no measurable effect on recovery, worsen incidence of intestinal injury, and increase anaesthetic complications in patients with SIRS (28,29). Corticosteroids have been shown to reduce circulating cytokine levels in patients undergoing cardiopulmonary bypass, some benefit in ventilation time, inotropic and vasopressor requirements and haemodynamic stability has also been reported (30,31). However a previous study in the same setting reported a lowering of pro-inflammatory cytokines, increased anti-inflammatory cytokines but more pulmonary dysfunction and a prolonged time to extubation (32).

- Serine protease inhibitors

The PROWESS trial (randomised double-blinded placebo controlled) demonstrated significant benefit in the use of activated protein C (Drotrecogin alfa) in reducing the mortality of patients with gram-negative sepsis, without a higher incidence of severe bleeding (33). However, a recent observational study involving 4374 patients undergoing coronary revascularization (with iatrogenic SIRS) compared a protesase inhibitor with placebo and found that it was associated with a 2-fold increased risk of renal failure requiring dialysis, a 55 % increase in the risk of adverse cardiac events and a 181 % increase in the risk of stroke or encephalopathy (34). Clearly this has caused marked concerns over the continued usage of these agents and thus, careful study design to protect future patients is required to determine its role in SIRS.

- Insulin

Insulin therapy reduces the in-hospital mortality and incidence of fatal infection in diabetic and non-diabetic critically ill patients. This may be related to the stimulatory effect of glucose on the production of pro-inflammatory cytokines without reciprocal increase in anti-inflammatory cytokines. Despite such positive reports, the data on insulin therapy in critically ill non-diabetic patients is conflicting (35).

- Antioxidant micro-nutrients

Berger et al., conclude Level A evidence for improvement in outcome with antioxidant supplementation in sepsis and SIRS. What remains to be elucidated are their exact influences on the dycytokinaemia (36). The main three agents of note are;

Selenium – administration of high dose Sodium Selenate IV (Se^{+}; n = 21) compared to placebo over 9days, to patients with SIRS

in ICU result in significantly improved APACHE scores and reduced the incidence of renal failure requiring haemodialysis (37).

Glutamine – oral glutamine supplements may increase gut permeability to endotoxin, but also reduce temperature, heart rate and leukocyte count (38). Data as yet is inconclusive.

Filtration and Adsorptive therapies

- Haemofiltration and Haemodialysis

The use of continuous haemofiltration and haemodialysis for the removal of cytokines in SIRS has gradually been entering the mainstream of management (39). Efficient filtering and clearance of cytokines by convection from activated blood, has been demonstrated in ex-vivo systems using large pore filtration membranes (40). Studies have revealed a 13% decreased in plasma TNFα using continuous veno-venous haemofiltration, compared to a 26% increase with haemodialysis (41,42). These results suggest that cytokines removal from the plasma is by an *adsorptive* process (rather than by a filtration process per se) and that membrane surface area may be the critical factor for cytokine removal (43).

- Cytokine Adsorption

The adsorptive removal of cytokines from blood is a logical progression from filtration devices; the technique of adsorption may offer a significant boon over filtration, dialysis and indeed drug based methods without adding to the chemical environment. The options for absorption include:

- Synthetic polymer resins

These are porous absorptive material with a surface area of ~1500m^2/g (44). A murine study using polystyrene divinyl benzene co-polymer beads showed that following administration of endotoxin, there was a benefit in survival time, reduction in IL-6 and IL-10 concentrations and also reduced liver NFκB DNA binding (45).

- Immobilised antibody systems

Such devices have demonstrate near-complete removal of TNFα from human plasma in an in vitro setting (46). The Lixelle column immobilised antibody system has shown much promise in clinical trial when cytokine levels (IL-1β, IL-1Ra, IL-6, IL-8 and TNFα) were reduced up to 70% following 5 minutes of use, in a haemoperfusion set up (47).

- Activated Carbon/ Charcoal

Activated carbon is a highly porous and extremely absorptive material, with surface area of 1500-2000m^2/g and a pore volume of 1.8cm^3/g (48); twice the magnitude of filtration/dialysis membranes (44). Most reports to date on activated charcoal absorption systems have agreed that charcoal can absorb almost 100% of plasma LPS, IL-Ra, IL-1β, IL-8, IL-1α and IFN-γ and 40% of TNFα (49-52). The BioLogic-DTPF (DeToxifier/Plasma Filter) System with activated powdered charcoal is as yet the fastest and most efficacious method of removing multiple cytokines from human blood (53), with each cartridge adsorbing 90% of IL-1β, 72% of IL-6, 100% of IL-8, and 7% of TNFα during each pass. A Phase 1 trial of the BioLogic-DTPF system with push-pull sorbent-based phoresis (the PF add-on module), for the treatment of both SIRS and MODS in 8 adult ICU patients reported resolution of sepsis in 5 of the 8 patients, although, there were only 2 long-term survivors. Although there were no negative effects in 7 patients, 1 patient died during treatment due to progressive cardiac failure (54).

- Polymyxin B immobilised fibre column haemofiltration system

This filtration system has been shown to protect mice from SIRS following endotoxin challenge (55). It has also been reported by Sato et al (2002) to decrease the levels of circulating TNFα, IL-6, IL-10 and PAI-1, and is currently licensed for the treat-

ment of SIRS in Japan. More recently, a RCT by Cruz et al (2009), involving 60 ICU patients with gram-negative-induced abdominal sepsis, compared patients receiving 2 sessions of conventional therapy plus polymyxin B haemoperfusion vs. conventional therapy alone. This group showed an increased mean arterial pressure (76 to 84 mmHg, $p = 0.14$), improved ventilation and reduced 28-day mortality (32 % vs. 53 %, adjusted Hazard ratio of 0.36, 95 % CI; 0.16 to 0.80) (56). However, this trial was unblinded and there was no mortality data beyond 28 days which would have been useful. We await further research into this promising device.

Accumulating studies relating to carbon are not able to yet provide definite proof of benefit but much promise if offered as it is an effective adsorbent for cytokines from human plasma with SIRS/ MODS.

Evidence based management of SIRS

Early Goal-directed therapy

Optimising cardiac pre-load, after-load and contractility ensures balanced oxygen delivery between heart and systemic tissues. The approach of aggressive goal directed management of haemodynamic parameters has been repeatedly shown to reduce morbidity and mortality in patients with severe sepsis, shock and SIRS (57).

Activated Protein C

Patients with severe sepsis and/or organ dysfunction fulfilling 4/5 criteria as proposed by Bone et al (58) for diagnosis of SIRS, benefit from a Recombinant Activated Protein C (Drotrecogin alfa) infusion within 48 hours of diagnosis. Exclusion criteria include; age less than 18 years, bleeding dyscrasia or increased bleeding risk (there are no validated guidelines for this), an epidural catheter in placement or a platelet count $\leq 30 \times 10^9$/L. The absolute risk reduction in mortality was reported to be 6.1 % by the PROWESS trial but this carries an uncertain bleeding risk. More recent guidelines on the treatment of severe sepsis suggest that Recombinant Activated Protein C (Drotrecogin alfa) infusion should only be commenced in eligible patients with evidence of infection and SIRS with MODS in addition to the most appropriate intensive care support (UCSF Guidelines for Usage of drotrecogin alfa (Xigris™), http://clinicalpharmacy.ucsf.edu/idmp/ucsf_specific/apcguide.htm). Outcomes from Activated Protein C in patients with patients with SIRS only as yet unclear and currently this agent is contra-indicated in patients with only SIRS.

Antioxidants

There is evidence to support the use of oral or infused Selenium, Glutamine and Eicosapentanoic acid in reducing bowel permeability and theoretically reducing endotoxaemia in patients with SIRS.

Future research

To date, there have been many agents and systems investigated and reviewed in an effort to find an effective treatment regime for the complex dyscytokinaemia found in SIRS. Some of the potentially beneficial agents under investigation and showing promise include:

- **BioLogic-DTPF** (detoxifier/plasma filter) – the most potent binder of TNFα, IL-1β and IL-6 showing promising results in Phase 1 human trials; it has the potential to bind all cytokines, is cheaper than immobilised antibodies systems and adds no chemical to the patient. We eagerly await results of Phase II clinical trials.
- **Protease inhibitors** – the efficacy of Activated Protein C (Drotrecogin alfa) in patients with severe sepsis has been established by the PROWESS trial, however, it carries a risk of haemorrhage,

cardiovascular events and its role in modifying circulating cytokines in SIRS without Gram-negative sepsis has yet to be shown. It may have a role in treating particular patients with SIRS and DIC (59) or MODS (60) but further clinical trials are needed.

- **sCD14** – this extracellular receptor protein which binds circulating endotoxin (LPS), has shown promise in in-vitro since 1991, by reducing circulating LPS, pro-inflammatory cytokines and modulating host cell reaction (61). Early human studies have displayed similarly exciting results and are the subject of current work (62) which we await the results of.
- **Polymyxin B immobilised fibre column haemofiltration system** – we have discussed this as a potentially useful system (63) which has provisionally shown promise in patients with severe gram-negative sepsis (64), although definitive evidence is still lacking and the device is currently the subject of intense research interest.
- **Phosphodiesterase inhibitors** – many agents within this class, including Pentoxifylline, HWA 138 and Amrinone have shown encouraging results in septic piglets and premature septic neonates with SIRS by reducing TNF, IL-6 and endothelin-1 and improving survival (65-67). These agents also improve cardiac function in hyperdynamic cardiac failure (68). Clinical trials are currently underway to determine their role in SIRS.

SUMMARY BOX

Treatments for SIRS

- **Physiological;**
  - "Early Goal Directed Therapy"
  - Blood sugar optimisation with insulin
- **Pharmacological;**
  - Monoclonal antibodies;
    Anti-TNFα
    Anti-TNF-R
  - Polyclonal antibodies;
    Anti-LPS (HA-1A and E5)
  - Activated Protein C (Xigris©)
  - Corticosteroids
  - Cholestyramine enterally
  - Antioxidants;
    Selenium
    Glutamine
    Ecosapentanoic acid
- Cytokine absorption/haemofiltration devices;
  - Activated carbon (BioLogic-DTPF)
  - Immobilised antibody systems
  - Synthetic polymer resins (sCD14)
  - Immobilised nano-fibre column haemofiltration (PMX)

CONCLUSIONS

The complexity of the instigating stimulus and subsequent inflammatory cascade has led to significant difficulties in the development of effective treatments for SIRS and MODS. Strategies targeting purported triggers, early mediators and even physiological responses to inflammation have largely been unsuccessful to date. There still remain many unanswered questions in this broad field and it is indeed one of great current interest. Some of the most prominent areas of research relates to the initiators of the pro-inflammatory cascade, how to modulate them, methods of extracting pro-inflammatory cytokines and how genetic polymorphisms may influences the natural history of SIRS in patients. It is clear that a great deal of work into this subject is still needed before clear answers will be forthcoming. However, some encouraging data exists with adsorptive strategies to attenuate the hyper-cytokinaemia associated with SIRS. The necessity for rapid and clinically significant reductions in the levels of cyto-

kines has prompted the search for a high surface area, selective solution to the adsorptive problem. Activated Charcoal and Polymyxin B haemofiltration systems have promising features in this respect, but we look forward to the generation of more exhaustive and definitive research in the future.

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