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Review

Neutrophils and inflammatory resolution in the mucosa

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ABSTRACT

Inflammatory diseases in mucosal organs as diverse as the lung, liver and intestine inevitably require the intimate interactions between neutrophils and epithelia. The physiologic consequences of such interactions often determine endpoint organ function, and for this reason, much recent interest has developed in identifying mechanisms and novel targets to promote the resolution of mucosal inflammation. Physiologically-relevant in vitro and in vivo model systems have aided in discovery of novel pathways to define basic inflammatory mechanisms and approaches to defining the concepts of inflammatory resolution. Here, we will review the recent literature regarding the contribution of neutrophils to inflammatory resolution, with an emphasis on the role of the tissue microenvironment, endogenous pathways for promoting resolution and the molecular determinants of neutrophil–epithelial cell interactions during ongoing inflammation. These recent studies highlight the dynamic nature of pro-resolving pathways and lend insight into the complexity of treating mucosal inflammation.

1. Introduction

The presence of neutrophils (polymorphonuclear leukocyte, PMN) at sites of tissue injury and infection has long been recognized as a hallmark of acute inflammation. It has become increasingly appreciated that the presence of PMNs at sites of injury do not necessarily prove causation to tissue damage, and in fact, may provide clues into the initiation of inflammatory resolution. In fact, history has suggested this to be the case. As an example, the Roman gladiatorial surgeon Claudius Galen (130–200 AD) made the observation that pus formation in wounds of his patients was associated with more successful wound healing. He became widely known for "*pus bonum et laudabile*", meaning "good and commendable pus" [1].

The contribution(s) of PMN to successful inflammatory resolution is an area of significant interest. Ongoing studies have revealed that infiltrating PMN communicate with the surrounding parenchymal tissues in ways which mold the microenvironment to promote tissue restitution, wound healing and homeostasis. In this review, we will summarize the current state of the art related to the role of PMN in the active resolution of inflammation, with a particular focus on the mucosa.

2. Inflammatory resolution: an active rather than passive process

The resolution of inflammation was historically conceived as a passive act of the healing process occurring independent of active biochemical pathways. This view has fundamentally shifted in the past decade [2–4]. It is now appreciated that uncontrolled inflammation is a unifying component in many diseases and new evidence indicates that inflammatory resolution is a biosynthetically proactive process [5]. These most recent findings implicate tissue decision processes wherein acute inflammation, chronic inflammation, or inflammatory resolution outcomes are dictated by endogenous processes employed to control the magnitude and duration of the acute response, particularly as they relate to the original cardinal signs of inflammation [6,7]. It has now become evident that the resolution program of acute inflammation remains largely uncharted, particularly at mucosal surfaces, and that a complete understanding of these critical pathways will unquestionably direct new therapeutic options.

The mucosa serves as an excellent model for which to define many features of inflammatory resolution. Whether it be the gastrointestinal (GI) tract, the lung or the skin, a primary function of the mucosa is to provide a selective barrier to the outside. At these same

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surfaces exist the potential for infection by pathogenic organisms and the necessity to control commensal microorganisms at homeostatic levels. In this regard, the tissue healing following injury occurs in conjunction with the constant flux of new antigenic material and require that the mucosal immune system appropriately dampen inflammatory and immunological reactions to harmless ingested antigens. The overlying epithelium plays an important role in coordinating both inflammation and resolution. The epithelium lies juxtaposed to the mucosal immune system and lines the entire gastrointestinal tract. Covering a surface area of approximately 300 m^2, the human adult intestinal epithelium consists of a monolayer of cells with intercellular tight junctions, a complex three dimensional structure and a thick mucous gel layer that provides a dynamic and regulated barrier to the flux of the luminal contents to the lamina propria [8,9]. It is widely understood that the gastrointestinal tract exists in a state of low-grade inflammation. Such a state results from the constant processing of luminal antigenic material during the development of oral tolerance and the priming of the mucosal immune system for rapid and effective responses to antigens or microbes that may penetrate the barrier [10].

There may also be significant differences between mucosal surfaces with regard to the contribution of PMN to the resolution process. For example, literature exists that the GI tract may differ from the lung in this regard. This particular aspect has been convincingly demonstrated in vivo. The depletion of circulating PMN using anti-Gr1 antibodies resulted in the exacerbation of symptoms in a number of different murine colitis models, strongly implicating PMN as a central protective factor in ongoing inflammation [11]. By contrast, the depletion of PMN in acute lung injury models appears to serve an anti-inflammatory role [12], although this idea has been revisited [13]. Nonetheless, these results suggest fundamental differences in mechanisms of inflammatory resolution between various mucosal organs. Below, we discuss some potential mechanisms that may contribute to the unique mucosal niche that drive the inflammatory resolution response.

3. Oxygen consumption by PMN as a driver of resolution during acute inflammation

Recent studies have indicated that a contributing factor to differences in the inflammatory resolution response between mucosal tissues is oxygen metabolism. The intestinal mucosa, for example, exhibits a particularly unique oxygenation profile, experiencing profound fluctuations in blood flow and metabolism. For instance, less than 5% of total blood volume is present in the gut during fasting, but following ingestion of a meal, approximately 30% of total blood volume is shunted to the gastrointestinal tract [14]. These fluctuations in blood flow result in a relatively low baseline pO_2 (<40 mmHg [15,16]) under physiologic conditions. By comparison to the lung where baseline pO_2 can be as high as 110 mmHg [17], it is perhaps not surprising that mucosal tissues have evolved a number of adaptive features to cope with these austere metabolic changes. Studies comparing functional responses between epithelial cells from different tissues have revealed that intestinal epithelia appear to be uniquely resistant to hypoxia and that even very low levels of O_2 within the normal mucosa (so-called "physiologic hypoxia") may be a regulatory adaptation mechanism to the steep oxygen gradient across the intestinal mucosa [18]. A key discovery was the observation that epithelial cells of the distal gut basally regulate hypoxia-inducible factor (HIF) [19], the master regulator of oxygen homeostasis [20]. Kelly et al., recently demonstrated that the low-O_2 conditions of the distal GI tract that enable microbial short chain fatty acid (SCFA) production (e.g. butyrate), promotes epithelial O_2 consumption to the extent that HIF is stabilized and functionally maintains mucosal barrier function [21] and the expression of certain antimicrobial peptides [22].

This aspect of oxygen metabolism is exacerbated during inflammation. It was recently demonstrated that during acute inflammatory disease, infiltrating neutrophils mold the tissue microenvironment in ways that significantly promote the stabilization of HIF [23] (Fig. 1). Microarray analysis of epithelial cells following PMN transmigration identified the induction of a prominent cohort of HIF target genes. Utilizing HIF reporter mice, $Gp91^{phox-/-}$ mice (lack a respiratory burst) and PMN depletion strategies in acute colitis models, these studies revealed that transmigrating neutrophils rapidly deplete the microenvironment of molecular oxygen in an NADPH-oxidase-dependent manner and "transcriptionally imprint" a molecular fingerprint that significantly reflects PMN induction of HIF target genes onto the surrounding tissue (Fig. 1). Importantly, this molecular signature promotes effective HIF-dependent inflammatory resolution. Indeed, $Gp91^{phox-/-}$ mice developed highly accentuated colitis relative to controls with exaggerated PMN infiltration, diminished inflammatory hypoxia and increased microbial invasion. In this regard, a clinical corollary to these findings have indicated that patients which lack a functional NADPH oxidase (i.e. chronic granulomatous disease) often present with an inflammatory bowel disease (IBD)-like syndrome [24]. Interestingly, chronic granulomatous disease (CGD) patients exhibit congenital defects in genes coding the subunits comprising the neutrophil NADPH oxidase complex (i.e. mutations in: CYBA, CYBB, NCF1, NCF2, NCF4, RAC1 and RAC2). This NADPH oxidase complex is responsible for the generation of reactive oxygen species (ROS) and used by innate immune cells (esp. PMN) to kill invading pathogens. Approximately 40% of CGD patients develop IBD-like symptoms [25]. Such clinical observations suggest that CGD-associated IBD could represent a failure to resolve acute intestinal insults.

Significant evidence indicates that the large amounts of localized oxygen consumption associated with acute inflammation signals epithelial restitution and inflammatory resolution through the stabilization of HIF [26] (see Fig. 1). Numerous studies have demonstrated that such "inflammatory hypoxia" stabilizes the transcription factor HIF [27]. Once stabilized, HIF triggers the transcription of a cohort of genes that enable intestinal epithelial cells to resolve defective barrier function [18,28–30]. Originally studies by microarray of intestinal epithelial cells subjected to low O_2 revealed profound influences on barrier-related genes [31] that have now been validated in a number animal models of intestinal inflammation [19,32–36] and in human intestinal tissues [23,37–39]. The functional proteins encoded by HIF targets genes include those that localize primarily to the most luminal aspect of polarized epithelia that contribute fundamentally to effective barrier function. These target genes include mucins [40], molecules that modify mucins (e.g. intestinal trefoil factor [18]), antimicrobials [22], xenobiotic clearance [28], and nucleotide signaling [30]/metabolism (e.g. ecto-5′-nucleotidase) [30,31].

It is noteworthy that one of the more prominent epithelial genes induced by PMN transmigration was cyclooxygenase-2 (COX-2) [23]. COX-2 contributes fundamentally to both inflammation and resolution [6,41]. During epithelial cell-PMN interactions, pro-resolving lipid mediators (e.g. lipoxins, resolvins and protectins) are amplified by transcellular biosynthesis through the interactions of two or more cell types, each contributing an enzymatic product [42]; in this case epithelial cell COX-2 generates 18-HEPE from dietary omega-3 fatty acids and PMN-expressed 5-LO to generate resolvins [3]. Such locally generated resolvins is then made available to activate surface expressed ChemR23 receptor, which in turn has been shown to activate a number of antimicrobial pathways within the mucosa [43].

The identification of HIF as a central component to the resolution of mucosal inflammation has guided the development pharmacologic compounds that function to stabilize HIF and drive the