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The role of the microcirculation in multiple organ dysfunction syndrome (MODS): a review and perspective

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Abstract Major advances in intensive care medicine during the past two decades have altered the spectrum of disease encountered by intensive care physicians, anaesthesiologists, traumatologists and pathologists. One of the most important manifestations of severe trauma or infections is the multiple organ dysfunction syndrome (MODS), a life-threatening condition that often ends in multiple organ failure (MOF) and death. Evidence gathered from clinical and morphological observations in humans, taken together with experimental animal studies and a vast accumulation of in vitro data, clearly indicate that the microcirculation lies at the centre of this complex process, which results in peripheral vascular insufficiency, inadequate oxygen delivery to vital organs, and hence, severe organ dysfunction. The multifunctional nature of the endothelium makes it a prime candidate for study of the pathomechanisms of MODS. This paper reviews the evidence for the hypothesis that the microcirculation, and in particular its endothelial component, has a central role in the pathogenesis of MODS. The evidence is reviewed principally from the standpoints of classical morbid anatomy and cell pathobiology.

Key words Multiple organ dysfunction syndrome · MODS · Sepsis · Microcirculation · Endothelium

Introduction and definitions

Multiple organ dysfunction syndrome (MODS) describes the broad spectrum of symptoms and signs that form the background against which multiple organ failure (MOF) develops. In many cases, MODS develops after a certain latency period in the context of a host reaction to a primary insult (e.g. trauma, burn injury, pancreatitis), this reaction being generally termed SIRS (systemic inflammatory response syndrome [20]. A further significant component is the frequent complication of sepsis or even septic shock. Although these processes involve the activation of numerous mediator cascades, the present paper will concentrate on the role of the endothelium, especially in the microcirculation, in responding to these mediators. Space constraints preclude a detailed discussion of the role of inflammatory mediators, which is presented elsewhere [89], although the most significant findings will be cited as necessary background to allow understanding of the alterations in the microcirculation in response to their release. Three main lines of evidence for the central role of the microcirculation in MODS have emerged – human studies, experimental animal investigations and studies on in vitro systems.

Dedicated to Professor Dr. Christian Mittermayer on the occasion of his 60th birthday

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Studies in humans

Evidence from clinical observations

Pathophysiological observations

The application of modern technology to intensive care medicine has led to numerous monitoring methods, both invasive and non-invasive, that facilitate the simultaneous determination of physiological variables in the central and peripheral circulation. These include data on flow, pressure vascular resistance and oxygen content, enabling the differential kinetics and hence the efficiency of the cardiovascular and respiratory systems to be evaluated accurately [6, 72]. The quintessential observation that can be made from these data is that strict control of peripheral vascular function is lost, leading to maldistribution of blood flow and markedly decreased oxygen uptake by the tissues [135, 174].

Pathobiochemical data

Numerous clinical studies indicate that both plasma and cellular inflammatory mediator systems are activated in the course of MODS. This occurs on the basis of SIRS, which is the generalized inflammtory response to injury [20]. The frequent association of sepsis and MODS [13] and the known ability of bacterial wall products to activate inflammatory mediator systems [123] has led to detailed monitoring of mediator levels with time (mediator mapping) in various states leading to MODS, with a view to pattern recognition, correlating with prognosis and thus serving as an objective instrument for therapeutic activity. What is rational and easily stated in theory turns out to be much more complicated in practice, for three principal reasons. First, severely ill or injured patients are not left to follow the natural course of events, but are treated by complicated invasive therapeutic regimens, which make controlled clinical studies difficult. Nevertheless, clinical research groups attempt to compensate for the heterogeneity of disease states by using scoring systems, such as the APACHE scores or the Injury Severity Score (ISS) [7, 47], which enable an acceptable level of standardization. Second, early events in the pathogenesis of MODS are difficult to observe, as many of the patients are victims of trauma. Nevertheless, attempts are being made to overcome the logistic problems of emergency medicine to gain a window on the acute phase (up to 3 h) after injury. Third, the mediators released in MODS are legion, so that unravelling primary from secondary cascades is problematic.

Cytokines and their pivotal role in sepsis and MODS have received much attention during the past decade, with particular interest being focused on tumour necrosis factor alpha (TNF-α) and on the interleukins 1, 2 and 6 (IL-1β, IL-2, IL-6) [27, 41, 50, 184], and more recently, IL-8 [63, 73, 194], all of which can be produced by cells of the monocyte/macrophage series [52, 137]. Casey et al. have shown that the three cytokines, IL-1β, Il-6 and TNF-α, as well as endotoxin (lipopolysaccharide, or LPS), can be detected in the circulation of patients with the sepsis syndrome regardless of blood culture status [34] and that a score derived from these levels correlates with survival. The initial euphoria about TNF-α, suggesting a correlation between TNF-α levels in the blood and the development of MOF [190], has been countered by other data indicating variously that *persistence* and not necessarily peak levels of cytokines predict poor prognosis [137] and that serum TNF-α levels are not significantly elevated in trauma patients who subsequently develop MOF [145]. Furthermore, although endotoxin is accepted as an essential component in the pathogenetic cascade in MODS and sepsis [160] and can elicit monocyte release of cytokines such as TNF-α [189], the therapeutic use of anti-endotoxin and anti-TNF antibodies has yielded disappointing results in some experimental studies and, especially, in the clinical situation [9, 53, 115].

Although much attention has been paid to the significance of cytokines in the pathogenesis of MODS, numerous publications indicate that other mediator systems are of importance. Among these are metabolites of the arachidonic acid cascade, such as the leukotrienes [117, 167], thromboxane A2 [139, 176] and prostacyclin [193], the complement system [179, 180], the coagulation cascade [142, 178], products of fibrinolysis [39], platelet activating factor (PAF) [2, 127, 128, 144], the kinin system [76, 183], reactive oxygen species [25] and cellular enzymes, such as granulocyte elastase [106, 185]. A detailed discussion is beyond the scope of this review, although important members of these mediator cascades will be alluded to in the context of modulation of endothelial cell function to be discussed below ("Studies in vitro").

One of the principal lessons to be learned from the diverse pathobiochemical studies both in man and in experimental animals is that no one mediator can be taken as a single prognostic parameter, and that the temporal sequence of production and duration of elevation of mediators [131], as well as their interactions with one another [43], must receive more attention. Furthermore, it needs to be stressed that infection and sepsis do not always accompany MODS following severe trauma, so that mediator systems activated by tissue damage and independent of bacterial products may present different mediator patterns [70, 199]. Future mediator studies will also have to consider the control of the balance between aggressive factors and defence mechanisms, including anti-enzyme systems, such as the anti-proteases, alpha 1-proteinase inhibitor and antithrombin III [85] and anti-oxidants, e.g. superoxide dismutase and glutathione peroxidase [207], and also stress proteins [196]. In addition, certain cytokines exert a down-regulating function in the systemic inflammatory response. IL-10 is a prime example of the latter and is able to block LPS-induced release of TNF and other cytokines [60, 66], as well as tissue factor production in monocytes [148], a trigger of disseminated intravascular coagulation (DIC). IL-4 is also able to down-regulate gene expression of TNF-α and IL-1 in monocytes [54].

Pathological anatomy

Morbid anatomical studies (at light and electron microscopical level) on the adult respiratory distress syndrome (ARDS), which is probably the best-studied element of MODS, gave the first real insight into the central role of the microcirculation [124]. Hydropic swelling followed by necrosis of the endothelial cells (EC) can be found in the capillary network. Impairment of perfusion is further complicated by neutrophilic granulocyte sequestration [158, 171] and microthrombus formation in the lumen of the microcirculation [165]. A cursory glance at these lesions might suggest that the humoral and cellular compartments of the blood would suffice to explain these pathological changes. This impression is misleading. The major advance in our understanding of the pathogenesis of MODS during the past 10 years has been the recognition of the essential role of the endothelium in maintain-

ing and amplifying the microcirculatory disturbances (see section: "Studies in vitro").

Studies in experimental animals

The complexity of the disease entities MODS, SIRS and sepsis, coupled with the relative unavailability of suitable human tissue for temporal studies, has promoted the development of numerous animal models in the hope of answering one or more of the following questions: (1) Can the pathophysiological and -morphological changes be reproduced? (2) What is the temporal sequence of mediator production? (3) Can new therapeutic strategies be tested? Considerable controversy surrounds the choice of animal for experimentation, which ranges from small rodents [61] and medium-sized animals, such as the rabbit [59, 84] and dog [82], over larger animals such as the pig [35] or sheep [107, 153] to primates [81, 118]. In addition, there are divergent views on the choice of initiating factor. The latter includes LPS, applied intravenously or intraperitoneally [24, 166], live bacteria, given by the intravenous, intraperitoneal or intramuscular route [33, 38, 58, 64], caecal ligation with perforation [157] and intravenous administration of TNF-α [48]. One of the major problems with some of these models is the discrepancy between the model and the human situation, not just in the choice of animal but, more importantly, in the relevance of the initiating factor. Thus, rodents, such as the rat, can tolerate relatively large doses of endotoxin [61] and also rapidly develop tolerance [108].

The close association between sepsis and MODS has led many research groups to study the pathomechanisms in vivo by infusing bacterial cell wall endotoxins into experimental animals to induce endotoxic shock. In morphological terms such models clearly reproduce the cardinal structural alterations found in human MODS. Thus, granulocyte sequestration and microthrombus formation are found in many vascular regions, including the liver, spleen [133] and lung of rats [176], and the canine lung [150, 151, 202]. Moreover, the early endothelial lesions are among the integral findings in such models. Thus, in the rat degeneration and necrosis of endothelial cells was described in the liver and spleen [133]. Swelling of the endothelium was also reported as a constant lesion in the cerebral capillaries in the rabbit and dog [140] and in the canine lung, liver and skeletal muscle [169].

One of the major challenges in animal models for MODS is the need to reproduce as many aspects of the human syndrome as possible. A critical view of the literature indicates that most animal models focus on one or at most a few variables, for example certain mediators, detectable in the systemic circulation, and that the initiating factor (e.g. endotoxin) is often used in high dosage. The result of such overwhelming insults is that the observation period is often a few hours, or at most a day. Our philosophy of animal experimentation is to choose a *relevant* model, which in the context of MODS implies the following:

1. The clinical context should be simulated. MODS is a disease complex in intensive care medicine, in which the patients, especially those with sepsis, are more often than not under artificial respiration.
2. The cardiovascular and respiratory systems should be as similar to the human situation as possible and should be monitored in a manner similar to ICU practice.
3. The time course of observation should be over days and not hours. ARDS, and in particular its later stage, with interstitial fibrosis, cannot develop in the context of an overwhelming insult, leading to death within a few hours.
4. The chosen initiating factor should aim at producing a disease state in which the pathophysiological, pathobiochemical and pathomorphological changes typical of human MODS are reproduced.

Such prerequisites for a relevant animal model have received attention by some research groups [121, 128, 173].

With these considerations in mind, we developed a porcine model of recurrent endotoxaemia, achieved by infusing a small dose (0.5 μg/kg) of endotoxin over a 60-min period. The animals were intubated and ventilated under conditions of invasive monitoring, enabling such variables, as pulmonary arterial pressure, pulmonary arterial wedge pressure, cardiac output and systemic vascular resistance to be measured [95]. In this 48-h model, the endotoxin infusion was given at 1 h and 22 h, conditions that permitted a sustained shock state. Therapeutic intervention was kept to a minimum, consistent with maintenance of vital functions. In a second model, aimed at monitoring inflammatory mediators, an 18-h observation period was chosen, with three endotoxin infusions at 0, 5 h and 10 h [96]. With this model it has been possible to obtain many of the cardinal features of the human septic shock state, including a hyperdynamic cardiovascular state, marked decrease of systemic vascular resistance, and a typical sequential mediator pattern, involving decreasing peak profiles of PAF, TNF-α and TXB_2, and post-endotoxin peaks of prostacyclin and IL-6. Moreover, this model demonstrated that reproducible morphological alterations could be achieved and included microvascular damage, such as endothelial swelling in the microvasculature of skeletal muscle accompanied by marked protein-rich intestitial oedema [77], a significant finding also described in a hyperdynamic septic state in a sheep model [80]. In addition, we found endothelial swelling in the pulmonary microcirculation with interstitial oedema [89]. The latter is the characteristic change in early ARDS in the human [124]. Further cardiovascular lesions included verrucous endocarditis, especially of the aortic valve, accompanied by endothelial regenerative activity [89] and cardiac muscle changes [78]. These morphological findings give further support to the hypothesis that the endothelium generally, and not just that in the microcirculation, is essentially involved in the septic shock state, which is so intricately associated with MODS and MOF.

Studies in vitro

The contribution of conventional morphological studies to the understanding of the pathogenesis of MODS has been the basis of identification of the central role of the microcirculation. During the last 15 years, cell biology has made a major contribution to deepening our understanding by demonstrating that essential mechanisms are initiated *prior to* the earliest morphological lesions and appear to centre on the endothelium. In this section, predominantly in vitro data will be reviewed, although some in vivo results will be presented to support the pathogenetic relevance of the in vitro model under discussion.

The concept of the endothelium as a passive barrier has been replaced by the concept of a multifunctional interface with numerous sensory and modulator functions, which make it a key player in various physiological and pathological responses. From a pathophysiological viewpoint, the essential sequence in MODS appears to be peripheral vascular insufficiency, leading to maldistribution of flow, which is combined with defective oxygen extraction. The cell pathobiological hypothesis concerning microcirculatory dysfunction is based on the multifunctional nature of the endothelium and regards events centring on this entity prior to the morphological changes (described above) as of major pathogenetic significance. In this view, the main tenet is, therefore, that *endothelial dysfunction* is a principal pathogenetic event in the syndrome complex involving SIRS, sepsis and MODS.

We will consider a number of discreet functions of the endothelium, the causative factors, especially the significance of bacterial wall components, and the roles of blood cells and of hypoxia. Particular emphasis will be placed on the paradoxical functions of the endothelium with respect to coagulation, vasomotor activity, the inflammatory response and mitogenic activity, all of which can be affected in MODS.

The endothelium as the face of Janus

To understand this concept, it is necessary to analyse the broad spectrum of functions with which the endothelium is equipped; flexibility of response enables the endothelium to react appropriately to injury. However, in the context of MODS, this same adaptability can spell disaster [8]. It is relevant to regard the endothelium as the face of Janus, with the following opposing elements: (a) anti- and prothrombogenic activity; (b) vasodilating and vasoconstricting functions; (c) modulation and amplification of inflammation; and (d) growth-inhibitory and growth-promoting functions.

Anti- and prothrombogenic activity

Table 1 lists the most important anti- and prothrombogenic substances produced by the endothelium. Space does not permit a detailed presentation, although some important aspects should be emphasized. The physiological situation in situ involves a predominance of the anticoagulatory activity of the endothelium [19]. It has been known for a long time that EC can rapidly synthesize and secrete prostacyclin [40], a potent inhibitor of platelet aggregation, a function also shared by NO [146]. In MODS, and in particular in the forms associated with sepsis, this delicate haemostatic regulatory activity is tipped in favour of the procoagulant functions of EC. Tissue factor production plays a central part in this alteration [163]. That such a disturbance will promote the development of microthrombi is evident. Numerous inflammatory mediators, including the cytokines, are able to up-regulate the prothrombogenic activity of the endothelium [17].

Table 1 Anti- and procoagulant activity of endothelial cells

Anticoagulant	Procoagulant
Prostacyclin	Tissue factor
EDRF	PAF
t-PA, u-PA (plasminogen activator of tissue or urokinase type)	Factor V
	Factor VIII
Heparan sulphate proteoglycans	Factor IX receptor
Thrombomodulin	Factor X receptor
Protein S	PAI-1

Endothelium and vascular tone

Intensive care physicians have long known the characteristics of peripheral vascular insufficiency. From a pathophysiological viewpoint, the striking feature is the altered function of smooth muscle activity in the microcirculation. EC appear to be instrumental in these changes on the basis of their production of vasodilating and vasoconstricting factors. The most important of the former are prostacyclin and NO (=EDRF, endothelium-derived relaxing factor), whilst the essential vasoconstrictor is the potent endothelin-1 (ET-1). One of the well-known aspects of septic shock is the marked reduction of peripheral vascular tone, implicating a predominance of vasodilator activity, although it must be taken into account that certain elements of the microcirculation can show increased smooth muscle activity. Thus, in the early phase of septic shock peripheral *venous* tone increases [3]. Furthermore, it is known that endotoxin can alter the sensitivity of arteriolar smooth muscle to vasoactive signals such as norepinephrine and vasopressin [5]. A further significant aspect is the heterogeneity of the microcirculation, so that the response to a stimulus in one vascular bed may be entirely different from that in another, even within a particular organ, this being possible without significant alterations in total organ blood flow [101]. In this respect the reduction of endothelial NO release in the splanchnic circulation as a result of ischaemia and reperfusion requires more attention [31]. Flow is a known stimulatory factor in NO production [99].

A delineation of the exact roles of EC factors is complicated by the fact that numerous other vasodilatory and vasoconstricting substances are produced by other members of the inflammatory orchestra, including blood cells and plasma mediator systems. Other vasodilators include kinins, serotonin, histamine and eicosanoids. In addition, it must be noted that NO overproduction by smooth muscle cells in various segments of the microvasculature by induction of the isoform II of NO synthase probably makes a major contribution to the often refractory vasodilatation that characterizes the septic shock state [62]. Important evidence for this is the finding that the cytokines, IL-1 and TNF-α, can induce smooth muscle NO synthase [26]. The latter activity would appear to overshadow EC NO production in the context of sepsis, as it has been shown that TNF-α actually *down*-regulates EC NO synthase [208].

Endothelium and amplification of inflammation

This very complex field of the shifting of a controlled inflammatory process to that of a self-perpetuating amplification of inflammation involves all major components of inflammation: mediators (including bacterial toxins, cytokines, arachidonic acid metabolites, kinins, complement factors and products of the coagulation and fibrinolytic cascades), blood cells (especially monocytes, neutrophilic granulocytes and platelets) and EC. Concerning the last, three aspects should be emphasized.

Autocrine loops for mediator synthesis. This interesting cell biological phenomenon of a cell producing a substance, stimulating further synthesis in the same cell, was shown as early as 1987 for IL-1 (interleukin 1-β) [198]. Thus, IL-1 can induce the synthesis of IL-1 in EC.

Priming effects of mediators. Priming action involves the ability of a biologically insignificant concentration of a mediator to prime a biological system, such as a cell, to respond dramatically to a second mediator at a concentration of the latter that alone evokes no significant activity or only minimal activity. Relevant priming agents with regard to the pathogenesis of MODS appear to be platelet-activating factor (PAF; 1-*O*-alkyl-2(*R*)-acetyl-glycero-3-phosphocholine) [144], which is produced by a variety of cells, including EC, monocytes and granulocytes [22], and also other cytokines [98] and endotoxin [181]. Braquet et al. cite various examples of ways in which PAF, TNF-α, IL-1 and GM-CSF interact with EC and blood cells so that one of the cytokines acts at very low concentration as a primer [23]. Thus, for example, TNF-α can prime human neutrophilic granulocytes to respond to PAF stimulation by a marked increase in superoxide anion release, a factor with damaging effects on EC.

Up-regulation of CAM expression. The regulation of the expression of both constitutive and inducible cell adhesion molecules (CAM) on the luminal surface of EC represents an integral part of the physiological response to injury by controlling the interaction between blood cells and the endothelium. That this is relevant to the pathogenesis of MODS is seen by recalling that a cardinal lesion of this entity is the sequestration of granulocytes in the microcirculation, in which the scene is set for granulocyte-mediated endothelial damage (see above). These CAM belong to two molecular families: the selectins, represented by E-selectin and P-selectin (CD62) and the immunoglobulin gene superfamily, represented by ICAM-1, ICAM-2 and VCAM-1 [14]. Important mediators produced in MODS, such as endotoxin, TNF-α and IL-1, can elicit a marked up-regulation of CAM on the EC surface [1]. This phenomenon can be well demonstrated on EC in vitro (Fig. 1).

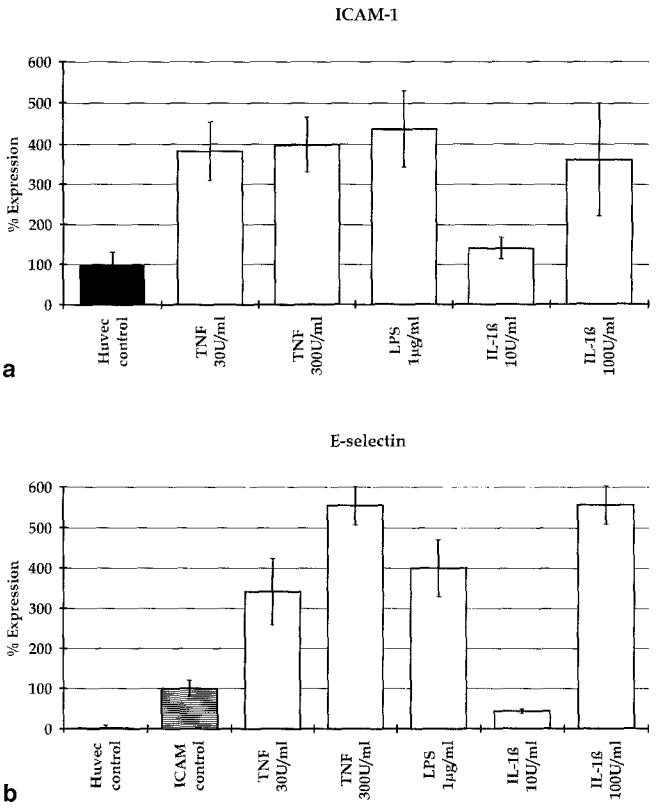

Fig. 1 Up-regulation of **a** ICAM-1 and **b** E-selectin on cultured human umbilical vein endothelial cells (HUVEC) in passage 2. In both histograms the constitutive expression of ICAM-1 on unstimulated cells was set at 100%, and all other values are given as relative percentages. The assay involved spectrophotometric measurement in a cell-EIA (enzyme immunoassay). In both **a** and **b** it can be seen that TNF-α (30 and 300 U/ml), LPS (1 µg/ml) and IL-1β (10 and 100 U/ml) all elicit a statistically significant ($P<0.001$) increase in CAM expression compared to unstimulated controls. Other experiments showed that LPS concentrations of as low as 1 ng/ml were able to stimulate CAM expression on HUVEC

The cytokine-induced up-regulation of CAM on the endothelial luminal surface serves to increase PMN and monocyte adhesion. There is, however, a concomitant activation of the blood cells, both by the present circulating inflammatory mediators and by locally derived activators

from the stimulated endothelium, for example IL-8 and PAF. These up-regulate important members of the integrin family of CAM on the blood cells, such as β2 and β1 integrins, which act as ligands for the CAM on the endothelial surface [14, 113]. Recently, Coughlan et al. were able to support in vitro data by an in vivo model of early (<5 min) neutropenia induced by endotoxin infusion in anaesthetized rats [37]. These investigations indicate that P-selectin, a CAM member responsible for the physiological "rolling" of leukocytes [105], and PAF are instrumental in the early, rapid phase of PMN-EC interaction.

Further convincing evidence that these considerations are not merely academic has been supplied by a baboon shock model. Redl et al. studied the expression of E-selectin in the vascular system of the baboon under hypovolaemic shock or in septic shock, the latter induced by intravenous injection of live *E. coli* bacteria [152]. Whilst in the former there was only minimal expression of E-selectin, the septic shock model gave marked expression in various vascular beds. The latter has been confirmed in a similar model, which also described differential expression of E-selectin, especially in the spleen [46]. LPS infusion in Cynomolgus monkeys induced E-selectin expression in the endothelium, especially in the lung and skin [51]. Preliminary studies from our own laboratory indicate that this principle also applies to the human situation. Thus, the pulmonary microcirculation in human sepsis also demonstrates a marked up-regulation of E-selectin expression (Fig. 2), whereas controls (without sepsis) give no detectable expression. Recently, Newman et al. [129] described a marked elevation of serum levels of a soluble form of E-selectin (sE-selectin) in patients in septic shock, compared with the very low levels detectable in normal individuals. Whether such parameters truly reflect microcirculatory damage and whether they possess any prognostic significance still requires much more detailed research.

Endothelium and growth-regulating signals

In the walls of blood vessels strict growth-regulatory control is exerted by both EC and smooth muscle cells (SMC). In this physiological situation, both cell types synthesize growth-inhibitory substances. However, under certain stimuli very potent growth-regulating molecules can be produced, including transforming growth factor beta-1 (TGF-β1), platelet-derived growth factor (PDGF), basic fibroblast growth factor (b-FGF), IL-1, insulin-like growth factor I (IGF-I) and various proteoglycan species. Cytokine signals appear to be able to up-regulate the synthesis of some of these mitogenic substances. Thus, for example, TNF-α can stimulate EC to produce PDGF [74]. In vitro studies indicate that neutrophilic granulocytes (PMN) induce the release of PDGF and b-FGF from EC [188]. The significance of this for MODS becomes apparent when one considers that ARDS, one of the hallmarks of MODS, involves fibroblastic proliferation and increased matrix synthesis in the lung.

The role of bacterial cell wall products

The close association of sepsis and MODS is well established [13]. This has led to intensive studies on the significance of substances produced by microorganisms, and, in particular, bacteria. Although much attention has been focussed on endotoxins (lipopolysaccharides, LPS) from gram-negative bacteria [147], there is accumulating evidence that peptidogylcans from gram-positive bacteria are also able to elicit critical pathogenetic reactions in the host organism [69]. Concerning LPS, it is now clear that LPS binds with high affinity via its lipid A moiety to a serum protein, LPS-binding protein (LBP). The LPS-LBP complexes then interact with membrane-anchored CD14, especially on blood monocytes, to induce activation, with subsequent production of cytokines, such as TNF-α and IL-1 [143, 189]. With respect to the endothelium, LPS exerts the following important effects.

Direct damage to EC

Using cultivated pulmonary artery EC, Meyrick et al. were able to show that *E. coli* endotoxin at concentrations as low as 1 ng/ml could elicit structural and functional alterations [122]. More recent studies using LPS from *Haemophilus influenzae* indicate that LPS is cytotoxic to brain microvascular EC by a CD14-dependent pathway [136].

Activation of mediator systems

This applies not only to cell-associated mediator cascades, but also to mediator systems in plasma. An essential step in activation of the latter is the activation of coagulation factor XII (Hagemann factor), which can be achieved both by LPS and by peptidoglycans [69]. LPS can also induce EC production of tissue factor [125], IL-6 [197] and von Willebrand factor [71]. Further significant actions of LPS are the suppression of EC thrombomodulin [126] and the induction of plasminogen activator inhibitor-1 [36, 192].

Up-regulation of CAM expression

The importance of this phenomenon has been stressed above. It should, however, be added that various CAM are involved at different times following stimulation. Thus, for example, P-selectin expression appears to play a vital role in the first few minutes after LPS stimulation and is responsible along with PAF for early neutropenia [37], whereas E-selectin reaches a maximal expression after 4–6 h [15]. However, ICAM-1 requires 8–12 h to peak and can remained at elevated levels of expression for more than 48 h [1, 162].

There are still many aspects of endotoxin-induced endothelial injury that are incompletely understood. Delin-

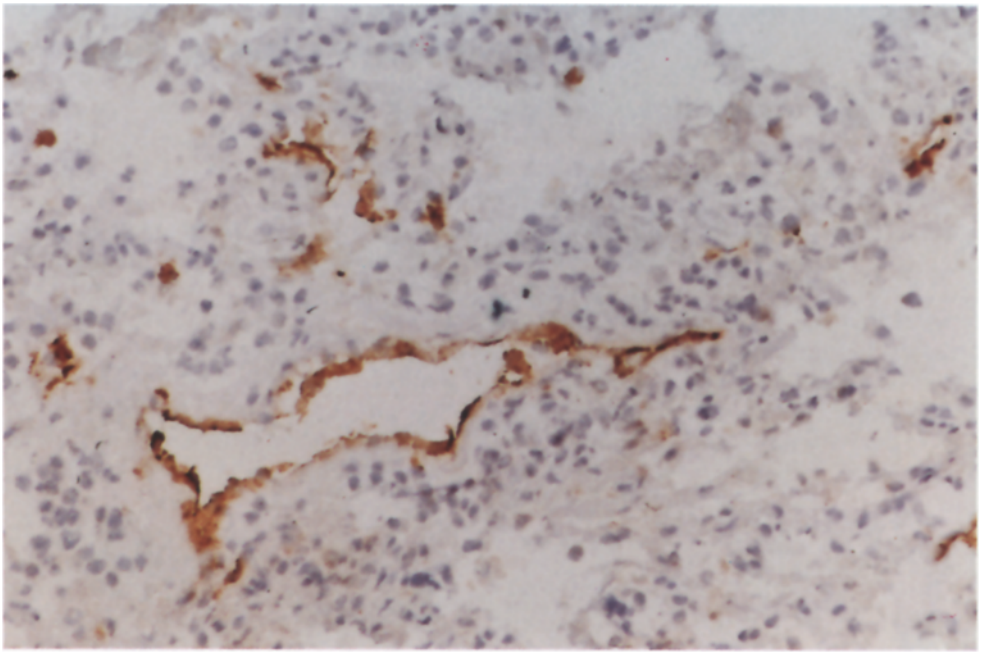

Fig. 2 Immunohistochemical detection of E-selectin in human ARDS associated with sepsis: 5-μm cryosections were air-dried for 4 h, followed by acetone fixation for 10 min at 4° C. Sections were treated with a mouse monoclonal antibody against E-selectin at a dilution of 1:100 and visualized using the avidin-biotin complex method. ×225

eation of the intracellular signal transduction pathways in EC in response to LPS and cytokines might offer feasible ways for therapeutic intervention to inhibit induction of amplification pathways, which perpetuate the inflammatory response and constitute self-aggression. The pathogenetic significance of CAM in regulating blood cell-EC interactions make investigation of their intracellular biochemistry attractive as a possible means of intervention. Fundamental research of this kind is under way [79] and indicates that, for example, TNF-α induction of E-selectin involves receptor-mediated endocytosis [21] but appears to be independent of cyclic AMP [44]. Other studies implicate tyrosine phosphorylated proteins in CAM expression [119] and members of the sphingomyelin pathway, the latter via mitogen activated protein (MAP) kinases and transcription factors such as NF-κB, in transduction of cytokine signals [97].

Future fields of investigation will also be the significance of endotoxin induction of heat shock proteins (HSP) [161], a highly conserved family of proteins which appear to equip cells with the ability to withstand severe stress [111]. This defence system, commonly referred to as the stress response and present in virtually all organisms, from bacteria to mammals, can be initiated by a wide variety of different agents, including ischaemia, several types of metabolic stress, hyperthermia and endotoxaemia [112, 130, 155]. This stress response involves the rapid synthesis of a small number of intracellular proteins, the so-called stress proteins, including HSP and metallothionein (MT). It has been postulated that stress proteins may be involved in the protection against cytotoxic factors and the repair of the vessel wall in the course of septic shock and SIRS.

The best studied are HSP and the heat shock response. Thus, recent studies indicate that these proteins protect and recover the functions of various protein com-

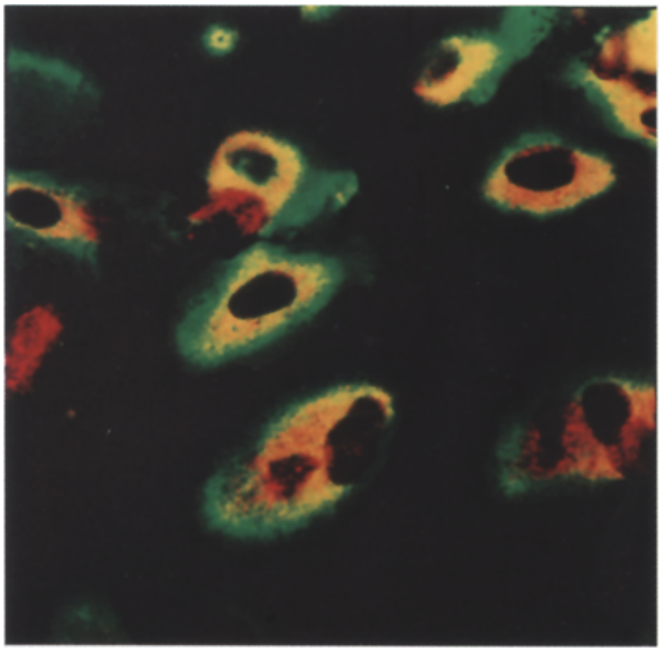

Fig. 3 Co-expression of HSP 70 (*red*) and metallothionein (*green*) in HUVEC, treated for 20 h with IL-6 (ng/ml), which up-regulates their expression. Double fluorescence immunocytochemistry using Texas red and fluorescein isothiocyanate (FITC). ×880

plexes during heat shock [172]. HSP 70 appears to be important in the unfolding, disassembly, and translocation of proteins, whereas HSP 60, and possibly HSP 90, participate in the folding and assembly of proteins. Several stress situations other than heat, such as viral infections, heavy metal exposure, ethanol, hypoxia, cytokines, the sulphydryl reagent sodium arsenite, and amino acid analogues also induce HSP [56, 104, 109, 156, 161, 187, 196, 209]. MT is another well-defined stress protein,

known to bind heavy metals, such as cadmium and zinc. In EC, cytokines, such as TGFβ, as well as IL-1β, IL-6 and TNF-α, and heavy metals, induce MT [86]. It has been shown that cytotoxicity accompanies MT induction by cadmium and TNF-α, but not by other cytokines, thus clearly indicating that induction of the protein is not a simple response to cytotoxicity in EC [86]. It is as yet unclear how such data could be utilized to develop new interventional concepts. However, the use of EC culture models could prove helpful in delineating the signal transduction mechanisms involved in HSP and MT expression and their relationship to expression of other important molecules within the endothelium. Antibodies are available which permit the in situ localization of these stress proteins, for example, within the EC cytoplasm (Fig. 3).

The role of blood cells

Although this aspect is discussed in the context of studies in vitro, it should be stressed that animal experimentation has provided essential data on the pathobiological significance of blood cells in the pathogenesis of MODS. The role of blood lymphocytes, including their cooperation with cells of the monocyte/macrophage lineage, will not be discussed here, as we believe that current thinking on pathomechanisms is dominated by the production of inflammatory mediators, and other tissue damaging effectors, by monocytes, polymorphonuclear granulocytes (PMN) and platelets. Activation of these cells has far-reaching effects on the microcirculation, and is thus an integral component of the present discussion.

The sequestration of granulocytes in the microcirculation is one of the hallmarks of ARDS [124, 170]. As well as a body of evidence from in vivo experiments [91], various groups have provided extensive data from research in vitro, indicating that this close association between PMN and endothelium is much more than a hindrance to microcirculatory flow; it leads to pathogenetically significant endothelial damage. This damage is thought to be mediated by products of activated PMN, such as oxygen radicals [164, 195] and enzymes released from the granules, such as elastase and cathepsin G [177]. Numerous activators of PMN are produced in the course of MODS, including complement components [164], PAF [65, 203], oligopeptides and lysins from bacterial walls [16, 90], and cytokines, including TNF-α [92, 186] and IL-8 [4]. The last is produced by various cell types, including macrophages [149] and EC [68] and is induced by the cytokines, TNF-α and IL-1β [182]. It has been shown recently that activated platelets can induce the endothelial secretion of IL-8 [87]. Taking these findings together, it is clear that there are numerous sources of IL-8 production in the context of MODS. It is probable that IL-8 activity in the microcirculation may explain why there is granulocyte sequestration and not emigration into the extravascular space, as IL-8 can inhibit PMN-endothelial interactions [68].

A further role for PMN in eliciting the characteristic changes in the microcirculation is the suppression of antithrombogenic activity [201] and concomitant up-regulation of procoagulant activity in the endothelium [138]. Future investigations will need to elucidate the role of new mediators not generally regarded as classical inflammatory mediators. An example is the potent vasoconstrictor peptide, endothelin-1 (ET-1), synthesized by EC [205] and elevated in the blood of patients in sepsis [120, 200]. Both in vitro and in a heart perfusion model, Farré et al. have shown that ET-1 can stimulate neutrophil adhesion to EC [57].

The role of monocytes (Mo) in the pathogenesis of MODS has tended to concentrate on their production of cytokines, which must be regarded as an essential element. Nevertheless, the local interactions between Mo and microvascular EC, as well as Mo and other blood cells, such as platelets and PMN, must be considered as further important pathomechanisms. In vitro models to simulate the biochemical and cellular microenvironment in the microvasculature in MODS/sepsis provide a wealth of relevant data to support the latter. Compared with PMN or lymphocytes, Mo demonstrate a relatively high level of binding to unstimulated EC [10, 55]. Cytokine-stimulated EC show an increased adhesion of Mo [12], an event which is mediated by various CAMs, including VCAM-1 and E-selectin on the EC, although it must be stressed that neither β2-integrins on the Mo or ICAM-1 on EC appear to be essential for this adhesion phenomenon [10, 32]. Furthermore, it seems that Mo employ the myeloid differentiation protein CD14, in addition to the conventional CAMS, for binding to cytokine-stimulated EC [11]. Other groups have shown that the adhesion-promoting effects of LPS (in a Mo-EC coculture system) occur rapidly for Mo, whereas an increase of adhesion via simulation of the endothelium requires a longer period of time [45].

Important effects of Mo in contact with the endothelium include the ability to increase EC production of the anti-fibrinolytic substance PAI-1 [75]. It should also be stressed that not all cytokines promote Mo adherence to EC. Thus, IL-4 is able to inhibit Mo adhesion [49].

To conclude this section it is necessary to emphasize that most of our data from in vitro studies derives from simplified cellular systems, designed to investigate the role of one, or at most two cell types, in a particular pathomechanism. That this is over-simplified is evident. However, this scientific approach is necessary to understand the fundamental processes involved in MODS and resembles the individual elements of a mosaic. The situation becomes much more complex as soon as more than two cell types are considered in the context of the permutations of interaction. This approach is nevertheless useful, even if fraught with interpretative problems. An instructive example is that of the cell-cell interactions involving Mo, PMN, platelets and EC from the viewpoint of PAF production [98]. TNF-α produced by endotoxin-stimulated Mo elicits PAF release by PMN [29], with a known deleterious effect on EC, especially

cytoskeletal changes and increased permeability of the monolayer [23], an integral component in the development of interstitial oedema. The effects are amplified by the ability of PAF to enhance TNF production by Mo [141]. A further pathomechanism is induced by the ability of TNF-α to release cathepsin G from PMN, which in turn involves platelets by stimulating them to produce PAF [154].

The role of hypoxia

Inadequate delivery of oxygen to and uptake of oxygen in tissues undergoing aerobic metabolism is a hallmark of MODS, so that a discussion of the pathogenesis of this life-threatening syndrome would be incomplete without consideration of the consequences of hypoxia for the microcirculation. It is evident that the effects of hypoxia on the various organs depend on their oxygen requirements, which means that tissue dysfunction arises if these requirements are not met. This extensive field is not, however, the topic under review here, but rather the specific role of the microcirculation. There are two principal aspects that need to be considered, namely the consequences of hypoxia alone and those of hypoxia combined with a phase of reoxygenation. It is evident that these pathologic conditions will exert effects on all elements of the microcirculation, including EC, smooth muscle cells and circulating blood cells and the various permutations of cell-cell interactions among these partners. Space limitations preclude a systematic and exhaustive discussion, so that here emphasis will be placed on endothelial reactions to these important modulating influences.

Hypoxia and the endothelium

From a study of the literature it is evident that no single model of the endothelium in vitro has been used, so that the effects described may or may not apply to the entire microcirculation. In fact, the vast majority of studies used macrovascular EC, of either venous or arterial type and from a variety of species, principally human or bovine. The present authors take the view that the structural and functional heterogeneity of the endothelium is such that many more comparative studies are required before extrapolation to the microcirculation is permissible.

Hypoxia in vitro has been shown to activate membrane phospholipids with subsequent release of fatty acids [18], increase prostacyclin production (measured as the stable metabolite, 6-keto-PGF1α) [116], as well as induce production of mitogens [42, 100], endothelin-1 [67], chemoattractant activity for granulocytes [56], and ACE (angiotensin-converting enzyme) [102]. In addition, fibrinolysis appears to be down-regulated [204]. In bovine adrenal microvascular EC, hypoxia decreases thrombomodulin, while inducing factor X activation and increasing permeability [132]. A further important aspect concerns the interaction between the coagulation cascade and hypoxia. Thus, Caplan et al. showed that hypoxia can increase ionophore- and thrombin-stimulated production of PAF [30]. The importance of the variability of response of the endothelium to hypoxia is underlined by the studies of Tretyakov and Farber [191], who demonstrated that chronic hypoxia causes the activity of phospholipase A2 to be increased in aortic EC, but decreased in pulmonary arterial EC (all bovine). Studies from our laboratory indicate that the endothelium can initiate protective mechanisms as a response to hypoxia. Thus, EC expression of cytokine-induced ICAM-1 and E-selectin is down regulated [94], and event that could be of biological significance, as potentially damaging EC-blood cell interactions would be inhibited.

Hypoxia/reoxygenation and the endothelium

Vasoregulatory mechanisms induced by hypoxia are aimed at restoring adequate blood flow. This means that hypoxia in many vascular beds is followed by a phase of reoxygenation. This field of investigation has received much attention, particularly with respect to ischaemia/reperfusion in the coronary circulation. However, the principle is of importance to the entire circulatory system, although it should be stressed that the literature presents conflicting results as well as controversies in their interpretation. In HUVEC, hypoxia followed by reoxygenation (H/R) appears to up-regulate the production of superoxide anion [168]. However, experiments with bovine aortic EC show that hydrogen peroxide release is oxygen concentration-dependent only over a narrow range [88]. Thus, >10% atmospheric oxygen leads to saturation of the cellular pathways, or in other terms, hypoxia, hyperoxia or H/R all failed to change hydrogen peroxide production at the intracellular site accessible to peroxisomal catalase.

Studies on myocardial ischaemia and reperfusion indicate that NO release by coronary EC is decreased and is associated with a marked increase in PMN adhesion [114]. (NO is known to be a potent inhibitor of PMN adhesion [103].) Thus, NO production and its down-regulation in H/R could be an important deleterious factor in the pathogenesis of MODS. Recent attention has focused on cell adhesion molecule (CAM) alterations in H/R. Palluy et al. described an increase in PMN adhesion to EC after 5 h H followed by up to 24 h R, and stressed a correlation with expression levels in the EC of P-selectin and E-selectin [134]. Using the same model (HUVEC), Yoshida et al. confirmed that anoxia followed by reoxygenation elicited increased PMN adhesion [206]. However, experiments of the latter research group with blocking antibodies indicated that E-selectin was *not* involved and that the effect appeared to be regulated at the *PMN* ligand level, as ICAM-1 expression was not up-regulated. It is evident that this complex field of investigation requires much more clarifying experimentation.

Problems and perspectives

Although there is accumulating evidence from in vitro and in vivo studies that the endothelium plays a central role in the pathogenesis of MODS, some essential questions still remain unanswered. These include the following:

1. Do systemic mediator levels reflect the local status? This important issue concerns not just better understanding of the pathogenesis, but also prognostic aspects.
2. What role does the heterogeneity of the microcirculation play? As a corollary to our hypothesis that the endothelium is central to the development of MODS, the role of organ-specific effects of the endothelium needs to be considered. In this context the barrier function of the hepatic endothelium against intestinal bacterial toxins requires more intensive investigation, although there are indications that sinusoidal EC may be less easily stimulated by cytokines to increase procoagulant activity [159]. It is also clear that the hepatic endothelium is not the only protective component in the liver. Evidence exists that Kupffer cells activated by LPS in a low-arginine microenvironment produce increased amounts of prostaglandin E2, which suppresses cytokine release [28].
3. What are the key events in endothelial dysfunction and what is their temporal sequence? These questions might appear at first glance to represent an academic exercise. However, the principle of targeted intervention presupposes detailed knowledge of the pathogenetic events. The application of monoclonal antibodies to the treatment of MODS has already reached an advanced stage, although the initial promise of success has only been partially fulfilled by the clinical trials conducted [115]. Of possible future significance is the use of genetic manipulation, for example by introducing antisense mRNA in order to regulate certain processes [25]. An example of such manipulation in an in vitro system was given by Itoh et al., who introduced and antisense oligonucleotide complementary to b-FGF mRNA into EC in vitro [83]. As a result, b-FGF production was reduced, with concomitant suppression of DNA synthesis. These experiments indicate that it is possible to specifically interfere with cellular synthetic activity, although numerous technical, not to mention ethical difficulties still need to be overcome.
4. How reliable are in vitro models of the endothelium as a mirror of in vivo processes? In a sense this is probably a rhetorical question, but one that is not only relevant to the research activity on the role of the microcirculation in MODS, but also to numerous fields of investigative pathology. The scientific community is very divided on this important issue, workers in clinical practice often being negatively critical of data collected in vitro. The present authors are protagonists of the view that in vitro techniques, provided they are strictly standardized and controlled with respect to the parameters being studied, act as an invaluable adjunct to in vivo studies, whether in experimental animals or in human tissue. These approaches are not mutually exclusive, but rather complement one another. It should also be seen as a positive point that many of the caveats come from research groups actively using in vitro methodologies. Liaw and Schwartz compared the expression of a variety of genes in endothelial cells directly following isolation from the bovine aorta with that following culture [110]. Their results showed that in culture overexpression of genes for important growth factors, such as bFGF and PDGF (B-chain), occurred, whereas vWF expression decreased and other genes, such as those for TGF-β and plakoglobin, remained unchanged. Our own studies underline the importance of standardizing the culture conditions and carrying out comparative studies between different endothelial cell types for the variables under investigation. Thus, we were able to show that to study basal and stimulated CAM expression, EC from early passages, preferably P1, should be used, and that the pattern of CAM expression is similar for human EC from the umbilical vein, the adult saphenous vein and the femoral artery [93]. Nevertheless, despite efforts to improve stringency of in vitro methods, the problem remains that much extrapolation takes place from macrovascular to microvascular endothelium, the latter being the prime site of pathologic change in MODS. At present we are in the process of establishing cultures of human pulmonary microvascular EC to extend our understanding of the pathomechanisms involved in ARDS.

Conclusion

In conclusion, it can be stated that the microcirculation, and in particular its endothelial component, has a polyvalent role to play in the pathogenesis of MODS and that many of the essential elements in this role offer the possibility of specific therapeutic intervention. The evidence presented is based on well-documented morbid anatomical, pathophysiological and biochemical data in vivo, combined with valuable evidence derived from in vitro investigations. Whilst there is no reason for euphoria, there is legitimate cause for hope that certain vital pathogenetic steps can be effectively regulated. This clearly remains a challenge for both basic sciences and clinical medicine. In both of these fields of endeavour pathology has a clear integrative role to play.

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