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Weird and weirder: how circulating chemokines coax neutrophils to the lung

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NEUTROPHIL TRAFFICKING to the lung and airways is not well understood. Intratracheal instillation of interleukin-8 (IL-8, CXCL8) or related CXC chemokines leads to rapid neutrophil accumulation. IL-8 is substantially elevated in bronchoalveolar lavage from patients with acute lung injury, and expression levels are positively correlated to neutrophil recruitment (9). Blocking IL-8 has been shown to ameliorate reperfusion-induced neutrophil influx (13). In rats, important chemokines for inflammatory neutrophil recruitment to the lung are cytokine-induced chemokines (CINC, similar to CXCL1, 2, and 3, called keratinocyte-derived chemokines or KC in mice) and macrophage inflammatory protein-2 (MIP-2, similar to CXCL1), both of which bind to and activate the chemokine receptor CXCR2. In a model of systemic inflammation induced by intraperitoneal injection of LPS, mRNA expression for both KC and MIP-2 is increased (1). In a model of lung inflammation induced by hindlimb ischemia-reperfusion, MIP-2 and CINC were upregulated (4). Similarly, hepatic ischemia and reperfusion cause increased expression of MIP-2 and KC in mouse lungs, and their neutralization reduces injury (17). CXCR2 chemokines are of medical significance, for example, in the setting of ventilator-induced lung injury (2).

Why are there several, seemingly redundant chemokines that bind to and activate CXCR2? Do they have similar or different functions? Quinton et al., in one of the current articles in focus (Ref. 11a, p. L465 in this issue), begin to shed some light on this question. In an elegant series of experiments, the authors convincingly demonstrate that CINC, but not MIP-2, appears in the systemic circulation when produced in the lung in response to LPS or when instilled into the airways. CINC is a low-affinity ligand for CXCR2, whereas MIP-2 is a high-affinity ligand. MIP-2 can desensitize for CINC, but the reverse is not true. In response to intratracheal LPS, CINC, but not MIP-2, appears in blood plasma, and even under baseline conditions a little CINC is found there. Both MIP-2 and CINC mRNA expression are restricted to the lung after intratracheal LPS, suggesting that both chemokines are produced in lung tissue and get into the circulation from there. Entry into the circulation happens quickly, reaching maximal concentrations at 15 min after intratracheal instillation of CINC. Coadministered MIP-2 never makes it into the circulation.

To confirm that the CINC truly comes from the airways rather than being produced elsewhere in the body, the authors (11a) use radiolabeled CINC and trace it from the airways to the blood space. As an additional confirmation, they even show that less CINC than MIP-2 is recovered from the airways by bronchoalveolar lavage after both are administered in equal amounts. Together, these data conclusively show that CINC, but not MIP-2, reaches the blood circulation after intratracheal instillation.

But the most amazing and perhaps the most weird finding is that coadministration of CINC and MIP-2 greatly enhances neutrophil recruitment into the bronchoalveolar lavage fluid. The effect seems to be synergistic rather than additive. This is completely unexpected, because circulating chemokines are supposed to desensitize their respective receptors, in this case, CXCR2, and curb cell recruitment. Indeed, in a rabbit model, intravenous injection of IL-8 was shown to drastically reduce neutrophil recruitment to a mesenteric site of inflammation (10), quite the opposite of what Quinton et al. (11a) report here for the lung. Even more amazing, intravenous injection of CINC, but not MIP-2, drives neutrophils into the bronchoalveolar lavage fluid. The authors looked for release of neutrophils from the bone marrow but did not find evidence under the experimental conditions studied. This suggests that the chemokines may largely affect neutrophil adhesion and transmigration, but bone marrow release should not be ruled out.

What does all this mean? First, there must be a transport mechanism for CINC from its site of production, i.e., the airway epithelium, alveolar macrophages, or endothelial cells. This mechanism for pumping chemokine into the blood is apparently selective for CINC and does not transport MIP-2. The nature of this transport system is completely mysterious at this time. Functionally, CINC appears to provide a priming signal of sorts to the neutrophils that are then attracted to the more powerful but local chemoattractant MIP-2.

These findings are fascinating for several reasons. First, neutrophil recruitment to the airways is not understood, and here we get a glimpse of a possible "two-hit" mechanism. Second, priming agents usually act through a receptor separate from the ultimate chemoattractant receptor, for example, integrins (7), LPS receptors (6), L-selectin (14), and many others. Here, the priming event, if it is one, is delivered through the same receptor, CXCR2, that delivers the recruiting signal. Alternatively, there could be another CINC receptor that remains to be discovered.

The implications of the paper by Quinton et al. (11a) for our understanding of neutrophil recruitment to the airways are profound. Their work suggests that preactivated neutrophils have an inherent tendency to end up in the airways. Of course, preferential homing of neutrophils to the lungs has been known for at least 50 years (3), and IL-8-induced neutrophil accumulation in the lung was described more than a decade ago (15). Reduced neutrophil deformability has long been suspected to be responsible for this so-called "margination" (16). However,

this "parking" of neutrophils was always considered to be an intravascular event, without transmigration into the interstitium or the airways. This is not so in the present study. Intravascular CINC clearly induces neutrophil recruitment to the airways and greatly enhances neutrophil recruitment in response to MIP-2. And, most surprisingly, the CINC produced in the lung gains access to the blood stream to exert its effect. The molecular mechanisms underlying the special recruitment paradigm to the airways remain to be determined. It is not clear which, if any, leukocyte adhesion molecules are involved or whether the neutrophils enter from pulmonary capillaries or the bronchial microvasculature. However, this fascinating study suggests a novel and highly unusual synergism between two CXCR2 chemokines that regulate neutrophil trafficking to the airways.

What does CINC do in the systemic circulation to induce and amplify neutrophil recruitment? CINC is very similar to mouse KC, which is known to bind to vascular endothelial cells where it can serve as an efficient arrest chemokine for monocytes (8) and probably for neutrophils. Human IL-8 can also bind to endothelial cell surfaces (12) and even be transported across endothelial cells to be presented at the luminal surface (11). Both IL-8 and KC are very effective at triggering integrin activation and arrest of rolling cells (5, 8). It is, therefore, reasonable to speculate that similar transport and presentation mechanisms may be operative for CINC, the rat ortholog of KC. If this is indeed the case, some of the circulating CINC may be immobilized on endothelial cells, perhaps preferentially in the pulmonary microcirculation, although this was not investigated in the present study. This is an attractive hypothesis because it would explain how the low-affinity chemokine CINC could trigger arrest without desensitizing the arrested cell for another chemokine, MIP-2, operating through the same receptor.

Although the functional details of how circulating CINC promotes neutrophil recruitment to the airways remains to be investigated in more detail, the study by Quinton et al. (11a) introduces a new paradigm and perhaps opens the door to a true understanding of neutrophil trafficking to the lung.

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