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New perspectives on basic mechanisms in lung disease· 2

Neutrophil traffic in the lungs: role of haemodynamics, cell adhesion, and deformability

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There has been increasing interest in recent years in the traffic of neutrophils in the lungs,[1-3] prompted by several factors. Neutrophils are the first cells to arrive at sites of infection or inflammation, and are present in the lungs in increased numbers in many conditions of acute and chronic lung injury.[4 5] They have thus been implicated in the pathogenesis of many lung diseases through the release, from activated cells, of reactive oxygen intermediates[6] and proteolytic enzymes.[7] Uniquely, the pulmonary capillary bed receives all of the circulating neutrophils and also contains a pool of temporarily non-circulating cells.[8] Influenced by various stimuli, these cells can either rejoin the circulating pool or migrate to an inflammatory site. It is in the pulmonary capillaries that neutrophils can interact with endothelial cells, with which they are in close contact. In addition, close proximity to the alveoli allows the transfer of messages between the air and vascular spaces.

In this review we will assess the influence of the factors that govern neutrophil traffic. Such knowledge is necessary for the complete understanding of conditions of lung inflammation and injury.

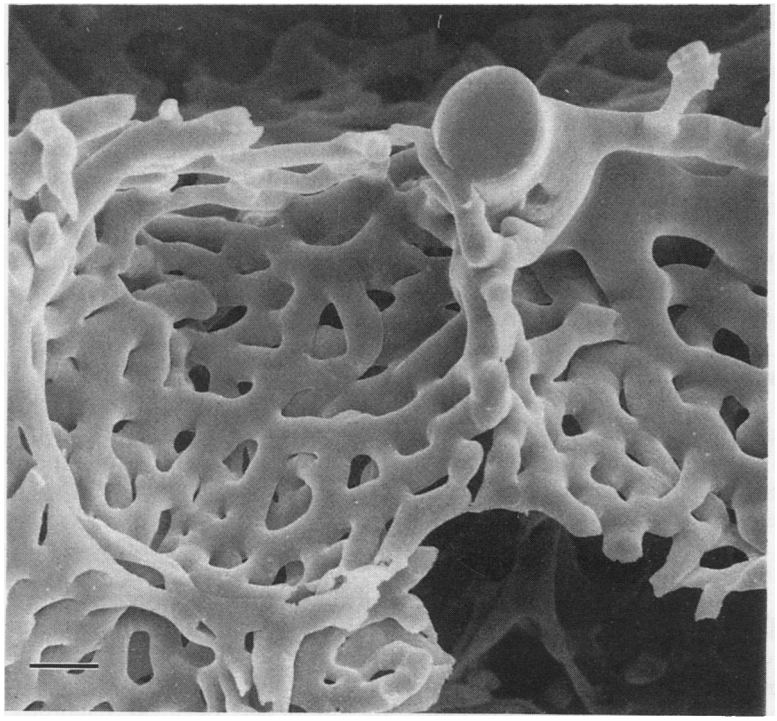

Figure 1 Scanning electron micrograph of a latex cast of the alveolar capillary bed of a rat lung, showing the interior view of the alveolar meshwork. The basic component is clearly cylindrical. The human pulmonary capillary bed is considered to have a similar arrangement,[10] but has yet to be so well illustrated. The bar represents 10 μm. Reproduced from Guntheroth et al[13] by courtesy of the American Physiological Society.

Anatomical and morphometric factors

In 1661 Malpighi[9] described the pulmonary microcirculation as a network of tubular capillaries (derived from the Latin *capillaris*: of hair, hair like). In his book on the morphometry of the human lung Weibel[10] extended this observation by describing a hexagonal arrangement of interconnecting tubules. This view was disputed by Fung and Sobin,[11 12] whose description of the microcirculation, based largely on light microscopy and the theory of fluid mechanics, was of sheets of endothelium separated by posts, between which blood flowed, like "a parking garage with floor and ceiling, and intervening posts." More recent studies, using latex casts and scanning electron microscopy, show convincingly that the alveolar microcirculation is composed of tightly matted intersecting tubules, rather than "sheets" (fig 1).[13] This configuration complicates the path taken by circulating neutrophils from arteriole to venule. The surface area of the alveolar capillaries in the human lung has been estimated to be of the order of 90 m^2, consisting of 296 $\times$ 10^6 alveoli and 277 $\times$ 10^9 capillary segments—that is, 1000 capillary segments per alveolus.[14] Hogg[1] calculated that the average pathway for a neutrophil from arteriole to venule has 60 capillary segments.

There is a discrepancy between the diameter of the circulating neutrophils, mean 7·03 (range 5–8) μm,[15] and that of the pulmonary capillary segments, mean 5 (range 1–16) μm).[14] Similar dimensions have recently been confirmed by morphometry of neutrophils and capillaries in situ in resected human lungs.[16] Capillary diameters are, however, influenced by alveolar pressure, which gives rise to zones of differing capillary recruitment.[17] Accordingly, the mean capillary diameter has been calculated to be 1·99 μm in zone II conditions and 5·78 μm in zone III in the rat lung.[18]

Obstruction of blood flow, by neutrophils that plug capillaries and impede the passage of erythrocytes, is well established in the systemic microcirculation and is enhanced by a reduction in blood flow.[19] This may be important in the pathogenesis of many ischaemic

diseases.[20] The transit of neutrophils through the pulmonary microcirculation is less well characterised, and for several reasons may differ from that of the systemic circulation. Pressures within the pulmonary circulation are much lower than in the systemic circulation[21] and flow is pulsatile.[22] In addition, the average diameter of the pulmonary capillaries[15] is probably less than that of the systemic capillaries (6 μm)[23] and the properties of endothelial cells may differ between the circulations. This means that the results of studies of neutrophil transit may also differ between the two vascular beds.

Margination, retention, sequestration, and migration

Neutrophils are present in three separate pools within the body—a bone marrow pool, an intravascular pool, and a pool within the tissues. Maturation within the bone marrow and release of neutrophils from it are not the subject of this review, but clearly factors that alter the release of neutrophils from the bone marrow will affect the intravascular pool of neutrophils.[24,25] The fate of neutrophils in the tissues is not well understood, but probably includes the process of apoptosis or "programmed cell death."[26]

Neutrophils spend a relatively short period of their total life span within the intravascular space ($T\frac{1}{2}$ = 7 hours). Early studies of neutrophils radiolabelled with diisopropylfluorophosphate ($DF^{32}P$, reviewed in refs 1 and 2) indicated that more than half of these cells rapidly disappeared from the blood on reinjection, forming a non-circulating pool of cells, which did not relate to the removal of cells damaged as a result of the labelling procedure.[25] Subsequent studies suggested that the circulating and the non-circulating pools contained roughly equal numbers of cells.[8] This non-circulating pool of neutrophils, with the pool of mature and immature cells within the marrow, can be released in times of stress.[27]

Cohnheim[28] first noticed that neutrophils appeared to "marginate" to vessel walls, while erythrocytes remained in the central stream of blood. This led to the idea that the intravascular non-circulating neutrophil pool consisted of marginating cells, temporarily withdrawn from the circulation. Schmid-Schonbein and coworkers[19] have shown that 94% of circulating neutrophils marginate in the postcapillary vessels in the systemic circulation, which occurs at the step change in vessel diameter between the capillary and the postcapillary venule, and has been shown to be flow dependent.[29] Whether true neutrophil margination occurs in the pulmonary circulation remains a subject of debate.[1,2]

More recent studies (reviewed in refs 1 and 2), using both labelled and unlabelled cells, supported the idea that the lungs contained a large pool of non-circulating neutrophils that were retained or sequestered within the pulmonary circulation. There is certainly evidence, however, that some harvesting[30] and labelling procedures[31] may be detrimental to cell function. Such ex vivo activation or even cell injury may account for the variation in the patterns of neutrophil kinetics observed after the reinjection of radiolabelled neutrophils in some studies in patients. Indeed, some authors claim that any retention of radiolabelled neutrophils in the pulmonary vasculature is due entirely to ex vivo cell activation or injury.[32] The same authors have calculated that the so called "marginating neutrophil pool" accounted for 54% of the total blood granulocyte pool and that the lungs contained 33% of the total marginating pool.[33] The debate over whether the lungs contain a substantial pool of non-circulating neutrophils is compounded by the misuse of the term margination; this correctly refers to neutrophils, located predominantly in the postcapillary venules in the systemic circulation, that roll slowly along vessel walls.[29,34] The term should not be used synonymously with neutrophil retention, which is the number of cells retained as a percentage of the cells delivered to an organ. We prefer to use the term sequestration to describe cells other than the freely circulating pool of neutrophils within the pulmonary vasculature. These include cells that are truly marginating, those that are trapped, those adherent to the endothelium, and those slowly moving through the microvasculature. The process of intravascular neutrophil sequestration in the lungs is critical to the subsequent migration of these cells, because to migrate cells in transit in the circulation must stop if they are to adhere to the endothelium before diapedesis.

Among the most convincing evidence that the lungs influence the passage of circulating neutrophils is that obtained by studies of unlabelled cells, where interventions such as the Valsalva manoeuvre cause increased sequestration of neutrophils in the pulmonary vasculature.[35] These findings are supported by morphometric data on animals, where more neutrophils have been found sequestered in the lungs than in the circulating pool.[36] Additional support comes from direct intravital observation in animal lungs of the delay of a proportion of neutrophils in transit within the pulmonary microvasculature.[37] Moreover, the study of the transit of radiolabelled neutrophils in the lungs seems justified because after their reinjection radiolabelled cells are distributed in the pulmonary vasculature in the same way as unlabelled cells,[36] and respond to signals to rejoin the circulating pool in a fashion similar to that of native unlabelled cells.[38–42]

Haemodynamic factors

A series of experiments, conducted over the past 10 years largely in animals and principally by Hogg's group in Vancouver,[1] have examined the influence of local haemodynamics on the sequestration of radiolabelled neutrophils in the lungs by comparing their transit with that of radiolabelled erythrocytes. Initial experiments in the dog suggested that

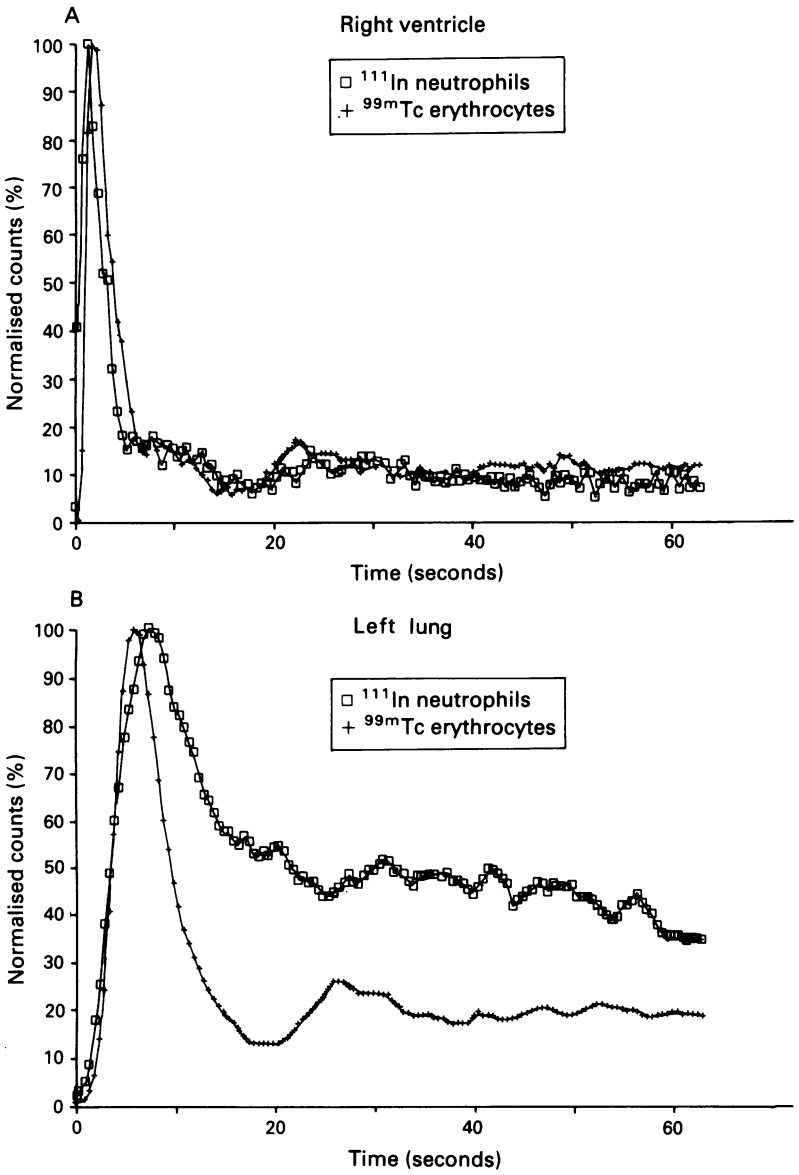

Figure 2 First pass time-activity curves from regions of interest assigned to the right ventricle (A) and left lung (B) after a bolus injection of indium-111 labelled neutrophils and technetium-99m labelled erythrocytes. All four curves are normalised to their peak counts and represent the first few seconds after reinjection. The two cell types pass through the right ventricle synchronously. Neutrophils, however, are slower in their transit through the lung than erythrocytes.

80–90% of reinjected radiolabelled neutrophils were removed in a single pass through the lungs.[38 39] A high initial rate of neutrophil sequestration in the lungs has been confirmed in other species, such as the rabbit[36] and pig.[43] In man there is also good evidence, obtained with a gamma camera that tracked the passage of an intravenous bolus injection of indium-111 labelled neutrophils and technetium-99m labelled erythrocytes, that neutrophils are delayed more than erythrocytes during their first transit through the pulmonary circulation before their recirculation (fig 2).

The regional retention of radiolabelled neutrophils, 10 minutes after reinjection, appears to depend on local haemodynamics, both in animals[41] and in man.[44 45] Reducing the cardiac output by inflating a balloon in the inferior vena cava in the dog produced an arteriovenous difference of unlabelled cells, fewer being present in systemic arterial blood than in venous blood.[38] These sequestered neutrophils are subsequently released if flow is increased.[39] If a reduced flow is maintained any arteriovenous difference in unlabelled neutrophils diminishes after 10 minutes as a new equilibrium is reached and cells are added from other pools in the body.[39] These studies support the concept of a dynamic equilibrium between circulating and non-circulating neutrophil pools within the pulmonary circulation. In support of this concept, Perlo and colleagues[46] showed that the ratio of white blood cells to red blood cells was greater in zone II than in zone III in isolated perfused lungs, though the increase in white blood cells was largely due to increased lymphocytes. They concluded that compression of the microvasculature in zone II may cause the alveolar vessels to act as a sieve for neutrophils. Recent studies in patients undergoing cardiac catheterisation seem to confirm this view because increasing alveolar pressure, causing compression of the alveolar vessels, produced a transient fall in neutrophil count in the left ventricular blood but not in the pulmonary arterial blood, as a result presumably of neutrophil sequestration in the lungs.[35] Moreover, the fall in neutrophils correlated with the proportion of the lung that changed from zone III to zone II.[35]

The passage of neutrophils through the pulmonary microcirculation depends on the balance between the forces that tend to retard their progress and the dispersive forces that act to move them through the vessels. The retardive forces are those of adhesion and friction between the neutrophil and the endothelial cell and those related to the deformation of the neutrophil. Deformation of the neutrophil is necessary as it has to achieve a smaller effective diameter to squeeze through the smaller diameter capillaries. The dispersive forces are those related to the shear stress of the vessel wall, which in turn depends on the local blood velocity.[47]

The effect of local blood velocity on neutrophil sequestration in the lungs can be studied by relating retention of neutrophils to the transit time of erythrocytes across the lungs. Staub and Schultz[48] have shown that there is no regional variation in the distribution of pathway lengths between different regions of the lung, at least in animal lungs. Thus the measurement of regional transit time of erythrocytes, either directly by intravital microscopy[49] or indirectly by obtaining time-activity curves for the transit of a bolus of radiolabelled erythrocytes across the lungs,[50] provides a measure of local blood velocity. In dogs,[51] rabbits,[36] and man[50] if markers that become lodged in proportion to flow (radiolabelled microspheres or macroaggregated albumin) are injected intraoperatively with a volume marker (radiolabelled erythrocytes) counts on the volume marker can be divided by counts on the flow marker in subsequently resected specimens of lung, and this enables the regional transit time of the erythrocytes to be calculated. These studies show a regional variation in the transit time of erythrocytes,

with longer transit times, and hence slower blood velocities, in the upper, more dependent regions.[36 50 51] This has been confirmed in man with a gamma camera technique.[44 50] There is also a correlation between the number of neutrophils retained 10 minutes after injection of radiolabelled cells and the erythrocyte transit time, so that the longer the erythrocyte transit time the more neutrophils are retained.[36 41 44 45 52]

These data seem to suggest that local blood velocity, and hence shear stress, are important determinants of the retention of radiolabelled neutrophils in vivo 10 minutes after their reinjection. This may be relevant to the increased sequestration of neutrophils in states of shock, where blood flow is reduced, which may be important in the pathogenesis of the adult respiratory distress syndrome (ARDS).[53] Indeed, peripheral blood neutropenia, due presumably to increased neutrophil sequestration in the lungs, has been shown to occur as a prodromal event in patients at risk of developing this syndrome.[54]

The physiological shear stresses calculated to occur in the pulmonary microcirculation (100–500 dyne cm^{-2}) may prevent the adherence of both normal and activated neutrophils to endothelium. Any decrease in flow, however, may alter this interaction.[55]

Furthermore, a reduction in the transit time of erythrocytes—for example, by treatment with adrenalin[40]—should decrease retention of neutrophils in the lungs. Erythrocyte transit time was reduced, and the exchange rate between the pools of circulating and non-circulating neutrophils increased, in the lungs of rabbits treated with adrenalin in regions with short erythrocyte transit times. There was no overall change, however, in neutrophil retention in the lungs.[40] This can be explained by decreased neutrophil retention in regions with short transit times, which was offset by the recruitment of underperfused capillary segments produced by this treatment, in which transit times were longer and hence neutrophil retention was greater.

The site of neutrophil sequestration in the lungs is relevant to the factors that control such sequestration. The relation between blood velocity and neutrophil retention[36 41 44 45 52] seems to suggest that true margination, which is flow dependent,[28 34] may occur in the normal lung. The geometric constraints imposed on the larger neutrophils in the smaller pulmonary capillaries, however, mean that such margination would be most likely to occur in the postcapillary venules, as in the systemic circulation.[28] As interventions that reduce erythrocyte transit time, such as infusion of adrenalin,[40] smoking,[44] and infusion of activated plasma,[56] are associated with an increase in neutrophil sequestration, this suggests that factors other than those related to local haemodynamics may influence the transit of neutrophils in the lungs. Indeed, studies in man indicate that, although the 10 minute neutrophil sequestration in the lungs correlates with erythrocyte transit time, blood velocity does not influence the initial or first pass sequestration.[57]

As described above, Doerschuk and coworkers,[36] using both morphometric techniques for unlabelled neutrophils and autoradiography in studies of radiolabelled cells, have convincingly shown that labelled and unlabelled cells have a similar distribution in the pulmonary circulation of the normal rabbit lung. In this study 74% of neutrophils injected intravenously were removed in the first passage through the lungs, and 70% of the injected cells could be located in the lungs, liver, or spleen at 10 minutes. Most of the cells in the lungs were located in the alveolar capillaries. Morphological studies show that the ratio of neutrophils to erythrocytes was almost 10 times greater in the alveolar capillaries than in the extra-alveolar vessels. From these studies the authors calculated that in the rabbit the size of the non-circulating pool of neutrophils in the lungs was at least twice that of the circulating pool.[36] This is a larger difference than that reported previously for man, in whom the total "marginating" pool of neutrophils was similar in size to the circulating pool.[8] Indeed, some authors have calculated that the lungs contribute a relatively small percentage to the total "marginating" pool.[33]

Using direct in vivo videomicroscopy to observe the transit of fluorescently labelled neutrophils through a transparent window in the periphery of the dog lung, Lien and coworkers[37] confirmed that neutrophils were sequestered exclusively in the pulmonary capillaries. None of the cells were delayed in the arterioles or in the venules and the authors did not observe any rolling or margination of cells in the pulmonary microcirculation. The distribution transit times of neutrophils in the capillaries was wide, ranging from under 2 seconds to over 20 minutes. Lien *et al*[58] also calculated that only a small number of obstructions (2%) were needed to stop most (71%) of the neutrophils in the capillaries. These neutrophils do not obstruct the passage of erythrocytes because the excess of capillary segments allows streaming of erythrocytes around them. Less than half of the neutrophils passed through with no delay from arteriole to venule. Most of the neutrophils that were delayed in the pulmonary capillaries stopped completely for varying lengths of time. Very few cells moved slowly throughout their transit.[37 58] Although in vivo microscopy of the lung is possible only for the subpleural microcirculation, capillary diameter, which is the critical dimension, is similar in the subpleural capillaries and in the capillary bed in the deep lung.[13]

In a further study using the same technique, Lien and colleagues[58] observed the effects of changes in pressure and flow on the transit of neutrophils in the pulmonary capillaries of the dog lung. The transit times of fluorescently labelled neutrophils in the pulmonary capillaries fell when adrenalin or hypoxia was administered: the two procedures increased pulmonary arterial pressure

by the same amount, adrenaline in addition causing an increase in cardiac output.[58] Balloon inflation in the vena cava, by contrast, reduced cardiac output by 41% without changing pulmonary arterial pressure and produced an increase in the transit times of neutrophils through the capillaries by shifting the distribution of transit times between the fastest and the slowest groups of cells.

Muir and coworkers[42] have shown an increase in the numbers of circulating radiolabelled and unlabelled neutrophils in response to adrenalin infusion or exercise in normal subjects, which appeared to result from the release of cells from a pool in the lungs. Recently, Peters *et al*[59] challenged the idea that the lung contributed cells to the leucocytosis of exercise. Different techniques were used in the two studies,[42 59] however, to calculate neutrophil sequestration in the lungs, and the exercise protocols were also different. Moreover, in the study by Muir *et al*[42] measurements were made within 15 minutes of simultaneous reinjection of simultaneously radiolabelled neutrophils and erythrocytes, whereas in the study by Peters *et al*[59] the two cell types were injected at different times and the measurements were made at a later time after reinjection, at a time of low activity within the lungs.

As described above, several studies have now shown that certain interventions that reduce erythrocyte transit time and should therefore decrease neutrophil sequestration in the lungs paradoxically increase sequestration. These studies suggest that factors other than local blood velocity may also influence the transit of neutrophils in the pulmonary microcirculation.

Neutrophil deformability

The influence of the mechanical properties of neutrophils on blood flow in the systemic microcirculation has been increasingly recognised.[47 60 61] Although present in smaller numbers in the blood than erythrocytes, neutrophils are not easily deformed,[62] which leads to entrapment in the capillaries. If subsequently activated,[63] with the release of oxygen radicals and proteases, these sequestered cells could contribute to the pathogenesis of ischaemic diseases[20] and to lung injury.[53 64] As both erythrocytes and neutrophils are larger than the pulmonary capillaries[14 15] the rheological properties of blood cells, particularly the ability of cells to deform in order to squeeze through the smaller capillaries, are likely to be important determinants of the transit of these cells in the lung.

The deformability of a cell depends on: (1) the viscoelastic properties of the cell membrane; (2) the viscoelastic properties of the cell cytoplasm; and (3) the surface area-volume relationship.[65] Neutrophils are 700 times less deformable than erythrocytes, though of a similar size.[65] The spherical shape of the neutrophil, however, is much less deformable than the biconcave disc shape of the erythrocyte. The neutrophil also contains a rigid nucleus and its granular cytoplasm is 1000 times more viscous than the cytoplasm of the erythrocyte.[65] Although the two cells are similar in size the neutrophil, being spherical, has twice the volume of the erythrocyte. Both neutrophils and erythrocytes have excess surface areas, greater than is necessary for enclosing their volume as a sphere. In the case of the neutrophil there is an 84% excess surface area,[65] due to cell membrane ruffling, which allows the cell to change shape and actively squeeze through tight junctions between endothelial cells as small as 1 μm in diameter. Passive deformation, which occurs during micropipette aspiration in vitro or during transit through capillaries of smaller diameter in vivo, requires a threshold pressure to unruffle the membrane followed by a slower deformation of the viscous cytoplasm.[66 67]

The deformability of white blood cells can be studied by several techniques. The mechanical properties of populations of neutrophils can be studied by cell filtration through a micropore membrane[68–70] and the influence of pore size, cell deformability, and hydrodynamic factors determined.[71] The average dimensions of the pulmonary capillary segments can be mimicked in vitro by this technique by using a micropore membrane with a pore diameter of 5 μm and a length of 11 μm. Several filtration techniques have been used. Cells can be filtered at a constant flow and the pressure developed over time within the filtration chamber measured.[68] Alternatively, the cells can be filtered at a constant pressure and a flow-time curve generated.[72] Neutrophils can also be radiolabelled and their retention in filters assessed. In this way the effects of varying both pressure and flow on neutrophil retention can be studied.[73] The technique used depends on the questions asked concerning the biophysical properties of the neutrophil.

More direct methods have also been used to study the deformability of individual cells. Cell aspiration with micropipettes of 5 μm internal diameter can be used to measure cell deformation time as a measure of deformability.[74] Probably the best method to assess cell stiffness or deformability, however, is the "cell poker" technique. This device indents the cell surface in a time dependent manner and measures both the force and the degree of cell indentation.[75 76]

With these techniques populations of leucocytes appear to vary in their stiffness, monocytes being less deformable than neutrophils.[77] The most important hydrodynamic factor influencing the retention of neutrophils during filtration through a micromembrane in vitro is the perfusion pressure and hence the shear stress, so that as the shear stress increases the retention of neutrophils in a filter decreases in a logarithmic fashion.[73] The shear stresses used in these studies of model capillaries (80–800 dyne cm^{-2})[73] are of the same order of magnitude as those calculated to occur in the pulmonary microcirculation in vivo (100–500 dyne cm^{-2}),[71] but are three

orders of magnitude greater than is necessary for inhibiting adherence of neutrophils to endothelium.[55 73] Five μm appears to be the critical pore size at which neutrophil retention in a filter increases substantially,[71] which is relevant to neutrophil retention in similarly sized pulmonary capillary segments. Monocytes and neutrophils have similar diameters and are retained when radiolabelled and reinjected in the rabbit lung to a greater extent than the smaller diameter lymphocytes.[78] These data suggest that cell size influences leucocyte retention in the lungs. Monocytes are retained to a greater extent than neutrophils, however, despite being of similar diameter,[78 79] indicating that factors other than cell size are also important in determining the transit of leucocytes in the lungs. Downey and coworkers[80] and Doerschuk *et al*[56] were both able to show that when neutrophils were artificially stiffened by fixation in glutaraldehyde substantially more were retained in rabbit lungs. Using both a filtration technique and the "cell poker" to measure deformability in vitro, Worthen *et al*[81] also showed that populations of neutrophils with differing deformability had differing retention rates in the lungs of rabbits in vivo: the more deformable the cells, the fewer were retained in the lungs.

Neutrophil adhesion, at least that mediated by the CD11/18 integrin (see below), does not have a major role in the retention of unstimulated neutrophils in micropore membranes. In support of this hypothesis, differentiated cells of the myelomonocytic HL60 cell line, which express CD11/18, were retained less than undifferentiated cells, which do express this antigen.[82] These differences in retention may be accounted for by differences in cell size and stiffness. Moreover, the decreased cell deformability that accompanies activation of neutrophils with *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) did not change when HL60 cells were treated with the monoclonal antibody 60·3 to the common beta chain of the CD11/18 integrin.[82] The decreased deformability induced by cell activation appears to be due to assembly of the cytoskeleton, particularly the microfilaments.[83 84] The decreased cell deformability induced by FMLP was completely abolished by treating HL60 cells with cytochalasin D, which disrupts actin.[82] Moreover, neutrophils activated with FMLP and treated with cytochalasin D were not retained in the rabbit lung more than cells treated with FMLP alone.[81] These studies confirm the role of cell deformability as a mechanism of neutrophil retention in the pulmonary microcirculation in vivo in animals. The importance of the cell's cytoskeletal assembly in the retention of neutrophils in the pulmonary microcirculation is emphasised by studies of cells of the HL60 line, which differentiate towards granulocytes.[82] During differentiation these cells change the organisation of their actin in response to stimulation with FMLP, though they are unable to increase their adhesiveness. Disruption of the cell microfilament organisation with cytochalasin D abolished the increase in retention in vitro of both undifferentiated and FMLP stimulated differentiated cells, confirming the important influence of microfilament organisation on the rheological properties of the neutrophil.[82]

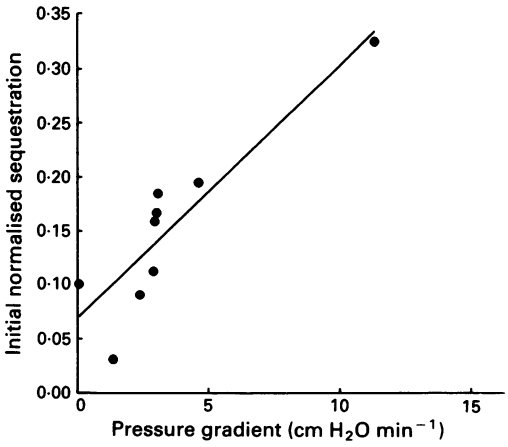

Figure 3 The significant linear relationship seen in normal subjects between the initial pressure gradient generated when neutrophils are filtered in vitro and first pass sequestration when autologous neutrophils are filtered simultaneously in vivo by the pulmonary vascular bed ($r = 0·9$, $p = 0·001$). Reproduced from Selby et al[57] by courtesy of the American Physiological Society.

These in vitro studies of neutrophil filtration through capillary sized pores have recently been applied to the problem of neutrophil sequestration in vivo in man. We showed a significant correlation between the deformability of neutrophils in vitro and their sequestration during their first transit in the pulmonary vasculature after reinjection in normal subjects[57] (fig 3). This study confirms the hypothesis that cell deformability is an important determinant of the normal sequestration of neutrophils in the lungs due to the geometric constraints imposed on neutrophils in transit in the pulmonary capillary bed. Such increased intravascular sequestration of neutrophils is enhanced in inflammation of the lung in animal models,[56] and by direct videomicroscopy has been seen to occur in the capillary bed rather than in the postcapillary venules,[85] in contrast with the systemic circulation.

Increased capillary sequestration of neutrophils occurs whether the inflammatory stimulus is administered via the airways or via the blood.[86 87] Using an animal model of neutrophil sequestration,[88] which has been verified by studies in man,[89] we have also shown that activated neutrophils are sequestered in normal lungs more than quiescent neutrophils in the inflamed lung. This increased sequestration is associated with a decrease in cell deformability[90] and with increased migration of neutrophils into the air spaces.[90] Moreover, sequestration of activated neutrophils in itself may be associated with both epithelial and endothelial injury.[86]

As might be expected from the preceding discussion, conditions where neutrophil deformability decreases in man should be associated with increased neutrophil sequestration in the lungs. This has been confirmed in patients with chronic obstructive lung disease during acute exacerbations, who have significantly greater retention of radiolabelled

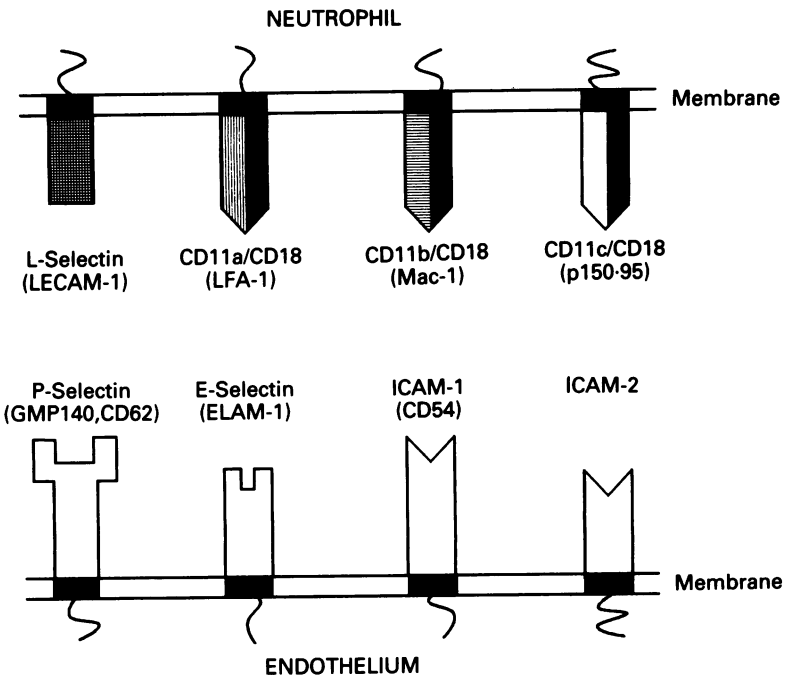

Figure 4 Adhesion molecules, currently considered to be important in neutrophil-endothelium interactions. The double solid lines are the respective plasma membranes, the black boxes representing the transmembrane domains. Cytoplasmic domains are represented by curved lines. The common (smaller) CD18 chain is represented by the filled portion of the arrow heads. Current (and previous or alternative) nomenclature is indicated.

neutrophils than clinically stable patients, and this increased retention is associated with a decrease in cell deformability measured in vitro.[91] These changes resolved when the patients became clinically stable.[57] Cigarette smoking is also associated with increased sequestration of neutrophils in the lungs.[44] Exposure of neutrophils to cigarette smoke in vitro results in decreased deformability associated with polymerisation of actin.[92] We have also recently shown that cigarette smoking is associated with an acute decrease in the deformability of neutrophils in arterial blood in vivo,[93] which is further confirmation of the important influence of changes in neutrophil deformability on cell sequestration in the pulmonary vasculature. Preliminary data also indicate that increased neutrophil sequestration during smoking results in increased elastase concentrations in rapidly sampled arterial blood,[94] which may increase the protease burden in the lungs and may be relevant to the pathogenesis of emphysema.[5 7] As described above, activation of neutrophils is associated not only with a rapid decrease in their deformability but also with changes in their adhesion to each other and to other cells. Decreased deformability seems likely to be the initiating event producing neutrophil delay or entrapment in the pulmonary microcirculation and allowing adhesive interaction between neutrophils and endothelial cells to proceed thereafter.

Cell adhesion

Three principal families of adhesion receptors have been identified in leucocyte-endothelial cell adhesion: the integrins, those belonging to the immunoglobulin supergene family, and the vascular selectins. Recent reviews have considered adhesion of leucocytes, including neutrophils, to both endothelial and epithelial cells[95-97] and their relevance to neutrophil emigration.[98]

Integrins are transmembrane heterodimeric glycoproteins comprising large α and smaller β chains. Among these are the leucocyte cell adhesion molecules (leuCAMs), which consist of a common β_2 chain; the CD18 antigen, associated with one of three α chains: the CD11(a-c) antigens.[96 99] CD18/CD11a and CD18/CD11b are constitutively present on the surface of human peripheral blood neutrophils.[96 99] When cells are activated augmentation of integrins occurs both by recruitment from intracellular sources, particularly from specific granules,[100] and through conformational changes, with the revelation of neoepitopes[101] and phosphorylation.[96] These changes are usually associated with increased cell adhesivity. There is, however, a dichotomy between increased CD18/CD11 expression and increased cell adherence.[102] Hence immunostaining of cell surface integrins does not provide a precise indicator of the functional state of cell adhesiveness. This section of the review focuses on intravascular neutrophil adhesion and we have therefore not discussed the β_1 or the β_3 families of integrins.[98]

LeuCAM-ligand interaction initiates a series of signal transduction mechanisms that are only now beginning to be unravelled; they include protein phosphorylation and protein kinase C activation with cytoskeletal conformational changes.[103]

The intravascular ligands for the leuCAM integrins CD11a/CD18 and CD11b/CD18 are single chain glycoproteins of the immunoglobulin supergene family known as intercellular adhesion molecules (ICAMs). Both ICAM-1[104] and ICAM-2[105] are constitutively present on endothelium (fig 4). ICAM-1 is also present on the basal surface of bronchial epithelial cells[106] and probably the luminal surface of type II alveolar pneumocytes.

Of the vascular selectins, L-selectin is present constitutively on circulating unactivated neutrophils[107] and a further two, E-selectin[108] and P-selectin,[109] are inducible on endothelial surfaces. In the systemic circulation neutrophil L-selectin interacts probably with P-selectin and an additional uncertain endothelial ligand, which mediate low level adhesion and the rolling of neutrophils along the endothelium.[110] Thereby shear stresses are reduced, which allows neutrophils to develop stronger adhesion to endothelium mediated by CD18/CD11-ICAM or E-selectin.[98] This brings the neutrophil to a halt in preparation for subsequent diapedesis, whereupon L-selectin appears to be proteolytically cleaved from the neutrophil surface.[111] There are few data on the role of L-selectin in the pulmonary circulation, but it would be facile and probably erroneous to postulate mechanisms

similar to those in the systemic circulation. Preliminary data suggest that L-selectin is not necessary for sequestration of neutrophils in the lung in response to the infusion of activated complement in the rabbit.[112] Neither is such sequestration associated with loss of L-selectin from the neutrophils.[112]

What of the other side of the intravascular equation, the endothelium? Treatment of endothelium with thrombin mobilises P-selectin within minutes from intracellular Weibel-Palade bodies.[114] This selectin can bind normal neutrophils,[110] probably via L-selectin, and initiate leucocyte rolling. Its role in the pulmonary circulation is not clear.

Activation of endothelium by proinflammatory mediators, such as the cytokines IL-1 and TNF, or by lipopolysaccharide induces the sequential expression of E-selectin[108] followed by ICAMs,[106] which is blocked by inhibitors of protein synthesis. This novel expression of E-selectin peaks around four hours after stimulation,[108] in contrast with the up regulation of vascular ICAM-1, which is maximal at around 24hrs.[106] The ligand for E-selectin on the neutrophil has yet to be conclusively characterised but is probably sialated Lewis X.[115]

The crucial role of neutrophil adherence, particularly the functioning of leuCAMs in vivo in man, is graphically illustrated by the leucocyte adhesion molecule deficiency syndrome, which results from a congenital inability to express functional CD18/CD11.[116] Neutrophils in these patients display impaired adherence in vitro, and an increased intravascular half life in vivo.[117] These neutrophils, however, sequester normally within the pulmonary vasculature and are released normally during vigorous exercise.[117] The syndrome is fatal, death being caused usually by overwhelming mucous membrane and skin infection, but rarely by pneumonia.[116]

CD18/CD11 does not appear to be necessary for the sequestration of normal neutrophils within a normal pulmonary vascular bed.[118] Thus the initial excess of neutrophils within the capillaries of the normal lung may result from the adverse geometrical constraints and deformability characteristics considered previously, which impede the transit of cells.

Neutrophil migration, however, but not intravascular sequestration within the lungs, in response to the inflammation induced by intrabronchial phorbol ester was inhibited by a monoclonal antibody to CD18. In contrast, intravascular sequestration and migration in the lungs in response to pneumococci instilled intrabronchially was not abolished, which was not the case when the same stimulus was applied to the skin.[119] Thus the mechanisms mediating neutrophil migration not only are stimulus specific but also differ in the pulmonary and systemic circulations. This may be due to differences in endothelial responsiveness or to the proximity of other effector cells, such as epithelial and phagocytic cells, which enhance recruitment of neutrophils within the lungs. It would indeed be unusual for CD18 alone to be a central pivot in such mechanisms. The rarity of pneumonia in patients lacking functional CD18/CD11 in the leucocyte adhesion molecule deficiency syndrome confirms alternative, CD18-independent, pathways for effective neutrophil recruitment.

Thus treatment with monoclonal antibodies directed against CD18 may be of limited benefit if not detrimental. In the few studies in man investigating this antibody in patients undergoing bone marrow transplantation little consistent success has been claimed.[120 121] There may be more hope with the development of monoclonal antibodies that can identify epitopes of activated CD18/CD11. Blocking endothelial ligands is an alternative approach. Monoclonal antibodies to ICAM-1 protect renal allografts in primate recipients[122] and have attenuated eosinophil infiltration and airway hyperresponsiveness in a primate model of asthma.[123] Short peptides have been found to block ICAM-1 function in vitro[124] and may be more attractive clinically.

Such treatment, however, may be effective for only a finite time window of opportunity.[53] Future study of these factors that control neutrophil traffic in the lungs will provide exciting insights into the mechanisms of lung inflammation and injury and indicate novel avenues of therapeutic intervention.

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