

Polymorphonuclear leucocyte traffic in lung inflammation

The margination of polymorphonuclear leucocytes (PMNs) in microvessels and their migration out of the vascular space is a multistep process that is regulated by a cascade of molecular events involving several families of adhesion proteins. Adams and Shaw¹ have recently reviewed the evidence for the hypothesis that this process begins with a selectin-mediated rolling of the PMNs on the endothelial surface of the post-capillary venules followed by a chemokine-induced triggering of a stronger, integrin-mediated adherence between the leucocyte and the endothelial surface that occurs in preparation for migration. The data that support this hypothesis are impressive, particularly for the systemic vessels where both margination and migration apparently occur in post-capillary venules. However, in the pulmonary circulation there are compelling reasons to believe that the circulating cells slow down, adhere, and migrate through the endothelium of the capillaries, and that only a fraction of the PMNs passing through an inflammatory site become significantly activated to migrate out of the vascular space.²

The progressive activation of PMNs is most easily investigated *in vitro*. Studies using a prototypic agonist of neutrophils, f-met-leu-phe (FMLP), have shown that low concentrations (10^{-12} – 10^{-10} M) are associated with reorganisation of the cytoskeleton to a configuration which allows the cells to move independently.³⁻⁵ As the FMLP concentration is increased, the specific granules release CD11b/CD18 onto the surface and L-selectin is shed.⁶ These events occur in association with increased intracellular levels of inositol trisphosphate and calcium.⁷ However, it is only at very high concentrations (10^{-7} – 10^{-6} M) that the major cytotoxic responses of azurophilic granule release and superoxide production are demonstrable.⁶ Other agonists that use structurally similar receptors (interleukin 8, platelet aggregating factor, C5a) have similar dose-dependent responses but these agonists can also prime the cell and amplify its cytotoxic response.^{8,9} Priming refers to an enhanced response that results from the sequential addition of priming agent and agonist.⁹ Priming of the superoxide response usually occurs at intermediate doses sufficient to cause increases in intracellular calcium and specific granule release, but just at or below the doses necessary to release superoxide.^{8,9} Other mediators such as the cytokines (interleukin 1, granulocyte-macrophage colony stimulating factor, tumour necrosis factor) and endotoxin are incapable of producing a full repertoire of neutrophil responses.

The relationship between the graded neutrophil response *in vitro* and their behaviour *in vivo* has only been partially determined. Their response within the vascular space where agonist concentrations are low is probably limited to the assembly of actin, a reduction in cell deformability, up-regulation of CD11b/CD18, and a loss of L-selectin from the cell surface. Once adherent to the endothelium, the cells move along chemotactic or haptotactic gradients centred at the site of injury. This allows the PMNs to encounter increasing agonist concentrations that first prime and then trigger a full cytotoxic response. Any abnormality in this sequence that allows large numbers of PMNs to become fully activated before leaving the vascular space puts the lung tissue itself at risk of injury.

Approximately 8640 litres of blood pass through the pulmonary circulation of an adult human in 24 hours. As each litre of blood contains 4.5×10^9 PMNs about

3.8×10^{13} PMNs pass through the pulmonary microvessels each day. The human PMN has a similar diameter to, but larger volume than, the erythrocytes,¹⁰ and the pulmonary capillary bed is formed by an interconnecting network of 10^{11} short segments with an average diameter of 7.5 (2.3) μm and an average length of 14.4 (5.8) μm .¹⁰⁻¹² The distributions of PMN diameters (6.8 (0.8) μm) overlap that of the capillary segments by approximately 38%, which means that circulating cells encounter segments that restrict their passage on each transit through the lung.¹² During this encounter the PMNs deform and come into close contact with the capillary endothelial surface.¹² Because the post-capillary venules have much larger diameters and a total endothelial surface area of only five square metres compared with the >100 square metres available in capillaries, there is a much greater opportunity for interactions to occur between PMNs and the endothelium in the capillaries than in the post-capillary venules of the lung.¹³

Direct observations of the pleural surface¹⁴ and indirect measurements from deeper lung regions¹¹ have shown that it takes leucocytes much longer than the erythrocytes to travel through capillaries. The slower movement of the PMNs compared with the erythrocytes is a result of the fact that they are about 300 times less deformable than erythrocytes and are held up for longer periods in narrow segments.^{12,15} The interconnecting network of segments that forms the capillary bed allows the erythrocytes to have median transit times of one second compared with 120 seconds for PMNs.¹¹

The concentration of PMNs with respect to erythrocytes that results from this difference in speed can be influenced by mechanical events affecting either the vessels or the circulating cells. Studies of patients undergoing simultaneous right and left cardiac catheterisation have shown that an immediate arteriovenous difference for PMNs developed when the lung capillaries were compressed by raising alveolar pressure and shifting lung from zone III to zone II conditions.¹⁶ Animal studies subsequently showed that the arteriovenous difference produced in this way disappears with time as a new equilibrium is established at the longer PMN transit times.¹⁷ PMNs rapidly accumulate in the lung when stimulated by the infusion of zymosan-activated plasma.¹⁸ This accumulation is a two-step process where reduced PMN deformability dominates the initial retention and CD11/CD18-mediated adherence plays a secondary role.¹⁹ Interestingly, the massive PMN margination produced by the infusion of activated plasma causes only minor changes in the epithelial permeability with no increase in extravascular lung water or protein.²⁰ This suggests that the plasma-derived stimuli that massively increase PMN margination fail to activate the cells to a point where they are capable of injuring lung tissue.

Downey *et al*²¹ showed that in the lung PMNs migrate out of capillaries rather than post-capillary venules and Doerschuk *et al*²² showed that this migration could be CD18 independent in the lung but not in the systemic circulation.²¹ Interestingly, only 1–2% of the cells delivered to an area of pneumonia actually migrate out into the airspaces² compared with the 60–80% migration observed *in vitro*.²³ If both lungs of an adult human weigh 1000 g and have a total air capacity of seven litres, the maximum available airspace would be 7 ml/g. If the entire lung receives about 10^{13} leucocytes in 24 hours, each gram will

receive 10^{10} cells and a 1% migration of the delivered cells – that is, 10^8 would occupy a volume equal to all of the available airspace. This suggests that PMN migration into inflammatory sites in human lungs must be closer to the 1% of delivered cells observed *in vivo* than the much higher values demonstrated *in vivo*.

In local streptococcal infections in rabbit lungs PMN migration into airspaces was limited to the site of inoculation, whereas PMN margination was increased throughout both lungs.² The partial activation of the PMNs responsible for this widespread margination in lung capillaries in the early stages of pneumonia is similar to the margination induced by the infusion of activated plasma²⁰ in that it causes little damage. However, full activation of this large intravascular pool of cells by a subsequent bacteraemia may result in complete PMN activation with the widespread destruction of the lung surface.

In summary, the current paradigms for migration of PMNs out of post-capillary venules in systemic vessels cannot be directly applied to the lung. The size of the PMNs relative to the microvascular segments ensures that there will always be close apposition of PMNs to the endothelium as they transit through lung capillaries. Their relatively slow speed in the pulmonary capillaries provides ample opportunities for contact between the PMNs and endothelium. The contact can be widespread and prolonged by the presence of inflammatory stimuli and may contribute to the increased risk of widespread lung injury associated with bacteraemia in pneumococcal infections.²⁴

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