

The Role of Lymphocytes in Eosinophilic Gastrointestinal Disorders

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KEYWORDS

- Eosinophilic esophagitis • Eosinophilic gastroenteritis
- Lymphocyte • helper T cell • Food allergy

Eosinophilic gastrointestinal disorders (EGIDs) encompass a variety of disorders named after the organ of involvement with inflammation and eosinophilia and its resultant symptoms. EGIDs include specific entities such as eosinophilic esophagitis (EoE), eosinophilic gastroenteritis (EG), and eosinophilic colitis.¹ Although the pathogenesis of EGIDs is still poorly understood, dietary food antigens have been shown to cause EGIDs through several short-term clinical studies. Various clinical trials of food elimination resulted in various degrees of clinical response and histologic improvement. These trials included amino acid-based formula therapy^{2–4} and elimination of suspected allergens based on testing⁵ or elimination of six major allergens.⁶ Reintroduction of the foods resulted in reoccurrence of symptoms,² proving a causative relationship of EGIDs to food antigens. This relationship of EGIDs with food allergy points to a potential breach of oral tolerance in EGIDs and to a potentially important role played by lymphocytes in responding to the oral food antigens. In this article, the concept of oral tolerance is discussed briefly, focusing on the role of regulatory T lymphocytes in the process. Discussion then centers on the available evidence for the role that lymphocytes play in the induction and pathogenesis of EGIDs, focusing specifically on T lymphocytes carrying the type 2 helper T-cell phenotype (T_H2), which is important in mediating allergic phenomena. The authors also summarize available evidence for a potential breach in oral tolerance in EGIDs. Among the EGIDs, EoE

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and EG are particularly discussed because they have been the most extensively studied and the most challenging subsets of EGIDs to clinicians and researchers alike.

ORAL TOLERANCE

The gastrointestinal tract is the largest immunologic organ in the body. Despite the large extent of dietary antigenic exposure, only a small percentage of individuals develop food allergy, which is due to the development of oral tolerance to dietary proteins. Oral tolerance refers to a state of active inhibition of immune responses to an antigen by means of prior exposure to that antigen through the oral route.⁷

Normally, dietary proteins that escape gastrointestinal luminal digestion and processing come in contact with the intestinal epithelium and the underlying mucosal immune system in various ways. Dietary antigens can be sampled by intestinal dendritic cells that extend processes into the lumen.⁸ Particulate antigens can be taken up by microfold cells overlying Peyer's patches and delivered to dendritic cells in the subepithelial dome region and then to underlying B-cell follicles of the Peyer's patches.^{9,10} Immunoglobulin (Ig)A switching occurs in these cells, mediated by transforming growth factor (TGF)- β -secreting T cells, hence contributing to oral tolerance.¹¹ Soluble dietary antigens may cross the intestinal epithelium through transcellular or paracellular routes to encounter T lymphocytes or macrophages in the lamina propria.^{12,13} In addition to dendritic cell-presenting antigens, intestinal epithelial cells are thought to act as nonprofessional antigen-presenting cells given that they constitutively express major histocompatibility complex class II molecules on their basolateral membranes^{14,15} and present antigen to primed T cells.

After the dietary antigen is in contact with immune cells, oral tolerance can be induced by two mechanisms, depending on the quantity of antigen (**Fig. 1**). High-dose oral tolerance is mediated by lymphocyte anergy,¹⁶ which occurs through T-cell receptor ligation in the absence of costimulatory signals¹⁷ or by lymphocyte deletion, which occurs by means of Fas-mediated apoptosis (see **Fig. 1B**).¹⁸ Low-dose tolerance is mediated by regulatory T cells. In addition to CD8⁺ T cells^{19,20} and natural killer T cells,²¹ various types of regulatory CD4⁺ T cells play a role in low-dose tolerance.^{22–24} These cells include T_H3 cells, which mediate suppression through secreted TGF- β ,^{25,26} T regulatory cells 1 that mediate suppression through secreted interleukin (IL)-10,²⁷ and CD4⁺CD25⁺ cells that mediate suppression possibly through surface-bound TGF- β (see **Fig. 1C**).^{28,29} A breach in any of these mechanisms has been demonstrated to result in loss of oral tolerance to an antigen in animal models and is hypothesized to lead to food allergies in humans.³⁰

EOSINOPHILIC ESOPHAGITIS

Lymphocytes are Increased in Number in Eosinophilic Esophagitis

Rare intraepithelial lymphocytes are normally found in the esophagus of healthy individuals.^{31–33} These cells were historically referred to as squiggle cells by histopathologists because of their irregular nuclear contours resulting from their intermingling with esophageal epithelial cells, as seen by microscopic examination of hematoxylin and eosin-stained sections of the esophageal mucosa.³³ Squiggle cells were found to be T lymphocytes.^{32,33} In the esophageal epithelial layer, suppressor T cells (CD8⁺) are more frequent than helper T cells (CD4⁺),^{31,34} whereas in the esophageal lamina propria, lymphocytes are comprised mainly of helper T cells.³⁴

The number of esophageal intraepithelial T lymphocytes is increased in patients who have EoE compared with healthy control subjects, as demonstrated by immunohistochemical staining for CD3⁺ cells.^{31,35,36} Both CD4 and CD8 subsets of the T lymphocytes were found to be increased in EoE, with maintenance of CD8

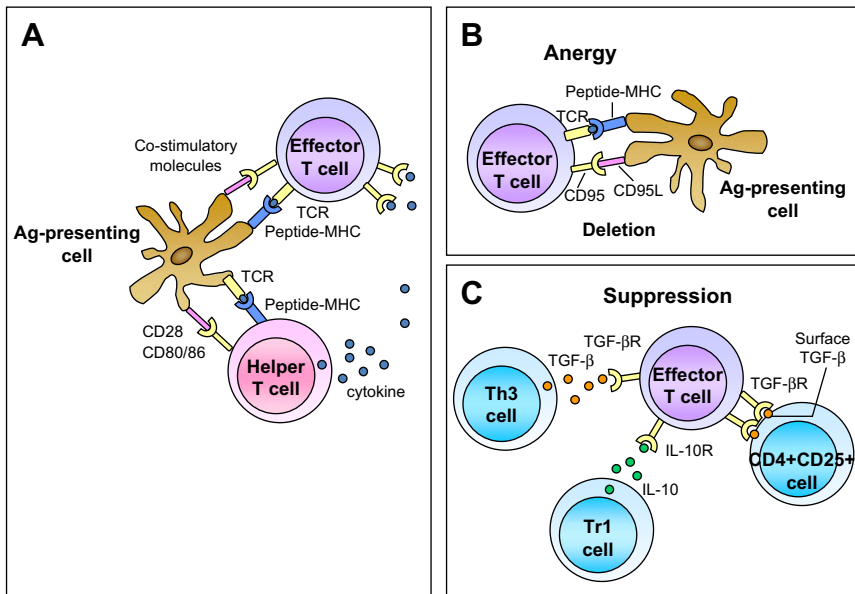


Fig. 1. Mechanisms of oral tolerance. (A) Generation of an immune response. (B) High-dose tolerance. (C) Low-dose tolerance. Ag, antigen; MHC, major histocompatibility complex; TCR, T-cell receptor; Tr1, T regulatory cells 1. (Modified from Chehade M, Mayer L. Oral tolerance and its relation to food hypersensitivities. *J Allergy Clin Immunol* 2005;115(1):3-12; with permission.)

predominating over CD4 cells.^{31,35} The number of lymphocytes and their characterization into subsets in the esophageal subepithelial lamina propria of patients who have EoE have not been examined.

As for B lymphocytes, although absent from the esophageal epithelium in healthy individuals,³¹ some were shown to be present in the subepithelial lamina propria along with helper T cells.³⁴ In EoE, B lymphocytes were found to infiltrate the esophageal epithelium, as demonstrated by immunohistochemical staining for the B-cell marker CD20, albeit in very small numbers.³¹

The Role of Lymphocytes in Induction and Pathogenesis of Eosinophilic Esophagitis

To examine whether lymphocytes play a role in the induction of EoE, Mishra and colleagues³⁷ used a murine model of EoE created by intranasal instillation of *Aspergillus fumigatus* antigen, which resulted in pulmonary and esophageal eosinophilia. Lymphocytes were found to be increased in the esophagus of *Aspergillus*-treated mice compared with saline-treated mice.³⁸ Specifically, a twofold increase in B cells and a fourfold to fivefold increase in CD4⁺ and CD8⁺ T lymphocytes were found. B and T lymphocytes were detected in the mucosal and submucosal regions of the esophagus. When *RAG-1* gene-deficient mice lacking B and T cells were intranasally challenged with *Aspergillus* antigen, no esophageal infiltration was found, indicating an important role for lymphocytes in EoE induction. Using B cell-deficient mice (IgH6) and T cell-deficient mice (Foxn1), the investigators were able to demonstrate that T cells, rather than B cells, were important in the induction of esophageal eosinophilia. Within T cells, CD4⁺ rather than CD8⁺ cells were shown to be important: CD4-deficient

mice were moderately protected from the induction of EoE, whereas CD8 α -deficient mice developed unaltered EoE.

In humans, the role of lymphocytes in the induction of EoE is less clear. B lymphocyte function in EoE has not been examined. Although slightly increased in number in the esophagus of EoE patients, it is not known whether these B cells bear IgE and hence contribute to the pool of IgE-positive cells (most of which are thought to be mast cells) found in the esophagus of patients who have EoE. B lymphocytes are known to be capable of holding antigens to their surface for recognition by specific T lymphocytes along with major histocompatibility complex class II molecules.³⁹ The potential role of B cells in antigen presentation in EoE remains to be tested.

The role of T lymphocytes, especially CD4 cells in the induction of EoE in humans, has not been directly investigated. Various descriptive studies, however, point to T_H2 adaptive immunity in EoE. The role of T_H2 cytokines in the induction of EoE was demonstrated by Mishra and colleagues⁴⁰ in a murine model of EoE. Intratracheal delivery of the T_H2 cytokine IL-13 to mice induces dose-dependent esophageal eosinophilia by an IL-5 and STAT6-dependent mechanism. These results strongly implicate T_H2-associated immune responses in the pathogenesis of EoE, indicating that the T_H2 phenotype in EoE is potentially crucial in its pathogenesis in humans.

In humans, aside from the many clinical short-term therapeutic studies linking the induction of EoE to food allergens^{2,3,5} and therefore pointing to a T_H2 type of immune response, various investigators have examined the predominant cytokines in the peripheral blood and the esophagus of patients who have EoE and found them to be consistent with a T_H2 phenotype.

In the peripheral circulation, Bullock and colleagues⁴¹ examined cytokine expression by peripheral blood mononuclear cells (PBMCs) and found that children who had active EoE had an increased percentage of CD4⁺ T cells expressing IL-5 compared with nonatopic control subjects. This percentage was lower in EoE patients in remission than in patients who had active disease. Straumann and colleagues³⁶ stimulated PBMCs of EoE patients with phytohemagglutinin, a nonspecific stimulant, and measured resultant cytokine secretion by the PBMCs. Increased release of the T_H2 cytokine IL-13 was found in 40% of the patients studied. Yamazaki and colleagues⁴² conducted similar experiments using stimulation with specific allergens. PBMCs from 15 adult patients who had EoE were incubated *in vitro* with food allergen extracts and aeroallergen extracts. Food extracts consisted of milk, egg, soy, wheat, and peanut. Compared with healthy control subjects, PBMCs from EoE patients secreted significantly more of the T_H2 cytokine IL-5, IL-13, or both in response to various allergens, even in the absence of clear-cut sensitization to the allergens when serum levels of allergen-specific IgE were measured. Whether the T_H2 response is secondary to the concomitant atopic features of the patients remains unknown, because 73% of the EoE patients in the study had concurrent allergic disease, a finding common to patients who have EoE.^{43,44} A study comparing EoE patients with non-EoE allergic control subjects is needed to confirm these findings.

In the esophagus, increased levels of the T_H2 cytokine IL-5 in biopsy samples of patients who had EoE were found, whether assayed at the messenger RNA (mRNA) level by real-time polymerase chain reaction (RT-PCR) or at the protein level using immunohistochemical staining of esophageal sections.^{36,45} Using microarray analysis, mRNA for IL-4 and IL-13, two other T_H2 cytokines, were not detected or up-regulated in the esophagus of patients who had EoE.⁴⁶ Using RT-PCR, a more sensitive technique, esophageal IL-13 was found to be increased in patients who had EoE compared with control subjects.⁴⁷ IL-13, in turn, likely plays an important role in EoE pathogenesis: when stimulated with IL-13 *in vitro*, esophageal epithelial cells produce

high levels of eotaxin-3,⁴⁷ a highly induced chemokine in EoE that is shown to regulate eosinophil responses *in vitro*⁴⁸ and to correlate with the severity of esophageal eosinophilia in EoE.⁴⁶

Identification of the specific inflammatory cells producing the T_H2 cytokines just described, however, has not been done. As a result, we do not know the exact extent of the lymphocytes' contribution to the production of these T_H2 cytokines, especially given that other immune cells can also secrete these cytokines. For example, when esophageal eosinophils of patients who had EoE were examined for T_H2 cytokine expression using immunohistochemical staining, 60% of these cells were found to express IL-4 and IL-13.⁴⁹ Further studies addressing the specific contribution of esophageal lymphocytes to the production of T_H2 cytokines, especially in response to specific dietary allergens, need to be performed.

No studies addressing regulatory T cells and their corresponding cytokines such as TGF- β in EoE have been published. The coexistence of EoE in patients who have other diseases such as celiac disease^{50,51} and Crohn's disease (Mirna Chehade, MD, Hugh A. Sampson, MD, unpublished data, 2008), both characterized by a predominant T_H1 rather than a T_H2 phenotype, points to the potential importance of abnormal regulatory cell function leading to the development of these diseases in the same patient and warrants further study.

EOSINOPHILIC GASTROENTERITIS

Lymphocytes are Increased in Number in Eosinophilic Gastroenteritis

Jaffe and colleagues⁵² counted the number of lymphocytes in gastric biopsy samples of patients who had EG characterized by moderate to severe gastric eosinophilia. A twofold to threefold increase in the number of T cells was found in gastric biopsy samples of patients who had EG compared with normal gastric tissue in control subjects using immunohistochemical staining. No further differentiation into CD4⁺ and CD8⁺ cells was performed. B cells were found only in the rare benign lymphoid aggregates and were absent in the epithelium and lamina propria of EG patients and control subjects. As with EoE, these findings also indicate a more prominent role for T lymphocytes and adaptive immunity than for humoral immunity in EG.

The Role of Lymphocytes in Induction and Pathogenesis of Eosinophilic Gastroenteritis

Using a murine model of EG, Hogan and colleagues⁵³ demonstrated that gastrointestinal eosinophil accumulation was antigen induced and associated with a T_H2 phenotype. Oral challenge of allergen-sensitized mice with enteric-coated beads containing the allergen resulted in marked serum allergen-specific IgE, increased splenic T lymphocyte secretion of the T_H2 cytokines IL-4 and IL-5 following *in vitro* allergen restimulation, and increased eosinophil accumulation in the blood and small intestine compared with placebo-challenged mice. Therefore, the accumulation of gastrointestinal eosinophils may be antigen induced and associated with a T_H2 phenotype.

In patients who have EG, mRNA levels for IL-5 are slightly increased in gastric mucosal biopsy samples compared with those of control subjects when assayed by RT-PCR. In contrast, mRNA for the T_H1 cytokine interferon (IFN)- γ was undetectable in the stomach of EG patients but demonstrable in that of normal control subjects.⁵² These results point to a T_H2 phenotype in EG in humans, as demonstrated for EoE. Similar to EoE, the cellular sources of the cytokines studied were not specifically determined, resulting in a lack of a direct evidence for the degree of tissue T lymphocyte contribution to this phenotype.

In the peripheral circulation, the role of T cells was more directly investigated in patients who had EG. T-cell synthesis of IL-5 was increased in patients who had EG compared with control subjects, especially the CD4 subset.⁵² When stimulated nonspecifically with the mitogen phytohemagglutinin, production of the T_H2 cytokines IL-4 and IL-5 was found to be markedly elevated in EG patients compared with control subjects.⁵² Mitogen-stimulated synthesis of the T_H1 cytokine IFN- γ by CD4 cells was normal. These results again point to a T_H2 phenotype and suggest a role for T lymphocytes in the pathogenesis of EG.

Beyer and colleagues⁵⁴ examined cytokine secretion by tissue lymphocytes in response to specific allergens in patients who had EG. In this study, duodenal biopsy specimens from patients who had EG and milk-induced symptoms and from control individuals were minced and cultured in vitro with nonspecific mitogens (phytohemagglutinin, phorbol myristate acetate, and irradiated PBMCs) or with milk proteins (α -casein, β -casein, α -lactalbumin, and β -lactoglobulin). Polyclonal expansion of mucosal T cells using mitogens resulted in predominantly T_H1 cells from the duodenum of EG patients and control subjects. After stimulation with milk proteins, milk-specific mucosal T-cell lines could be established from most patients who had EG but from none of the control subjects. In contrast to nonspecific polyclonal expansion, milk-specific expansion showed a clear T_H2 cytokine profile, with T lymphocytes releasing predominantly IL-5 and IL-13 following in vitro restimulation with milk proteins. These results show that intestinal allergen-specific T lymphocytes release T_H2 cytokines following allergen stimulation, potentially playing an important role in the pathogenesis of EG.

In both studies,^{52,54} the T_H1 proinflammatory cytokine IFN- γ was also found to be excessively produced by peripheral blood and intestinal T lymphocytes of patients who had EG following in vitro stimulation, although to a much lesser extent than the T_H2 cytokines.

Equally important in Beyer and colleagues'⁵⁴ study was the lack of production of the regulatory cytokines TGF- β and IL-10 by duodenal milk-specific T lymphocytes of patients who had milk-allergic EG when stimulated in vitro with milk proteins.⁵⁴ These findings point to a lack of a tolerogenic response by lymphocytes in response to food allergens in patients who have EG and warrant further investigation for future therapeutic dietary interventions.

SUMMARY

EGIDs appear to be antigen-driven diseases, with an increase in the number of T lymphocytes detectable in the tissue involved with inflammation and eosinophilia. Ex vivo examination reveals a T_H2-predominant phenotype of involved tissues using biopsy samples or following antigenic stimulation of peripheral or tissue lymphocytes. The exact role of these lymphocytes in the induction and pathogenesis of EGIDs in patients is still not confirmed, however. Although T lymphocytes were shown in murine models to have an essential role in induction of EoE, little is known about their exact role in human EGIDs. More studies are needed to determine sites of antigen absorption, inductive sites for T lymphocytes, and homing molecules for these T lymphocytes back to the diseased tissues. These studies will help tailor development of targeted therapies for EGIDs. In addition, the contribution of T lymphocytes in the pathogenesis and severity of EGIDs still needs to be fully investigated. The T_H2 cytokine IL-13 was shown to be important in disease pathogenesis in human EoE; however, the extent of T-lymphocyte contribution to the production of this cytokine and other T_H2 cytokines still needs to be elucidated. Furthermore, the effect of elevated T_H2 cytokines on tissue eosinophilia needs to be studied. Results from these studies may help determine

whether concomitant therapies are needed in some patients, especially when using therapies specifically targeting eosinophils. Finally, mechanisms leading to a breach of oral tolerance in patients who have EGIDs are still preliminary. So far, we know only that tissue antigen-specific T cells from patients who have EG do not produce T regulatory cytokines on in vitro restimulation. Because the sensitization potential for dietary antigens cannot be identified using standard diagnostic techniques (eg, prick skin tests or serum food allergen-specific IgE levels), the potential for achieving tolerance to these foods cannot be predicted in patients who have EGIDs. Therefore, studies dissecting mechanisms of oral tolerance and subsequent ways to monitor for the development of tolerance in patients who have EGIDs are crucial, because they may help dictate timing of dietary antigen re-introduction in patients on restricted diets and thus improve the quality of life of patients suffering from these diseases.

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