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ROUS-WHIPPLE AWARD LECTURE

Neutrophil-Epithelial Interactions

A Double-Edged Sword

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In recent years, it has become clear that innate immune cells termed neutrophils act as double-edged swords by playing essential roles in clearing infection but also causing tissue damage, yet being critical for wound healing. Neutrophil recruitment to sites of injured tissue or infection has been well studied, and many of the molecular events that regulate passage of leukocytes out of the microcirculation are now understood. However, after exiting the circulation, the molecular details that regulate neutrophil passage to end targets, such mucosal surfaces, are just beginning to be appreciated. Given that migration of neutrophils across mucosal epithelia is associated with disease symptoms and disruption of critical barrier function in disorders such as inflammatory bowel disease, there has been long-standing interest in understanding the molecular basis and functional consequences of neutrophil-epithelial interactions. It is a great honor that my work was recognized by the Rous-Whipple Award this past year, giving me the opportunity to summarize what we have learned during the past few decades about leukocyte interactions with epithelial cells. *(Am J Pathol 2016, 186: 1404–1416; http://dx.doi.org/10.1016/j.ajpath.2016.02.001)*

Role of Neutrophils in Pathogen Clearance and Bystander Tissue Damage

Neutrophils function as double-edged swords, representing the critical first line of defense against invading pathogens while simultaneously having the potential to cause substantial local tissue injury. The pathogen killing function of neutrophils encompasses several steps. Microbial killing begins with receptor-mediated uptake of invading pathogens into an intracellular phagosome, followed by generation of highly toxic reactive oxygen species. The final step in this process is the fusion of neutrophil granules (containing an arsenal of neutrophil antimicrobial mediators) into the phagosome. Early studies demonstrated potent microbicidal activities in neutrophils derived from the ability to produce large quantities of hydrogen peroxide dependent on a membrane-bound superoxide-generating NADPH oxidase.[1] Defects in components of the NADPH oxidase were shown to be present in various forms of a life-threatening immune deficiency termed chronic granulomatous disease.[2] One of the many consequences of this defect is that some patients with chronic granulomatous disease develop chronic intestinal inflammation and have defective intestinal barrier function and symptoms similar to those observed in individuals with ulcerative colitis and Crohn's disease.[3] In addition to producing reactive oxygen species, neutrophil granules contain numerous important antimicrobial and proteolytic agents, including the antibacterial enzyme myeloperoxidase, as well as serine proteases, including neutrophil elastase and cathepsin G,[4–6] defensins, β glucuronidase, proteinase 3, and bactericidal permeability increasing protein. Other subsets of neutrophil granules contain lactoferrin

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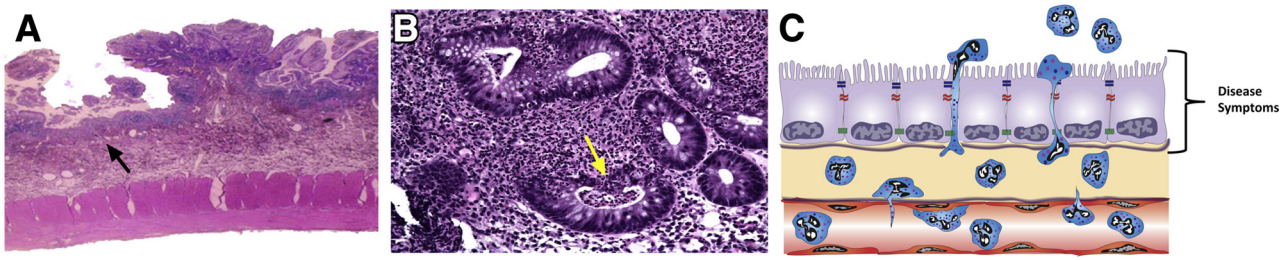

Figure 1 Neutrophil infiltration of intestinal mucosa is associated with tissue injury, disrupted barrier, and disease symptoms. Low (**A**) and high (**B**) magnification images of inflamed colonic mucosal resection and biopsy specimens depicting ulceration (**black arrow**) and neutrophils migrating across the epithelium that results in formation of a crypt abscess (**yellow arrow**). Adapted from Chin et al,[24] with permission from *Annual Reviews*. **C:** Schematic outlining sequential stages of neutrophil migration from the circulation across vascular endothelium followed by migration through the interstitium and terminating in neutrophil transepithelial migration. Disease symptoms in conditions such as inflammatory bowel disease correlate most strongly with the presence of neutrophil transepithelial migration and crypt abscess formation.

(an antibacterial iron chelator), lysozyme, and numerous metalloproteinases (MMPs), including MMP-8, MMP-9, and MMP-25. These granule constituents are essential for pathogen killing but also cause significant bystander tissue damage. Detailed reviews of neutrophil killing functions can be found elsewhere.[7]

Given this arsenal of destructive power, it is remarkable that recruited neutrophils can efficiently enter tissues and destroy invading pathogens (a process culminating in the resolution of infection/inflammation), usually with little residual tissue damage. In addition, it is well documented that infiltration of inflammatory cells, including neutrophils, macrophages, and lymphocytes, is necessary for the process of mucosal wound healing.[8,9] Typically, neutrophils begin arriving at wounded sites within minutes of injury and persist for several days before being cleared by macrophages. During this time, neutrophils are an important source of proinflammatory cytokines, including IL-1α, IL-1β, tumor necrosis factor α,[10] and others. More recently, it has been demonstrated that neutrophils at wound sites also produce (or contribute to the production of) growth factors, including vascular endothelial growth factor and proresolving lipid mediators derived from Ω 3 fatty acids, as well as arachidonic acid metabolites, including lipoxin A4, protectin D1, and resolvin E1.[11,12] Resolvin E1 and protectin D1 decrease neutrophil recruitment and increase macrophage phagocytosis of apoptotic neutrophils.[13] Furthermore, neutrophils have been shown to be actively involved in inflammation resolution through the phagocytosis of cell debris accumulated at sites of mucosal wounds.[14] Proof of the importance of neutrophils in wound repair is highlighted in experiments demonstrating that depletion of neutrophils results in impaired wound healing.[15–17]

Neutrophil Trafficking and Inflammatory Diseases

Although neutrophil migration into tissues is an essential component of host defense and wound repair, dysregulated transmigration across mucosal surfaces in multiple organs is the hallmark of many inflammatory diseases that are characterized by persistent or intermittent bursts of active inflammation. In the gut, for example, neutrophil transepithelial migration is characteristic of disease flares in individuals with inflammatory bowel disease (IBD). This debilitating disorder affects well over a million individuals in the United States and Western Society, and is composed of both ulcerative colitis and Crohn's disease.[18] Patients with ulcerative colitis and Crohn's disease most commonly have an undulating clinical course with bouts of remission interspersed with disease flares. Characteristic histological features of intestinal biopsies or resections during disease flares include disordered architecture, transepithelial migration of neutrophils with crypt abscesses, and large areas of mucosal ulceration associated with infiltration by massive numbers of neutrophils[19–23] (Figure 1, A and B). In other organ systems, there are multiple inflammatory conditions that are similarly associated with neutrophil transepithelial migration during the symptomatic phase of disease. Specifically, in the urinary tract, colonization with *Escherichia coli* is associated with large-scale migration of neutrophils across the urothelium.[25,26] Under certain conditions, this can contribute to development of pyelonephritis. In the respiratory system, transepithelial migration of large numbers of neutrophils is associated with a plethora of pulmonary infections, chronic bronchitis, and allergic responses, as seen in asthma.[27,28] In the skin, migration of neutrophils through the squamous epithelium is characteristic of the disorder psoriasis.[28,29] Given the strong link between inflammatory disease activity and neutrophil transepithelial migration (as observed in the above examples), this article will highlight some key aspects of what we have learned through ongoing investigations in this generally understudied area.

Characterization of Neutrophil Transepithelial Migration *in Vitro*

Figure 1C highlights the pathway taken by neutrophils as they sequentially exit the circulation through the vascular