THORAX

Editorials

Inflammatory predictors for the development of the adult respiratory distress syndrome

The adult respiratory distress syndrome (ARDS) is a catastrophic form of acute inflammatory microvascular lung injury which is a central component of multiple organ failure.[1] Within the intensive therapy unit ARDS continues to exert a substantial financial and human burden with mortality rates of 50–70%.[2] Trials of several anti-inflammatory therapies in established ARDS have shown little benefit,[3–6] but this is perhaps not surprising when the complex pathological picture of established ARDS is considered. Most current definitions of ARDS are based on chest radiographic and blood gas parameters which may represent relatively crude indices of advanced lung injury. In many patients the diagnosis is not established before the patient requires mechanical ventilation, by which time the histological features have progressed from pulmonary microvascular injury to widespread epithelial injury, type II cell proliferation, and even the deposition of new scar tissue matrix proteins.[7 8]

In many cases of ARDS, particularly those which occur in response to "indirect" insults – for example, sepsis or pancreatitis – there is usually a delay of several hours or days between the initiating insult and the development of respiratory failure. This "latent period" provides special opportunities for clinical research and the delivery of interventional therapy, since in most other inflammatory diseases of the lungs no such delay exists and pathological processes are usually well advanced at the time of initial presentation. The presence of systemic markers of inflammation early in the period of ARDS risk suggests that early lung injury could be evolving at a subclinical level. Definition of the systemic and pulmonary inflammatory events during this "latent period" could therefore facilitate the identification of individuals at high risk of progression to ARDS. This "latent period" may then represent a "window of opportunity" during which mechanism-based therapies could be delivered with the aim of attenuating or even aborting lung damage at a much earlier stage of inflammation in the tissue.

The ability to predict progression to ARDS on clinical criteria is variable – for example, 5–8% of cases of multiple trauma and up to 35% of cases of Gram negative sepsis progress to ARDS,[2] but it is not currently possible to predict which patients will develop ARDS. The administration of interventional therapy to such large populations of patients may have a low cost-benefit ratio and it would clearly be advantageous to identify subgroups of patients at very high risk of this catastrophic disease who could then be targeted more effectively.

Disease pathogenesis

A substantial body of evidence implicates the neutrophil in the pathogenesis of the microvascular lung injury of ARDS[9]; thus, at some time before the clinical appearance of ARDS significant neutrophil recruitment to the pulmonary microvasculature must have occurred. The explosion of knowledge regarding mechanisms of neutrophil-mediated microvascular injury should facilitate the identification of key predictive parameters and possibly therapeutic targets. Key events in neutrophil recruitment include the generation of specific chemotactic agents and the expression of adhesion molecules on the neutrophil and endothelial surface which promote neutrophil sequestration in the pulmonary capillaries.

A new family of peptides has been described which function as potent chemokines for specific inflammatory cells. Interleukin 8 (IL-8), a proinflammatory cytokine and a prominent member of the C-X-C subgroup of this cytokine family, has emerged as a major neutrophil chemoattractant and activator important in initiating and perpetuating the inflammatory response in the lung.[10] We have found that the presence of a high level of IL-8 in the initial bronchoalveolar lavage (BAL) fluid of patients considered "at risk" of ARDS is strongly correlated with subsequent progression to ARDS (fig 1). We have now

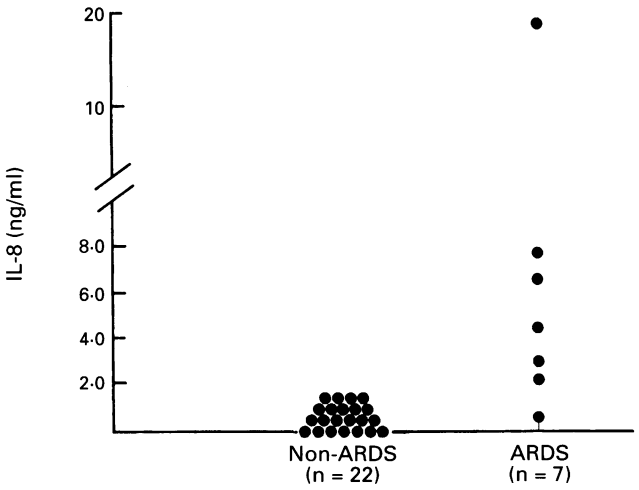

Figure 1 Interleukin-8 concentrations in samples of bronchoalveolar lavage fluid of patients who subsequently progressed to ARDS compared with those who did not ($p<0·001$).

enrolled 65 patients and continue to report this highly significant association; indeed, retrospective analysis suggests that this single lavage mediator possesses a positive predictive index for subsequent ARDS progression of more than 80%. These data further support the pivotal role of the neutrophil in the pathogenesis of ARDS, but also provide important evidence for the development of a localised pulmonary inflammatory response present very early in the risk period (sometimes within two hours of the initiating insult), prior to the clinical presentation of acute lung injury.

Whilst bronchoalveolar lavage is safe in patients "at risk" of ARDS and in those with established ARDS,[11 12] the technique requires expertise and may not be appropriate in all clinical settings. Predictive entities from less invasive investigations such as plasma markers or the non-invasive assessment of pulmonary microvascular permeability could prove more clinically relevant.

CIRCULATING FACTORS

Cytokines, markers of endothelial injury, and neutrophil products

The inflammatory cascade involves the participation of a complex network of mediators with many examples of redundancy. Attempts to identify a final common mediator which impinges on neutrophils and other inflammatory cells to induce abnormal sequestration and release of histotoxic products within the pulmonary microcirculation has not met with much success. Blood borne mediators such as C5a[13] and TNF[14–16] have not been successful at predicting progression to ARDS. We have recently examined levels of circulating neutrophil elastase in 61 patients with trauma and found that the plasma elastase level on initial hospital presentation was significantly higher in those who progressed to ARDS than those who did not ($p<0{\cdot}01$), and that the level correlated with the degree of subsequent lung injury.[17]

Given that injury to the pulmonary microvasculature is likely to be the earliest event in ARDS (resulting from indirect lung insults), markers of endothelial damage have been explored. Rubin *et al* found that plasma levels of von Willebrand antigen (vWf-Ag), a marker of endothelial damage, were raised in patients "at risk" of ARDS and demonstrated that plasma levels of vWf-Ag more than 450% above control values were 87% sensitive and 77% specific for predicting the development of acute lung injury in the setting of non-pulmonary sepsis[18]; however, subsequent investigators have been unable to confirm this observation.[19]

The level of angiotensin converting enzyme (a dipeptidyl carboxpeptidase found in lung capillary endothelial cells) is depressed in patients with ARDS but probably lacks the specificity that would be required to be of any predictive value.[20–22] Levels of circulating thrombomodulin (an endothelial surface glycoprotein released into the circulation specifically by endothelial damage)[23] are raised in patients with ARDS,[24] but we have not found samples taken from "at risk" patients to correlate with the subsequent development of acute lung injury (unpublished data).

Adhesion molecules

The process of neutrophil adhesion to pulmonary microvascular endothelium is a prerequisite for neutrophil emigration into the lung and is necessary for neutrophil-mediated neutrophil endothelial injury. The adhesion process is likely to be a multistage event involving a complex interplay between different groups of neutrophil and endothelial surface adhesion molecules.[25] It is thought that molecules of the selectin family of adhesion molecules are responsible for the earliest stage of neutrophil adhesion,[26] whereas those of the integrin family determine the later "tight" adhesive phase which is a prelude to neutrophil transmigration of the capillary endothelial layer. Constitutively expressed neutrophil L-selectin interacts with its corresponding ligand on the endothelium and mediates a key early stage in the process of neutrophil transendothelial migration – namely, a low level of adhesion allowing the neutrophil to decelerate and "roll" along the endothelium under conditions of heightened shear stress.[27] Blocking the expression of L-selectin with specific monoclonal antibodies in vitro results in a significant inhibition of early neutrophil/endothelial adhesion and a consequent reduction in neutrophil transendothelial migration.[28] Following this transient adhesive event, specific upregulation of neutrophil integrin adhesion molecules – for example, CD11b/18 – leads to a firmer adhesion, bringing the circulating neutrophil to a halt and facilitating neutrophil/endothelial transmigration.[29] During the transition to "tight" adhesion a cleaved form of L-selectin is shed from the surface of the neutrophil, probably by proteolytic action. It can then exist in the circulation in soluble form (sL-selectin) which continues to exert biological activity by retaining the capacity to bind to activated endothelium.[30] Thus, circulating levels of sL-selectin should reflect a key adhesive event occurring between neutrophils and endothelial cells in the microvascular bed, and we speculate that the plasma levels in the ARDS risk period may be of significant predictive value.

In collaborative work we have shown that patients who progress to ARDS have significantly lower levels of circulating sL-selectin than those who do not (fig 2). Furthermore, the level of sL-selectin is of prognostic significance, strongly correlating with semiquantitative indices of lung injury (time spent on a mechanical ventilator,

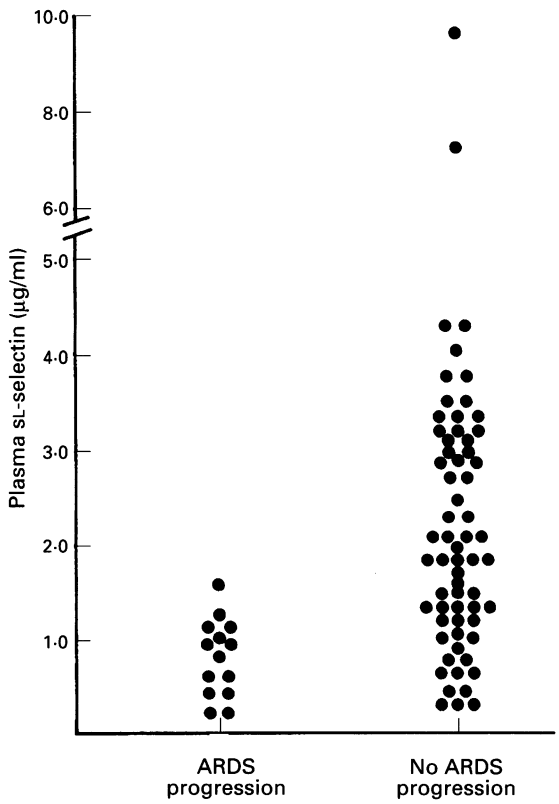

Figure 2 Initial plasma sL-selectin levels in patients who subsequently progressed to ARDS compared with those who did not ($p=0{\cdot}0001$).

the lowest Pao_2/Fio_2 ratio) and with subsequent mortality.[31] We propose that this low level of circulating sL-selectin reflects widespread endothelial activation and binding of the circulating receptor to its endothelial ligand. Thus, this circulating marker may reflect important events occurring at the alveolar-capillary interface implicating endothelial activation in the early ARDS disease process, and assist in the identification of that subgroup of patients at "high risk" of ARDS progression. Furthermore, in a retrospective analysis we have shown that the combination of plasma sL-selectin, elastase, and IL-8 can assign patients to a high risk of ARDS progression with a sensitivity of 86% and specificity of 95%.[32]

MEASURES OF PULMONARY PERMEABILITY

The pathophysiological consequence of the inflammatory response is injury to the endothelium with consequent breakdown of the alveolar-capillary interface resulting in the leakage of a protein-rich fluid from the circulation into the pulmonary interstitium and alveolar air spaces. The chest radiograph is notoriously insensitive at detecting significant degrees of "lung leak".[33] Techniques have been developed to detect this increased "lung leak" by recording the rate of accumulation of radiolabelled proteins within the lung by external scintillation counters and provide a sensitive and specific measurement of non-hydrostatic pulmonary oedema.[34] Strum *et al* have shown an early increase in lung permeability after trauma, within the risk period, but did not appraise the technique as a method of assessing ARDS progression.[35] However, Braude *et al* have reported an increase in the protein accumulation index prior to the appearance of chest radiographic abnormalities in a patient who subsequently developed ARDS.[36] Thus, the protein accumulation index may provide an earlier marker of impending severe lung injury.

Need for prospective studies

If the goal of delivering therapy prior to the development of established lung injury is to be realised, the strength of these measurements at predicting impending lung injury/organ failure must be affirmed in the setting of prospective studies. Several potential markers relating to neutrophil recruitment and endothelial damage exist and, as more are being described, it would seem reasonable to begin to focus on several of the more likely beneficial candidates in order to determine their positive and negative predictive values.

Therapy

Now that we have begun to elucidate the early events involved in acute lung injury, it offers us the potential of developing novel mechanism-based therapies that could be delivered during the "latent period" before the clinical manifestation of extensive established tissue damage. Since IL-8 is such a key chemoattractant and activator of neutrophils, it seems a particularly plausible target; animal models of lung injury support this. Sekido *et al* have shown that monoclonal antibodies, directed against IL-8 and delivered at the onset of a reperfusion injury, prevented both neutrophil infiltration and tissue injury.[37] It may prove feasible to develop IL-8 receptor blockers or other drugs which interrupt IL-8 signalling. Interesting work by Mulligan *et al* highlights the potential of interrupting neutrophil adhesion demonstrating that, in a cobra venom model of lung injury, antibodies directed against selectins reduce vascular permeability and neutrophil accumulation.[28] Ganert *et al* have recently demonstrated, in a rabbit meningitis model, the application of sugar moieties to inhibit L-selectin reduced leucocyte rolling; leucocytosis in skin and cerebrospinal fluid was profoundly inhibited with a reduction in blood-brain barrier permeability.[38]

Justifiable excitement must, however, be tempered with the awareness that the inflammatory response is primarily concerned with host protection against infection and although, in the context of ARDS, the balance of the system has swung towards tissue destruction, the interruption of key early events in the imflammatory cascade may have the potential to diminish the host's capacity to fight infection. Further elucidation of the temporal stages involved in the pathogenesis of the ARDS disease process will, however, hopefully demonstrate periods in the inflammatory cascade when one or several mediators are more important in terms of tissue destruction than host defence.

Conclusions

The application of cell biology techniques to patients within the ARDS risk period has led to a new understanding of the factors governing the development of lung inflammation. We have now begun to identify markers that may alert us to the development of ARDS and multiple organ failure before the clinical appearance of the condition as it is currently defined. Further elucidation of the temporal stages involved in the cellular and humoral events mediating tissue damage, coupled with refinements in techniques to detect subclinical lung injury, may therefore allow us to identify and target those "high risk" patients with evolving lung injury and deliver novel mechanism-based anti-inflammatory therapies at this early point, with the hope of attenuating or even aborting the extensive lung injury.

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