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Review

The role of eosinophils in eosinophilic esophagitis

[1]Loredana Saveanu, [2]Liliana Adriana Dina
[1] ATIP-AVENIR Team, U1149 INSERM, CRI Bichat, Faculté de Médecine Xavier-Bichat, Paris, France; [2] Department of Internal Medicine, IIIrd Medical Clinic, Faculty of Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.

Abstract: Eosinophiles, while well characterized by their specific morphology and staining properties, remain relatively poorly understood in terms of their physiologic role and potential in the development of pathologic conditions. Eosinophilic esophagitis is a pathological situation exacerbated, in many instances, by allergens and its study could give rise to new therapeutic strategies for other diseases in which eosinophiles play a pathogenic role. We present current knowledge on the role of eosinophiles and associated immune cells in the pathogeny of esophagitis and in the development of the complex but rather unspecific clinical signs and symptoms of this disease. Both clinical and mouse knock-out strain studies have been critical to establish the role of eosinophiles, and especially the Eotaxin-3 and its interaction with the eosinophile-specific CCR-3 receptor in the disease. The ability to correctly diagnose this disease is essential for devising an effective treatment strategy. While non-specific immune suppression through glucocorticoid treatment is, for the moment, one of the most effective treatments, development of specific inhibitors of eosinophil multiplication and activation is an active area of research.

Key Words: eosinophilic esophagitis, eosinophils.

Corresponding Authors: L. A. Dina, email: dina_a_lili@yahoo.com.

Eosinophilic esophagitis – definition, history and diagnosis

Eosinophilic esophagitis (EoE) has been first described in 1993 (Attwood et al 1993) as a clinical syndrome of dysphagia with high esophageal intra-epithelial infiltration of eosinophils and normal values for 24 hours monitoring of esophageal pH. The relatively easier access to esophageal biopsies in comparison with other gastrointestinal specimens, the EoE became the most studied and the best characterized disease in the group of Eosinophilic Gastrointestinal Disordes (eosinophilic gastritis, gastroenteritis and colitis). This explains the clarity of diagnostic criteria published initially in 2007 (Furuta et al 2007) and updated in 2011 (Dellon 2011). The first diagnostic guidelines include the presence of clinical symptoms of dysphagia, the detection of more than 15 eosinophils per high-power microscopy field (hpf), a normal esophageal pH during 24 hours monitoring or the persistence of symptomatology after 8 weeks of treatment with high doses of proton pump inhibitors. Additionally, in 2011, the concept of EoE responsive to proton pump inhibitors was introduced, suggesting that some of EoE patients may partially respond to this treatment. The response to proton pump inhibitors seems to be only partial and transient, as demonstrated by the publication of a small case series in which inflammatory infiltration of esophageal tissues was found to decrease 3 months after initiating the treatment with proton pump inhibitors but had recurred after 9 months despite continuous treatment (Dohil et al 2012). The partial response to proton pump inhibitors of some patients indicate that EoE and gastro-esophageal reflux might co-exists and can influence each other in a complex way. Understanding the etiology and pathogeny of EoE is important because its diagnostic can easily be confused with a common gastro-esophageal reflux syndrome. However, the differential diagnosis is important for the ensuing treatment strategy that is completely different in these two cases.

The clinical presentation of the illness varies according with the age of the patient (Winter et al 1982). Young children usually show frequent regurgitations, abdominal and chest pain, with slower than usual weight gain. Older children and adults usually show food intolerance and dysphagia. Physical examination findings are non-specific and there is no laboratory marker characteristic for EoE. Endoscopy could be normal or might show esophageal rings and linear furrows, a thickened and pale mucosa and sometimes white exudates. Erosions and ulcerations are more suggestive of gastro-esophageal reflux than of EoE. Radiography is not recommended for EoE diagnosis, except in situations in which a stricture is suspected and the caliber of the esophageal lumen can be fully investigated only by radiography. Since no laboratory markers or clinical signs are pathognomonic for EoE, the crucial clinical step in EoE diagnosis remains endoscopy, which provides the esophageal biopsies allowing histological characterization of esophageal inflammation.

The esophageal inflammation that leads to fibrosis and subsequent esophageal dysfunction in EoE is promoted by a variety of immune cells observed in epithelia: eosinophils, T cells, B cells and mast cells. While all of these cell types are present, the histological hallmark of the disease is undoubtedly the important

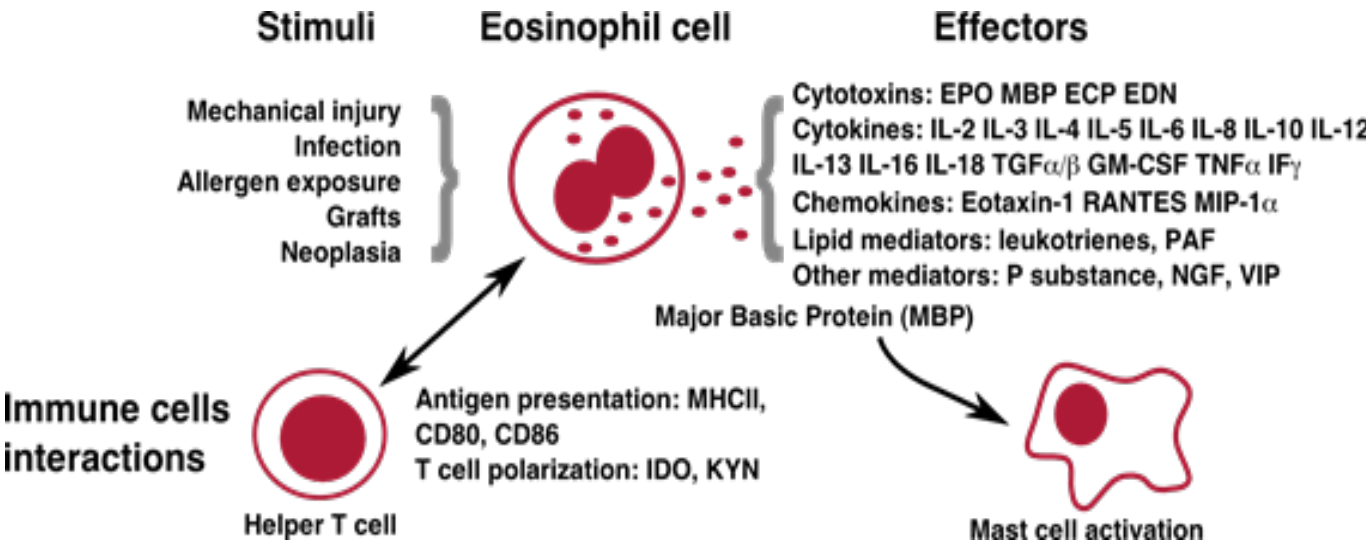

Figure 1. Complex roles of eosinophils in the immune response. Different stimuli activate degranulation of eosinophils, leading to the release of many effectors. Cooperation with mast cells or helper T cells participates to the overall eosinophil-dependent effects.

infiltration of esophageal biopsy specimens with eosinophils, culminating with the formation of eosinophil micro-abscess.

Eosinophils – friends or foes during digestive tract inflammatory disease?

Eosinophils are rare granulocytic blood cells that increase in number in blood and tissues during helminthic infections and allergic inflammation. They develop in the bone marrow from myeloid precursor cells in response to the cytokines interleukin 3 (IL-3), interleukin 5 (IL-5) and granulocyte macrophage-colony stimulating factor (GM-CSF). In healthy individuals, the relatively small numbers of eosinophils produced by bone marrow are trafficking principally to gut mucosa where they are involved in the regulation of host interactions with gut microbiota (Jung and Rothenberg, 2014). Eosinophil density in mucosal tissues strongly increases during specific inflammatory conditions. Their recruitment is coordinated by several cells including helper T cells, B cells and mast cells via the cytokines and chemokines that these cells release when they are activated.

Conventionally, the role of eosinophils was restricted to immune defense against helminthic parasites and to allergic reactions. The relatively recent development of mice strains lacking eosinophils allowed revisiting the role of these cells in immunity. For example, mice lacking eosinophils show an inhibition of parasitic multiplication rather than increased parasite load (Swartz et al 2006) and an unexpected high sensitivity to infections with respiratory viruses (Percopo et al 2014) although traditionally the eosinophils were never associated to anti-viral immunity. These new findings designated the eosinophils as an enigmatic cell type in the immune response. The large panel of cytokines and chemokines produced by eosinophils themselves suggest indeed a complex role of these cells in the regulation of immune responses. In addition to approximately 10 cytokines and chemokines that are stored in eosinophil crystalloid granules, the eosinophils are able to synthetize and secrete under cell-specific stimulation up to 35 cytokines and growth factors (Figure 1 and for a complete review see (Davoine&Lacy 2014)). Therefore, the immune-modulatory potential of eosinophils is very high and is coordinated by the immunological context in which the cells are activated. Depending on the type of stimulation, eosinophils may produce pro-inflammatory cytokines and chemotactic factors that recruit other immune cells in the tissues or they are able to secrete IL-10, the prototype of an immunosuppressive cytokine that limits immune cells recruitment and inflammation. Considering these diverse immune-regulatory capacities of eosinophils, it is not surprising that in different digestive inflammatory disease, the eosinophils have opposite roles. Thus, in EoE, the eosinophils are critical for disease pathogenesis as strongly suggested by the fact that eosinophil deficient mice develop only a mild disease, without collagen deposition and without esophageal dysfunction (Mishra et al 2008). Contrary to their role in EoE, the eosinophils have a clear protective effect in experimental acute mouse colitis, via production of anti-inflammatory lipid mediators (Masterson et al 2014).

The pro-inflammatory role of eosinophils – a key factor in the physiopathology of EoE

The eosinophils store in their granules a series of pre-formed active mediators that are released under cell stimulation and are toxic to the surrounding tissues. Among these compounds, the most abundant are eosinophilic cationic protein, major basic protein, eosinophil protein X, eosinophil derived neuroendotoxin and eosinophil peroxidase. The damage to surrounding tissues is caused by formation of pores across the cell membranes, hyper-contraction of smooth muscles and generation of reactive oxygen species. In addition to direct tissue damage, these compounds activate also the mast cells and promote their degranulation, thus amplifying cellular destruction. Fibrosis is induced mainly through eosinophil secreted TGF-β a pro-fibrotic factor (Malhotra&Levine 2014), and is responsive for esophageal strictures and dysfunction.

Physiological homing of eosinophils in the digestive tract is a consequence of constitutive expression in the gastro-intestinal tract of eotaxins, the most powerful chemo-attractants for eosinophils. Eotaxins are the ligands for CCR3, a receptor highly expressed by eosinophils. The increased eosinophil recruitment to esophageal mucosa during EoE is governed by

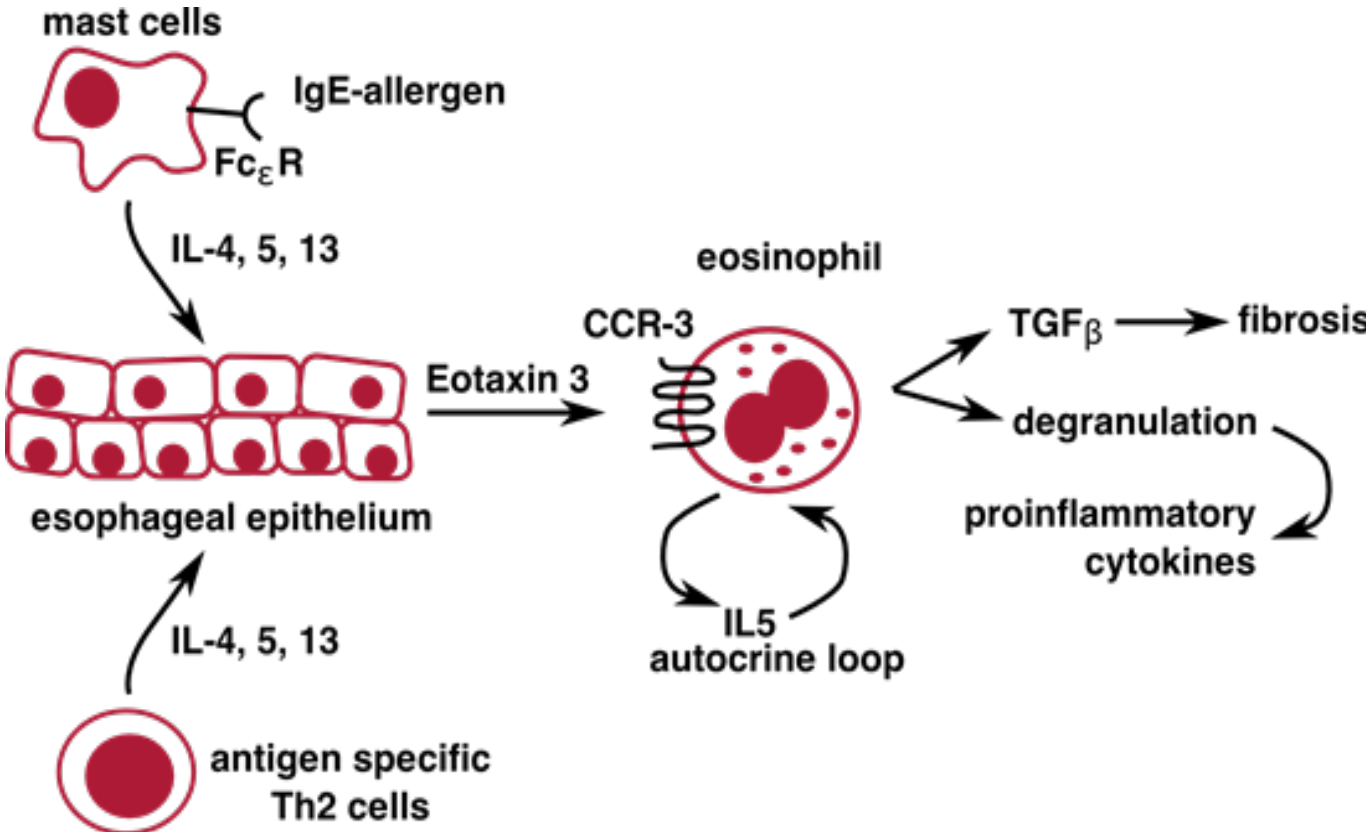

Figure 2. Pathogenesis of EoE. Eosinophils are recruited to esophageal tissues as a consequence of IL-5 production by mast cells activated by IgE-allergen complexes. IL-5 triggers eotaxin-3 secretion by esophageal epithelial cells and increases simultaneously CCR3 expression on eosinophils. Alternative sources of IL-5 are antigen specific helper T cells.

eotaxin-3(CCL26)/CCR3 interaction, as demonstrated by animal models and human pathology. A gene expression analysis that used DNA microarrays found that eotaxin-3 was the most highly induced transcript in EoE and a direct positive correlation could be established between disease severity and eotaxin-3 messenger RNA levels. Moreover, a single nucleotide polymorphism in eotaxin-3 gene was associated to disease susceptibility. As expected from these results obtained from human samples, mice deficient for CCR3 are resistant to experimentally induced EoE (Blanchard et al 2006).

Factors triggering eosinophils recruitment and activation into esophageal tissues

A strong correlation between atopic disease and EoE (Simon et al 2005), with approximately 75% of EoE patients having allergic rhinitis or asthma, positive reactions to allergen by skin tests and specific IgE in the serum, pointed out an allergic etiology for EoE (Noel et al 2004). Sometimes, the allergic reactions are triggered by pollen and EoE exacerbations have a seasonal springtime recurrence. When food allergens are involved, the most frequent allergens are cow's milk, wheat, soy, egg or meats. Allergens identification is beneficial since the disease is ameliorated by specific allergen-free diet or specific desensitization treatments (Ridolo et al 2011).

Similar to other atopic diseases, mast cells activated by IgE-allergen complexes are involved in the initiation of the inflammatory process in EoE. Once stimulated by IgE-allergen complexes binding to the high affinity FcεR, the mast cells secrete IL-4, IL-5, and IL-13 and induce eosinophils differentiation from their precursors, as well as eosinophils recruitment by activation of epithelial cells that secrete eotaxin-3 (Figure 2).

Twenty-five to 30 percents of EoE patients are negative for IgE and no relevant allergens are identified by skin tests (Carr&Watson 2011). These cases of IgE negatives EoE are usually characterized by a T cell mediated hypersensitivity. Following activation of antigen specific T cells several cytokines are produced and recruit inflammatory cells. Among them, Il-5 produced by Th2 CD4 helper cells is the most important for EoE pathogenesis. IL-5 is able to induce eosinophil differentiation from bone marrow precursors, to promote survival of eosinophils and to induce their activation. Mice deficient for IL-5 are resistant to EoE induction (Mishra et al 2008), while transgenic mice overexpressing IL-5 spontaneously develop hyper-eosinophilia (Rothenberg and Hogan, 2006).

In conclusion, EoE is an auto-immune disease elicited by IgE mediated allergy or T cell mediated hypersensitivity. Among the complex pathogenic events, Il-5 secretion by T cells and mast cells and eotaxin-3/CCR3 interaction are the molecular players responsive for eosinophil recruitment in the esophageal tissues and their activation.

Current treatment of EoE and future directions for research

Although very complex in terms of pathology and sometimes difficult to correctly diagnose, the concerted effort of clinicians, pathologists and researchers led to clear diagnosis and therapeutic guidelines in EoE. An overview of these guidelines is represented in Figure 3 (Carr&Watson 2011).

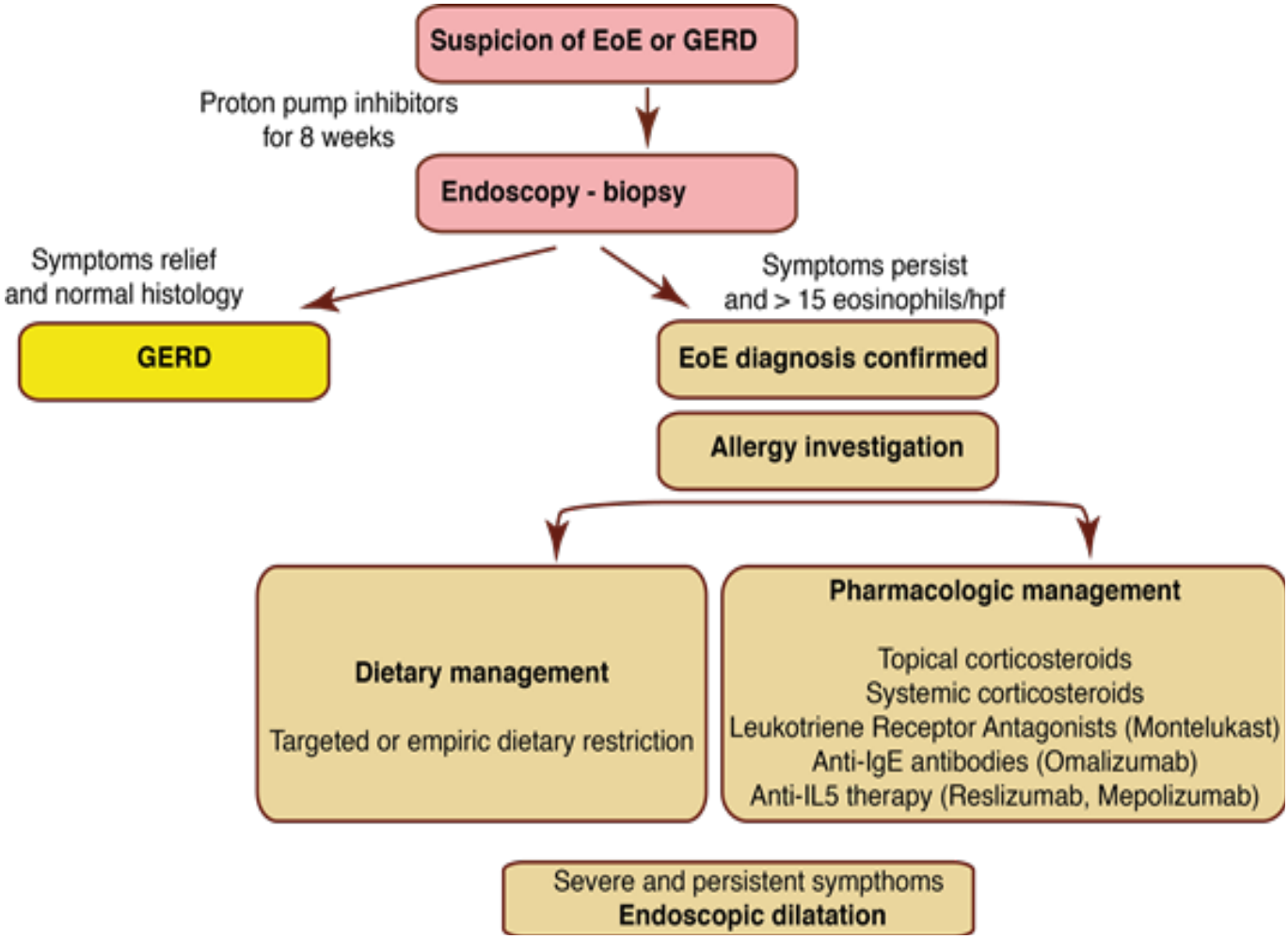

Figure 3. Main steps in the diagnosis and management of EoE (adapted from Carr&Watson 2011)

As in most allergen-triggered disease, the EoE treatment involves the exclusion, if possible, of allergenic stimuli by specific diet and simultaneous glucocorticoid treatment. Glucocorticoids are the most effective treatment for reducing eosinophilia. Their action consists in the suppression of transcription of many genes involved in the inflammatory response. These include the genes for IL-3, IL-4, IL-5, GM-CSF, and eotaxins. Very effective in the clinical response, the glucocorticoids remain a compromise solution, due to their adverse effects (Rothenberg and Hogan, 2006). Therefore, the development of more specific therapies that target eosinophil multiplication, their survival and their activation remain a hope for the future, and is an active field of research.

Overall, EoE is a complex disease that illustrates how much fundamental knowledge of immune cells function and interactions is needed but still lacking. Future efforts in understanding the disease and devising efficient treatment strategies will depend on new data on allergy, T cell, eosinophile and mast cell physiology.

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Authors

•Loredana Saveanu, ATIP-AVENIR Team, U1149 INSERM; CRI Bichat, Faculté de Médecine Xavier-Bichat, 16 rue Henri Huchard, 75018 Paris, France, EU, email: loredana.saveanu@inserm.fr

•Liliana A. Dina, Department of Internal Medicine, IIIrd Medical Clinic, Faculty of Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, 5 Constanta Street, 400158, Cluj-Napoca, Cluj, Romania, EU, e-mail: dina_a_lili@yahoo.com

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