

Eosinophils in Inflammatory Bowel Disease

a report by

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The etiology and pathogenesis of inflammatory bowel disease (IBD) is still unclear. It is generally believed that IBD results from abnormal interactions between gut microflora and the deregulated host's immune response in genetically susceptible individuals.¹⁻³ There is an increase in the number of inflammatory cells in inflamed gut mucosa in patients with IBD, including plasma cells, T-lymphocytes, macrophages, mast cells, neutrophils, and eosinophils. Accumulated evidence suggests that eosinophils play an important role in IBD. Histopathological observations demonstrate eosinophils and their related proteins in increased quantities in the mucosa of IBD patients. Accumulation of eosinophils is also seen in asthma, allergy, parasitic infections, and certain neoplasms.⁴ While the pathogenetic role of eosinophils in allergy⁴ and asthma⁵ has been extensively studied, the contribution of these cells in the pathogenesis of IBD is still under investigation and remains controversial.

Clinical Evidence of the Diagnostic and Prognostic Values of Eosinophils in IBD

An increased number of eosinophils have been found in the gut mucosa and luminal exudates in patients with IBD⁶⁻¹⁰ as compared with healthy individuals.^{11,12} Further studies have shown that exacerbations of ulcerative colitis (UC) are frequently accompanied by an increase in the number of circulating eosinophils and tissue eosinophilia,¹³ and an elevated eosinophil count may precede relapse.¹⁴ These eosinophils in the inflamed mucosa are largely degranulated on electronic microscopy.¹⁵

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The diagnostic and prognostic roles of tissue eosinophilia in IBD are still debatable. Clinical investigations have been performed to assess association between eosinophil infiltration and disease activity. Sarin et al.¹⁶ showed rectal eosinophilic cell counts to be significantly higher in active UC than in quiescent UC and healthy controls, but there was no correlation between the eosinophil cell counts and disease severity. Jeziorska et al.¹⁷ reported an increased number of eosinophils only in active IBD, while much less in chronic quiescent disease. In contrast, Korelitz et al.¹⁸ described low mucosal eosinophil counts in active untreated UC. There was no correlation between the number of eosinophil in lamina propria and treatment response to sulfasalazine. The presence of tissue eosinophils corresponded to the general tendency for the condition to deteriorate and require intense medical therapy. Lampinen et al.¹⁹ further established that the activity of eosinophils was high in active disease. During this inactive phase of UC, patients had a higher number of eosinophils while being asymptomatic both clinically and histopathologically. These observations have sparked even more controversy, implying eosinophils might be involved also in the resolution of inflammation and repair of damaged tissue, which has also been elicited in allergy and asthma.^{20,21}

The number of eosinophils in intestinal mucosa appears to be dependent on the diagnosis, disease activity, and type of drug therapy. Conflicting research on eosinophil inflammation and response to drug therapy has been reported. Heatley et al.²² discovered high eosinophil counts in rectal biopsy indicated a good prognosis in UC in such cases, being self-limited or easily controlled with medical therapy. Patients with low counts had a poor prognosis. Conversely, Binder et al.¹² showed the degree of eosinophil infiltration did not predict response to medical therapy or who required proctocolectomy.

Proteins in eosinophilic granules participate in the inflammatory process associated with IBD. Dvorak²³ reported that major basic protein (MBP), purified from the core of eosinophils, is a key element in the cell injury of degranulated cells in Crohn's disease (CD) patients. Peterson et al.²³ suggested using eosinophil protein X (EPX) as the measurement of inflammation based on observing a clear relationship between disease activity in CD and fecal level of EPX. Higher fecal eosinophil cationic protein (ECP) and EPX concentrations were associated with clinical relapse within three months in CD, but not in UC. Fecal EPX and ECP might reflect disease activity in some patients, while not in others.²⁴ The study still conceded that the measurement of these eosinophilic-derived proteins in feces was a useful way of evaluating disease activity and predicting relapses. Studies have found a positive correlation between

ECP concentrations and inflammatory activity in terms of endoscopic and histopathological grading, but the relationship to treatment effect is unclear.⁵⁹

Some investigations have also shown increased levels of these eosinophilic proteins in other gastrointestinal disease, including chronic gastritis, celiac disease, and eosinophilic gastroenteritis.^{24,25} Hallgren et al.²⁶ observed increased tissue levels of both myeloperoxidase and ECP in CD patients as well as in patient with celiac sprue compared with controls. There was more prominent extracellular release of ECP in relation to ulcers and fistulas in CD patients on immunohistochemistry compared with sprue.

Role of Tissue Eosinophils and Their Related Proteins in the Pathogenesis of IBD

The role of eosinophils in the etiology and pathogenesis of IBD remains unclear. Tissue eosinophilia in UC may have a detrimental effect on the structural integrity of the intestine.²⁷⁻²⁹ Eosinophils produce a variety of cytotoxic inflammatory mediators, ribonucleases, and peroxidases, which can cause toxic pores to membranes, smooth muscle vagal receptor dysfunction, degranulation of mast cells and basophils, generation of leukotrienes, and amplification of other inflammatory pathways.⁴ On the other hand, some authors have alluded to a potential anti-inflammatory function to eosinophils, such as the release of histaminase, aryl sulphatase B and phospholipase D, as well as the ability to defend the colonic mucosa from enteric bacterial infections.³⁰⁻³²

Eliciting the molecular process involved in eosinophil production and transit is important in understanding the role of these cells in immune surveillance and the pathogenesis of IBD. Eosinophils are generated in the bone marrow from pluripotent stem cells. Interleukin (IL) 3, IL-5, tumor necrosis factor (TNF) alpha, and granulocyte macrophage-colony stimulating factor (GM-CSF) regulate their development, maturation, accumulation, and function.³³ Of these cytokines, IL-5 is thought to be the most specific to eosinophil lineage, mainly responsible for its selective differentiation.³⁴⁻³⁷ IL-5 messenger ribonucleic acid (mRNA) has been highly expressed in tissue with pronounced eosinophil infiltration in inflamed areas of CD patients.³⁸ Upregulation of IL-5 in transgenic mice causes profound eosinophilia,³⁴ while genetic deletion causes a marked reduction in blood and lung eosinophils after an antigen challenge.³⁹

Eosinophil migration into tissue is a complicated process that involves several steps regulated by adhesion molecules and chemoattractants. Eotaxin, a β -chemokine, with preferential chemotactic capacity for eosinophils plays a critical role in these cells' recruitment.⁴⁰⁻⁴² Intestinal eosinophilia in mice deficient in eotaxin are rarely encountered, and the number of the cells shown to be significantly lower compared with wild type mice.⁴⁰ C-C chemokine receptor 3 (CCR-3), a main receptor of eotaxin, while not expressed on neutrophils or monocytes, is highly concentrated on eosinophils.⁴³ T-lymphocytes also express this CCR-3 receptor, suggesting that eotaxin/CCR-3 may represent an additional mechanism for T-lymphocyte recruitment.⁴⁴ In addition to chemotaxis, eotaxin also induces eosinophilic aggregation and increases adhesion molecule expression, leading to transmigration of eosinophils into inflammatory tissue.⁴⁵

Eotaxin in serum is elevated in patients with active and inactive IBD compared with subjects with irritable bowel syndrome, colonic cancer, or healthy individuals.^{42,46,47} Eotaxin alone appears not to be sufficient to lead to inflammatory cascade,⁴¹ since the mucosa of the upper gastrointestinal tract expresses abundant eotaxin mRNA, but has a small number of eosinophils. In this study, mice genetically deficient in eotaxin with elevated IL-5 have an increased number of circulating eosinophils, while having a significant reduction in gastrointestinal eosinophils compared with wild-type mice. This highlights the unique role of eotaxin in regulating eosinophil accumulation in the gastrointestinal tract at baseline, even in the presence of elevated levels of IL-5.⁴¹

Eosinophils' biologic function is mainly attributed to the secretion of their pro-inflammatory granules. The histological observation in a few IBD studies showed that intestinal mucosa in some patients had decreased or normal eosinophil counts, which may be contributed to degranulation. Attempts have been made to quantify eosinophils by their products. Eosinophils' cytotoxic mechanism of action is thought to be variable and mediated by a number of different mechanisms. Eosinophils have a potent armamentarium of inflammatory mediators such as histamine, proteolytic enzymes, cationic proteins, and other metabolites.⁴⁸⁻⁵⁰ The protein content of their granules is dominated by highly cationic proteins, including major basic protein, ECP, eosinophil peroxidase, and eosinophil-derived neurotoxin/EPX.⁵¹ These proteins have been shown to be elevated in allergic and other inflammatory diseases.⁵¹⁻⁵⁵ Higher concentrations of these proteins have also been reported in the stool specimen,⁵⁶⁻⁵⁸ colorectal perfusion/lavage fluid,^{30,59,60} and tissue biopsy specimens³⁰ in IBD patients. Berstad et al.⁵⁷ demonstrated a 14-fold increase in ECP in IBD patients compared with healthy individuals. Two of these patients showed a gradual and distinct fall in ECP concentrations in parallel to marked clinical improvement. Another study showed a 300-fold increase in ECP and 10-fold increase in eosinophils in active UC compared with healthy controls.⁶¹

Eliciting the molecular process involved in eosinophil production and transit is important in understanding the role of these cells in immune surveillance and the pathogenesis of IBD.

IBD, Allergy, and Environmental Factors

The presence of tissue eosinophilia implies that some 'allergic type' immune mechanisms may contribute to the disease mechanisms for IBD. The early findings of tissue eosinophilia in IBD prompted speculation that IBD and allergic disorders may show some common pathogenesis. D'Arienzo et al.⁶² studied 50 patients with UC and found that these patients frequently had personal and/or family history of allergy, positive skin prick tests, positive patch tests, and serum eosinophilia compared with healthy controls. It was found that patients with UC or CD had significantly more allergic and respiratory symptoms, abnormal pulmonary function tests, and skin prick tests than controls.⁶³ There was an increased number of eosinophils in the induced sputum of patients with CD.⁶⁴ Additional evidence supporting the concept of IBD related to allergy is an increased incidence in atopy and

immunoglobulin E (IgE) levels in patients with IBD versus the general population.⁶⁵ Moreover, IBD patients have been shown to have favorable, but also debatable, responses to some allergy and asthmatic therapy, which will be discussed later.

Environmental factors likely play an important role in the pathogenesis of IBD. In early 1960s, Taylor et al.⁶⁶ described circulating antibodies to milk proteins in UC patients. The concept of cows' 'milk allergy' was introduced some years before in 1925.^{67,68} Patients were placed on various diets with different time courses. More patients on milk-free diets remained 'well,' and had more mild inflammation on rectal biopsy, although these findings have not been confirmed by other investigators.⁶⁹⁻⁷¹ Glassman et al.⁷² found a significantly greater number of UC patients with a history of milk sensitivity during their youth than controls. These patients were also diagnosed with UC at a younger age. The cow's milk theory for etiology of IBD was further specified to malabsorption of lactose⁷³ and more recently malabsorption of long chain triacylglycerides in IBD patients.⁷⁴ Breast feeding is thought to be a protective factor against IBD in various case reports since the 1960s. It has been speculated that immunoglobins may decrease the risk of microbial contamination of human milk compared with formula and stimulate early development and maturation of infant gastrointestinal tracts.⁷⁵

Eosinophilia—A Therapeutic Target?

The clinical and experimental research that has been presented has provided key targets for beneficial intervention, as well as the need to investigate other medication employed in other eosinophilic disorders. Corticosteroid therapy is effective for the treatment of IBD. One of its therapeutic mechanisms is believed to be associated with reduction of eosinophils.^{4,76} Corticosteroids inhibit eosinophil activation and release of pro-inflammatory mediators.¹⁷ These agents also suppress genes for inflammatory mediators and various chemokines, such as eotaxin, by reducing these proteins' half-lives and inhibiting cytokine-dependent survival of eosinophils.^{77,78} However, there are some patients with asthma that maintain high eosinophil counts despite high doses of corticosteroids.⁷⁹ These 'glucocorticoid-resistant' patients, also seen in other hypereosinophilic syndromes, require drugs like interferon-alpha, or

myelosuppressive agents like cyclosporin A, hydroxyurea, and vincristine as therapy.⁷⁹ These drugs work by inhibiting eosinophil degranulation and function, and by blocking transcription factors to cytokines produced by eosinophils.^{80,81} No studies have been done specifically looking at how these other agents affect eosinophils in IBD patients.

Agents used in the management of other inflammatory conditions could have potential benefit in IBD based on their effect on eosinophils. Lidocaine and sulfonylureas have been shown to mimic the effects of corticosteroids, by reducing eosinophils and inhibiting IL-5.^{82,83} Antibodies against IL-5 have also been effective in treating allergic airway disease in animal models.^{84,85} Leukotriene (LT) antagonists and inhibitors, like zileuton, have been shown to block the rate-limiting step in LT synthesis and inhibit the generation of LTB₄, an eosinophil chemoattractant.⁷⁶ There are on-going trials of apparently safe agents in allergic rhinitis that block eotaxin, such as anti-eotaxin-1 antibody, as well as blockers to eotaxin receptors, CCR3 and Siglec-8.⁸⁶ Some third-generation antihistamines like cetirizine inhibit vacuolization and accumulation of eosinophils after allergic challenge and directly inhibit eosinophils *in vitro*.⁸⁷⁻⁸⁹ Rampton et al.⁹⁰ showed disodium cromoglycate, a mast cell stabilizer used in patients with asthma, to reduce eosinophil content and stool frequency in the exudate of patients with active UC, but the therapeutic effect was unimpressive. Ketotifen, another mast cell stabilizer, demonstrated a decrease in the number of eosinophils and showed symptomatic and endoscopic improvement in two of 10 children with active UC.⁹¹ Still, there are few data utilizing these medications in IBD. More research is needed to investigate eosinophil-targeting agents in IBD.

In summary, we need to further explore the role of eosinophils in IBD. Methods to assess eosinophil activation in the gut have not been validated. The reproducibility of these studies is hampered by the heterogeneity of IBD patients. The reviewed literature provides data that seem to support the principle that eosinophils actively participate in the inflammation of IBD. Further experimental work and prospective clinical studies, using validated methods in new untreated patients, may further discover functions of these eosinophils in IBD, leading to further understanding and therapy for this disease. ■

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