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The role of polymorphonuclear leukocyte trafficking in the perpetuation of inflammation during inflammatory bowel disease

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Abstract

The inflammatory bowel diseases (IBD; Crohn's disease and ulcerative colitis) are chronically relapsing, inflammatory disorders of the intestine and/or colon. The precise etiology of IBD remains unclear, but it is thought that a complex interplay between various factors including genetic predisposition, the host immune system, and the host response to luminal microbes play a role in disease pathogenesis. Further, numerous lines of evidence have implicated the accumulation of large numbers of PMN in the mucosa and epithelial crypts of the intestine as a hallmark of the active disease phase of IBD. Massive infiltration of PMNs is thought to be instrumental in the pathophysiology of IBD with the degree of PMN migration into intestinal crypts correlating with patient symptoms and mucosal injury. Specifically, migrated PMN have been implicated in the impairment of epithelial barrier function, tissue destruction via oxidative and proteolytic damage, and the perpetuation of inflammation through the release of inflammatory mediators. This review highlights the multifactorial role of PMN egress into the intestinal mucosa in the pathogenesis of IBD, as it represents an important area of research with therapeutic implications for the amelioration of the symptoms associated with IBD.

1. Introduction

Inflammatory bowel disease (IBD), an important cause of life-long morbidity with a prevalence of greater than 1 in 1,000 (1, 2), is characterized by chronic uncontrolled inflammation of the small intestine and/or colon, presenting in affected individuals as multiple symptoms including bleeding, weight loss, abdominal pain and recurrent diarrhea. Historically, IBD has been most evident in western civilizations, however its prevalence in developing countries has increased in recent years. At present, no cure exists and available treatments are limited in terms of efficacy. In addition, it has been reported that up to 75% of individuals with Crohn's disease and 25-33% of individuals with ulcerative colitis will require surgery for therapy-unresponsive disease during their lifetimes (3). The major forms

of idiopathic IBD, ulcerative colitis and Crohn's disease have been extensively studied by clinical, pathological, endoscopic and radiological analyses (4). Ulcerative colitis is characterized by diffuse mucosal inflammation that is restricted to the colon, beginning at the rectum and spreading proximally in a continuous fashion. In contrast Crohn's disease can affect any part of the gastrointestinal tract, though it is most commonly associated with non-continuous transmural inflammation or 'skip lesions' of the terminal ileum or the perianal region.

The pathogenesis of IBD remains incompletely understood, though complex multifactorial interactions between enteric commensal bacteria and the host immune system, as well as the underlying host genotype, are thought to influence its development (5-7). It has however, been well documented that disease activity in IBD is linked to the transepithelial influx of polymorphonuclear leukocytes (PMN) into the mucosal epithelium (cryptitis) and subsequently into the intestinal lumen. Indeed the extensive homing of PMN into areas of mucosal inflammation has facilitated the development of imaging techniques where radiolabeled PMN are used for the semi-quantitative assessment of disease activity in IBD (8, 9). Co-incident with massive luminal influx of PMN is extensive mucosal and/or transmural injury including edema, loss of goblet cells, decreased mucous production, crypt cell hyperplasia, erosions, ulceration and crypt abscess formation (Fig. 1) (8-12). In this review, we highlight the role of PMN trafficking in the perpetuation of inflammation in IBD, as this represents an important area of research with novel therapeutic implications for the amelioration of the symptoms associated with both ulcerative colitis and Crohn's disease.

2. PMN Trafficking Cascade

As the first responders of the innate immune system, PMN must execute a rapid, exquisitely orchestrated, cascade of migratory events encompassing transendothelial, transmatrix and transepithelial navigation (Fig. 2), terminating in the engulfment and phagocytosis of invading pathogens and subsequent resolution of inflammation.

Transendothelial Migration

PMN migration from the vasculature begins with free-flowing PMN tethering to the endothelial wall, followed by PMN rolling along the surface of the microvascular endothelium. These processes are mediated through weak, reversible interactions between E-, P- or L-selectin, and their carbohydrate ligands including P-selectin glycoprotein ligand-1 (PSGL-1) (13-15). Specifically, the binding of endothelial P-selectin to PMN PGSL-1 is thought to be important for the initial tethering of PMN to the endothelium (16), with binding to E-selectin being more relevant to the subsequent process of PMN slow rolling (17). Rolling PMN remain in close approximation with the vascular endothelium which facilitates detection by PMN of chemokines bound to heparin sulfate proteoglycans on endothelial cell membranes. PMN-chemokine interactions, in turn, prime PMN for firm adhesion to the endothelium (18). Activation of PMN by chemokines such as IL-8 and chemoattractants including C5a, leukotriene LTB₄, platelet activating factor (PAF) and bacterial-derived formyl peptides induce firm adhesion through high affinity, integrin-mediated binding. This high affinity PMN binding to the endothelium is mediated by well established interactions between PMN expressed-Mac-1 (CD11b/CD18) and Lymphocyte

Function Associated antigen-1 (CD11a/CD18) and members of the immunoglobulin super-family, such as intracellular adhesion molecule-1 (ICAM-1). More recently it has been proposed that the PMN integrins $\alpha 4\beta 1$ and very late antigen-4 (VLA-4), as well as endothelial expressed ICAM-2, vascular cell adhesion molecule-1 (VCAM-1) and the receptor for advanced glycation end products (RAGE) can participate in the high affinity binding of PMN to the endothelium (19, 20).

Following firm adhesion, PMN crawl along the endothelial cell surface to reach the point of transmigration, which proceeds in a CD18 integrin-dependent manner (21). The next step in the PMN migration cascade is diapedesis through the endothelium, which proceeds predominantly through a paracellular route, with transcellular migration reported to occur only under certain circumstances (22). It has recently been observed that preferential PMN paracellular, transendothelial migration takes place at tricellular corners and endothelial junctions positioned above areas with low levels of expression of basement membrane components such as laminin 10, collagen IV and nidogen-2 (23). The paracellular migration of PMN between endothelial cells is mediated in part by active involvement of various endothelial junctional molecules such as platelet/endothelial cell adhesion molecule-1 (PECAM-1), CD99, ICAM-2, endothelial cell-selective adhesion molecule (ESAM) and members of the junctional adhesion molecule family (JAM-A, B, and C) (19, 24). To facilitate the completion of transendothelial migration, PMN extend lateral protrusions in order to locate suitable sites in the vascular basement membrane and pericyte sheath that allows further movement out of the extravascular tissue and through the interstitium. However, before approaching the interstitium, it has been reported that leukocytes must detach their tails/uropods from the basolateral side of the endothelial monolayer and/or basement membrane in a CD18 integrin dependent manner (25).

The importance of targeting PMN trafficking as a therapy for IBD is highlighted by the number of novel biomolecules that have been designed to specifically modulate leukocyte intestinal recruitment and retention. Oral metronidazole is one of the most commonly used antibiotics in the treatment of Crohn's disease. In addition to acting as an antimicrobial, metronidazole also exerts anti-inflammatory effects on the intestinal endothelium. Specifically it has been demonstrated that metronidazole inhibits PMN trafficking by preventing the firm adhesion of leukocytes to the endothelium in the postcapillary mesenteric venules of rats (26). One open study reported a decrease in pain and tenderness in up to 80% of individuals with perianal Crohn's disease upon treatment with oral doses of metronidazole (27), with an additional study demonstrating healing of fistulas in one-third to one-half of subjects treated (28). Another molecule that has been investigated as a possible therapeutic reagent for IBD is ISIS 2302 (Alicaforsen), a human anti-sense oligonucleotide that alters the local inflammatory environment of the vascular endothelium by blocking ICAM production. Alicaforsen initially showed promise in a phase I placebo controlled trial for Crohn's disease (29), but failed to meet more definitive primary endpoints in subsequent human trials (30), and as yet, this compound has not been further developed for use in IBD therapeutics. Natalizumab is a human recombinant monoclonal antibody directed against the $\alpha 4$ integrin subunit which targets VCAM mediated leukocyte-endothelial interactions. Though primarily a lymphocyte associated integrin, it has also been reported that human neutrophils, under inflammatory conditions, can express the $\alpha 4$ integrin subunit (20).

Natalizumab is the first selective adhesion molecule inhibitor approved for treatment of Crohn's disease. Clinical trials testing the efficacy of natalizumab have demonstrated improved C-reactive protein levels, improved quality of life and an improvement in the rates of clinical remission in individuals with active Crohn's disease (31, 32).

Transepithelial Migration

Following migration out of the inflamed endothelial vasculature and across the interstitium, migrating PMN next encounter the mucosal epithelial barrier. As with migration across the microvascular endothelium, PMN transepithelial migration is a multi-step process. However the sequence of interactions that occur between infiltrating PMN and the mucosal epithelium remains incompletely characterized despite the strong correlation between PMN transmigration and symptomatic disease. The initial binding contact as PMN migrate across the epithelium is mediated through CD11b/CD18-dependent recognition of counter-ligands on the basolateral surface of epithelial cells. The importance of CD11b/CD18-dependent interactions in PMN transepithelial migration has been well documented with antibodies to CD11b and CD18 blocking almost all migration to the bacterial peptide fMLF (33). However, it has also been reported that PMN migration to other chemoattractants such as C5a, IL-8, and LTB₄ can also occur in a CD11b/CD18 independent fashion (34). Specific epithelial ligands for PMN CD11b/CD18 have not been identified to date, however a role for fucosylated, but not sialylated, epithelial proteins in this binding interaction has been reported (35).

Following initial adhesion to the basolateral surface, migrating PMN must next navigate epithelial intracellular junctions encompassing basally-located desmosomes, followed by adherens and apically localized tight junctions. Together, tight and adherens junctions associate with the actin cytoskeleton to form the apical junction complex (AJC) which, in addition to forming a formidable barrier to infiltrating PMN, is critical in determining polarization of epithelial monolayers and regulation of paracellular fluid and solute transport. Despite the importance of the role of PMN transepithelial migration in epithelial barrier disruption and IBD pathophysiology, the molecular details facilitating PMN transepithelial migration across the AJC are far from understood. It is however widely accepted that PMN migrate between epithelial cells within the paracellular space and that epithelial proteins are important in the regulation of this process. Specifically, interactions between PMN-expressed signal regulatory protein α and the basolaterally expressed epithelial CD47 have been implicated in the regulation of the rate of PMN migration across polarized intestinal epithelium (36, 37). In addition numerous members of the cortical thymocyte of *Xenopus* (CTX) protein family including nectins, Coxsackie and adenovirus 5 receptor protein (CAR), A33 antigen, and the junctional adhesion molecules (JAMs) have been implicated in PMN epithelial interactions. Specifically, it has been demonstrated that protein binding interactions between PMN expressed JAM-like protein (JAM-L) and epithelial expressed CAR play an important role in modulating PMN migration across epithelial tight junctions (38).

Following passage through the epithelial tight junctions, a late stage in the PMN transepithelial migratory response entails interactions between PMN and the apical aspect of

the intestinal epithelium. Through these binding interactions, PMN remain in close approximation with intestinal epithelial cells (IECs). Under these conditions, PMN are exposed to a complex milieu of bacterial and host-derived stimuli, resulting in a sustained cycle of PMN stimulation and activation. Crypt abscesses (a classic feature of active IBD), represent collections of massive numbers of activated PMN that have migrated across the epithelium before accumulating within the colonic epithelial crypts. This retention of PMNs correlates both with deformation of crypt architecture (Fig. 1) and, also with IBD disease symptoms (7).

Not surprisingly, the intestinal epithelium expresses receptors that interact with PMN that have migrated across the AJC. These epithelial proteins therefore play an important functional role in mediating PMN detachment and clearance from the luminal surface of the intestinal epithelium. It has been previously reported that Intracellular Adhesion Molecule 1 (ICAM-1), one of the known ligands for the PMN adhesion molecule CD11b/CD18, is primarily expressed at the apical surface of the intestinal epithelium but only under inflammatory conditions. In addition antibody mediated blockage of CD55, which also localizes to the luminal surface of the intestinal epithelium, results in the accumulation of PMN at the apical epithelial surface (39, 40). Most recently a role for the epithelial transmembrane glycoprotein CD44v6 in the detachment of PMN from the apical aspect of the intestinal epithelium has been reported. Specifically, it was demonstrated that a CD44v6 binding mAb blocked PMN transepithelial migration across T84 IECs by preventing both the shedding of the extracellular domain of CD44v6 and the detachment of PMN from the apical aspect of the epithelium (41). The relevance of these apical epithelial proteins and the role they play in the regulation of PMN trafficking in disease is highlighted by previous reports demonstrating that the expression of CD44v6 is up-regulated in the colonic mucosa of individuals with IBD (41, 42).

In support of the importance of PMN transit through the intestinal epithelium contributing to the pathogenesis of disease, it has been reported that in the intestine, PMN transjunctional migration is associated with transient decreases in both transepithelial resistance (43, 44), and epithelial barrier integrity (12). In addition, one of the consequences of continuous PMN infiltration, as is seen with IBD, is mucosal ulceration accompanied by massive disruption of epithelial barrier function and enhanced entry of luminal contents and microorganisms into the submucosa. Further, intense PMN egress has been shown to alter the expression levels of tight junction proteins including zonula occludens -1 (ZO-1), Claudin 1, JAM A, β -Catenin and E-cadherin in epithelial cells resulting in compromised barrier function and increased intestinal permeability (12).

3. PMN Reactive Oxygen Species and IBD

In addition to disruption of epithelial barrier caused by PMN migration, recruited PMNs become activated and release large amounts of cytotoxic mediators, such as reactive oxygen and nitrogen species, into the intestinal mucosa where they can trigger bystander tissue injury and continued barrier disruption. Upon PMN activation by agonists including bacterial formylated peptides, cell membrane localized nicotinamide adenine dinucleotide phosphate (NADPH) oxidase transfers electrons from NADPH to molecular oxygen,

generating superoxide radicals (O_2^-) (Fig. 3). O_2^- is rapidly converted to hydrogen peroxide (H_2O_2), either by spontaneous dismutation or through catalysis by the enzyme superoxide dismutase (SOD). In addition, in the presence of Fe^{2+} , superoxide radicals can be converted, through a non-enzymatic secondary process, into highly reactive hydroxyl radicals (OH), which are known to depolymerize gastrointestinal mucin, peroxidize lipids, oxidize proteins and carbohydrates, and promote DNA degradation (45). Superoxide radicals can also react with nitric oxide, which is found at high concentrations at sites of intestinal inflammation, generating the strong oxidant peroxynitrite ($ONOO^-$). Further, in the presence of a halide such as Cl^- , superoxide radicals are readily converted into hypochlorous acid (HOCl) or N-chloramines (RNHCl) in reactions catalyzed by the PMN granular enzyme myeloperoxidase (MPO) (46). Hypochlorous acid is both a chlorinating and oxidizing agent that is 100-1000 times more toxic than O_2^- or H_2O_2 and acts nonspecifically and rapidly on a range of substrates including sulfhydryls, polyunsaturated fatty acids (PUFAs), DNA, pyridine nucleotides, aliphatic, and aromatic amino acids, and nitrogen containing compounds (47). In addition to its role in the generation of ROS, MPO has recently been reported to be an important regulator of the production of neutrophil extracellular traps (NETs), the end product of a distinct form of programmed cell death called Netosis (48). Further, production of NETs by PMN has the potential to participate directly in the perpetuation of intestinal inflammation in IBD, through bystander tissue damage, as it has been reported previously that vascular deposition of NETs is linked to small-vessel vasculitis, hepatitis, thrombosis and lupus nephritis and that pulmonary deposition of NETs in the lungs has been linked to the pathogenesis of cystic fibrosis (48, 49).

PMN produced ROS can also have particularly deleterious effects on the intestinal mucosa as the plasma membranes of IECs contain large amounts of PUFAs, which are highly susceptible to damage by oxidative radicals. Degradation of PUFAs within the cell membranes of the intestinal epithelium by lipid peroxidation results in the subsequent disruption of the cell membrane and increased mucosal permeability (50). However, under normal circumstances, human tissue contains sufficient amounts of protective enzymes such as catalase and glutathione peroxidase as well as non-enzymatic anti-oxidants such as thiols, ascorbate and α -tocopherol. Thus, most of the potentially injurious oxidizing agents that leak into the tissues are neutralized, thereby limiting tissue damage. However uncontrolled ROS release by transmigrated PMN, as occurs in the mucosa during active episodes of IBD, is thought to overwhelm these protective mechanisms, resulting in oxidative damage to intestinal tissue, and more specifically, damage to cells responsible for maintaining colonic epithelial barrier function (45, 51, 52). In support of the potential for PMN-derived ROS to cause tissue damage during intestinal inflammation, it has been reported that PMN from individuals with IBD generate increased levels of superoxide when compared to PMN from healthy volunteers (53, 54). In contrast it has also been reported that PMN isolated from individuals with Crohn's disease have a reduced respiratory burst (55). However it should be noted that several of the affected individuals included in this recent study had a profound defect in superoxide production, similar to that seen in individuals with chronic granulomatous disease (CGD), suggesting that other underlying factors affecting ROS generation might be influencing the data in this study. It has also been demonstrated through chemiluminescence studies that the inflamed colons from humans with IBD or mice with

experimental colitis contain much higher amounts of ROS than control or uninvolved colons (56, 57). In addition, biopsies from individuals with active IBD have increased levels of lipid peroxidation products suggestive of enhanced ROS generation (52, 58). Furthermore, levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG), the primary DNA modification produced by ROS, are significantly increased in the mucosa of individuals with ulcerative colitis (59). In addition multiple experimental animal models of IBD have demonstrated increased production of ROS (superoxide, hydrogen peroxide, hypochlorous acid) that correlates with both disease severity and progression (47, 51).

In addition to the directly toxic effects of ROS mentioned above, these active metabolites can also mediate epithelial and mucosal damage indirectly by altering the protease/anti-protease balance within the intestinal interstitium. Specifically PMN derived hypochlorous acid can inactivate the α 1 protease inhibitor and also α 2 macroglobulin, facilitating dysregulated proteolysis of the mucosal interstitium and the intestinal epithelium by phagocyte derived elastase (60). Also, PMN use the MPO-H₂O₂-chloride system to generate reactive oxidants, activating metalloproteinases that can in turn go on to hydrolyze both the α 1 protease inhibitor and collagen (60). It has also been reported that, even at non-toxic concentrations, certain PMN derived oxidative metabolites significantly enhance intestinal and colonic Cl⁻ secretion. Thus, these reactive metabolites may affect the pathogenesis of inflammation-induced diarrhea and the depressed intestinal contractility associated with IBD (61, 62).

The involvement of ROS in the pathogenesis of IBD is further supported by the protection against chemically induced colitis in mice afforded by application of free radical scavengers and other antioxidants (63-65). Finally evidence for a cause and effect relationship between reactive oxygen species and IBD is derived from animals modified chemically or genetically to alter the expression of endogenous colonic antioxidant enzymes. Specifically, it has been demonstrated that either transgenic over-expression of Zinc superoxide dismutase (ZnSOD) or *ex vivo* extracellular SOD gene transfer resulted in attenuation of DSS-induced colitis in mice (an acute injury ulcerogenic colitis model which histologically mimics ulcerative colitis) (66, 67). It has also been reported that intragastric administration of a manganese superoxide dismutase (MnSOD)-producing recombinant *Lactobacillus casei* BL23 strain attenuated DSS-induced colitis in mice (68). Conversely inhibition of glutathione biosynthesis has been reported to result in the development of colitis in mice (69), and mice with targeted disruption of both glutathione peroxidase-1 (GPx1) and GPx2 also develop spontaneous colitis (70).

Despite their well characterized role in driving the ongoing inflammatory response associated with IBD, in a normal self-limited immune response, PMN are also key players in the resolution of inflammation and the restitution of the intestinal epithelial integrity (71). Therefore constant transmigration of PMN into the inflamed intestinal mucosa, during dysregulated immune responses, where the local environment mimics the acute phase of inflammation, results in a bias towards release of PMN derived ROS and pro-inflammatory cytokines and not the PMN mediated release of anti-inflammatory agents such as vascular endothelial growth factor, lipoxins, resolvins and protectins that would facilitate mucosal healing (72). As such, rather than participating in the removal of inflammatory mediators

and cell debris from the tissues, during active episodes of IBD, PMN production of tissue damaging reactive oxygen metabolites and other pro-inflammatory mediators is excessive, thus contributing to the perpetuation of an acute inflammatory response and delaying the restitution of intestinal homeostasis.

4. Role of PMN-derived inflammatory mediators in IBD

Though previously thought of as being poorly biosynthetic and largely devoid of *de novo* transcriptional activity, PMN have more recently been reported to synthesize and secrete a wide variety of pro-inflammatory cytokines, chemokines, growth factors and other inflammatory mediators in a largely stimulus-dependent manner (Table 1). Therefore, in addition to secreting cytotoxic metabolites at sites of inflammation, PMN have the capacity to contribute to the broader inflammatory response through the release of specific inflammatory mediators. Also PMN are the predominant infiltrating cells present in inflamed tissues, so despite their generally lower synthetic capacity, PMN nevertheless represent an important source of inflammatory mediators produced and secreted into inflamed tissues. In view of the broad spectrum of activities exerted by PMN-produced pro-inflammatory mediators, it can reasonably be inferred that migrated PMN play an important role in eliciting and sustaining the inflammation of the intestinal mucosa that is characteristic of IBD. Indeed, it is also therefore possible that increased amounts of certain pro-inflammatory mediators in the mucosa and feces of individuals with IBD may be produced in large part by migrated PMN (73). A complete characterization of all the cytokines/inflammatory mediators produced by PMN is beyond the scope of this current manuscript, and this topic has been previously and extensively reviewed elsewhere (74, 75). However an understanding of the production and release of specific IBD-associated cytokines by PMN is important in terms of better understanding the potential for PMN produced mediators to influence the perpetuation of the inflammatory phenotype seen in IBD.

PMN-derived Cytokines

It has been reported that one of the most abundant PMN produced cytokines is Interleukin-8 (IL-8) (76). PMN-derived IL-8 is likely to be relevant to the pathogenesis of IBD as analysis of resected bowel segments from individuals with active ulcerative colitis or Crohn's disease demonstrates strong and specific IL-8 expression in the affected mucosa that correlates with the histological grade of active inflammation. IL-8 expressing cells were found to be mainly macrophages, PMN and epithelial cells, however it has been suggested that PMN represent the main IL-8 expressing cell population at the base of ulcers, in inflammatory exudates on the mucosal surfaces, in crypt abscesses, and at the borders of fistula tracts (77).

PMN have also been shown to produce and secrete other potent chemokines. Growth-related gene product α (GRO α), like IL-8 is a potent PMN chemoattractant and activator. Secretion of IL-8 and GRO- α by PMN at sites of intestinal inflammation facilitates the ongoing recruitment of PMN and triggers the release of reactive metabolites into the intestinal mucosa, leading to ongoing tissue damage and the continuation of the cycle of neutrophilic attraction and activation. The ability of activated PMN to secrete Macrophage Inflammatory Protein-1 α (MIP-1 α /CCL3) and MIP-1 β (CCL4) has also been well documented (78).

MIP1 α acts as a potent chemotactic/activating factor for monocytes, eosinophils and subpopulations of T and B lymphocytes in addition to activating several effector functions of macrophages and PMN. These effector functions include the generation of H₂O₂, and the secretion of IBD associated cytokines including tumor necrosis factor- α (TNF- α), IL-1 α and IL-6 (76, 79). The ability of PMN to secrete MIP-1 α suggests that once PMN arrive at an inflammatory site, they not only support the ongoing trafficking of more PMN but also promote the subsequent accumulation and activation of monocytes, macrophages, eosinophils, and lymphocytes.

PMN also produce and release TNF- α , IL-1 α and IL-1 β at sites of inflammation, with PMN implicated as being one of the cellular sources of TNF- α in colonic biopsies obtained from individuals with Crohn's disease (80). TNF- α is a pro-inflammatory mediator which exerts effects on monocytes, macrophages, lymphocytes and eosinophils as well triggering increased PMN adhesion to the vascular endothelium, enhanced PMN phagocytosis, increased PMN survival at sites of inflammation, increased PMN degranulation and increased PMN mediated release of reactive oxygen metabolites (81). IL-1 α and IL-1 β can also exert immunomodulatory effects on PMN, leading to enhanced priming and activation, and therefore these PMN-produced cytokines may also contribute to disease pathogenesis observed during a dysregulated immune response. Indeed it has previously been reported that both TNF- α and IL-1 β are implicated as important mediators in the initiation and perpetuation of intestinal inflammation associated with IBD (73). In addition, peripheral PMN from individuals with IBD secrete increased amounts of TNF- α and IL-1 β compared to PMN from normal volunteers, suggesting that these PMN have an enhanced ability to induce PMN recruitment, activation, and prolonged survival at inflammatory sites, suggestive of an important role in perpetuation of the inflammatory response seen in IBD.

Non-cytokine inflammatory mediators produced by PMN

In addition to cytokines, PMN produce other important non-cytokine inflammatory mediators relevant to IBD pathogenesis, several of which are highlighted below. Human neutrophil peptides (HNP) 1-4, otherwise known as α defensins, are cysteine-rich, cationic antimicrobial peptides responsible for oxygen dependent killing of microorganisms. These peptides constitute 30-50% of the total protein content of PMN azurophilic granules (82). It has previously been reported that plasma concentrations of HNP 1-3 are significantly elevated in individuals with active IBD compared to healthy controls (83). In addition, HNP 1-3 are secreted by PMN in the lamina propria and crypt abscesses of active IBD mucosa but not in the lamina propria of normal mucosal tissue (84). This secretion of α defensins in the inflamed intestinal mucosa by activated PMN has implications for perpetuation of the inflammatory response associated with IBD. This is particularly true as HNP have potent chemotactic potential for macrophages, dendritic cells and T lymphocytes (85, 86). In addition α -defensin-mediated stimulation of IL-8 production by epithelial cells has been reported. This, in turn, would lead to ongoing recruitment of more PMN to sites of intestinal inflammation (87).

PMN also produce and secrete calprotectin (S100A8/S100A9); a calcium and zinc binding protein that constitutes 40%-60% of the total cytosolic protein content of mature neutrophils.

PMN therefore are the main source of calprotectin which is secreted into tissues at high concentrations during inflammation of intestinal mucosa such as occurs during flares of IBD (88). In addition to having anti-microbial and anti-proliferative effects, calprotectin, which can be used as a biomarker for active IBD, has other effects relevant to the pathology of inflammation. Specifically, it has been reported that murine calprotectin has potent chemotactic activity for PMN and macrophages and that it enhances scavenger receptor and Mac-1 expression on murine macrophages (89, 90). Further, calprotectin-mediated activation of PMN surface CD18 integrin and subsequent enhancement of integrin mediated PMN adhesion to fibrinogen has also been reported (91).

Another IBD relevant mediator produced by PMN is the serine proteinase elastase, which is normally stored in the azurophilic granules of quiescent PMN. Activation of PMN by potent triggers such as engagement of opsonized bacteria can trigger the release of elastase into the intestinal mucosa. This released elastase can then contribute directly to the perpetuation of inflammation by facilitating the ongoing migration of immune cells through the tissues, in addition to acting indirectly via the proteolytic activation of other inflammatory mediators. Functionally elastase is capable of degrading a large range of proteins including collagen, elastin, fibrin, fibronectin, platelet IIb/IIIa receptor and epithelial cadherins (92). Detection of elastase, which can be used as a marker for activated PMN, is increased in the stools and intestinal mucosa of individuals with IBD (93). Further it has recently been reported that PMN transmigration across lung epithelium induces elastase-mediated cleavage of E-cadherin with associated up-regulation of β -Catenin signaling (94). It has also been reported that elastase secretion correlates both with the Crohn's disease activity index and also with a numerical activity index in ulcerative colitis (95). In addition anti-elastase therapy ameliorates the disease activity both in DSS and TNBS induced colitis in rats (96), suggesting a role for this PMN produced serine proteinase in mediating the deleterious effects of colitis, *in vivo*.

PMN are also known to produce and secrete azurocidin 1, also known as heparin binding protein (HBP) and cationic anti-microbiocidal protein 37 (CAP37), a multi-functional heparin binding microbiocide stored mostly in PMN primary granules. Azurocidin is a highly basic member of the serprocidin family of serine protease homologues, which includes the glycoproteins elastase, cathepsin G and proteinase-3. Together these proteins represent a major constituent of the azurophil granules of PMN (1-2 μ g protein/10⁶ PMN). Azurocidin 1 can contribute to the cycle of inflammation characteristic of IBD as it is a potent chemoattractant for monocytes and, also a less effective chemoattractant for PMNs. In addition azurocidin 1 triggers an enhanced release of IBD associated cytokines including TNF- α and IL-6 from monocytes as well as triggering enhanced macrophage activation (97).

More recently PMN have been shown to synthesize human PMN lipocalin (HNL), a protein mainly stored in PMN secondary granules. It has been suggested that HNL could contribute to the pathogenesis of IBD as it has been reported that high levels of HNL are detected in the colonic epithelium of individuals with ulcerative colitis but not in normal healthy volunteers. It has also been reported that HNL is secreted by PMN simultaneously with pro matrix metalloproteinase-9 (proMMP-9) and, that HNL can activate latent MMPs, including MMP-9/Gelatinase B, in a concentration dependent manner resulting in the generation of

catalytically competent enzymes (98). Several MMPs, including MMP-9, have been reported as being up-regulated, both in animal models of colitis and in individuals with IBD (99). Further MMP-9 has specifically been implicated as having a role both in mediating tissue damage during colitis, presumably through the inhibition of wound healing (100, 101).

5. Conclusion

Uncontrolled PMN trafficking is implicated in the pathogenesis of many inflammatory mucosal disorders including IBD. In addition to a reduction in intestinal epithelial barrier integrity caused by uncontrolled PMN influx and the physical damage to the epithelium caused by PMN-derived reactive metabolites, PMN also function as important proinflammatory effector cells capable of secreting mediators that influence other immune cells thereby delaying barrier restitution and fostering epithelial dysfunction and mucosal injury associated with IBD. Accordingly increased understanding of PMN recruitment and PMN function in the inflammatory environment represent important targets for pharmaceutical approaches aimed at ameliorating the consequences of dysregulated inflammation in IBD.

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Figure 1. Mucosal epithelium from patient with ulcerative colitis showing (A) PMN transepithelial infiltration and binding to the apical epithelial surface (red arrow) and (B) characteristic crypt abscess caused by luminal accumulation of migrated PMN (black arrow).

Figure 2.
Sequential PMN trafficking cascade.

Figure 3.

The integrins are $\alpha\beta$ heterodimers; each subunit crosses the membrane once. Mammalian integrins form subfamilies that share common subunits that bind distinct ligands. Illustrated in color are the integrins that have been targeted for the treatment of human diseases. RGD: arginine-glycine-aspartic acid sequence found in some integrin ligands. C3bi: Complement 3b inactivated.

Table 1
Neutrophil-derived Inflammatory Mediators

Immunoregulatory cytokines
IFN- α
TNF- α
IL-12
IL-22
IL-1 α
EL-1 β
MIF
IL-17
C-C chemokines
CCL2
CCL3
CCL4
CCL17
CCL18
CCL19
CCL20
CCL22
C-X-C chemokines
GRO- α
GRO- β
CXCL1
CXCL2
CXCL3
CXCL4
CXCL5
CXCL6
CXCL8
CXCL9
CXCL10
CXCL11
Growth factors
GM-CSF
G-CSF
M-CSF
IL-3
TGF- β
Noncytokine inflammatory mediators
Peptides
HNP1

HNP-2
HNP-3
Other granule enzymes
Calprotectin
Elastase
Azurocidin
Lipocalin

TGF, tumor growth factor; GRO, growth-related gene product α ; MIF, migration inhibitory factor; GM-CSF, granulocyte-macrophage colony stimulating factor; G-CSF, granulocyte colony stimulating factor; M-CSF, macrophage colony stimulating factor.