Eosinophils in the gastrointestinal tract : friends or foes ?

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Abstract

Eosinophils play an important role in the mucosal immune system of the gastrointestinal tract under resting and under inflammatory conditions.

Under *steady-state conditions*, the mucosa of the digestive tract is the only organ harboring a substantial number of eosinophils, which, if need be, get activated and exert several effector and immunoregulatory functions. The precise function of these late-phase inflammatory cells is not yet completely understood. Nevertheless, it has recently been demonstrated that lipopolysaccharides from gram-negative bacteria activate eosinophils to rapidly release mitochondrial DNA in the extracellular space. Released mitochondrial DNA and eosinophil granule proteins form extracellular structures able to bind and inactivate bacteria. These findings suggest a novel mechanism of eosinophil-mediated innate immune responses that might be important in maintaining the intestinal barrier function.

Moreover, eosinophils also play a crucial role in several *inflammatory conditions*, such as intestinal infections, immune-mediated inflammations and hypersensitivity reactions. Under chronic inflammatory conditions, the ability of the eosinophils to induce repair can lead to pathological sequelae in the tissue, such as esophageal remodeling in eosinophilic esophagitis. It is established that the uncontrolled eosinophilic inflammation induces fibrosis, esophageal wall thickening and strictures leading to damage that results in a loss of esophageal function. One potential mechanism of this remodeling is so-called 'epithelial mesenchymal transition', which is triggered by eosinophils and is potentially reversible under successful anti-eosinophil treatment.

Therefore, eosinophils may act either as friends or as foes, depending on the microenvironment. (Acta gastroenterol. belg., **2012**, 75, **310-315**).

Key words : eosinophils, eosinophilic esophagitis, eosinophil traps, mucosal barrier function, remodeling.

Abbreviations

CCR-3	= C-C chemokine receptor type 3
CD	= Cluster of differentiation
EMT	= Epithelial mesenchymal transition
EoE	= Eosinophilic esophagitis
GERD	= Gastro esophageal reflux disease
GI tract	= Gastrointestinal tract
GM-CSF	= Granulocyte macrophage colony stimulating factor
IL	= Interleukin
TGF-β	= Transforming growth factor beta
TNF-α	= Tumor necrosis factor alpha
VCAM-1	= Vascular cell adhesion molecule 1

Introduction

The human gastrointestinal tract is the only non-hematopoietic organ, which harbors eosinophils under *basal conditions* (1). Moreover, eosinophils are crucially involved in the development of several intestinal *inflammatory conditions* (2). Therefore, the eosinophils and the gastrointestinal tract are partners in an intimate and enigmatic relationship. A few decades ago this relationship was thought to be quite straight forward, with the eosinophils protecting the gastrointestinal tract by fighting parasitic infections. However, in the current context of dramatically diminished parasitic infections in industrialized countries, we must look with a fresh eye on the role of eosinophils in mucosal immunity and disease. Today, we have to admit that, despite a flood of new insights, many pieces of the puzzle that would give us a good view at the eosinophil function are still missing. However, at least one thing is for certain : eosinophils in the gastrointestinal tract exert both beneficial and detrimental effects. In the following sections, we will first provide a brief overview of the function of both partners and then will specifically discuss one example each of a beneficial and a detrimental interaction between the gastrointestinal tract and eosinophils.

The first partner : the eosinophils

Eosinophils in history

In 1879, Paul Ehrlich, a German hematologist and immunologist, first identified eosinophils as a distinct type of blood leukocytes (2). At that time, the distinguishing feature of these cells was their distinctive staining with the acidic dye eosin. This tinctorial property visible by light microscopy is a consequence of the binding of eosin to cationic proteins present in the granules, which these cells are armed with. While more than 80 markers with CD designations and at least 20 unclustered molecules have been identified on the surface of eosinophils, no single eosinophil-specific surface marker has been described (3). Thus, the staining characteristic in histologic studies has remained the primary distinguishing feature for identification of these terminally-differentiated leukocytes in blood and in tissues (3).

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Eosinophil differentiation and trafficking

Eosinophils reside predominantly in three anatomical compartments : bone marrow, blood vessels and mucosal tissues. Eosinophils originate from pluripotent stem cells in the bone marrow (4). Their differentiation process is mainly orchestrated by interleukin 3 (IL-3), IL-5 and granulocyte-macrophage colony stimulating factor (GM-CSF). In the bone marrow, eosinophils are differentiated to a fully granulated state before they migrate to the vascular space. IL-5, in particular, mediates a myriad of eosinophil lineage-specific functions including the release of eosinophils from the bone marrow and their survival in target tissue.

It is well-established that eosinophils reside in the hematopoietic and lymphatic organs, such as the bone marrow, spleen, lymph nodes and thymus. However, under basal conditions, eosinophils also reside in gastrointestinal tract (1). It has also been demonstrated that the distribution of the eosinophils throughout the gastrointestinal tract is not homogeneous. While the cecal and appendiceal regions have the highest density of cells (1), the esophagus is the only segment of the digestive tract that is completely devoid of eosinophils under non-inflammatory conditions (7). Moreover, it has been shown in animal models that eosinophils home to the gastrointestinal tract as early as during the embryonic development. Eosinophil homing to the GI tract was observed even in embryos from germ-free adult animals. Therefore, this homing is likely independent of the presence of the intestinal flora (8). Eotaxin-1 has been identified as the primary regulator of eosinophil gastrointestinal homing under non-inflammatory conditions as it binds the C-C chemokine receptor type 3 (CCR-3) on eosinophils (Fig. 1) (8).

As determined primarily in studies of allergic inflammation models, the process of eosinophil migration from the vascular space into tissues is mediated by a variety of adhesion molecules on endothelial cells, such as P-selectin, intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 (VCAM-1), and corresponding counter receptors/integrines on eosinophils, such as P-selectin glycoprotein ligand-1, $\alpha_L\beta_2$ integrin and $\alpha_4\beta_1$ integrin. Th2 cytokines, such as IL-4 and IL-13, induce expression of b-integrin family ligands on the surface of eosinophils and the corresponding receptors on endothelia that include VCAM-1 (5). We are yet to learn if these homing patterns apply to those in gastrointestinal tract. Various chemoattractants, released within local mucosal environments, including leukotriene B_4, platelet activating factor, chemokines (eotaxins) as well as bacterial products stimulate eosinophil migration into the tissue (6).

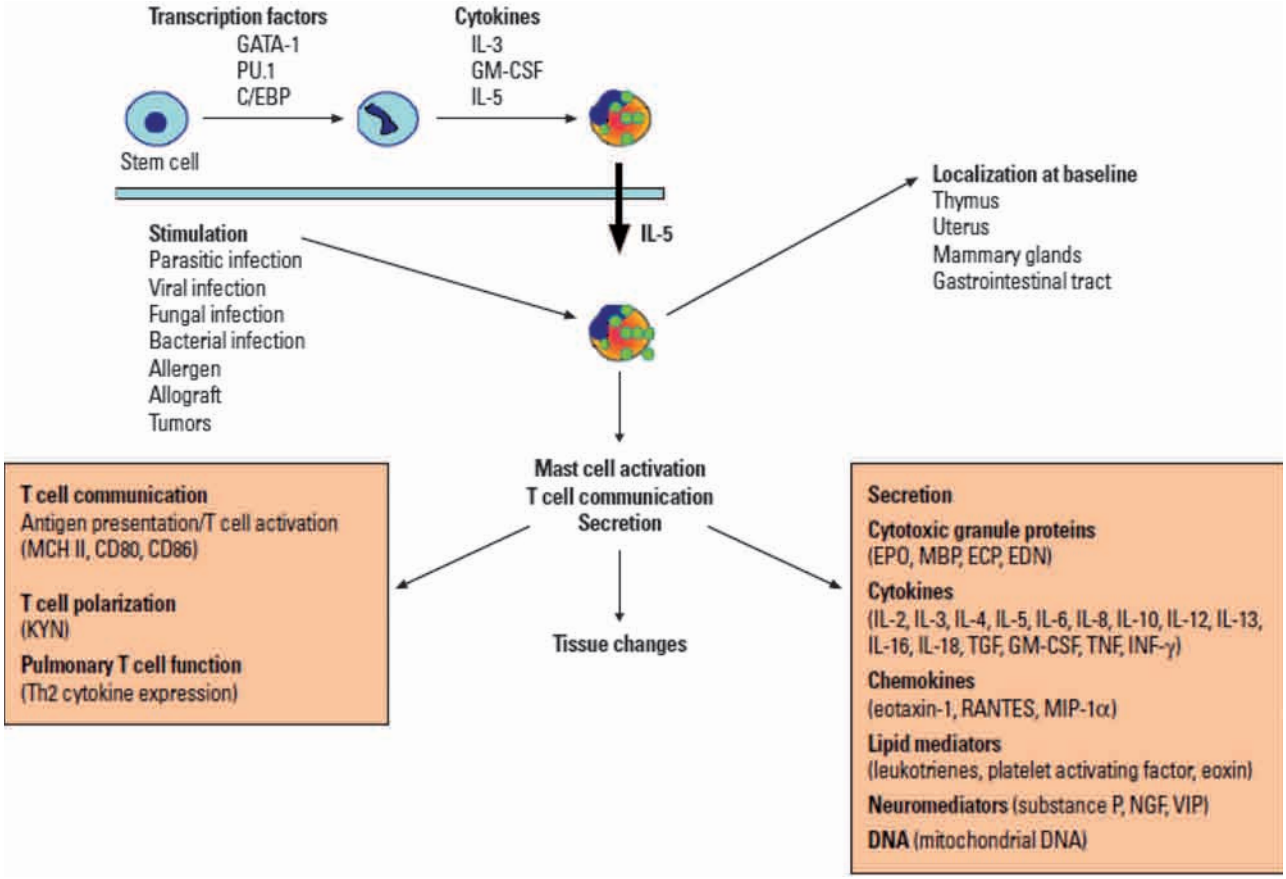

Fig. 1. — Eosinophils : from the hematopoietic stem cell to the mature, end-stage-differentiated leukocyte (figure courtesy of Bruce Bochner).

Eosinophils functions

Eosinophils are pro-inflammatory leukocytes with a wide spectrum of powerful *effector functions* (9). While destruction of helminthic parasites is one example of a beneficial effect of eosinophils in GI tract, tissue damage caused during a chronic inflammatory response is undoubtedly detrimental. Activation *via* different mechanisms leads to, among others, the release of the preformed granule proteins and the production of leukotrienes (9). In order to exert their effector functions, such as degranulation and/or inflammatory mediator synthesis, eosinophil susceptibility towards inflammatory stimuli must be increased. Such 'priming' of eosinophils is mediated by cytokines, such as IL-5 and GM-CSF (10).

In addition to their role as effector cells, eosinophils appear to have important *immunoregulatory properties* (11). The question of whether eosinophils actually possess the ability to produce cytokines has long been debated. Blood and tissue eosinophils are terminally differentiated cells ; therefore, these cells were thought to have a limited capacity to synthesize new proteins. However, based on results of multiple studies, it is now clear that eosinophils generate a wide spectrum of inflammatory and regulatory cytokines, such as TNF-α, TGF-β, GM-CSF, IL-4, IL-5, IL-8, and IL-13 (11). Some of these cytokines, such as TNF-α, function in amplifying immune responses during an inflammatory reaction, which increases the risk of enhancing tissue damage. Others, such as IL-4 or TGF-β, may influence T cell differentiation or tissue repair processes, respectively.

In addition, eosinophils can also act as *antigen presenting cells* under certain pathologic conditions (12). However, eosinophils are not as efficient at this function when compared to professional antigen-presenting cells (13).

The second partner : the gastrointestinal tract

The morphology of GI tract

The basic task of the digestive tract is to supply the body with all required nutrients. In order to establish and maintain normal body structure and functions, nutrients must be absorbed, at least in a minimal quantity. This goal is effectively achieved by maximizing the functional absorptive surface area by presence of folding, plicae, villi and microvilli. Together, all these 'extensions' lead to an enormous enlargement of the total absorptive surface to approximately 20'000 m^2 (14).

The barrier function of the GI tract

Not only nutrients, but also billions of microorganisms and potentially immunogenic macromolecules are present in the intestinal contents. Under non-inflammatory conditions, the intestinal microbiome contains more than 500 different species of bacteria and harbors about 10^{11} or 10^{12} bacteria per gram of luminal contents (15). Of note, the human body contains 10 times more microbial cells than human cells, and the majority of bacteria are located within the lumen of the digestive tract. Therefore, the human gastrointestinal tract represents the body's largest host-environment interface, where the epithelial surface is exposed to an overwhelming load of diverse microorganisms as well as dietary products. The mucosal immune system is an integral part of the GI tract, and its task is to constantly distinguish between 'self' and 'non-self' as well as between 'pathogenic' and 'non-pathogenic'. The integrity of the immunological barrier is maintained by a variety of different mechanisms. The degradation of potential immunogenic substances to low- or non-antigenic particles, *e.g.* proteins to amino acids, as well as the reduction of the intestinal bacterial load by gastric acid and enzymes are two important mechanisms that help to maintain the integrity of the GI tract along the longitudinal axis. In the transmural direction, the innate immune system mechanisms, including secretion of mucus and antibacterial peptides as well as the presence of tight junctions for maintenance of epithelial integrity, provide excellent defense to the GI tract. Furthermore, as demonstrated in animal studies, interactions between intestinal bacteria and immune cells of both the innate and the adaptive arms of the immune system play an important role in maintenance of immune homeostasis (16). While the function of the T cells in this subtle balance between up- and down-regulation of immune responses has been extensively evaluated, the role of the eosinophils in this interplay is less clear.

In the following sections, the interplay between eosinophils and the digestive tract will be discussed. We will also provide one example of each : a protective and a deleterious effect mediated by eosinophils in the GI tract.

Eosinophil trap formation : a friendly relationship between the two partners

Resident eosinophils are considered to be important in defending the GI tract against parasites. However, we are just beginning to understand the role that eosinophils play in contributing to innate immunity (2,9). Recently Yousefi *et al*. have shown that lipopolysaccharide from Gram-negative bacteria activates purified and IL-5-primed eosinophils to release mitochondrial DNA *in vitro* (17). This process of DNA release occurred swiftly within less than one second. The released DNA was able to inactivate and kill Gram-negative bacteria *in vitro*. Moreover, using immuno-staining and confocal microscopy, the authors found string-like extracellular structures consisting of DNA and eosinophil granule proteins in the close proximity to intestinal eosinophils (Fig. 2). In addition, these string-like structures were surrounded by bacteria in biopsies taken from patients suffering from bacterial GI infections (17).

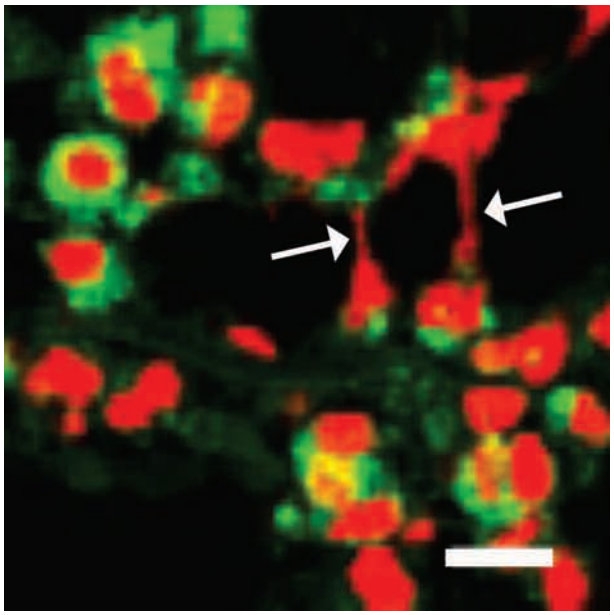

Fig. 2. — String-like structures (as indicated by white arrows) containing DNA and granule protein ECP in a biopsy specimen of inflamed colonic mucosa. (confocal microscopy, double staining with anti-ECP and anti-DNA antibodies).

These findings were confirmed in IL-5 transgenic mouse models and suggest a novel mechanism of eosinophil-mediated innate immune responses that might be important in maintaining the intestinal barrier function. While studies, such as this, improve our understanding of the role of eosinophils in the gastrointestinal immunity, further studies are needed to better understand the functions of the mucosa-residing eosinophils in the gastrointestinal tract.

Esophageal remodeling and epithelial mesenchymal transition in eosinophilic esophagitis : an unfriendly relationship between the two partners

Eosinophilic esophagitis (EoE) represents a chronic inflammatory, immune-mediated, esophageal disease characterized clinically by symptoms related to esophageal dysfunction and histologically by an eosinophil-predominant inflammation (18,19). It is important to note that esophageal eosinophilia is not exclusive to EoE and can also associated with other diseases, such as gastro-esophageal reflux disease (GERD), Crohn's disease, vascular diseases, connective tissue disorders, infectious esophagitis, drug-induced esophagitis and eosinophilic gastroenteritis (18). Therefore, exclusion of other diseases is mandatory before the diagnosis of EoE can be established (19).

Clinical observations as well as natural history studies have shown that an untreated EoE can lead to narrowing of esophageal lumen and formation of strictures (20) (Fig. 3). Under these conditions, the esophageal wall is

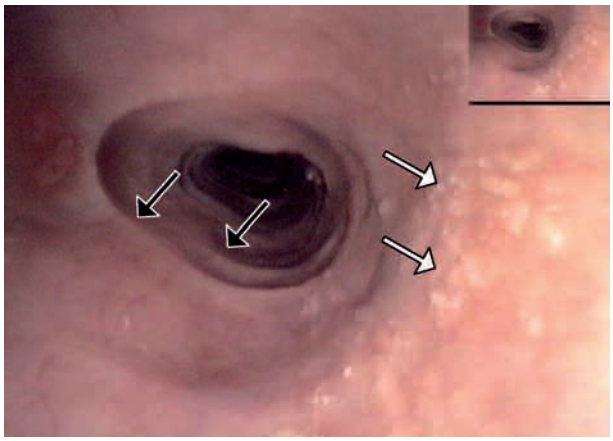

Fig. 3. — Endoscopic picture taken from a patient with confirmed EoE, showing signs of active inflammation, such as white exudates and furrowing (indicated with white arrows), and signs of remodeling, such as circular rings, leading to a moderate stenosis (indicated with black arrows).

fragile, rigid and prone to tears (21). This so-called 'remodeling' alters the function of the esophageal body and the lower esophageal sphincter resulting in dysphagia and secondary reflux, respectively (18). The development of these sequelae is one of the major concerns in untreated EoE (19).

The eosinophils are late phase inflammatory cells that help potentiate tissue repair (2,9). For instance, TGF-β1 is a prominent pro-fibrotic cytokine produced by eosinophils (11). Therefore, it was speculated that the remodeling process could be attributed to the presence of eosinophils in the esophageal tissue. Indeed, several pediatric and adult studies have demonstrated a significant increase in fibrotic tissue in the subepithelial compartment of the esophagus in patients with EoE, but not in healthy individuals (Fig. 4). While modest increase in fibrotic tissue is observed in other inflammatory esophageal diseases, such as reflux esophagitis, such a robust increase in fibrotic tissue is a hallmark feature of EoE (22-24). Studies in animal models have shown that remodeling is mediated by IL-5, a key cytokine that mediates myriad of eosinophil-associated functions (25). Furthermore, several therapeutic trials with corticosteroids have shown that the expression of fibrosis-associated biomarkers and the amount of fibrosis in the tissue correlate with the density of the eosinophilic infiltration. The other important result of all these studies was that successful treatment with corticosteroids was able to diminish the amount of fibrosis in the tissue (23,24,26). The mechanisms underlying the process of tissue remodeling are not fully understood. Recently, Kagalwalla *et al*. have evaluated the role of epithelial mesenchymal transition (EMT) in EoE tissue remodeling. EMT describes a series of events during which the esophageal epithelial cells loose many of the epithelial characteristics, including polarity, expression of epithelial markers,

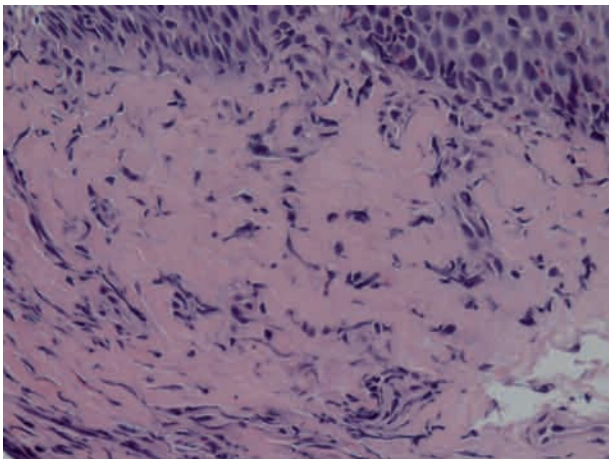

Fig. 4. — Esophageal biopsy from a patient with EoE with a highly increased amount of fibrotic tissue in the lamina propria. (HE-staining ; original magnification 40 × 10).

and tight junctions, and acquire properties of mesenchymal cells, including motility, loose cell adhesion, and depolarized cytoskeletal arrangements (27). First, the authors demonstrated that exposure of esophageal epithelial cells to TGF-β1 in an *in vitro* model induces up-regulation of mesenchymal genes, such as N-cadherin, vimentin and fibronectin (27). Kagalwalla *et al*. then compared the signs of EMT in esophageal biopsies from patients with EoE, patients with reflux disease and healthy control patients. The authors found that the signs of EMT were only present in the biopsies of EoE patients, and that the magnitude of EMT positively correlated with the eosinophil count in the biopsies. Following therapy with either diet or corticosteroids, EMT decreased significantly as the eosinophil infiltration resolved (27).

In summary, under prolonged inflammatory conditions, eosinophils induce considerable tissue damage with subsequent connective tissue deposition, altering esophageal structure and function. Fortunately, there is good evidence to suggest that this organ-damaging process might be reversible upon successful treatment.

Final comment

Today, we are faced with a flood of information about the relationship between eosinophils and the gastrointestinal tract. Eosinophils may act as friends or foes, depending on the local microenvironment. However, the results of studies to date are but pieces of jigsaw puzzle. While some of these pieces come together to form a fragment that gives us glimpse at a part of the picture, others pieces still lay scattered. Indeed, we are yet to complete a puzzle and to clearly understand the function of gastrointestinal eosinophils under physiological and pathological conditions.

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