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Neutrophils and the Inflammatory Tissue Microenvironment in the Mucosa

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Abstract

The interaction of neutrophils (PMNs) and epithelial cells are requisite lines of communication during mucosal inflammatory responses. Consequences of such interactions often determine endpoint organ function, and for this reason, much interest has developed around defining the constituents of the tissue microenvironment of inflammatory lesions. Physiologic *in vitro* and *in vivo* models have aided in discovery of components that define the basic inflammatory machinery that mold the inflammatory tissue microenvironment. Here, we will review the recent literature related to the contribution of PMNs to molding of the tissue microenvironment, with an emphasis on the gastrointestinal (GI) tract. We focus on endogenous pathways for promoting tissue homeostasis and the molecular determinants of neutrophil-epithelial cell interactions during ongoing inflammation. These recent studies highlight the dynamic nature of these pathways and lend insight into the complexity of treating mucosal inflammation.

Keywords

metabolism; hypoxia-inducible factor; inflammation; nucleoside; nucleotidase; mucosa; colitis; epithelium

Introduction

The recruitment of neutrophils (polymorphonuclear leukocyte, PMN) to sites of tissue injury and infection has long been recognized as a hallmark of acute inflammation. It is 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 to healing. The contribution(s) of PMN to the molding of the tissue microenvironment is an area of significant interest. These studies have shown that recruited PMN communicate with the surrounding parenchymal tissues in ways that fundamentally change the milieu to promote tissue restitution, wound healing and homeostasis. In this review, we will summarize recent

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literature related to the contribution of PMN to the tissue inflammatory microenvironment, with a particular focus on the GI mucosa.

The microenvironment of the intestinal mucosa

An especially unique feature of the GI microenvironment is the presence of large numbers of microbes on the luminal surface. The mammalian GI tract harbors trillions of bacteria, where a finely balanced mutualism exists (1). Lined by epithelial cells, a primary function of the intestinal mucosa is to provide a selective barrier to the outside world. At these same surfaces exists 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 antigenic material and requires 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 wound healing. The epithelium lies juxtaposed to the mucosal immune system and lines the entire gastrointestinal tract. Covering a surface area of nearly 300 m², 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 (2, 3). It is widely understood that the gastrointestinal tract exists in a state of low-grade inflammation, resulting from the constant sampling and 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 (4).

The GI tract may be somewhat unique in the manner of handling PMN. For example, the depletion of circulating PMN using anti-Gr1 antibodies results in the exacerbation of symptoms in a number of different murine colitis models, strongly implicating PMN as a protective factor in ongoing inflammation (5). By contrast, the depletion of PMN in acute lung injury models appears to serve an anti-inflammatory role (6), although this idea has been revisited (7). Nonetheless, these results suggest fundamental differences in mechanisms of inflammation between various mucosal organs. Below, we discuss some potential mechanisms that may contribute to the unique mucosal niche established by infiltrating PMN.

Contribution of infiltrating PMN to the inflammatory microenvironment

The role of PMN as a first line of defense against invading pathogens has been long recognized as a primary function of PMN at sites of inflammation. PMN diapedesis, during which PMN migrate from the circulatory system to areas of tissue damage and inflammation, results in priming of PMN to express and release a number of factors that drive inflammation in the microenvironment. Activated PMN release multiple pro-inflammatory cytokines, including IL-1, IL-6, TGF- β and TNF- α (8). Chemokines such as CXCL8, CXCL1, CXCL9, CCL2, and CCL3, and other chemoattractants are also produced, resulting in further autocrine and paracrine activation of inflammatory pathways and recruitment of leukocytes (8). Lipid mediators, including leukotriene B₄ and prostaglandin

E₂, as well as key enzymes in arachidonic acid metabolic pathways are also produced by activated neutrophils (9). Proteases such as PMN elastase, gelatinase, and collagenase are released during the process of PMN degranulation and can lead to further activation of cytokines and cause tissue damage (10). Further collateral tissue damage in the microenvironment may occur through the production of reactive oxygen species (ROS) by PMN. Thus, as PMN infiltrate inflamed tissues, they have the ability to cause further tissue injury as well as perpetuate inflammation through multiple mechanisms. While most studies indicate a protective role for PMN in colitis (5, 11), some reports suggest that PMN exacerbate inflammation (12). Seemingly paradoxical to their proinflammatory potential, the preponderance of evidence in the GI mucosa suggests that PMN promote the resolution of chronic inflammation. The remainder of this review will focus on mechanisms by which PMN create a microenvironment that dampens the inflammatory response and promotes tissue healing.

Oxygen consumption by PMN as a driver an hypoxic microenvironment

Recent studies have shown that a contributing factor to inflammatory resolution in the GI mucosa is the unique nature of oxygen distribution and metabolism. The intestinal mucosa, for example, exhibits a particularly unique oxygenation profile, experiencing profound fluctuations in blood flow and metabolism. These fluctuations result in a steep oxygen gradient from the lumen to the lamina propria in the human intestinal tract. Breathable air at sea level contains a partial O₂ pressure (pO₂) of ~145 mmHg (approximately 21% O₂). Measurements of the healthy lung alveolus have revealed a pO₂ of 100–110 mmHg (13). By contrast, the most luminal aspect of the healthy colon exists at a pO₂ below 10 mmHg (14–16). Such differences reflect a combination of O₂ sources, local metabolism and the anatomy of blood flow (17). The pO₂ drops precipitously along the radial axis from the intestinal submucosa to the lumen, largely driven by the vast number of luminal microbes (18). It is perhaps not surprising that the GI mucosa has evolved a number of adaptive features to cope with these profound metabolic conditions. 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 O₂ within the normal mucosa (so-called “physiologic hypoxia”) may be a regulatory adaptation mechanism to the steep oxygen gradient across the intestinal mucosa (19). A key discovery was the observation that epithelial cells of the distal gut basally regulate hypoxia-inducible factor (HIF) (15), a master regulator of oxygen homeostasis (20). Kelly et al, recently demonstrated that the low-O₂ conditions of the distal GI tract that enable microbial short chain fatty acid (SCFA) production (in particular butyrate), promotes epithelial O₂ consumption to the extent that HIF is stabilized and fundamentally contributes to the expression of some antimicrobial peptides (21) as well as maintenance of epithelial barrier function (22).

Metabolic demand for oxygen is prominently enhanced during inflammation. It was recently demonstrated by Campbell et al., (11) that during acute inflammatory disease, infiltrating neutrophils shape the tissue microenvironment in ways that significantly promote the stabilization of HIF. Guided by unbiased microarray analysis of epithelial cells following PMN transmigration, these studies identified a prominent induction of a cohort of HIF target genes. Utilizing PMN depletion strategies, HIF reporter mice and transgenic mice lacking a

respiratory burst (Gp91^{phox-/-}) in acute colitis models, these studies discovered that transmigrating neutrophils rapidly deplete the microenvironment of molecular oxygen in an NADPH-oxidase-dependent manner. As such, it was shown that transmigrating PMN “transcriptionally imprint” a molecular signature that reflects PMN-mediated induction of HIF-target genes onto the surrounding tissue. Notably, this molecular signature promotes effective HIF-dependent inflammatory resolution. Indeed, Gp91^{phox-/-} mice developed highly amplified colitic responses compared to controls. These differences included exaggerated PMN infiltration, diminished inflammatory hypoxia and increased microbial invasion. Clinical corollaries to these findings have revealed that patients which lack a functional NADPH oxidase (i.e. chronic granulomatous disease) often present with an IBD-like syndrome (23). CGD-associated IBD results from a number of single nucleotide polymorphisms that impact the NADPH oxidase complex are IBD candidate genes that can result in a disease termed very early onset IBD (i.e. occurs in the first 6 years of life) (24, 25). This NADPH oxidase complex is responsible for the generation of reactive oxygen species (ROS) and used by innate immune cells (esp. PMN and macrophages) to kill invading pathogens. Interestingly, chronic granulomatous disease (CGD) patients exhibit congenital defects in genes coding the subunits comprising the neutrophil NADPH oxidase complex and approximately 40% of CGD patients develop this IBD-like syndrome (26). A recent retrospective clinical study examined all CGD patients screened at the National Institutes of Health between 1990 and 2010. The authors observed that CGD patients with colonic involvement, 74% had skip lesions (indicative of Crohn’s disease) and 93% presented with anorectal disease (commonly associated with ulcerative colitis). Thus the authors propose that CGD-inflammatory bowel disease is a distinct entity (27). Most CGD patients exhibit mutations in gp91^{phox} (CYBB) and p47^{phox} (NCF1), with fewer exhibiting defects in p22^{phox} (CYBA) and p67^{phox} (NCF2) (28). More recently identified is a rare mutation in a fifth subunit, p40^{phox} (NCF4), involved in phagocytosis-induced superoxide production. The patient presented with CGD and a p40^{phox} mutation exhibited intractable IBD, distinct from infectious complications observed with other mutations (29). Taken together, these findings suggest that CGD-associated IBD represents a separate form of IBD which could represent a failure to resolve acute intestinal and colonic inflammatory insults.

Fine tuning ROS generation and dismutation at sites of injury or infection is likely a critical pivotal juncture in influencing either resolution of acute inflammation or progression towards a chronic inflammatory state. Aside from phagocyte oxidases, other enzymes in this family are expressed throughout the body and respond to inflammatory stimuli to generate ROS, including Nox1, Nox3, Nox4, Nox5, Duox1 and Duox2 (30). Both Nox1 and Duox2 are expressed at high levels within the colonic epithelium. Nox1 is expressed cytosolically, conversely Duox2 is localized to the apical epithelial membrane (31), as such, Nox1 is more likely involved in intracellular signaling, whereas Duox2 likely elicits extracellular ROS release. Proinflammatory stimuli such as IFN- γ (32) and LPS challenge (33) lead to up-regulation of Nox1, whereas Duox2 has been shown to be increased in a cohort of Crohn’s disease patients (34), intestinal staining was negative in the epithelial compartment. Moreover, murine Duox2 has been implicated in restricting *Helicobacter felis* colonization by secretion of H₂O₂ into the gastric lumen(35). In a recent study the authors suggest that mucosal oxidases perform in a sequential fashion during inflammation, prior to recruitment

of Nox2-expressing phagocytes. Here the authors examined the influence of epithelial Nox1/Duox2 on the virulence of *Campylobacter jejuni* and determined that epithelial-derived ROS (36). Furthermore, novel mutations in both Nox1 and Duox2 have been identified and implicated in very early onset IBD (37). Studies by Jones et. al., have implicated a protective role for epithelial-expressed NOX1 generating ROS in response to symbiotic microbes, including *Lactobacilli* species (38). They subsequently demonstrated that this epithelial cytoprotection is most likely due to xenobiotic-induced regulation of the Nrf2 pathway (39). Most recently they have demonstrated that wounded epithelia rely on Nox1 expression to facilitate wound healing, which is significantly induced by mucosally-associated microbes, including *Akkermansia mucinophilia* (40). Taken together these studies implicate mucosally-expressed oxidases in mediating homeostasis and highlight the intimate association between microbiota, epithelium and underlying immune system. Moreover, they emphasize the active role attributed to the epithelium in responding to environmental conditions and coordinating adaptive changes in both the mucosally-associated microbiota and immune response.

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 (41). Once stabilized, HIF triggers the transcription of a cohort of genes that enable intestinal epithelial cells to resolve defective barrier function (19, 42–44). Originally studies using microarray analysis of intestinal epithelial cells subjected to low O₂ revealed influences on barrier-related genes (45) that have now been validated in a number animal models of intestinal inflammation (15, 46–50) and in human intestinal tissues (11, 51–53). The functional proteins encoded by these HIF targets include those that localize to the most luminal aspect of polarized epithelia that contribute fundamentally to effective barrier function, including tight junction proteins (e.g. claudin-1(54)), mucins (55), mucin modifiers (e.g. intestinal trefoil factor (19), antimicrobials (21), xenobiotic clearance (42) and nucleotide signaling/metabolism (e.g. ecto-5'-nucleotidase)(44, 45)).

It is also notable that one of the more prominent epithelial genes induced by PMN transmigration was cyclooxygenase-2 (COX-2) (11). COX-2 contributes fundamentally to both inflammation and resolution (56, 57). During epithelial-PMN interactions, pro-resolving lipid mediators (e.g. lipoxin and resolvins) are amplified by transcellular biosynthesis through the interactions of two or more cell types, each contributing an enzymatic product; in this case epithelial cell COX-2 generates 18-HEPE from dietary omega-3 fatty acids and PMN-expressed 5-LO to generate resolvins (58). These locally generated lipid mediators bind to and activate surface expressed receptors (e.g. ChemR23), which in turn has been shown to upregulate a number of antimicrobial peptides in the mucosa (59).

HIF activity has also been shown to directly impact PMN function. In original studies by Cramer (60) and Peyssonnaud (61), these authors provided an essential role for HIF-1 in innate immunity of myeloid phagocytes, particularly related to bacterial killing. Utilizing conditional deletion of *Hif1a* in myeloid populations (lysM-Cre) revealed profoundly decreased bactericidal capacity and a failure to restrict systemic sepsis when myeloid phagocytes lacked functional *Hif1a*.

It is important to note that the majority of these studies on neutrophil and epithelial oxidases and the roles of HIF have utilized animal models, thus extending conclusions to humans should be done cautiously. All things considered, these studies have provided new insight into how PMN communicate with tissues during active inflammation. The described “transcriptional imprinting” response of select molecular pathways, notably HIF, provides mucosal memory of trafficked PMN that elicits functional responses that may be central to tissue homeostasis. While PMN accumulation as crypt abscesses has long served as a hallmark of mucosal inflammation, these findings reveal that PMN accumulation, coinciding with the surrounding tissue response, is likely a necessity for appropriate wound healing.

Neutrophils in the tumour microenvironment

Inflammation has long been recognized as an important feature of cancer development and progression. The DNA damaging and pro-mutagenic conditions that exist during chronic inflammation have long been suspected to initiate and promote tumor development. As such, significant evidence exists that neutrophils play an important role in tumor promotion (62). This topic was recently reviewed in detail by Liang and Ferrara (63), but some relevant points are noteworthy here.

While PMN are typically thought of as short-lived immune cells, their recruitment to tissues and exposure to microenvironmental factors such as GM-CSF (64) or hypoxia (65) prolong PMN lifespan and regulate their function. The tumor microenvironment provides a plethora of factors that recruit PMN and support the activity of PMN function (Figure 1). PMN are initially recruited to tumors through the local generation of several chemokines, but the primary signal appears to be CXCL8 (human IL-8, murine KC) (66). The reservoir for these CD11b⁺ Ly6G^{hi} cells appears to be the spleen (67). It remains unclear exactly what signaling cascade elicits tumor-derived IL-8/KC and whether this signaling is tumor-specific. Nonetheless, early in tumor development, these tumor-associated neutrophils (TAN) migrate to the periphery of the tumor, where they remain adherent to the tumor stroma (68). As tumors grow and develop, TANs appear to penetrate and associate among the tumor cells, even deep into the tumor bed. Some studies have shown that while at the tumor site, some TANs may phenotypically differentiate. For example, analogous to the macrophage M1 and M2 dichotomy, TANs appear to develop a more protumorigenic (N2) phenotype in untreated tumors, driven largely by the presence of tumor-derived TGF- β (69). Blocking TGF- β with neutralizing antibodies convert TANs to a more antitumor (N1) phenotype (70), termed N1 TANs that produce higher levels of mediators such as nitric oxide, hydrogen peroxide, and TNF- α and appear to be tumor cytotoxic (71). It is also interesting to note that the depletion of peripheral neutrophils with anti-Gr-1 antibodies resulted in decreased expression of tumor pro-angiogenic factors (e.g. VEGF, MMP9, CXCR4) and inhibited overall tumor growth (70). It is notable that the majority of pro-angiogenic factors are direct HIF target genes (72) and from this perspective, it is intriguing to speculate that PMN oxygen consumption/HIF stabilization within the tissue microenvironment (see above), may function to amplify the production of pro-tumorigenic factors within this niche to enhance tumor growth and/or tumor metastasis. Considering the disparate actions of M1 versus M2 polarized macrophages in tumor biology compared with inflammation, it is tempting to speculate on the presence of distinctly polarized PMN in

inflammatory conditions. The first report to characterize different subsets of PMN based on resistance or sensitivity to methicillin-resistant *Staphylococcus aureus* (Figure 1) (73). The authors identified distinct subsets of PMN with differential surface antigen expression, macrophage activation potential and cytokine/chemokine production. Aside from PMN that polarize in situ, recent findings indicate that subpopulations of PMN may preexist in circulation and respond to disparate chemoattractive stimuli. Massena et al recently demonstrated a CD49d⁺CXCR4^{high}VEGFR1^{high} population of PMN that specifically migrate to sites of hypoxia and promote angiogenesis (74). CD49d is a differentiation marker downregulated on mature PMN. Given the relatively low percentage of circulating PMN that exhibit this phenotype, it is unclear if this population represents an immature PMN population or a distinct VEGF-responsive PMN that responds selectively to hypoxic injury (75). On the polar end of the maturation spectrum, immature PMN progenitors have been characterized to adopt a hybrid PMN/dendritic cell phenotype when exposed to GM-CSF, capable of both antimicrobial activities and professional antigen presentation (76). Furthermore, this novel subset of PMN has been identified in vivo, demonstrated to accelerate clearance of *Escherichia coli* strains and present antigen to CD4 T cells (77). Placed in context, consideration for divergent PMN functions and polarities should not be disregarded in future investigations.

Purine nucleotides and neutrophils in the microenvironment

Neutrophils are an important source of extracellular nucleotides found within the microenvironment of inflammatory lesions (78). A number of studies have revealed that PMN serve as a prominent reservoir for adenosine precursors that are made available for metabolism to adenosine (79). During inflammation, PMN actively release ATP, ADP and AMP (80). Release of ATP occurs in an activation-dependent manner through a mechanism involving connexin 43 (Cx43) hemichannels expressed on the surface of PMN (80). Other studies have shown that human PMNs release ATP along the leading edge of their cell surface as a mechanism to amplify chemotactic signals and direct cell orientation by feedback through P2Y2 nucleotide receptors (81).

It is now well documented that the phosphohydrolysis of ATP and AMP by surface apyrases (e.g. CD39) and ecto-5'-nucleotidase (CD73), respectively, represents the major pathway for accumulation of extracellular adenosine (44, 45, 82). Once liberated in the extracellular space, adenosine is either recycled (e.g. through dipyridamole-sensitive carriers) or interacts with cell surface Ado receptors (83). Presently, four subtypes of G protein-coupled Ado receptors exist, designated AA1R, AA2AR, AA2BR or AA3R and are classified according to utilization of pertussis toxin-sensitive (A1 and A3) or insensitive (A2A and A2B) pathways (83). Recent work has specifically implicated the AA2BR in mucosal healing responses, wherein activation of this receptor elicits potent inhibition of inflammatory signaling cascades mediated by NF- κ B (84). Work in various cell types has shown that adenosine inhibits NF- κ B activation through a number of distinct mechanisms. First, adenosine signaling results in elevation of intracellular cyclic adenosine monophosphate (cAMP) and activation of protein kinase A (PKA), blocking I κ B phosphorylation and thus inhibiting NF- κ B activation(85). In addition, adenosine signaling leads to inhibition of phosphorylation and degradation of I κ B α , resulting in attenuated NF- κ B activation due to

increased SUMO-1 modification of I κ B α (86). Lastly, NF- κ B activity is also secondarily inhibited by a reduction in tumor necrosis factor (TNF)- α , which leads to a decrease in nuclear translocation of active NF- κ B without influencing I κ B phosphorylation or degradation(87).

Platelets also provide a rich source of extracellular ATP during mucosal inflammation. Platelets release nucleotides at high concentration upon activation by collagen or ADP through dense granule release (88). It was shown that interactions between platelets and PMN provide important signals for the resolution of intestinal inflammation and fluid transport via in an adenosine-dependent manner (89). Indeed, these studies showed that platelets migrate across intestinal epithelia in a PMN-dependent manner. Furthermore, platelet-PMN comigration was observed in intestinal tissue derived from human patients with IBD. The translocated platelet-PMN clusters were found to release large quantities of ATP, which was sequentially metabolized to adenosine via a 2-step enzymatic reaction involving CD73 and CD39-like molecules expressed on IEC(90). These studies identified a mechanism involving adenosine-mediated activation of electrogenic chloride secretion, with concomitant water movement into the intestinal lumen (91). This physiologic response has been demonstrated to serve as a protective flushing mechanism for mucosal-associated bacteria(92).

Given its long history, it is somewhat surprising how little is known about the molecular mechanisms of adenosine responses. The signal transduction through the various adenosine receptors (ARs) has been well characterized, however, less is known about the post-receptor events. One potentially important mechanism has revealed that adenosine stabilizes HIF (93) and inhibits NF- κ B (84, 94) through actions on Cullin neddylation pathways. Neddylation is a form of post-translational protein modification, whereby Nedd8 (neutral precursor expressed, developmentally downregulated) moieties are added to proteins(95). Cullin proteins are key components of the ubiquitin ligase complexes, regulating protein ubiquitination and proteasomal processing. Neddylation of cullins is essential for functional ubiquitin ligase complexes, similarly, deneddylation of cullins acts to inhibit ubiquitination(95). Original studies implicated commensal bacterial inhibition of NF- κ B through Cullin-1 (Cul-1) deneddylation (96). Bacterial fermentation products (e.g. butyrate) can increase intracellular ROS and lead to impaired neddylation of Cullins thus influencing NF κ B signaling, implicating an important role for the commensal intestinal flora for the regulation of inflammatory processes(97). An analogous signaling complex exists upstream of HIF- α proteins, which is comprised of Cul-2, Elongin B/C and the von Hippel-Lindau protein (pVHL). Similar to the Cul-1-I κ B-NF- κ B axis, neddylation of Cul-2 is required for the ubiquitination of hydroxylated HIF-1 α in normoxic conditions. HIF- α subunits are hydroxylated by prolyl hydroxylases (PHDs) to target the HIF subunits for degradation. In the absence of oxygen, the HIF- α subunits are not targeted for degradation and therefore translocate into the nucleus to dimerize with constitutively expressed HIF-1 β subunits, turning on targeting genes(78).

Recently, pharmacological inhibition of Cullin neddylation has been employed using a small molecular inhibitor, MLN4924. Interestingly, MLN4924 is a structural analog of adenosine monophosphate (AMP) and functions by inhibiting the Nedd8-activating enzyme, thereby

inhibiting the neddylation of Cullin proteins (98). The protective role of HIF and the contribution of the neddylation pathway were confirmed in a murine DSS-colitis model. Colitic mice exposed to MLN4924 displayed an overall diminished inflammation, reflected as decreased weight loss, decreased colonic shortening and decreased histologic injury (99). Identification of the Nedd8-specific protease DEN1/SEN8 has provided new insight into this emerging field. DEN1 contains dual isopeptidase activity capable of directly deneddylating Cullin targets (100, 101). Knockdown of DEN-1 in vascular endothelial and intestinal epithelial cells using lentiviral shRNA approaches revealed decreased Cul-1 and Cul-2 neddylation and significantly abrogated barrier dysfunction in vitro (93, 94).

Therapeutic considerations of PMN and the tissue microenvironment

Given the central role of HIF in mucosal inflammation, substantial efforts have been made in the development of pharmacologic compounds that stabilize HIF and enhance the expression of HIF target genes (102). In most instances, the pharmacologic approach to achieve HIF stabilization in normoxia has involved the inhibition of HIF prolyl-hydroxylases (PHDs), originally described as products of genes related to *C. elegans* egl-9 (103) and later cloned in mammals as three isoforms of PHDs (PHD1, PHD2 and PHD3) that hydroxylate HIF- α and promote degradation through VHL and proteasome-dependent pathways (103, 104). Targeting the catalytic domain of PHDs was initially achieved by a screen of molecules that interfere with critical cofactors such as the 2-oxoglutarate mimicry (e.g. dimethylxalylglycine, DMOG) (105). The original studies using PHD inhibition in mucosal inflammation demonstrated a protective role for HIF stabilizers in different models of intestinal inflammation. The use of DMOG for the treatment of intestinal inflammation in chemically-induced colitis proved highly effective in abrogating mucosal inflammation, at least in part through blocking epithelial apoptosis (46). A parallel study published at the same time utilized a HIF stabilizer (FG-4497) that was based on a screen to identify erythropoietin enhancers. Similar to DMOG, FG-4497 blocks the active site of PHDs (105). In both cases, HIF stabilization was associated with the abrogation of mucosal inflammation, most prominently related to enhanced barrier function (46, 49). More HIF-1 selective stabilizers, such as AKB-4924, have shown significant protection in colitis models as well as anti-microbial activity in stimulating the killing of the pathogens (11, 106, 107). Results from these studies in pre-clinical models indicate that IBD may be one of the more promising applications for PHD inhibitor-based therapies.

Conclusion

The migration of PMN into sites of injury and infection represents one of the most significant changes to the tissue microenvironment. In the mucosa, restitution of the epithelial barrier defines a critical determinant of the healing response. Recent studies investigating changes within the microenvironment of acute inflammation have revealed important new signaling cascades initiated by activated PMN. Of particular recent interest is the shift in tissue oxygenation toward hypoxia and its relevance to HIF-target genes that are strongly associated with promoting barrier function and altering tissue bioenergetics. Studies in animal models have demonstrated an overall protective and anti-inflammatory influence

of prolyl hydroxylase inhibition (i.e. HIF stabilization), identifying the mucosa as a strong candidate for targeted HIF-based therapy.

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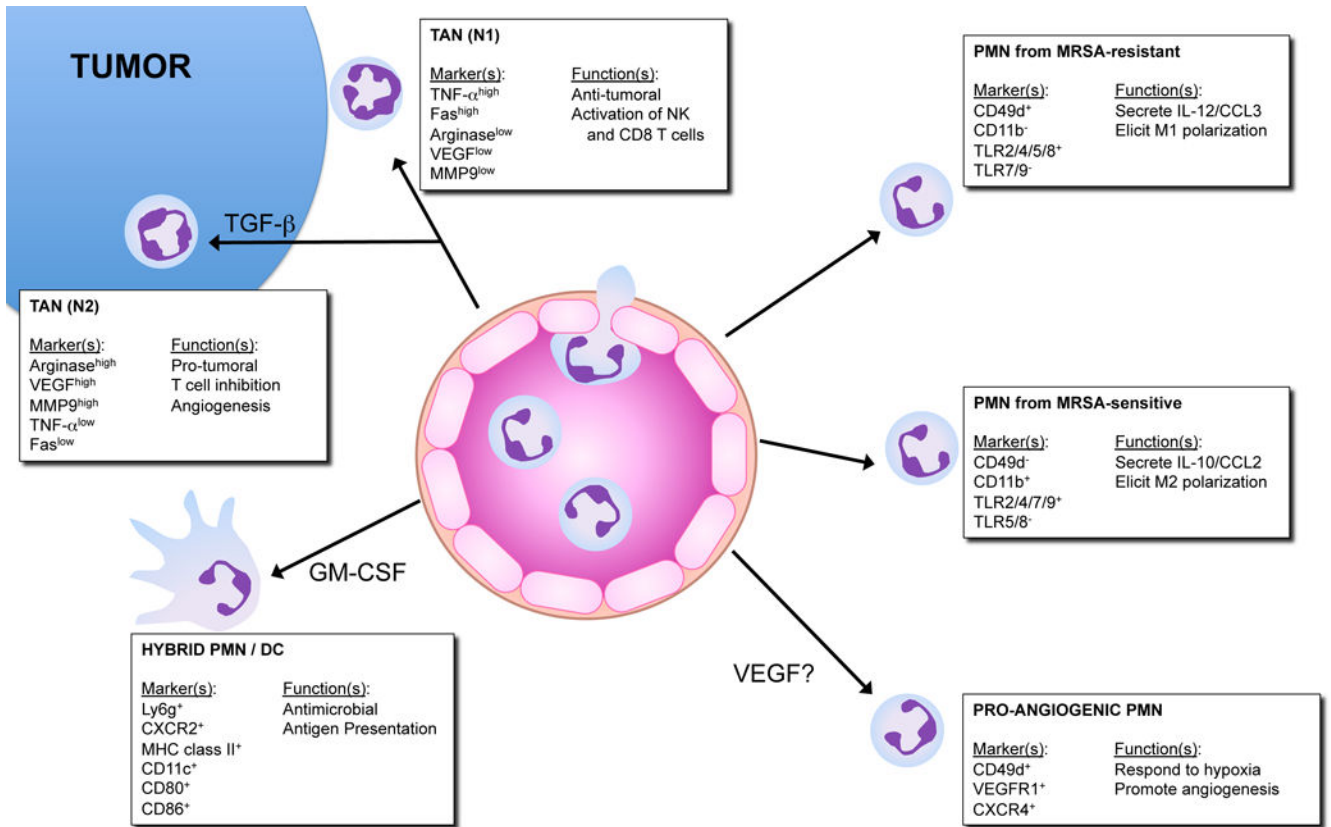


FIGURE 1. Neutrophil polarization in response to microenvironmental reprogramming
 PMN respond to environmental cues to elicit differential polarization and function. Counter-clockwise from the top: **1)** TAN-N1 migrate to tumor periphery, their nuclei exhibit a “hypersegmented” appearance and they mediate anti-tumoral activities. **2)** TAN-N2 respond to tumor-derived TGF- β , their nuclei appear “circular” and they elicit pro-tumoral role. **3)** Hybrid PMN/DC represent an immature PMN precursor exposed to GM-CSF and adopt a hybrid antimicrobial and antigen presenting phenotype. **4)** Pro-angiogenic PMN may be derived from longer-lived mature PMN and respond specifically to sites of ischemia/hypoxia. **5)** PMN isolated from MRSA-sensitive hosts secrete anti-inflammatory IL-10 and promote alternative activation in macrophages. **6)** PMN isolated from MRSA-resistant hosts secrete IL-12 and influence classical activation in macrophages.
 TAN: tumor associated neutrophil; DC: dendritic cell; MRSA: methicillin-resistant *Staphylococcus aureus*.