The majority of deaths amongst critically ill patients requiring intensive care are attributable to sepsis and its sequelae: septic shock, the systemic inflammatory response syndrome (SIRS) and the acute respiratory distress syndrome (ARDS). Patients within the ICU who develop these conditions and fail to survive succumb to multiple organ dysfunction syndrome (MODS). ARDS is considered to be the pulmonary component of MODS and is characterized by pulmonary hypertension, often in the setting of systemic hypotension. Endothelial cells, normally responsible for modulating vascular tone, becomes dysfunctional in sepsis. Pro-thrombotic, pro-inflammatory and vasoactive mediators are released including nitric oxide (NO), endothelins (ETs) and products of cyclo-oxygenase metabolism. It is probably the disordered production of these mediators in vascular beds that results in MODS. This review highlights recent research in this area with particular emphasis on possible therapeutic options.

Sepsis and its associated syndromes (Table 1) represent a formidable problem in many areas of clinical practice, afflicting more than 1% of hospital patients and leading in some 40% of cases to circulatory failure or septic shock\(^1\). An identifiable microbiological source of infection is found in less than 50% of patients with the clinical manifestations of sepsis\(^2\), the remainder displaying the systemic inflammatory response syndrome (SIRS). Many authorities consider that sepsis, septic shock and SIRS represent a continuum of severity of the host response to non-infective and infective insults, the extent of which may hold prognostic significance. Thus, mortality rises from around 7% for patients with SIRS to 50–90% for those with septic shock\(^3,4\), and is usually attributable to multiple organ dysfunction syndrome (MODS)\(^5\). Recently, investigators have, therefore, focused on the circulatory failure associated with sepsis and related conditions in the hope that the pathogenesis of MODS might be elucidated.

Historically, the circulatory manifestations of SIRS/sepsis have been recognised and investigated to a varying degree in individual organs.
The endothelium and sepsis

Table 1 Definitions of the systemic inflammatory response syndrome (SIRS), sepsis, septic shock and multiple organ dysfunction/failure (adapted from the American College of Chest Physicians, Crit Care Med 1992; 20: 864–74).

Systemic Inflammatory response syndrome (SIRS)

Two or more of the following clinical signs of systemic response to endothelial inflammation:

- A temperature of > 38°C or < 36°C
- An elevated heart rate 90 beats/min
- Tachypnoea, manifested by a respiratory rate of > 20 breaths/min or hypoventilation (PaCO₂ < 4.25 kPa)
- An altered white blood cell count (> 12 x 10⁹/l, or < 4 x 10⁹/l, in the presence of more than 10% immature neutrophils)

In the setting (or strong suspicion) of a known cause of endothelial inflammation, such as:

- Infection (Gram-negative or Gram-positive bacteria, viruses, fungi, parasites, yeasts or other organisms)
- Pancreatitis
- Ischaemia
- Multiple trauma and/or tissue injury
- Haemorrhagic shock
- Immune-mediated organ injury

Sepsis

The systemic response to infection, manifest by two or more of the following as a result of infection:

- A temperature of > 38°C or < 36°C
- An elevated heart rate > 90 beats/min
- Tachypnoea, manifested by a respiratory rate of > 20 breaths/min or hypoventilation (PaCO₂ < 4.25 kPa)
- An altered white blood cell count (> 12 x 10⁹/l, or < 4 x 10⁹/l, in the presence of more than 10% immature neutrophils)

Septic shock

Sepsis-induced hypotension (systolic blood pressure < 90 mmHg or a reduction of 40 mmHg from baseline) despite adequate fluid resuscitation

Multiple organ dysfunction syndrome

Presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention

Thus, the acute respiratory distress syndrome (ARDS) is now regarded as the pulmonary manifestation of MODS, but was first described as an isolated phenomenon over 30 years ago. ARDS is characterised by non-hydrostatic pulmonary oedema and refractory hypoxaemia and complicates up to 25% of cases of SIRS/sepsis. Pulmonary hypertension with increased pulmonary vascular resistance (PVR) is common, even in the setting of the lowered systemic vascular resistance (SVR) that characterises SIRS and sepsis. From the late 1980s, ARDS was known to be associated with endothelial dysfunction and disruption, which was recently characterised in vivo using non-invasive radioisotopic techniques. Moreover, the recognition that the refractory hypoxaemia of
ARDS was attributable to a loss of hypoxic pulmonary vasoconstriction in these patients highlighted the importance of vascular control mechanisms in determining the clinical characteristics of the syndrome and possibly the development of organ failure. Indeed, changes in vascular control have been documented in both experimental models and patients with sepsis uncomplicated by ARDS, and are characterised by systemic hypotension unresponsive to pressor agents and inotropes, possibly mediated through changes in the production of endothelially-derived vasomotor agents. The hypothesis that such substances play a significant role in modulating both systemic and pulmonary vascular tone under physiological conditions was proven by the early 1990s, which emphasised further the potentially crucial significance of endothelial barrier and endocrine functions in determining the clinical manifestations of SIRS/sepsis, with particular reference to the development of MODS.

The endothelium and sepsis

There are several comprehensive reviews concerning the pathogenesis of sepsis and related conditions, but the uniformly negative results of clinical trials in this area over the past 5 years has changed the way these syndromes and MODS are viewed. Thus, the clinical manifestation of a given insult may be determined by the extent to which exogenous influences activate endogenous pro- and anti-inflammatory...
Table 2  Substances produced by the endothelium

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<td>Thrombomodulatory</td>
<td>Thrombomodulin</td>
<td>Tissue plasminogen activator</td>
<td>Heparan sulphates</td>
<td>Von Willebrand factor</td>
<td>Ecto ADPases</td>
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<tr>
<td>Vasoactive</td>
<td>Prostacyclin, thromboxane</td>
<td>other prostanoids</td>
<td>Nitric oxide</td>
<td>Endothelins</td>
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<tr>
<td>Adhesion molecules</td>
<td>E-selectin</td>
<td>ICAM 1 and 2</td>
<td>VCAM</td>
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<tr>
<td>Inflammatory molecules</td>
<td>Platelet activating factor</td>
<td>Cytokines: IL-6, IL-8, MCP-1</td>
<td>Class II MHC molecules</td>
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Abbreviations: ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; IL, interleukin; MCP, monocyte chemoattractant protein.

Endothelial cell activation

The endothelium is the intimal layer of the vasculature, comprising simple squamous cells that provides a continuous, fluent surface for circulating blood. Endothelial cells exert active control over vascular tone, supply thromboresistance and determine the extent to which the vasculature is permeable to cells and molecules through the synthesis and release of a wide variety of substances (Table 2, Fig. 2A). Under inflammatory conditions, endothelial cell activation occurs, leading to a loss of vascular integrity, increased expression of leucocyte adhesion molecules, a change in phenotype from anti- to pro-thrombotic, cytokine production and an up-regulation of HLA molecules. Two stages of activation occur. The first is endothelial cell stimulation or Type I activation, which does not require de novo protein synthesis nor genotypic up-regulation. Endothelial cells retract from each other, express P-selectin leading to increased neutrophil adhesion, and release von Willebrand factor which regulates platelet adherence to the sub-endothelium. Type II activation processes, and which ultimately becomes the dominant force. Although these processes may be generated locally by tissue trauma, or systemically by an infective organism undergoing haematogenous spread; pro-inflammatory influences, principally mediated via cytokines, interact initially with the vascular endothelium.
Fig. 2 (A) The endothelial cell maintains vascular homeostasis by the tonic release of mediators such as nitric oxide (NO) via constitutive nitric oxide synthase (eNOS), prostacyclin (PGI2) via cyclo-oxygenase-1 (COX-1) and endothelin-1 (ET-1) from the increased transcription of preproET (ppET), conversion to proET (pET) and conversion to endothelin by endothelin converting enzyme (ECE). BK and CYT represent stimuli such as bradykinin and cytokines respectively.

(B) In sepsis the presence of endotoxin and early inflammatory cytokines (TNF, IL-1) results in endothelial cell activation and disruption. The activated cells produce larger quantities of NO via inducible NOS (iNOS), prostaglandins (PGs) via COX-2 and endothelin (ET). The smooth muscle appears to have an important, if not the most important, contribution under these conditions. The relative proportions of NO, PGs and ET determine the vascular response in the affected area.
requires up-regulation of mRNA expression and \textit{de novo} protein synthesis, particularly of cytokines and adhesion molecules. There appear to be common intracellular control mechanisms involved in this process, such as those mediated through the intracellular messenger nuclear factor kappa beta (NF-\kappa B)\textsuperscript{14}. The endothelium produces vascular cell adhesion (VCAM-1) and intercellular adhesion (ICAM-1 and ICAM-2) molecules and E-selectin, facilitating the binding of leukocytes. Simultaneously, activated neutrophils express a complementary sequence of surface adhesion molecules termed integrins, the most significant of which is the CD11/CD18 complex, which determine the migration of neutrophils into the interstitium. This adhesion cascade is reviewed in detail elsewhere\textsuperscript{15}, but is associated with increased expression of endotoxin/cytokine inducible genes that are significant in determining vasomotor control, particularly those encoding for the production of nitric oxide (NO), endothelins (ETs) and cyclo-oxygenase (COX) products (Fig. 2B).

\textbf{Endothelially-derived vasoactive mediators}

\textbf{Nitric oxide: production and regulation}

In 1980, an endothelially-derived relaxant factor (EDRF) was proposed as the mediator responsible for the vascular smooth muscle relaxation caused by acetylcholine, a process that required an intact endothelium\textsuperscript{16}. EDRF was later shown to be pharmacologically identical to NO. NO stimulates guanylate cyclase to form cyclic GMP (cGMP) leading to a reduction in intracellular calcium, thereby modulating dilation in both arterial and venous vascular smooth muscle (Fig. 3). NO is synthesised from the terminal guanidine nitrogen of the semi-essential amino acid L-arginine by a group of flavin-containing enzymes collectively called NO synthases (NOS), a process that can be inhibited by L-arginine analogues such as \textit{\textit{N}}\textit{G}-monomethyl-L-arginine (L-NMMA)\textsuperscript{17}. There are at least three different NOS isoforms all requiring NADPH and tetrahydrobiopterin as cofactors. Endothelial NOS (eNOS) and neuronal NOS (nNOS) are constitutive, and calcium- and calmodulin-dependent enzymes. A third isoform (iNOS) is induced by pro-inflammatory substances such as lipopolysaccharide (LPS) and interleukin (IL)-1 and is calcium- and calmodulin-independent. All three isoforms have been purified, cloned, sequenced and expressed. The basic pathway of metabolism of L-arginine to NO and L-citrulline is well conserved and all isoforms are inhibited by L-arginine analogues\textsuperscript{18}. Although endothelial cells were the first shown to produce NO in mammals, it is now known to be produced in a wide variety of sites. NOS inhibitors cause a rapid increase in systemic blood pressure and changes in regional blood flow in normal animals and man, suggesting that NO is continuously released.
in order to regulate blood flow under physiological conditions\textsuperscript{6}. Specifically, the quantity of NO produced depends on basal vascular tone. Increased flow (and, therefore, shear forces) can augment agonist-evoked endothelium-dependent relaxation\textsuperscript{1}. In the pulmonary vasculature, NO production is reduced under hypoxic conditions and as such may modulate hypoxic pulmonary vasoconstriction (HPV)\textsuperscript{19}. In rats, the transfer of constitutive eNOS genetic material using viral vectors reduces HPV\textsuperscript{20}.

**Nitric oxide: production in experimental sepsis**

In animal models of sepsis up to 1000 times the physiological concentration of NO have been identified\textsuperscript{18}. Endotoxin, and the cytokines IL-1, interferon-\(\gamma\) (IFN-\(\gamma\)) and tumour necrosis factor (TNF) induce iNOS in vascular smooth muscle\textsuperscript{21}. Interleukin-2 administered therapeutically to patients with malignant conditions also induces the production of NO metabolites\textsuperscript{1}. The sequence of events that actually leads to increased NO production in such circumstances is more difficult to characterise. Moreover, iNOS induction has not yet been identified clearly in human beings. Nevertheless, mRNA encoding for iNOS production is detectable in rat pulmonary artery within 20 min of exposure to endotoxin \textit{in vivo}\textsuperscript{22}, but the production of protein almost certainly takes considerably longer. Thus, hyporeactivity to noradrenaline starting within 60 min of the administration of an infective insult to a rodent is too rapid to be explained by the production of NO by iNOS alone. It is possible that the early and relatively modest increased production of NO is attributable to the activity of constitutive NOS; although the availability of tetrahydrobiopterin (BH\textsubscript{4}) may be rate-limiting. Nevertheless, 3 h after an endotoxic insult there is a massive increase in NO production as a result of the appearance of iNOS activity in both endothelium and vascular smooth muscle. An intact endothelium appears to be required for maximal NO response, such that its removal causes a significant delay in the onset of vascular hyporesponsiveness (6 h compared with 4 h) in the rat aorta.
exposed to lipopolysaccharide (LPS) fraction of endotoxin in vitro. The response is prevented by pretreatment with dexamethasone, which limits the expression of mRNA encoding for iNOS at a transcriptional level. Mice lacking iNOS, although unable to increase their nitrate production, are not protected against the lethal effects of LPS.

The lung is a major site of iNOS expression in sepsis. Inducible NOS mRNA has been demonstrated in the pulmonary arteries of rats treated with LPS, in which NO release is responsible for the hyporesponsiveness to constrictor agents seen in this tissue. The induction of iNOS in lung tissue and pulmonary arteries is inhibited by dexamethasone. Inhibition of iNOS with the specific inhibitor aminoguanidine also protects against LPS-induced pulmonary hypertension but further impairs HPV with obvious implications for the application of such therapy in patients with ARDS and elevated PVR. Recent work in rodent models has also suggested that eNOS may be down-regulated, increasing endothelial dysfunction. Under these conditions, the underlying smooth muscle is a major source of NO via increased iNOS expression.

Endothelins: production and regulation

Endothelins (ETs) are the most potent naturally occurring vasoconstrictors identified to date. Three isoforms have been identified, ET-1, ET-2 and ET-3, each of which is a 21 amino-acid peptide related to the snake venom sarafotoxin. ETs are formed following cleavage of so-called ‘big-endothelin’ by an endothelin-converting enzyme (ECE). Big-endothelin is formed by cleavage of a pre-pro-peptide (ppET). Endothelin converting enzyme is a membrane-bound glycoprotein metallopeptidase, of which there are now known to be several different isoforms. Most evidence suggests that ETs, of which ET-1 is the predominant isoform in the human vasculature, are not stored but synthesised de novo. ET-1 is thought to be produced predominantly in the endothelium but is also found in vascular smooth muscle and cardiac myocytes. A wide number of factors stimulate ET-1 release including vessel wall shear stress, hypoxia, endotoxin, TNF, interferon, adrenaline, angiotensin, thrombin, activated platelets and some prostanoids. ET-induced smooth muscle contraction occurs via several secondary messenger pathways. However, phospholipase C activation leading to increased inositol triphosphate and diacylglycerol synthesis is thought to be the principal effector system, although protein kinase C is also involved.

Two ET receptor types have so far been cloned and expressed. ETA has a higher affinity for ET-1 than ET-2 and ET-3 and is expressed widely, especially on vascular smooth muscle cells. ETB is non-selective and binds all three ETs equally. ETB receptors are found both on the endothelium and smooth muscle in some vascular beds. It is thought generally that ETA stimulation is responsible for the direct constrictor effects of ET-1.
and that ETB stimulation results in the release of the vasodilator NO and the vasoconstrictor and dilator COX products thromboxane (Tx) A and prostacyclin (PGI₂). However, ETB receptors also exist on some vascular smooth muscle cells and can mediate contraction directly, and ETA receptors are capable of releasing prostanoids. A bolus injection of ET-1 administered to man or animals causes a transient fall in blood pressure, which can be antagonized by L-NMMA pre-treatment, followed by a sustained vasopressor response. This latter response can be attenuated by an ETA receptor antagonist¹,²,²⁹.

Genetically-modified mice with deletions for ET-1, ET-3, ECE, ETA and ETB have been produced. ETA and ET-1 deficient mice have a similar phenotype with distinct craniofacial abnormalities and malformed thoracic blood vessels. Homozygous mice die soon after birth due to respiratory failure. Heterozygous mice are (unexpectedly) hypertensive³¹. ET-3 and ETB are also phenotypically similar and characterised by toxic megacolon and piebald spotting³². This implies a role for ET-3 and ETB in the migration of neural crest cells. However, these models have not to date provided data that define the vasoactive roles of the ETs and their receptors.

ET-1 is cleared predominantly in the pulmonary vasculature. This process seems to be ETB receptor mediated, at least in rats, as ET-1 levels are increased after treatment with an ETB antagonist⁶. Defects in the ratio
of clearance to production of ET-1 by the lungs, and not the systemic circulation, can result in increased circulating levels of the peptide\textsuperscript{33}.

**Endothelins: production in experimental sepsis**

ET-1 receptor (ETA and/or ETB) antagonists reduce the rise in PVR induced by hypoxia\textsuperscript{34} in rodents. ECE inhibition attenuates the pulmonary hypertension seen after cardiopulmonary bypass in the piglet\textsuperscript{35}. Additionally, ET-1 can induce the expression of platelet-derived and transforming growth factors in cultured smooth muscle cells, suggesting it is important in modulating vascular remodelling\textsuperscript{36}. The effects of ET-1 on vascular tone are probably the result of autocrine and paracrine activities, especially since the release of ET-1 from endothelial cells is polarized abluminally, that is towards the smooth muscle. Circulating ET levels may not be a true reflection of local tissue concentrations. In endotoxin-treated rats pre-pro-ET-1 mRNA is increased in several tissues (heart, lung, aorta and pulmonary artery) although not seen in kidney or skeletal muscle\textsuperscript{37}. ET-1 mRNA and peptide production are increased in human vascular smooth muscle cells when exposed to cytokines and LPS\textsuperscript{38}. This would suggest that, under inflammatory conditions, the vascular smooth muscle as well as the endothelium may be a site of significant ET-1 production.

The role of these peptides in sepsis remains unclear. It would be tempting to speculate that ETs contribute to the pulmonary hypertension and reduced blood flow to the kidneys and splanchnic circulation seen in sepsis despite systemic hypotension. Isolated pulmonary artery rings from rats treated with LPS are hyporesponsive to exogenous ET-1, an effect potentiated by removal of the endothelium and by both thromboxane A\textsubscript{2} and ETB receptor antagonists\textsuperscript{39}. Again, in pulmonary arteries removed from rats rendered endotoxaemic, ETA receptor blockade attenuates ET-1 induced contraction. ETB receptor activation causes only vasodilation via the release of NO, an effect lost after treatment with endotoxin\textsuperscript{40}. Rats treated with endotoxin exhibit an attenuated depressor phase of the hemodynamic response to ET-1 but no attenuation of the pressor phase\textsuperscript{41}. The combined ET receptor antagonist bosentan administered prior to endotoxin challenge attenuates the second phase of elevation of PVR\textsuperscript{42}. In conscious rats undergoing LPS infusion over 24 h, the non-selective ET receptor antagonist, SB209670 enhances the fall in mean arterial blood pressure, and converts the mesenteric vasoconstriction to LPS infusion alone to significant vasodilation\textsuperscript{43}. Finally, despite demonstrating raised levels of ET-1, the combined ET receptor antagonist bosentan did not affect the resting haemodynamics in endotoxemic rats compared to controls\textsuperscript{44}. These findings suggest that the effectiveness of ET antagonists is unpredictable in sepsis and suggests that further investigation is required aimed at elucidating the receptor and secondary messenger pathways in septic animals.
Apart from its vasoconstrictor effects, ET-1 is a smooth muscle mitogen and may contribute to the vascular remodelling and pulmonary hypertension seen in acute lung injury. Under these inflammatory conditions, endothelial dysfunction results in reduced release of the NO and prostacyclin formation induced by ET-1, contributing further to an overall constrictor effect. Increased levels of circulating ET-1 seen in sepsis may be due to reduced clearance or increased production. Studies suggest that both mechanisms may occur, the lung being the probable site for both.

Cyclo-oxygenase products: production and regulation

Prostaglandins are important regulatory mediators of cardiovascular and pulmonary functions. The known synthetic pathways are summarised in Figure 5. Of the various derivatives, prostacyclin and thromboxane are probably the most important. Prostacyclin (PGI₂) is a potent vasodilator, acting on G-protein coupled receptors to increase intracellular cAMP levels. It is also an important inhibitor of platelet aggregation. Thromboxane (TXA₂) is a potent constrictor of pulmonary arterioles after endotoxin infusion, and is also capable of increasing
capillary permeability. Conversely, it causes the aggregation of platelets. The most important enzyme appears to be cyclo-oxygenase (COX). COX has two isoforms (COX-1 and COX-2) which are encoded by separate genes. COX-1 is constitutively produced and is thought to contribute to the maintenance of physiological homeostasis, whereas COX-2 is expressed at high levels upon induction\(^7\). This induction is rapid and the gene encoding COX-2 has been termed an immediate/early or primary response gene. Many of the agents that cause induction of COX-2 act on receptors that have an intracellular tyrosine kinase domain, activation of which results in the phosphorylation of proteins. COX-2 leads to the release of a large amount of prostaglandin and thromboxane. Absent from normal tissue, COX-2 is expressed at sites of inflammation and in monocytes and macrophages stimulated with lipopolysaccharide or interleukin-1. Expression of COX-2 is inhibited by anti-inflammatory glucocorticoids both in vivo and in vitro, and by anti-inflammatory cytokines such as IL-4 and IL-10\(^8\). COX-1\(^9\) and COX-2\(^5\) deficient knock-out mice have provided little data on the vasoactive roles of these mediators. ET-1 stimulates the release of the vasodilators prostacyclin and prostaglandin E\(_2\), as well as the vasoconstrictor, thromboxane, possibly via ET-1 induced activation of protein kinase C\(^1\). In isolated human internal mammary artery, prostacyclin reverses ET-1 induced vasoconstriction\(^1\). There are obviously parallels between the COX and NOS enzyme systems which raises the question of possible ‘cross-talk’. Interestingly, endogenously released NO has been shown to both inhibit and activate COX-2 and possible ‘feedback’ mechanisms may be important in vivo\(^7\).

The exact role of cyclo-oxygenase products under conditions of hypoxia is not clear. That they are important is demonstrated by the observation that indomethacin augments HPV. However, flurbiprofen, a different NSAID, worsened HPV in isolated human pulmonary arteries. Furthermore, the actions of prostaglandins appears to vary with the size of artery\(^6\).

Isoprostanes are a newly described group of prostaglandin-like compounds which can be produced independently of the COX pathway under conditions of oxidative stress. 8-iso prostaglandin F\(_{2\text{-alpha}}\) (8-isoPGF\(_{2\text{-alpha}}\)) is increased approximately 3-fold in patients with non-insulin dependent diabetes mellitus, a condition associated with increased oxidative stress and endothelial dysfunction. 8-IsoPGF\(_{2\text{-alpha}}\) has been shown to have both constrictor and dilator functions in rat pulmonary arteries. The constrictor action is inhibited by thromboxane receptor antagonists. Vasodilatation appears to act via NO and the predominant action of 8-isoPGF\(_{2\text{-alpha}}\) is, therefore, concentration-dependent and linked to the level of endogenously produced NO. This would imply that 8-isoPGF\(_{2\text{-alpha}}\) is a dilator under the normal conditions of a reducing environment with low oxidant stress, but may reach concentrations
sufficient to produce vasoconstriction during the pro-oxidant conditions that pertain in sepsis\textsuperscript{51,52}.

**Cyclo-oxygenase products: production in experimental sepsis**

Bacterial endotoxin, cytokines and mitogens have been reported to induce COX-2 mRNA or protein expression, whereas anti-inflammatory cytokines IL-4 and IL-10 inhibit expression\textsuperscript{48}. Several respiratory cells such as alveolar macrophages, lung fibroblasts, pulmonary microvascular endothelial cells and alveolar or trachobronchial epithelial cells express COX-2 mRNA and/or protein \textit{in vitro} when stimulated with endotoxin or cytokines. COX-2 induction and corresponding increases in PGI\textsubscript{2} have been demonstrated around 6 h following IL-1 therapy, which may represent a protective response to the pulmonary hypertension seen with sepsis. COX-2 induction is also seen in cultured human endothelial cells after exposure to endotoxin\textsuperscript{6}.

Rats treated with LPS \textit{in vivo} display differential regulation of COX-1 and COX-2 gene expression. LPS up-regulates COX-2 with a corresponding down-regulation of COX-1. Neither the change in expression is affected by pre-treatment with dexamethasone, although expression of iNOS induced by LPS is markedly inhibited in the same tissues\textsuperscript{53}. The loss of the ‘cyto-protective’ COX-1 effect as well as the up-regulation of COX-2 may lead to a loss of vascular control and organ failure in patients with sepsis. In rat aortae in culture treated with LPS, COX-2 and iNOS are co-induced in intact vessels, the vascular smooth muscle being the main site of mediator production. COX and NOS pathways appeared to act independently in that L-NAME has little effect on the activity of COX, and indomethacin does not influence NOS activity\textsuperscript{54}.

**Manipulation of the inflammatory response in patients with sepsis**

Mortality associated with septic shock remains high. Most treatment aims at stabilising and supporting the patient. To date there is very little evidence that manipulating the individual elements of the inflammatory cascade or vasoactive mediators affect mortality. The few trials that have been carried out are under-powered and non-randomised.

**Nitric oxide production and manipulation**

The loss of vasoconstriction induced in the pulmonary and systemic circulations by NO may be clinically significant in patients with ARDS.
and sepsis, respectively. There are numerous reports of increased levels of
the nitrogen oxides in both adult and pediatric patients with sepsis. If
NO is responsible for the systemic vasodilation that characterises sepsis,
levels of circulating nitrogen oxides might be expected to correlate with
the extent of haemodynamic disturbance. Few studies have been
performed in this area, but a mixed group of trauma victims and
patients with sepsis were found to display a significant reduction in SVR
when NO concentrations fell more than 1 SD outside the mean control
value. Other studies have used hemodynamic measurements or
dependency upon pressor agents to quantify vasodilation in this context.
Methemoglobinemia may be induced by inhaled NO at high
concentrations and theoretically by increased local (i.e. endothelial)
production. High methemoglobin levels have been identified in children
with septic shock. Tissue concentrations of cGMP have been used as
markers of activity of the L-arginine NO pathway, and higher levels have
been identified in critically ill patients with septic shock compared to
appropriate controls. Levels of exhaled NO seem to be reduced in
patients with both SIRS induced by cardiopulmonary bypass and full
blown ARDS, despite evidence of inflammatory cell and endothelial
activation. Whether this apparent anomaly is attributable to changes in
NO scavenging or do not accurately reflect events in the pulmonary
circulation is unclear.

Both NOS isoforms can be inhibited in a competitive manner by
analogues of L-arginine. Examples are L-NMMA and L-NAME (N\textsuperscript{G}-
nitro-L-arginine methyl ester). It would appear logical that inhibition of
NOS should help sepsis-induced hypotension. Administration of NOS
inhibitors to patients with sepsis or animals treated with LPS results in
a rise in SVR. However in a porcine model of endotoxaemia NOS
inhibition, given as a continuous infusion, caused a decrease in cardiac
output and an exacerbation of pulmonary artery pressure. In a recent
study in septic humans, similar findings were described. Thus, it may
be detrimental to inhibit both the constitutive and inducible forms of
NOS. Specifically, cNOS produces NO which has anti-thrombotic and
protects against microvascular leakage and cell adherence, regulating the
blood flow in the microcirculation.

More recently, agents have been developed that selectively inhibit the
inducible form of NOS. Aminoguanidine, is 7-fold more selective for
iNOS than L-NMMA, and caused a dose dependent increase in
phenylephrine-induced tension in intact and endothelium-denuded
pulmonary artery rings from endotoxin-treated rats, but had no effect
on sham-treated rats. However, its toxicity limits its use in humans and
newer less toxic agents are awaited.
**Endothelin production and manipulation**

ET-1 expression is increased in the plasma\(^6^2\) and lungs\(^6^3\) of patients with pulmonary hypertension developing in association with a variety of conditions including ARDS\(^4^6\). ET-1 levels are also elevated in critically ill patients, which is to an extent related to the severity of illness and correlates to outcome\(^6^4\). Although there has been little data describing manipulation of endothelin in such patients, bosentan, an endothelin-receptor antagonist, has been used to lower blood pressure significantly in patients with essential hypertension\(^6^5\).

**COX production and manipulation**

Patients with ARDS display increased serum levels of TXA\(_2\). TXA\(_2\) is a vasoconstrictor and its inhibition has been shown to diminish the early pulmonary hypertension in an experimental model of endotoxin-induced sepsis\(^6\). Thus, the local production of vasodilator (PGL\(_2\)) and vasoconstrictor (TXA\(_2\)) agents contributes to the vascular tone in that area. The differential regulation of COX-1 and COX-2 genes raises the possibility that increased COX-2 and decreased COX-1 activity may contribute to the loss of microvascular control that leads to organ failure in patients with sepsis. In addition, downregulation of COX-1 with non-steroidal anti-inflammatory agents leads to gastric ulceration and renal dysfunction. This may be due to loss of the cytoprotective effect of PGE\(_2\). This may also be true in patients with sepsis\(^4^7\). Ibuprofen, a non-selective inhibitor has not shown benefit in trials to date\(^6^6\). Therefore, attempts have been made to develop selective COX-2 inhibitors and clinical trials are underway.

**Steroid therapy in sepsis**

The molecular mechanisms underlying the anti-inflammatory effects of glucocorticoids are not fully understood, but there is increasing evidence that they inhibit the action of transcription factors such as AP-1 and NF-kB\(^6^7\). Unfortunately, theoretical benefits of this application in sepsis have not been shown in practice. Two meta-analyses of trials of high-dose glucocorticoids were published recently\(^6^8,6^9\) and both concluded that there was no evidence of beneficial effect in patients with sepsis. Indeed there was overall a higher mortality in the treatment arms of these studies.
The endothelium and sepsis

Non-steroid therapies

In a recent review of trials of non-glucocorticoid agents in sepsis, 18 studies published from 1989 to 1997 were included that contained survival data and included a control group. Interventions included interleukin-1 receptor antagonist (IL-1ra), anti-bradykinin antibodies, anti-platelet activating factor, anti-tumour necrosis factor antibodies, soluble TNF receptor and anti-prostaglandin (ibuprofen). Meta-analysis revealed a small beneficial effect that was not statistically significant. Reassuringly, nearly all control groups had a similar mortality rate (36%). More alarmingly the high molecular weight (P 80) soluble TNF receptor demonstrated an increase in mortality in the treatment group. The beneficial effect of non-steroid interventions in sepsis is, therefore, likely to be small. Large numbers of patients may be needed to show a significant difference. The results of high doses of P 80 soluble TNF receptor and glucocorticoids suggest that high doses of anti-inflammatory agents in sepsis may even be detrimental.

Potential therapies

A review of potential therapies has recently been published by the American-European Conference on ARDS and included anti-oxidant therapies, anti-proteases, antibodies directed against adhesion molecules, pro-inflammatory approaches and genetic manipulation. Few of these have reached clinical trials despite promising results in animals.

Conclusions

The endothelium is a metabolically active layer of cells that in health is able to maintain local blood flow and homeostasis. Under conditions of sepsis such balance is lost leading to a pro-thrombotic, pro-inflammatory state with production of vasoactive mediators such as NO, endothelin and cyclo-oxygenase products. Much of the production of such mediators is performed by the underlying smooth muscle. The relative production of vasodilators (such as NO and prostacyclin) or vasoconstrictors (such as endothelin and thromboxane) may determine the local pattern of vascular response and hence organ dysfunction. As such, the endothelium plays a central part in the modulation of the vascular response to sepsis. Research in this area has led to the production of potentially valuable clinical treatments such as specific inhibitors of iNOS and COX-2 and ET receptor antagonists. Results of clinical trials are eagerly awaited.
Acknowledgement

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