The Role of Innate Lymphoid Cells in Food Allergy

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Catarina Carvalho Naia

Dedicatória

Para a minha avó Idalina, a pessoa mais bondosa que conheci.

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Resumo

A alergia alimentar é uma doença causada por uma reação imunológica exacerbada, que ocorre em resposta a substâncias alergénicas presentes em determinados alimentos, tais como os frutos secos, leite, peixe e marisco. A sua prevalência atual estima-se entre 1-20% da população europeia, sendo estes valores variáveis consoante a metodologia diagnóstica utilizada. Recentes revisões sistemáticas sobre esta temática têm vindo a confirmar uma prevalência crescente, o que supõe um importante problema de saúde pública.

A sintomatologia resultante é diversa, podendo afetar os sistemas dermatológico, respiratório, cardiovascular e neurológico. A intensidade dos sintomas é muito variável, desde reações leves até reações graves e potencialmente fatais, como é o caso da anafilaxia.

As células linfoides inatas são células do sistema imunitário que diferem dos linfócitos T e B principalmente por não possuírem recetores específicos de antigénios na sua superfície. As células linfóides inatas do tipo 2 respondem a células previamente ativadas, libertando citocinas que promovem inflamação e o desenvolvimento de sintomas alérgicos.

A Esofagite Eosinofílica é uma doença que cursa com inflamação crónica da mucosa esofágica, com infiltrado predominantemente eosinofílico. 70% dos doentes diagnosticados com Esofagite Eosinofílica têm uma história prévia de alergia alimentar. Nestes doentes, existe um número aumentado de células linfóides inatas na mucosa esofágica sugerindo que estas células, ao promover a inflamação, possam também contribuir para o desenvolvimento desta patologia.

Pretende-se então, com esta revisão bibliográfica, esclarecer e especificar as funções das células linfóides inatas, que através da produção de citoquinas e da interação com outras células imunitárias, podem contribuir para a fisiopatologia da alergia alimentar e influenciar o seu tratamento.

Palavras-chave

Alergia Alimentar; Células linfóides inatas; Esofagite Eosinofílica; Imunidade Inata

Abstract

Food allergy is a disorder caused by an exacerbated immunologic reaction, which occurs in response to specific allergenic substances, present in certain foods, such as tree nuts, milk, fish, and shellfish. It is estimated to affect around 1-20% of the European population, with its prevalence varying according to the diagnostic methodology used. Moreover, in the last years, systematic reviews have indicated an increasing prevalence, which suggests an important public health problem.

Clinical manifestations of food allergy are wide-ranging and can affect the dermatologic, respiratory, cardiovascular, and neurological systems. The intensity of the symptoms is highly variable, ranging from mild reactions to severe and potentially fatal reactions, such as anaphylaxis.

Innate lymphoid cells are immune cells that differ from T and B lymphocytes mainly for not possessing receptor-specific antigens on their surface. Type 2 innate lymphoid cells respond to previously activated cells, releasing cytokines that promote inflammation and the development of allergic symptoms.

Eosinophilic Esophagitis is a disorder characterized by chronic inflammation of the esophageal mucosa, with a predominantly eosinophilic infiltrate. 70% of the patients diagnosed with Eosinophilic Esophagitis have a previous history of food allergy. In these patients, the number of Innate Lymphoid Cells is increased in the esophageal mucosa, suggesting that these cells, by promoting inflammation, can also promote the development of Eosinophilic Esophagitis.

In conclusion, this review aims to clarify and detail the functions of Innate Lymphoid Cells which, through the production of cytokines and interactions with other immune cells, may contribute to the pathophysiology of food allergy and influence its possible treatments.

Key Words

Food Allergy; Innate Lymphoid Cells; Eosinophilic Esophagitis; Innate Immunity

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List of Acronyms

AIT	Allergen-specific Immunotherapy
COX-1	Cyclooxygenase-1
DCs	Dendritic Cells
EpCs	Chemosensory Epithelial Cells
FDA	Food and Drug Administration
G-CSF	Granulocyte colony-stimulating factor
GM-CSF	Granulocyte macrophage colony-stimulating factor
GITRL	Glucocorticoid-induced TNF receptor ligand
HB-EGF	Heparin-Binding Epidermal Growth Factor–like Growth Factor
ICOS	Inducible Costimulator
ICOS-L	Inducible Costimulator Ligand
Id2	Inhibitor of DNA binding 2
IFN-γ	Interferon Gamma
Ig	Immunoglobulin
IHST	Immediate Hypersensitivity Skin Test
IL	Interleukin
ILCs	Innate Lymphoid Cells
Lin-	Lineage Negative
LTis	Lymphoid Tissue-inducer cells
MDSCs	Myeloid-derived suppressor cells
MHC	Major Histocompatibility Complex
NK	Natural Killer
NMU	Neuromedin-U

OFCs	Oral Food Challenges
OIT	Oral Immunotherapy
PD-1	Programmed Cell Death Protein 1
PG	Prostaglandin
PLZF	Promyeloid Leukaemic Zinc Finger
ROR	Related Orphan Receptor
TCF-1	T-cell Factor 1
TCR	T-cell Receptor
TGF-β	Transforming Growth Factor Beta
Th2	T-helper type 2
TL1A	TNF-like ligand 1A
TLR	Toll Like Receptor
Tregs	Regulatory T cells
VIP	Vasointestinal Peptide

1. Introduction and aims

Food allergy is defined as an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food (1). It is a common disorder that affects between 1-20% of the European population. The prevalence rates vary depending on the food allergy diagnostic method (2).

Food allergy can be responsible for a reduced quality of life, also causing an important economic burden. A cohort study estimated that food allergy in children is responsible for a total economic burden of $25 billion a year, in the United States, mostly as a consequence of indirect costs and lifestyle changes, rather than direct medical care. Moreover, people with food allergies need to be constantly aware, to avoid exposure to offending foods, making significant daily changes (3).

There are hundreds of foods that are known to cause allergic reactions. However, the most common ones include milk, eggs, whey, peanuts, tree nuts, soy, fish, and shellfish. These are known as the “Big 8” group (4).

The symptoms of food allergy can affect multiple systems and range from mild itching, stomach pain, and rash to severe anaphylaxis. Most allergic reactions are not severe. However, they can be life-threatening if there is respiratory and/or cardiovascular distress (5).

Food allergies involve an immunologic reaction (6). On the contrary, food intolerance is a non-immune reaction that includes metabolic, toxic, pharmacologic, and other undefined mechanisms (7). On that account, there are several different types of food reactions. They can be divided into toxic and non-toxic reactions. Toxic reactions include, for example, food poisoning, whereas nontoxic reactions are furthermore divided into immunologic and nonimmunologic reactions. Non-immunologic reactions include lactose intolerance. There are two main types of immunologic reactions: Immunoglobulin (Ig) E-mediated reactions, or non-IgE-mediated (figure 1) (8).

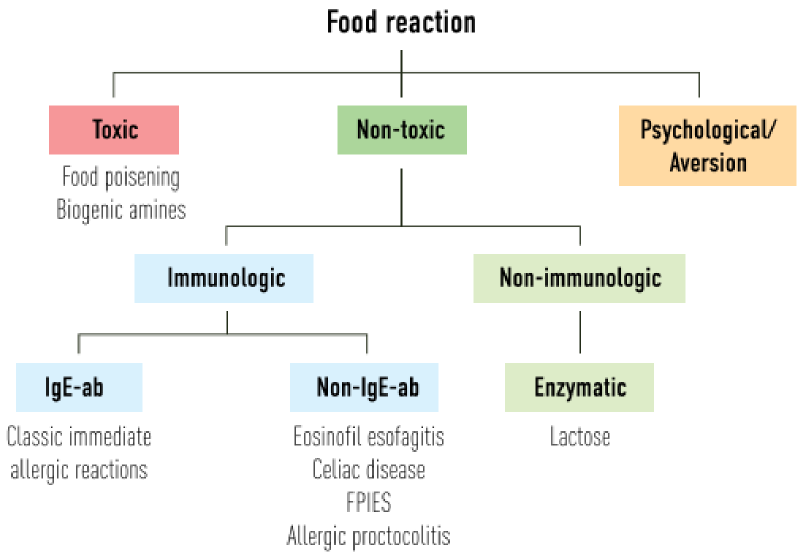

Figure 1: Types of adverse reactions to food. Adapted from (8).

IgE-mediated food allergy develops as a type 2 immunologic reaction. Innate lymphoid cells (ILCs) amplify the allergic reaction, responding to previously activated cells by releasing cytokines. This type of cell can be found on several barrier surfaces, such as the respiratory tract, gastrointestinal tract, and skin. This characteristic makes them an important link between innate and adaptive immunity (9).

In conclusion, adverse reactions to food are quite common events, with an important impact on people's lives, causing anxiety and concern, and ultimately leading to very strict diets. Therefore, people with food allergy and their families deal with constant risk and must maintain vigilance to prevent exposure to food allergens (10).

Considering this, the present review aims to analyse the most important evidence on how ILCs contribute to the pathophysiology of food allergies and if these cells can influence possible treatments. The main objectives of this review are to:

1. Characterize food allergy disorder.
2. Define the main characteristics of ILCs.
3. Detail the functions of ILC2s and how they influence the pathophysiology of food allergy.
4. Describe Eosinophilic Esophagitis and the role of ILCs in this disorder.
5. Detail the current knowledge regarding how ILC2s may influence potential food allergy treatments.

2. Methods

For the present narrative review, a search was conducted between June 2023 and October 2023, on 3 electronic databases: PubMed, Scopus, and Web of Science. The PubMed search was used as a model for the other databases, and used the following terms, MESH words, and bolean operators:

((food allergy[MeSH Terms]) OR (eosinophilic esophagitis[MeSH Terms])) AND (innate lymphoid cells[MeSH Terms])

The database search was restricted to articles written in English and Portuguese and published within the last 10 years (January 2013 to October 2023). The following article types were included: review, systematic review, clinical trial, meta-analysis, randomized control trial, original research articles, and guidelines.

Between the 3 databases, there was a total of 88 articles; from those, 32 were selected. In this review, pertinent references from the selected articles were also included, as well as international guidelines. Therefore, a total of 55 references were used in this review.

3. Food Allergy

3.1 Epidemiology

Food allergy prevalence has been increasing in the last 2 to 3 decades. Epidemiologically, food allergy seems to disproportionately affect people in westernized and developed countries (6).

However, it is difficult to precisely determine the prevalence of food allergy. This is due to the wide range of manifestations of food allergy, that differ in levels of intensity and severity. Moreover, there are several definitions of food allergy, and different studies evaluate different study populations, focus on specific foods, and use different methods (2,6).

Therefore, food allergy prevalence is influenced by geographic variations, and different diets, also depending on several factors such as age, race, and ethnicity (6). It is estimated to affect between 1-20% of the European population (2). Moreover, in a study conducted in the United States, a population of 40 443 adults was analysed (≥ 18 years of age). This research aimed to estimate the prevalence of food allergy in adults, in the United States. This study showed that 10.8% of the people were food allergic at the time of the survey. However, 19% of the adults included in this research, believed that they were food allergic (11). In fact, studies show that up to one-third of the population believes they have a food allergy (7).

Food allergy reactions can have different intensities. It is estimated that 40% of people diagnosed with food allergy need assistance in the emergency room, at least once, at some point in their lives (12).

Acute allergic reactions are more common in children than in adults. It is estimated that 5% of adults and 8% of children have food allergies (7). Regarding children, a population-based study conducted in Australia concluded that the prevalence of IgE-mediated food allergy was 11% at 1 year old, and 3.8% at 4 years old (13). Another three similar population-based studies conducted in our country, estimated this values in 1.4% (between 3- 11 years old), 1.41% in adolescents and 0.8% in adults (14–16).

Food allergy usually begins in the first 2 years of life. Some food allergies are often outgrown, for example, cow's milk and eggs, that often resolve later in childhood. Others, such as tree nut and peanut allergies, are less likely to resolve, persisting into adulthood. (5).

3.2 Pathophysiology

Immune responses to nonmicrobial environmental antigens develop with the production of type 2 cytokines IL-4, IL-5, and IL-13 produced by T-helper type 2 cells (Th2) cells and ILC2s. This type of response originates the production of IgE, and activation of mast cells and eosinophils, and can lead to various allergic disorders such as asthma, allergic rhinitis, atopic dermatitis, and food allergy (13,17).

In healthy individuals, the immune system normally tolerates food antigens, perceiving them as non-pathogenic. However, in some people, food allergies develop due to the breakdown of immunologic tolerance that prevents food antigens from being recognized as pathogens (7).

Immune tolerance develops as a lack of specific immune response to antigens representing the capacity of the immune system to adapt to innocuous antigens such as food proteins. These antigens are detected in the intestinal epithelium and lamina propria within minutes after consumption. Thus, the gastrointestinal tract has a key role in oral tolerance induction (18).

After food ingestion, food proteins are captured by Antigen-Presenting Cells (APCs) such as Dendritic Cells (DCs). APCs migrate to draining lymph nodes to present food antigens to naïve T cells. In healthy individuals, this leads to the induction of suppressive cytokines, such as IL-10, and in turn results in the differentiation of naïve T cells into CD4+ regulatory T cells (Tregs) (7,19).

On that account, induction, and maintenance of tolerance to food antigens requires active generation of food antigen specific Treg cells. If there is a disruption in this mechanism and oral tolerance fails to develop, adverse reactions such as IgE-mediated food allergies can occur (7).

In allergy, innocuous antigens are recognized as foreign invaders. Thus, in genetically predisposed individuals, and due to specific environmental signals, such as a disrupted intestinal barrier, inflammatory cytokines are released. This leads to the activation of DCs into phenotypes that are normally acquired during the defence against pathogens (7).

Dendritic cells present the antigens to Th2 cells. This leads to the production of type 2 cytokines: IL-4, IL-5, and IL-13, by Th2 cells, and also ILCs and T follicular cells. (figure 2) (17).

Active Th2 cells induce B cell class switching to IgE. IgE produced by B cells binds to the Fc receptor on mastocytes, causing mastocyte sensitization. In subsequent exposures, antigen-specific IgE binds to the IgE receptor on mast cells and basophils. This process may result in the release of inflammatory mediators, such as histamine, leukotrienes, and cytokines, that may cause increased vascular permeability, vasodilation, and bronchial and visceral smooth muscle contraction. Thus, allergic reactions require the previous production of allergen-specific IgE by B cells, and binding of IgE to mast cells (figure 2) (17).

Allergic sensitization occurs with the production of IgE. In food allergy, sensitization to food allergens most likely occurs in the gastrointestinal tract (13).

Type I hypersensitivity develops rapidly after exposure to the antigen, in a previously sensitized individual. Besides the immediate response, there is a late-phase reaction, that develops slower, and curses with the accumulation of eosinophils and neutrophils (17).

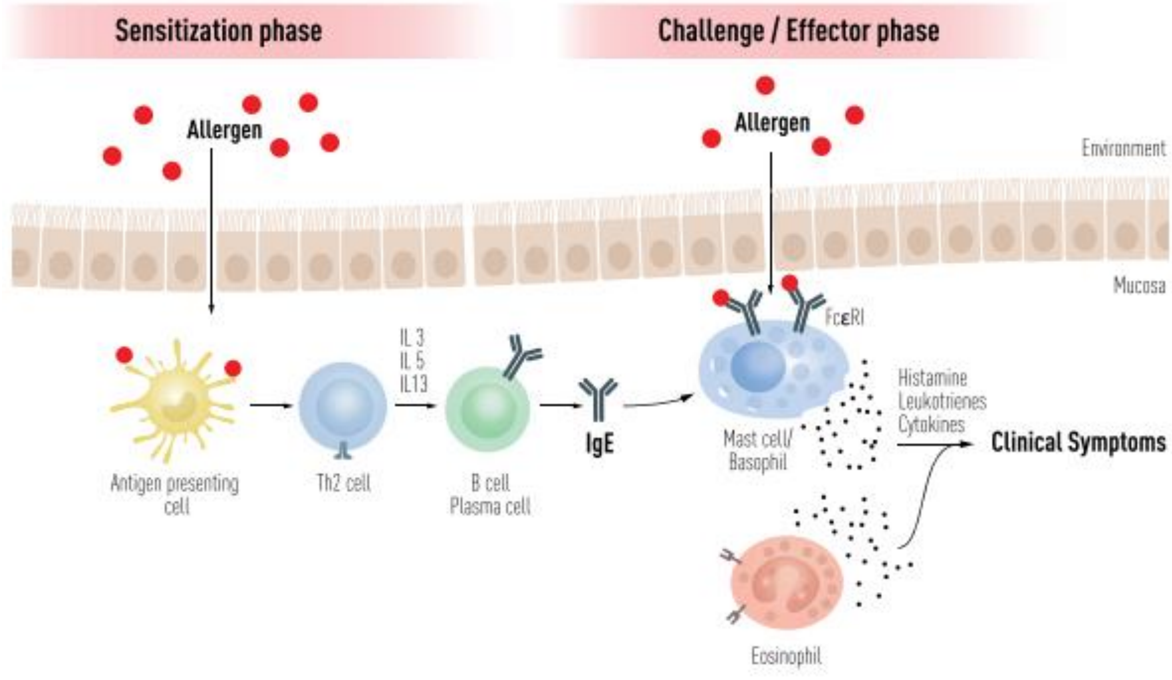

Fig 2. Pathophysiology of IgE-mediated food allergy. Adapted from (8).

Food allergies can be divided into two broad categories: IgE-mediated reactions or non-IgE-mediated disorders, such as Eosinophilic Esophagitis (6,7).

IgE-mediated reactions are typically of rapid onset, and clinical symptoms usually develop within minutes to a few hours after food ingestion. Whereas non-IgE-mediated disorders develop hours to days after food ingestion and usually have a chronic course (7).

3.3 IgE-Mediated Food Allergy Symptoms

Table 1: Food Allergy Symptoms in mild, moderate, and severe reactions. Adapted from (7).

Symptoms	**Mild Reactions**	**Moderate**	**Severe**
Cutaneous Rash	Mild Erythema.	Erythema.	Generalized marked erythema (more than 50% of the body surface).
Urticaria and Angioedema	Less than 3 areas of hives. Mild lip edema.	More than 3 areas of hives. Lip or face edema.	Periocular swelling. Generalized swelling in the face, lips, or eyelids.
Pruritis	Occasional scratching.	Continuous scratching.	Continuous scratching with excoriations.
Sneezing/itching	Occasional sniffing.	Frequent sniffing. Intermittent rubbing of nose or eyes.	Persistent rhinorrhea. Continuous rubbing of nose or eyes.
Wheezing	Expiratory Wheezing.	Expiratory and Inspiratory Wheezing.	Audible wheezing. Use of accessory muscles to breathe. Increased work on breathing
Laryngeal manifestations	Cough. Throat tightness.	Frequent Cough. Hoarseness.	Stridor.
Abdominal Pain	Mild Abdominal Pain.	Moderate Abdominal Pain.	Notably distressed due to GI symptoms with decreased activity.
Nausea/Emesis and Diarrhoea	Nausea 1 episode of emesis or diarrhea.	2-3 episodes of emesis or diarrhea.	More than 3 episodes of emesis or diarrhea.
Cardiovascular and Neurological manifestations	Subjective weakness; dizziness; tachycardia.	Blood pressure decrease; Mental status changes, such as anxiety, or confusion.	Unconsciousness; Cardiac arrest.
Other manifestations	-	Loss of bladder control.	Pelvic pain.

Food hypersensitivity reactions develop after food intake and can be caused by several different mechanisms. The symptoms are described in Table 1 and range from mild itching, stomach pain, and rash to severe anaphylaxis (7).

In IgE-mediated food allergy, the release of inflammatory mediators causes an increased mucus production and vasodilation. It also causes increased vascular permeability that leads to edema, stomach pain, vomiting, diarrhea, urticaria, and even bronchial obstruction and anaphylaxis. The immunopathogenic mechanisms that lead to non-IgE-mediated food allergies are not well defined yet (8).

It is extremely important to recognize anaphylactic reactions since anaphylaxis represents a life-threatening condition. An international study was conducted from July 2007 to March 2015 to collect data about episodes of anaphylaxis in children. This research was published in 2016, and the results were that: 92% of children had skin symptoms during the anaphylactic episode, including angioedema, urticaria, pruritus, and erythema; 80% showed respiratory symptomatology; 45% had gastrointestinal symptoms; 41% exhibited cardiac symptoms and in 26% of children, signs of neurological impairment were found (20).

3.4 Diagnosis

Food allergy diagnosis is based on a thorough clinical history. The patient should be asked to detail the symptoms and to say which food, cooked or uncooked, is supposedly causing them. Other questions include the amount of food ingested, the time from ingestion to the development of symptoms, factors such as viral illnesses, medications, and exercise that may have contributed to the reaction, and reproducibility following a subsequent exposure (7).

Double-blind-placebo-controlled oral food challenge is the gold standard procedure for the diagnosis of food allergy. In this test, the patient, in a blinded foodstuff process, during several hours consumes the “culprit food” in gradually increasing amounts. This procedure requires medical supervision and aims to diagnose or exclude a food allergy. However, Oral Food Challenges (OFCs) are not very commonly performed because they are time-consuming, require highly trained personnel, and can cause an acute allergic reaction, so they should be performed with caution (21,22).

In addition, if an IgE-mediated food allergy is suspected, there are approved tests, used to come up with a diagnosis, such as Immediate Hypersensitivity Skin Test and serum tests. However, it is also important to consider that ordering large panels of tests can result in false-positive results and unnecessary dietary eliminations (7).

An Immediate Hypersensitivity Skin Test screens for food-specific IgE antibodies bound to cutaneous mast cells. The skin surface is scratched using a device coated with commercial food extract (Prick-Test method) or fresh food (Prick-by-Prick method). These procedures allow the introduction of food protein through the skin, leading to antigen binding to specific IgE on cutaneous mast cells, followed by their degranulation and release of histamine. This process produces a wheal and flare reaction, with local swelling of the skin at the site of allergen introduction (7).

A positive skin test is characterized by a wheel whose diameter is superior to the negative control in 3mm or more. A positive skin test suggests that clinical reactivity is likely. However, a positive test only indicates the presence of specific IgE antibodies bound to mast cells. It is not diagnostic of clinical reactivity. Sensitization happens when there are specific IgE antibodies without clinical reactivity. Therefore, caution should be made when interpreting results, and only suspected food allergens should be tested, because food skin testing has a low specificity. In cases of anaphylaxis, it is recommended to wait for a period of 4 to 6 weeks before skin testing is performed (7).

Another test consists of the measurement of IgE, using a technique of fluorescence enzyme labelling. In this method, the patient’s serum is incubated with a surface fixed allergen, and a food-specific IgE from the patient’s blood binds to the allergen. Using this test, we can measure the circulating allergen-specific IgE levels. Levels correlating with a 95% positive predictive value can correlate with a reaction to a certain food. Although levels above 0.35 KUI/L are usually considered as “positive”, there is no clear consensus about this “positive cut-off” between different foodstuffs, patient´s age, or food reaction´s symptomatology (7).

The Basophil Activation Test (BAT) is a method that utilizes flow cytometry to measure the activation of basophils in the blood, by assessing their surface expression of activation markers, such as CD63, after stimulation with allergen or controls (23). Research shows that the BAT may be useful in nut-allergic patients (23,24). Since these patients are often IgE-sensitized to various nuts and seeds, this situation should involve, as previously referred, several OFCs (one for each food) performances before ensuring that the nuts are safe to consume and can be introduced into their diet. However, OFCs are time-consuming and risky procedures. Studies show that the BAT to hazelnut, cashew nut, sesame, almond, and peanut differentiates between allergic and nonallergic children, to the respective nut or seed. Using the BAT reduced the number of total OFCs by 5% to 15% and had a diagnostic accuracy of 96% to 100% (24)

There are several other tests, such as allergen-specific IgG or IgG4 testing, lymphocyte stimulation, cytotoxicity assays, mediator release test, provocation neutralization, applied kinesiology, or hair analysis testing. However, they are not recommended as valid diagnostic methods for IgE-mediated food allergies. Patients should not be subjected to these tests, because they are costly and could result in unnecessary diet restrictions (7).

3.5 Treatment

Currently, food allergy is managed by strict allergen avoidance (8).

Drug treatment is provided to patients, for cases of accidental exposure to offending foods. These treatments can include adrenaline autoinjectors, antihistamines, corticosteroids, and Beta-2-agonists. (8).

In the last years, allergen immunotherapies in respiratory diseases have been researched with the aim of reaching tolerance or desensitization. Several different routes have been studied, such as subcutaneous, sublingual, epicutaneous, and oral immunotherapy (OIT). OIT showed the best results in food allergy patients (8).

Allergen-specific immunotherapy (AIT) can induce and maintain long-term clinical tolerance against allergens. AIT induces CD4+ Treg Cells to produce regulatory cytokines such as IL-10, IL-35, and Transforming Growth Factor Beta (TGF-β). Through the production of regulatory cytokines, Tregs are able to inhibit Th2 cells, eosinophils, and basophils. IL-10 produced by Tregs is able to induce regulatory B cells that express IgG4 instead of IgE. IgG4 acts together with igG2, competing with IgE for binding of allergens on mast cells and basophils. Moreover, Tregs express suppressor surface molecules such as CTLA-4 and PD-1, that contribute to immune tolerance. Furthermore, regulatory Natural Killer (NK) cells and regulatory ILCs also have a role inducing tolerance, on account of producing IL-10 (figure 3) (25).

OIT research aims to find a treatment effective and safe. Recently, it has shown promising results with the oral peanut immunotherapy drug: Palforzia ®. Similar studies have been conducted regarding OIT in wheat, egg, and milk allergy. However, Palforzia ® is currently the only commercialized immunotherapy drug for food allergy that is approved by the United States Food and Drug Administration (FDA). This drug was approved for patients between 5 and 17 years old (8,26)

OIT is believed to be more effective and safer in young children. However, oral immunotherapy cannot be used in all the patients, on account of the risk of reactions and also because it is an expensive and time-consuming procedure (8).

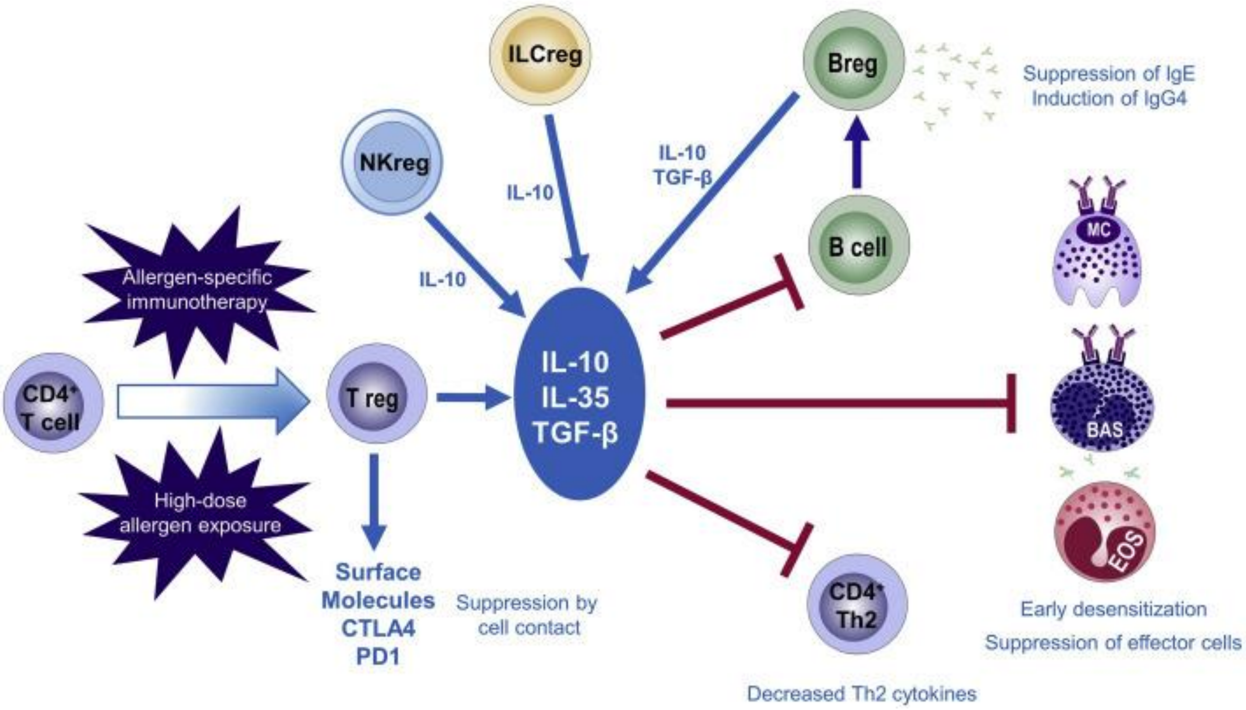

Figure 3: Mechanisms of Antigen-specific Immunotherapy. Adapted from (25). (Acronyms: BAS-basophils; EOS- eosinophils; MC-mast cells).

OIT primarily induces a state of desensitization, and a minority of patients have been shown to achieve sustained unresponsiveness. During OIT, there is an initial increase in allergen-specific IgE. As OIT advances, IgE levels decrease, and there is a gradual increase in allergen-specific IgA and IgG4. Other findings consist of basophil hypo responsiveness and decreased skin prick test wheal size, within the first year of OIT, although these findings are controversial. Their persistence after completing OIT is associated with sustained unresponsiveness. Regarding T cells, initially there is an expansion, which is then followed by a decreased number and activity of Th2 cells (26).

Regarding OIT, patients included in these studies need to be motivated and follow a strict protocol. The ideal dose of each allergen and the optimal duration of the treatment are still being researched (8).

Moreover, epicutaneous immunotherapy consists of using a daily patch, which releases the allergen through the skin. Studies concluded that it is safe and tolerable, but OIT has been shown to be more effective (8).

4. Innate Lymphoid Cells

ILCs are lymphoid cells that are different from T and B lymphocytes, mainly due to not expressing specific antigen receptors. There are 5 types of ILC, that produce different substances and are found in different organs of the body. There are 5 types of ILCs: NK cells, ILC1s, ILC2s, ILC3s, and lymphoid tissue inducer cells (LTis) (27,28).

Non-cytotoxic ILCs were discovered in the early 2010s. They represent an early source of cytokines during infection while the adaptive response develops to eradicate the pathogens. These cells also contribute to maintaining homeostasis and tissue repair. Due to its similarities, ILCs have been considered an innate counterpart of T lymphocytes. However, these cells also possess unique characteristics (27). Firstly, ILCs exist mainly in mucosal tissues but can be present in almost all organs and tissues, for example, the lungs, liver, stomach, intestine, pancreatic islets, adipose tissue, spleen, and lymph nodes (27,29). Furthermore, ILCs can develop in the absence of Recombination-Activating Genes (RAG) 1 and RAG-2. This means that ILCs do not express the antigen receptors characteristic of T and B lymphocytes (27).

ILC1s contribute to a first-line response against viruses and intracellular bacteria, whereas ILC3s influence the immune response against extracellular bacteria. Moreover, research reveals that type 2 ILCs contribute to tissue integrity. However, when exposed to inflammatory stimuli, ILC2s can initiate and amplify type 2 inflammation, leading to eosinophilia, and Th2 differentiation, with consequent mucus production (30,31).

4.1 Classification

ILCs have a similar origin to lymphocytes. All lymphocytes arise from a common lymphoid progenitor (CLP). CLP then differentiates into precursors, originating different cell lineages, for example B and T cell lineage. ILCs also differentiate from a common lymphoid progenitor (32).

CLP derived committed ILC precursors can develop into all ILC types, including NK cells. These precursors seem to be similar to CLPs, having the same phenotype, but contrary to CLPs, they also express the integrin $\alpha4\beta7$. T Cell Factor 1 (TCF-1) is a transcription factor important for T-cell differentiation, and critical for the development of all ILC subsets (32,33).

Downstream of ILC/NK precursor, there is a precursor that expresses the transcriptional inhibitor of DNA binding 2 (Id2) and can develop into all ILCs, including LTi cells. There is another precursor that expresses Id2 and Promyeloid Leukaemic Zinc Finger (PLZF). This cell can develop into ILC1s, ILC2s and ILC3s, but not LTis. Moreover, the expression of Id2 in these precursors prevents T-cell lineage commitment (figure 4) (32,33).

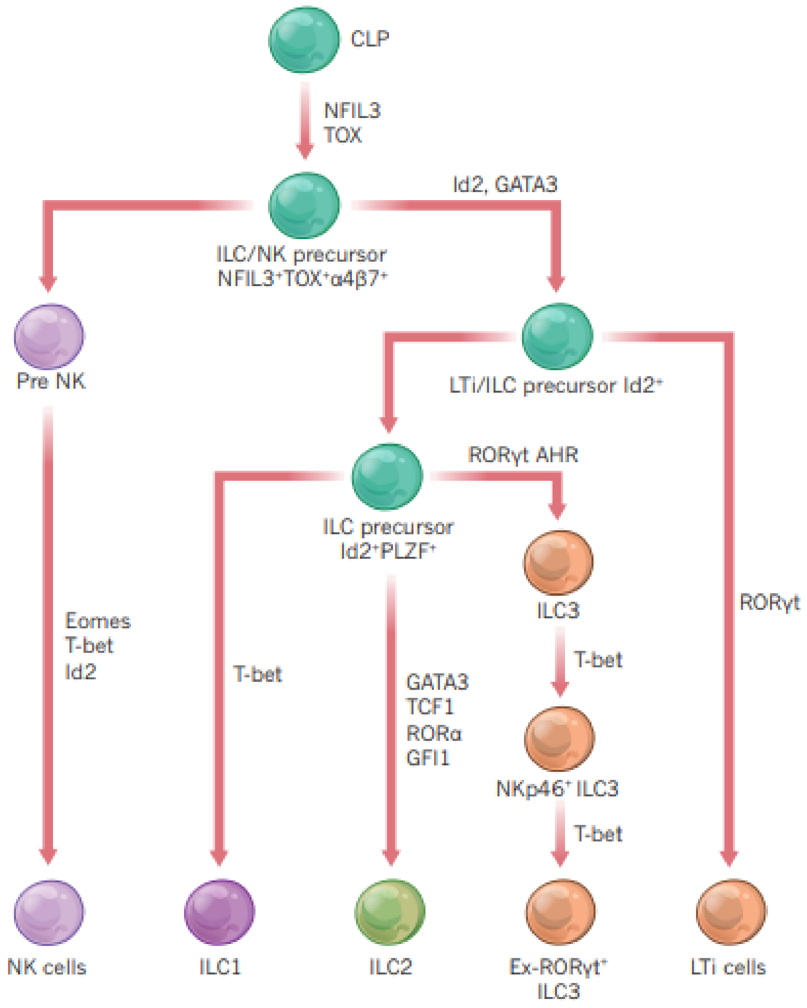

Figure 4: Pathways of Innate Lymphoid Cells' development. Adapted from (33).

NK cells are a cytotoxic type of ILC. They are found on the blood and lymphatic tissue and act upon tumour cells, or in cases of viral infection. (27). In contrast, ILC1s reside in tissues such as the liver, adipose tissues, intestines, and salivary glands. ILC1s level increase after exposure to viruses and intracellular bacteria, and their function is a first line response against infection by the production of cytokines. ILC1s are activated by soluble cytokines such as IL-15, IL-12, and IL-18, and produce Gamma Interferon (IFN-γ) (4,27).

ILC2s are found on mucosal tissues such as lungs, gastrointestinal tract, tonsils, and skin. They are early effector cells in type 2 immune reactions, releasing type 2 cytokines IL-4, IL-5, and IL-13 (27). Furthermore, ILC2s are also capable of producing IL-6, IL-9, IL-10, IL-13, Granulocyte macrophage colony-stimulating factor (GM-CSF), and amphiregulin. Amphiregulin has been shown to regulate tissue repair and remodelling, in cases of acute epithelial injuries and asthma. It is also important for wound healing, by stimulating keratinocyte proliferation. This suggests that ILC2s have functions of tissue repair and remodelling (31).

ILC2s express the transcription factor GATA3, which is necessary for their differentiation. Therefore, besides its significant role in general ILC development, GATA3 is additionally necessary for ILC2 development and functions (34). Furthermore, the transcription factor RORα is also required for ILC2 development, and it is not needed by ILC1 and ILC3. Although ROR-α is restricted to the ILC2 subset, it is also expressed by some Th17 cells. Another transcription factor, Gfi1, has also been found to control the development, activation, and specification of a subset of ILC2 (31).

Mice ILC2s are lineage negative cells (Lin-) because they do not express the surface markers usually associated with major hematopoietic lineages. Nonetheless, they have been shown to possess several surface markers, expressing CD25, the alpha chain of IL-2 receptor (IL-2Rα), CD69, CD90 (Thy1), CD117 (c-Kit), CD127 (IL-7Rα), Sca-1, and CD278 (ICOS), T1/ST2 (IL-33R), and IL-17RB (IL-25R). Human ILC2s are usually identified as Lin- cells that express CD25, CD127, CD161 (KLRB1), prostaglandin D2 receptor CRTH2, and T1/ST2. However, these markers may not be expressed equally since they depend on the tissue, and activation state (31) (figure 5).

ILC2s need survival factors, also known as costimulators, to maintain their normal functions. Thus, the lack of costimulators causes their dysfunction. There are two main groups of survival factors: γ chain family, and TNF superfamily. The γ chain family includes IL-2, IL-7 and IL-9, regulatory factors which are necessary for ILC2 survival and development. The TNF superfamily includes TNF-like ligand 1A (TL1A) and Glucocorticoid-induced TNF receptor ligand (GITRL), factors necessary for ILC2 proliferation and for cytokine release. IL-2 and IL-7 are needed for ILC-2 activation in mice, whereas human ILC2 can only be activated if IL-2 is present. Moreover, IL-2 enables ILC2 to release IL-9. Signals from IL-9 are critical for the survival of ILC2s (figure 5) (9).

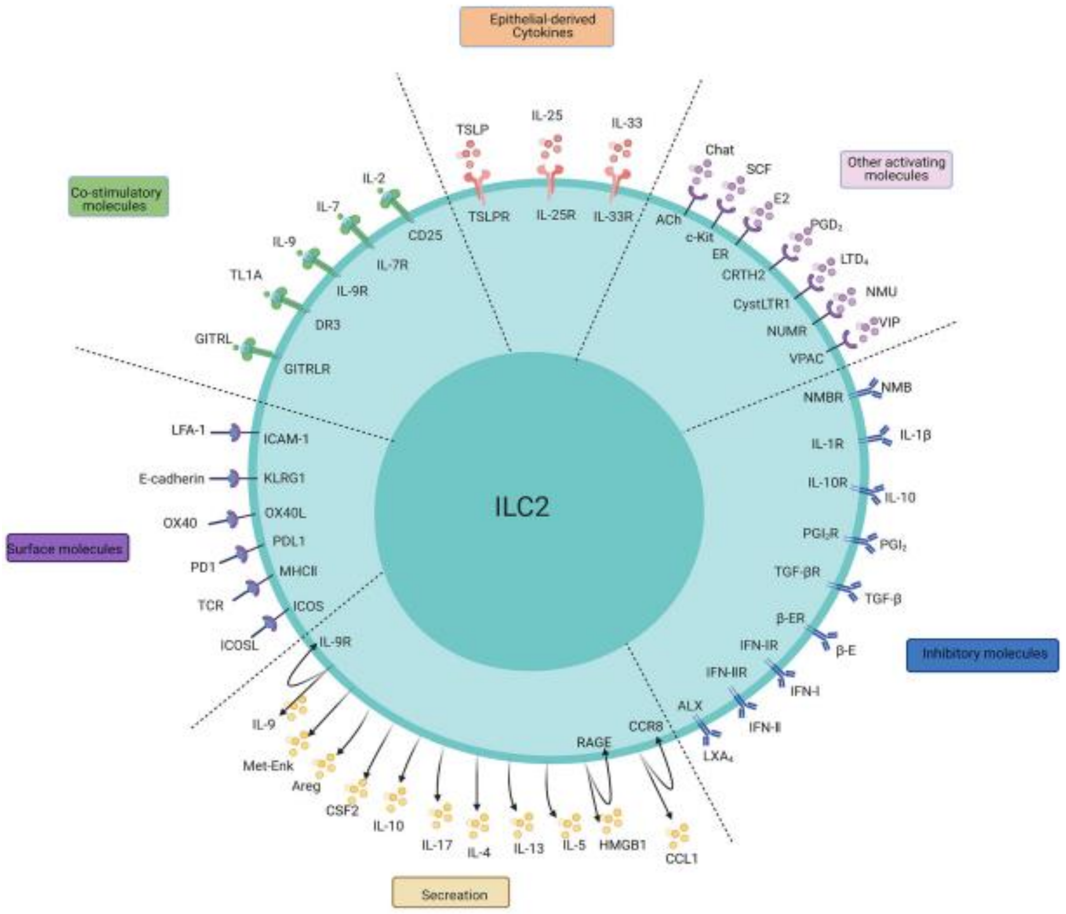

Figure 5 – Surface molecules, regulatory factors, and secretions of ILC2. Adapted from (9).

ILC3s reside on the intestinal mucosa, skin, lungs, and mesenteric lymph nodes. They contribute to immune responses against extracellular microbes, also regulating tissue homeostasis. These cells are defined by the expression of RORγt (Related Orphan Receptor Gamma). ILC3s include 2 subgroups, based on expressing or not the natural cytotoxic receptor NKp44. Both types of ILC3 produce IL-22, IL-17, INF-γ, TNF-α, and growth factor HB-EGF (heparin-binding epidermal growth factor–like growth factor) (27). The characteristics of ILC1s, ILC2s, and ILC3s are characterized in Table 2.

The final type of ILC is lymphoid tissue-inducer cells (LTis). Similarly to ILC3s, LTis also express RORγt. These cells derive from a fetal liver progenitor. During embryogenesis, they promote lymphoid tissue development, contributing to the formation of secondary lymph nodes (27,35).

ILC types are correlated to T cells. NK cells correspond to CD8+ T cells, ILC1s, ILC2 and ILC3 correspond to Th1, Th2, Th17, respectively. The related ILC and T cell subgroups have similar cytokine production profiles and similar regulatory pathways (9). Both NK cells and CD8+ T cells contribute to granular cytotoxicity, by the presence of lytic granules composed by perforin granzymes and granulosin. These substances act on target cells to induce cellular apoptosis. ILC1 and Th1 are grouped together because they both produce IFN-γ and TNF-α. ILC2 and Th2 are similar since they both produce IL-5, IL-13, and IL-4. Moreover, GATA3 is required for the development of both ILC2 and Th2. ILC3 subsets and Th17 have similarities regarding the production of cytokines IL-22 and IL-17. Additionally, they are both associated with immunity to extracellular pathogens and fungi, also contributing for tissue homeostasis. RORγt is required for the development of both Th17 and ILC3. (36).

Table 2: Main characteristics of ILC types. Adapted from (27,28,33).

ILC type	Activated by	Produces	Functions	Location
ILC1	IL-12, IL-15, IL-18	IFN-γ	Response against viruses and intracellular bacteria	Liver, adipose tissues, intestines, and salivary glands
ILC2	IL-25, IL-33, TSLP	IL-4, IL-5, IL-13, amphiregulin	Effectors in type 2 immune responses; Tissue homeostasis	Mucosal tissues: gastrointestinal tract, lungs, tonsils, and skin
ILC3	IL-1, IL-23	IL-17, IL-22, TNF-α, GM-CSF	Responses against extracellular microbes; Tissue homeostasis	Intestinal mucosa, skin, lungs, and mesenteric lymph nodes

4.2 Properties of ILCs

In the allergic response, ILC2s mediate a faster and antigen-independent response, in comparison with T and B cells. ILC2s release cytokines that act directly on blood vessels, mucosal epithelia, or nerves, enhancing local inflammation and further promoting the responses of T cells and DCs (9).

Unlike T cells, ILCs reside in non-lymphoid organs, particularly on mucosal surfaces. They are rarely found in lymphoid tissues and in the circulation. These cells do not express the antigen receptors characteristic of T and B lymphocytes, being activated by cytokines and other mediators released by epithelial cells, macrophages and DCs. ILCs are equipped with receptors that sense host-derived signals, responding to dietary metabolites, microbial products, hormones, neuropeptides, and cytokines (27,33).

Besides its roles in protection against pathogens and in allergic disorders, evidence also shows that ILC2s contribute to the homeostasis of their residing organs (27).

An important characteristic of ILCs is their plasticity. They are considered plastic cells on account of changing their phenotype and their function, as a response to changes in their microenvironment. These phenotype changes can occur for example in settings of inflammation. In these situations, ILCs of one subset preferentially expand or partially adopt the phenotype of another subset. This means that ILC2s can become ILC1-like or ILC3-like cells, for example (4,30).

Therefore, in response to IL-12, ILC2, and ILC-3 can start expressing the growth factor T-bet, similarly to ILC1s. Alternatively, IL-4 can convert ILC1s in cells that express ILC2 characteristics. Moreover, IL-1, IL-23, and TGF-β transform ILC2 in cells that produce IL-17, similarly to ILC3 (4).

To sum up, ILC plasticity contributes to a rapid and flexible immune response also promoting tissue integrity. However, plasticity might also lead to alterations in tissue homeostasis, resulting in persistent organ dysfunction (30).

Another important property of ILC2s is their ability to migrate to diverse tissues, in response to cytokines or in the presence of microbes. In these cases, ILC2s enter the lymphatic tissue and blood circulation and accumulate in peripheral tissues (33).

Furthermore, ILC2s are believed to possess memory responses. Studies in mice stimulated with TSLP or IL-33 showed an increased number of ILC2s for nearly 4 months and could react more quickly after the next antigen stimulation (9).

Another important property of ILCs is their heterogeneity. Analysis of human and mice ILC2s showed transcriptional differences between the ILC2s of different tissues, such as bone marrow, fat, lungs, intestine, and skin. For instance, research in mice deficient for all key tissue-derived cytokines for ILC2s, such as IL-33, IL-25, and TSLP, revealed that the number of IL-5 producing ILC2s was reduced in lungs, fat, and intestine, but was sustained in the skin. Contrary to the ILC2s of other organs, skin ILC2s expressed the receptor for IL-18 and produced IL-5 in response to IL-18. Moreover, in this research, lung ILC2s were affected by airway exposure to allergens or cytokines, changing their phenotypes. Therefore, the combination of these results suggests that ILC2s can express different surface molecules on different organs and inflammatory conditions. Therefore, can use heterogeneity to adapt to changes in their environment (33).

The mechanisms responsible for ILC2 heterogeneity are partially explained by ILC2 development. Research in mice revealed that ILC2s first appear during late gestation. Nonetheless, peripheral ILC2s are mostly formed during the neonatal period, leading to the expression of tissue-specific genes. For that reason, this period is critical for the expansion and differentiation of ILC2s. Consequently, tissue environment during neonatal period may influence the risk of developing allergic diseases later in life. However, this hypothesis needs to be examined further (33).

The heterogeneity of ILC2s has also been recognized in humans. Earlier, human ILC2s were identified by their expression of prostaglandin D2 receptor, CRTH2, and a lectin-type receptor, CD161. Subsequently, other molecules, such as CD127, ST2 and CD25, have been used to identify ILC2s in human peripheral blood and tissues (33).

5. Activation and Regulation of ILC2s

5.1 ILC2 activation

Epithelial and endothelial cells, at mucosal sites and the skin, react to tissue perturbations or physiological changes by secreting alarmins, such as IL-33, IL-25, and TSLP. These cytokines are released by epithelial cells in response to cellular stress, damage, or nonprogrammed cell death (figure 6) (30,31,37). These alarmins activate ILC2s which expand to exert reparative functions, through the secretion of IL-5, IL-13, and amphiregulin (37). By activating ILC2s, IL-25, IL-33, and TSLP can induce type-2 immunity responses, causing eosinophilia, IgE secretion, and type-2 cytokine production. In addition, IL-33 contributes to acute reactions to food by acting directly on mast cells and enhancing IgE-mediated activation (4).

For their survival and proliferation ILC2s need the cytokines IL-25, IL-33, and TSLP. Moreover, survival signals from cytokines IL-2 and IL-7 may also be important for their proliferation (9). Studies in mice suggested that mouse ILC2s can only be activated in the presence of IL-2 and IL-7. In humans, studies show that ILC2s need IL-2 for their activation. IL-2 induces ILC2s to release IL-9. Self-released IL-9 functions as a survival factor for ILC2s (9).

Mast cells can be found at mucosal barriers, having an important sentinel function. They have been associated with early induction of type 2 immune responses in food allergy. Mast cells and basophils produce several cytokines, including IL-4 and IL-9. These cytokines contribute to Th2 expansion and Treg cells inhibition in the intestinal mucosa, therefore leading to the induction of allergic adaptive immune responses. Research in mice revealed that IgE-activated mast cells drive intestinal ILC2 expansion in food allergy and reveal that ILC2, in turn, can enhance responsiveness to the mediators of anaphylaxis, such as histamine, produced by mast cells (35,38). In addition, mast cells produce prostaglandin D2, a lipid mediator that activates ILC2s (figure 6).

Moreover, research on mice revealed that mice with overexpression of IL-25 were prone to develop food allergy whereas mice with no expression of IL-25 were resistant to experimental food allergy. This happened because IL-25 induced the production of IL-13 by ILC2s, leading to the development of food allergy (34). Furthermore, studies in mice sensitized by intragastric administration of peanut extract and cholera toxin as an adjuvant, showed that IL-33 had a significant role in the development of systemic anaphylaxis as well as expansion of intestinal ILC2s (33).

To note, T cells are also able to activate ILC2s. ILCs interact directly with CD4+ T cells. Research using an in vitro cell culture system documented interactions between ILC2 and T cells in a cell-cell contact dependent way, which promoted Th2 polarization (39).

ILC2s have been shown to express major histocompatibility complex (MHC) class II. Moreover, ILC2s have been recognized as APCs, because of their ability to process and present peptide antigens, modulating the activation of naïve CD4+ T cells in a contact-dependent manner (figure 6) (39). Because of MHC-II expression, ILC2s were able to receive activating signals by T cell-derived IL-2, which caused the secretion of IL-13. This suggests that ILC2s and T cells can also communicate in an antigen dependent manner. However, it is still not clear if ILC2s have a significant role as APCs during Th2 response (39). Studies in mice revealed that ILC2s found at mediastinal lymph nodes, spleen, and small intestines express MHC-II. Those ILC2s could directly induce proliferation and IL-2 production by T cells in an MHC-II-dependent manner (33).

Moreover, a few studies suggest interactions between ILC2 and neuropeptides. An example of this is the neuropeptide VIP (Vasoactive Intestinal Peptide) (figure 6). VIP is expressed in intestinal cells, being released in response to food intake. Together with IL-7, VIP co-stimulates a receptor on ILC2s, leading to an increase in IL-5. The release of IL-5 by ILC2s activates eosinophils (9).

Neuromedin U (NMU) is another neuropeptide that is believed to have regulating functions of type 2 innate immunity. NMU is produced by mucosal neurons and acts on ILC2s through a NMU receptor - Nmur1 (figure 6). NMU induces ILC2 activation, proliferation, and secretion of type 2 cytokines (9,40). This receptor is expressed by ILC2s in human blood and intestine. Research in mice revealed that NMU alone could stimulate mice ILC2s to produce type 2 cytokines (33).

A subset of epithelial cells is also able to provide neuronal regulation of ILC2s. These cells are known as chemosensory epithelial cells (EpCs), and they can be found in mucosal tissues. EpCs found in the intestine are referred to as Tuft cells. Research in mice showed that tuft cells can activate gastrointestinal ILC2s to promote a type 2 response against helminth infection (figure 6) (33).

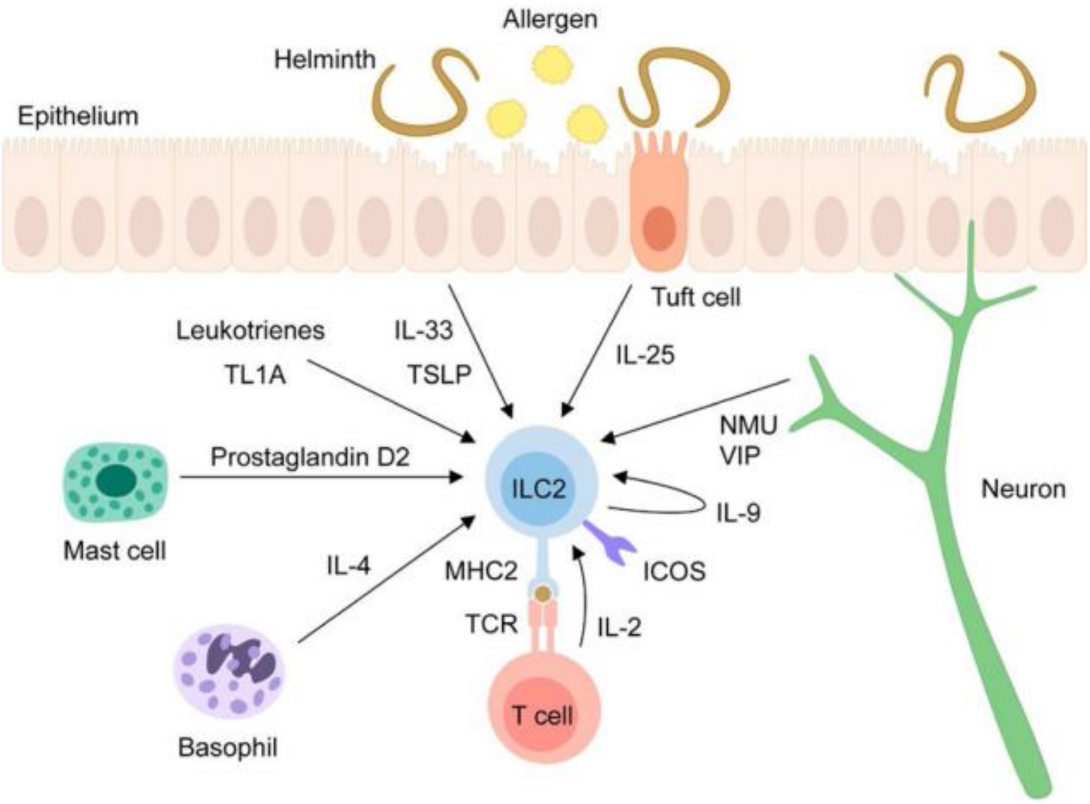

Figure 6 – Pathways of ILC2 activation. Adapted from (30).

5.2 Inhibitory Mechanisms

The mechanisms for suppressing ILC2s are still not completely understood. However, studies show that type 1 cytokines such as IFN-γ, IFN-β, and IL-27 inhibit ILC2s (figure 7). Additionally, lipid mediators such as prostaglandin I2 (PGI2) can also decrease ILC2 activity. Moreover, some of the molecules expressed by ILC2, such as Programmed Cell Death Protein 1 (PD-1), can regulate ILC2's functions (figure 7). Stimulation of ILC2s by IL-33 induces the expression of PD-1 in mice lung ILC2s and human peripheral blood ILC2s. Research in mice showed that the engagement of PD-1 suppressed viability and downregulated the effector functions of ILC2s, by altering their metabolism (33).

Interactions between ILC2s and other immune cells may also suppress ILC2s. Research in mice revealed that the administration of agonists of the Toll-Like receptor (TLR), such as TLR7/8 agonist R848, and TLR9 agonist CpG, leads to an inhibition of the production of IL-33. Therefore, there was an inhibiting of allergen induced type 2 inflammation in mice lungs (33). DCs are able to inhibit ILC2s. DCs contribute to the suppression caused by TLR agonists because DCs can rapidly produce IFN-α, leading to

ILC2 apoptosis. DCs are also able to inhibit ILC2s indirectly, on account of promoting IFN-γ production by NK cells (33).

Several neuropeptides promote type 2 inflammation by activating ILC2 responses. However, an inhibitory role of the nervous system has also been described. ILC2s in both mice and humans express the receptor for norepinephrine, β2AR. Studies in mice revealed that norepinephrine inhibits ILC2 proliferation and type 2 cytokine secretion (figure 7) (30).

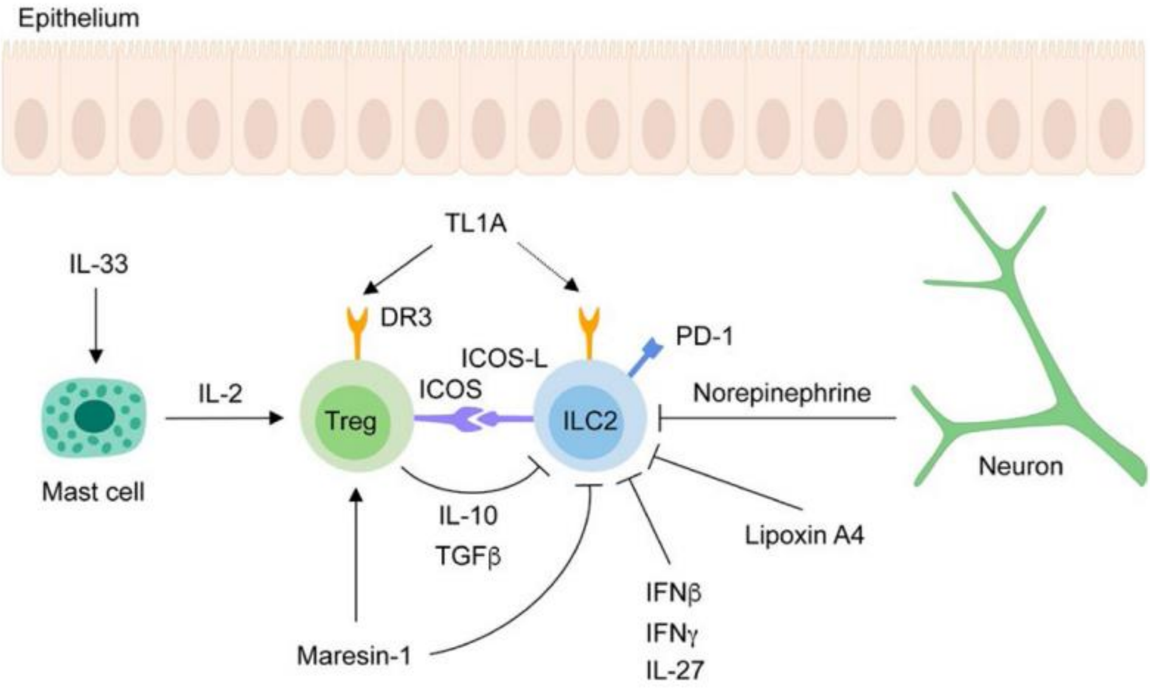

Figure 7 – Pathways of ILC2 inhibition. Adapted from (30).

Treg cells are also believed to downregulate ILC2s, because an increase in Tregs leads to a decrease in the production of ILC2 cytokines, on account of the release of TGF-β and IL-10. In contrast, the production of IL-4 by ILC2s inhibits Treg cells and promotes food allergies (9). Maresin-1 is a lipid mediator that increases the inhibitory functions of Tregs. Additionally, IL-2 produced by mast cells also promotes Treg functions. Studies in mice revealed that IL-33 activated mast cells to secrete IL-2, which led to an increase in the number of Treg cells. The suppressive effect of Tregs is also mediated by the interaction of Inducible Costimulator (ICOS) expressed on Tregs with Inducible Costimulator-Ligand (ICOS-L) expressed on ILC2s. Tregs also express DR3, the receptor for TL1A, and TL1A contributes to ILC2's activation. ILC2s and Tregs compete for the binding of TL1A to DR3 contributing to the downregulation of ILC2 activation (figure 7) (30).

Furthermore, neutrophils are able to inhibit ILC2s by decreasing the circulating levels of Granulocyte colony-stimulating factor G-CSF, decreasing the production of type 2 cytokine production by lung ILC2s, in a negative feedback mechanism (33).

Myeloid-derived suppressor cells (MDSCs) are immune cells that suppress adaptive immune responses in cancer. In vitro and in vivo studies revealed that MDSCs were able to inhibit type 2 cytokine production by ILC2s. This was achieved by a cyclooxygenase-1 (COX-1) pathway-dependent mechanism (32).

Sex hormones affect several immune cells that mediate allergic diseases. For instance, in mice, testosterone has been shown to inhibit ILC2 functions, while estrogen was able to mediate the expansion of ILC2 in female reproductive organs. It was also discovered that ILC2s from the lungs of female mice produce more type 2 cytokines in response to IL-33 than ILC2s from male mice. Furthermore, ILC2s from female mice were more metabolically active compared to male mice. Therefore, sex differences in allergic diseases might be partially explained by differences in number and activity of ILC2s (33).

6. Effector functions of ILC2 and their role in food allergy

Human IIC2s generally produce large quantities of IL-5 and IL-13 while a much lower amount of IL-4 is typically produced (30).

IL-5 binds to the IL-5 receptor and promotes eosinophilopoiesis in the bone marrow and peripheral eosinophil survival. Together with eotaxin, IL-5 is also able to recruit eosinophils to the inflammation site. Furthermore, IL-5 production by ILC2s promotes expansion of B cells and production of IgM by B cells (figure 8) (30).

IL-13 promotes mucus production by goblet cells and hyperplasia of tuft and goblet cells. IL-13 induces DCs' migration to lymph nodes, where they promote Th2 differentiation. Another function of IL-13 is the induction of CCL17 production by CD103+ DCs. This leads to the recruitment of memory T cells to the inflammation site (figure 8) (30).

Research suggested that mice exposed to peanut flour particles by inhalation produced IgE antibodies to peanuts and developed systemic anaphylaxis. During this process, lung ILC2 production of IL-13 leads to activation and migration of DCs, which then promotes T follicular helper cell development, which induces IgE class switch in germinal centre B cells (33).

Furthermore, it was discovered that IL-4 impaired oral tolerance by disrupting the differentiation of naïve T cells into Treg cells. Therefore, production of IL-4 compromises Treg cell function and increases mast cell activation (41).

Therefore, IL-4 produced by ILC2s has a suppressing effect on regulatory T-cells, in several organs such as the skin, lungs, and intestine. Activation of ILC2s ultimately suppresses regulatory T cells, contributing to the development of the allergic cascade, which may happen in response to food allergens. Research in mice suggested that ILC2s produce considerable amounts of IL-4 and found that this cytokine was indispensable to allergic sensitization to peanuts (4,41).

Innate lymphoid cells contribute to Th2 cell differentiation by cytokine secretion. Research using cell cultures showed that IL-4 produced by ILC2s increased Th2 cell proliferation and Th2 cytokine production when these cell types were cultured together (39).

In vitro studies revealed that stimulation of ILC2s with IL-9 leads to the production of IL-5 and IL-13, while neutralization of IL-9 in lung inflammation inhibited the production of IL-5 and IL-13. This finding suggests that IL-9 contributes to type 2 cytokine production. Following helminth infections, ILC2s produce IL-9 and also express the IL-9 receptor, which suggests an autocrine activation (figure 8) (30).

Research in mice suggests that IL-9 induces intestinal mastocytosis and promotes mast cell activation. IL-9 is one of the type 2 cytokines, secreted by CD4+ TH2 cells, to promote type 2 immune responses. Moreover, ILC2s potentiate food allergy on account of also producing IL-9. Research suggests that this interleukin is highly increased in children with peanut allergy (4,42).

Intestinal overexpression of IL-9 induces an experimental intestinal anaphylaxis transcriptome and phenotype, leading to the conclusion that intestinal expression of IL-9 predisposes to intestinal anaphylaxis (42,43).

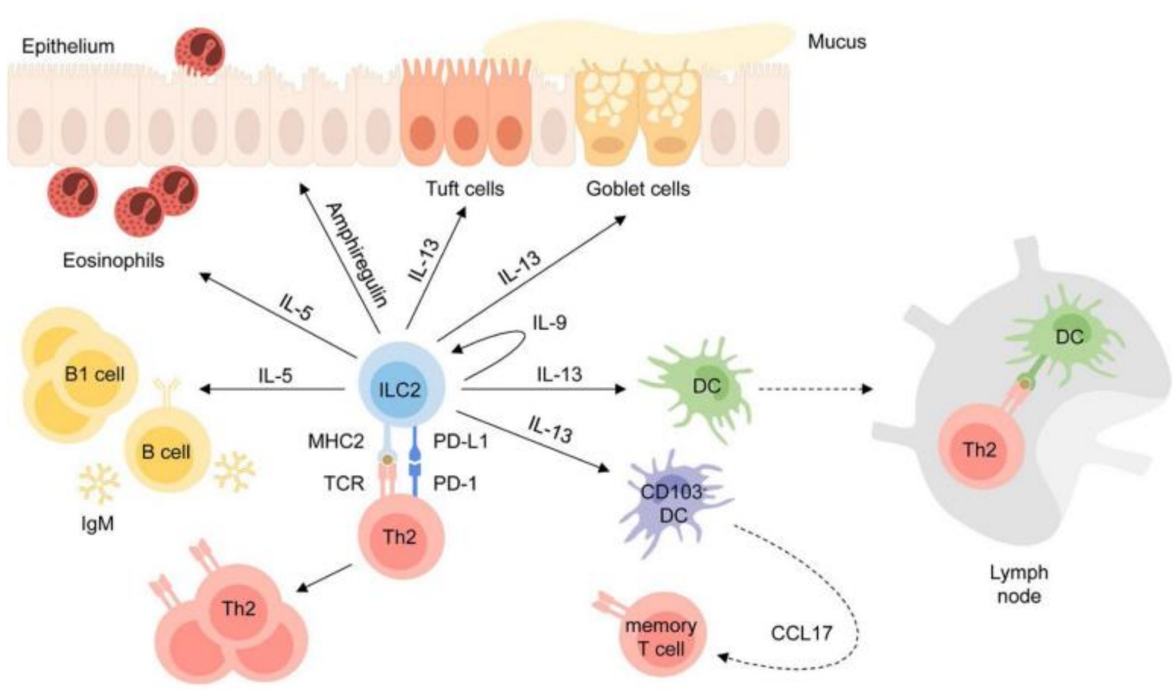

Figure 8 – Effector mechanisms of ILC2. Adapted from (30).

ILC2s also express certain characteristic chemokine receptors that play roles in the homeostatic distribution of lymphocytes to specific organ sites, most prominently CXC-chemokine receptor 6 CXCR6, CXCR4, and CC-chemokine receptor 9 (31).

The interaction between MHC-II and T-cell Receptor (TCR) activates T cells to produce IL-2, which induces ILC2 proliferation and production of type 2 cytokines, such as IL-13. The Interaction between ILC2s and Th2 cells via MHC2s and PD-L1 promotes Th2 differentiation and effector functions (figure 8). Moreover, a study using in vitro stimulation of human DCs with TLSP revealed an upregulation of OX40L and Th2 cell polarization of naïve CD4+ T cells. Research shows that a cellular interaction between OX40-OX40L in ILC2 and naïve CD4+ T cells increased the expression of type 2 cytokines in ILC2s and T cells (33).

Furthermore, stimulation of OX40L by ILC2s expanded effector Th2 cells while also increasing the number of GATA3+ Treg cells that express OX40. This finding suggests that ILC2s may be able to promote or suppress type 2 immunity depending on the context (33).

Moreover, IL-13 produced by ILC2 contributed to Th2 cell responses by promoting migration of CD40+ DCs to the draining LNs and by enhancing priming and differentiation of naïve T cells. Thus, the information so far suggests that ILC2s may promote or modulate type 2 immunity by multiple mechanisms (33).

The interactions between ILC2s and B cells have not been fully described. However, research shows that ILC2s located at fat-associated lymphoid clusters can promote IgA production and induce the division of innate B1 cells. This may happen since ILC2s produce IL-5 and IL-6. These cytokines act as B-cell growth and maturation factors, also contributing to Ig class switching (31).

Inducible costimulatory (ICOS) is a costimulatory molecule and one of the surface markers of ILC2s. ICOS engages ICOS ligand (ICOS-L) expressed by B-cells. The interaction between ICOS and ICOS-L also leads to the production of cytokines IL-2, IL-4, and IL-10 by B cells which have important roles in germinal centre responses and Ig class switching (31).

Moreover, research in mice revealed that IL-4 produced by gastrointestinal ILC2s and mast cells in response to epithelial IL-33 was necessary for IgE antibody production by B cells and consequent development of systemic anaphylaxis (33). Production of IL-4 induces isotype switching to IgE in B cells (4).

7. Eosinophilic Esophagitis

Eosinophilic Esophagitis (EoE) is a chronic, antigen-mediated inflammatory disease of the esophagus. EoE is associated with food allergy, since 70% of patients who have been diagnosed with EoE have a previous history of food allergy or positive skin prick test, to one or more foods. Besides IgE-mediated food allergy, evidence shows that ILC2s also contribute to the pathophysiology of EoE (44,45).

EoE can affect both children and adults, although there is a peak incidence between the third and the fifth decade of life (33,46). The prevalence of EoE has increased rapidly in the last few years, mostly in Westernized countries, affecting around 50/100,000 people (44). EoE is more prevalent in Caucasian ethnicity and in males, with a male-female ratio of 3:1 (46).

EoE develops as a chronic, hypersensitivity reaction to food allergens (33). This disorder clinically manifests with esophagic dysfunction, whereas histologically, there may be inflammation with eosinophil infiltrate. It often curses with other allergic disorders and with IgE-mediated hypersensitivity to food allergens (45).

Studies concluded that most children with EoE were sensitized to milk, eggs, soy, wheat, beef, or peanuts while 91% of adults with EoE, were sensitized to food and inhalant allergen components (45).

EoE develops as a non-IgE-mediated hypersensitivity reaction, therefore it is not immediate and can take several days to weeks to develop, after antigen exposure (45).

7.1 Pathophysiology

EoE has a multifactorial etiology and depends on a complex interaction between genetic and intrinsic factors, environmental factors, and antigenic stimuli (46).

Considering environmental exposure, the development of EoE has been associated with early life exposures (47). Factors such as caesarean delivery and the use of antibiotics or acid suppressants in the first years of life have been demonstrated to induce changes in the microbiome, altering its diversity and abundance of microbiota. Changes in the microbiome may also occur later in life but are usually temporary and self-limited. Several case-control studies have been conducted with the aim of clarifying the impact of early life factors in the development of EoE. Results indicated an association between early life exposures and the development of EoE. Among these factors, antibiotic use in infancy had the strongest correlation with the further development of EoE. (48).

Regarding genetic factors, EoE occurs in family members in a non-Mendelian pattern, indicating a complex heritability. Familial studies show a 44% concordance of EoE between monozygotic twins and 30% concordance in dizygotic twins (46).

Inflammation in Eosinophilic Esophagitis presents as a type 2 immunological response. After an initial stimulus, such as exposure to food antigens or barrier dysfunction, epithelial cells begin to express IL-25, IL-33, and TSLP, epithelial-derived cytokines, known as alarmins. This leads to the activation of ILC2s and basophils, that release the type 2 cytokines IL-4, IL-5, IL-9, and IL-13. Antigen sensitization develops in a parallel way, with dendritic cell processing and presentation of the antigens. Consequently, there is an activation of Th2 cells and NK cells, that also produce type 2 cytokines. Th2 cells activate B cells, leading to antibody production (figure 9) (49).

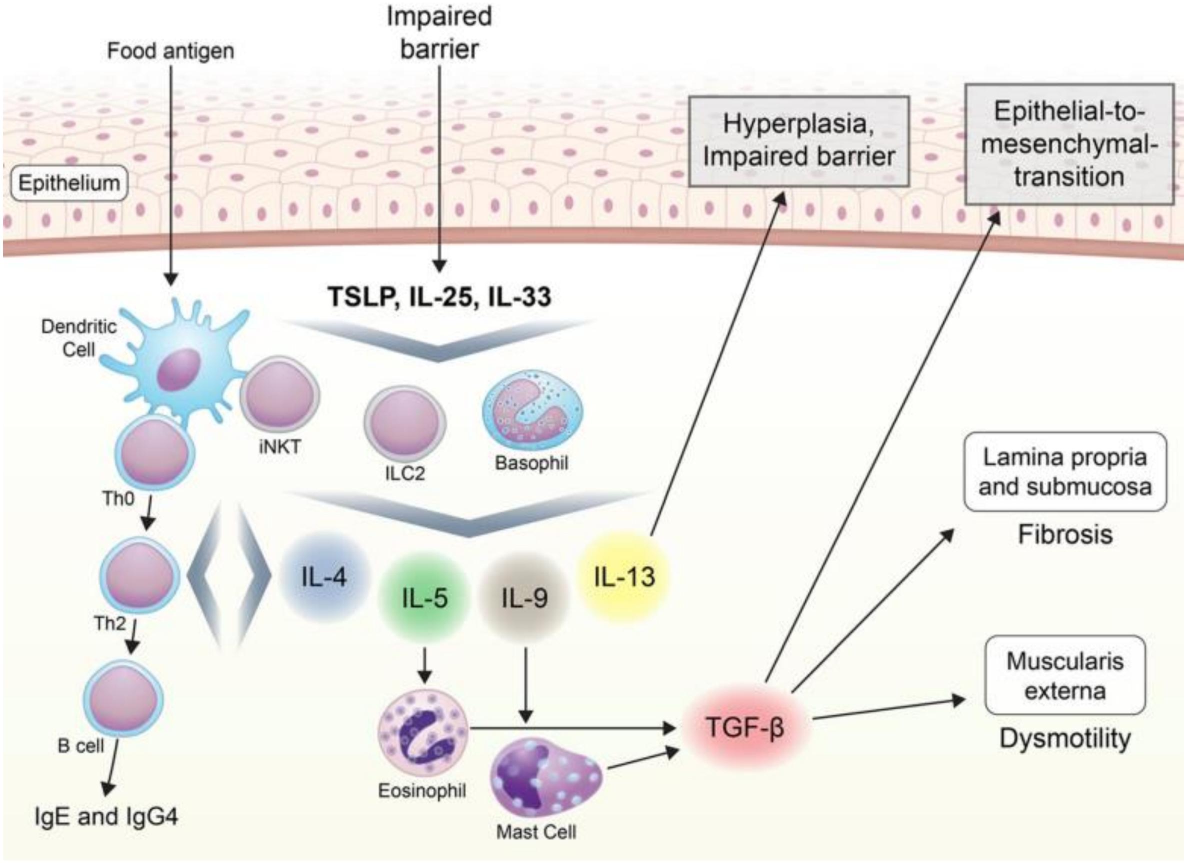

Figure 9 – Pathophysiology of Eosinophilic Esophagitis. Adapted from (49).

Although the inflammation characteristic of EoE is predominantly due to eosinophil infiltration, there is also an increase in the number of mastocytes, basophils, and T cells, and also increased levels of IL-5 and TNF-α. Eotaxin and IL-5 are important to eosinophil recruitment, accumulation, and activation in the esophagus (46). Inflammation resulting from infiltration of eosinophils and other cells in the mucosa leads to loss of integrity of epithelial cells, resulting in epithelial cell hyperplasia, and consequently damage to the esophagic mucosa, which may result in fibrosis and smooth muscle dysmotility (figure 9) (47,50). These changes lead to food impactions and strictures as well as symptoms of chronic pain, feeding intolerance, and dysphagia (50).

7.2 Symptoms

Clinical symptoms of Eosinophilic Esophagitis are related to the age of the patient. In young children and babies, it is common to find unspecific signs and symptoms, such as growth delay, food avoidance, and vomits. On the other hand, in teenagers and adults, the most common feature is esophagic fibrosis, leading to dysphagia and food impaction (47).

Importantly, 50% of patients who go to the emergency room with food impaction, and require endoscopic remotion, have EoE (47).

Clinical research on patients has shown that, the longer the inflammation lasts, the bigger the prevalence of esophagic fibrosis, dysphagia, and food impaction (47) A Cohort study was performed in the Netherlands, with a study population of 721 people diagnosed with EoE. It was concluded that, of patients diagnosed 21 years or more after the development of inflammation symptoms, 52% had esophagic stenosis and 57% had had previous food impaction episodes (51).

Because of causing dysphagia, patients might think that the last food eaten is causing the allergic response. However, usually, there are a few specific foods that can be responsible for most of the food-related inflammation symptoms. The more likely causal triggers are milk, wheat, egg, and soy (6).

In 15% of EoE patients, there is a history of previous anaphylactic reactions. This indicates a potential progression from IgE-mediated food allergy to EoE, on exposure to the same food allergen (49).

7.3 The Role of ILC2s in Eosinophilic Esophagitis

IL-5 and IL-13 have been implicated in the pathogenesis of human EoE. These cytokines are produced by several immune cells such as NK cells and ILC2s, suggesting that ILC2s may have a role in promoting this disorder. IL-5 is overexpressed in the esophagus of patients with EoE and has a role in the regulation of eosinophil maturation, activation, and survival. In EoE patients, the levels of IL-5 correlate with esophageal eosinophilia and disease activity (46).

Culture studies were conducted using lymphoid aggregate cells from the human esophagus. Results concluded that ILC2s are increased in esophageal biopsies from patients with active EoE when compared to patients with inactive disease, and healthy controls (50).

Another study was conducted based on the results from esophagic biopsies, in children. The outcomes were similar, revealing an increased number of ILC2 in those who had active EoE. Results also showed a correlation between the increased ILC2 number and eosinophilia. Tissue biopsies of children with EoE had increased levels of TSLP and IL-33, which promoted the activation and expansion of ILC2 (33).

7.4 Treatment of Eosinophilic Esophagitis

An important part of managing EoE is a food-elimination diet. This procedure consists of avoiding contact with the antigens, when identified. Although some authors reported that about 70-99% of their patients showed an improvement in disease symptoms, this strategy was found to have a limited success according to others. Conversely, the reintroduction of food allergens leads to a relapse in almost every patient, which proves that EoE is a chronic food antigen hypersensitivity disorder (49).

The first line therapy for EoE is swallowed topical steroids, such as fluticasone propionate and budesonide, and they are used in both children and adults. Systemic steroids are reserved for advanced cases, that may curse with severe dysphagia and weight loss (46).

Swallowed topical steroids are generally safe. However, side effects include superficial esophageal candidiasis, which may occur in up to 10% of patients, but responds to specific treatment. Adrenal axis suppression, bone demineralization, and diminished growth are other rare side effects (46).

Glucocorticoid treatment reduces the transcription of IL-13 and consequently reduces the eosinophilic and T cell infiltrate. Therefore, it helps decrease fibrosis and to re-establish esophageal motility (46).

Studies disclosed that the use of Mepolizumab and Reslizumab, monoclonal antibodies against IL-5, in EoE patients, decreased the eosinophil level in both peripheral blood and esophageal tissue. However, there was no significant reduction in disease symptomatology. The use of Dupilumab, an antibody against IL-4 receptor alpha, in EoE treatment was approved by the FDA in 2022, for its role in decreasing both eosinophil count and disease symptomatology. Similarly, Cendakimab, an antibody anti-IL-13 has shown a reduction in eosinophil count and symptoms (52).

8. Impact of ILCs on food allergy treatment

Type 2 inflammation has a major role in the pathophysiology of both IgE-mediated and non-IgE-mediated food allergies. ILC2s are important inducers of type 2 inflammation on account of their known ability to produce type 2 cytokines. Thus, cytokines produced by ILC2s may be targeted with the aim of suppressing type 2 inflammation. In this context, glucocorticoids are used to treat several allergic disorders and they possess a known ability to suppress ILC2s (4).

Research in patients with nasal Polyps showed ILC2s were reduced by 50% in patients treated with systemic corticosteroids. The use of topical corticosteroids did not suggest a reduction in the ILC2 number (53). Moreover, a study evaluated the impact of glucocorticoid therapy on blood ILC2s, in asthma patients. Results suggested a decrease in blood ILC2 levels, and also a reduction of IL-5, IL-13, and IL-9 produced by ILC2s. After 3 months of therapy with glucocorticoids, ILC2 levels almost reached normal levels (54).

Mepolizumab and dupilumab have been found useful in treating allergic diseases in humans. Dupilumab is able to inhibit the production of IL-4 and IL-13, which are two of the cytokines produced by ILC2s, and has shown results in controlling symptoms in allergic disorders such as EoE, asthma and atopic dermatitis (4,52).

Cytokines that activate ILC2s may also be targeted in order to prevent type 2 inflammation. By targeting IL-25 and IL-33, ILC2 proliferation can be inhibited. Cicaprost, an analog of PG I2 has been able to inhibit IL-33-induced ILC2 proliferation in mice. In humans, it was shown to decrease the levels of IL-5 and IL-13, type 2 cytokines. TSLP also activates ILC2s and has been associated with resistance to steroids. By inhibiting TSLP with Tezepelumab, eosinophilic induced inflammation was reduced in targeted tissues (4).

Regulatory immune cells can also be a potential target for food allergy treatment. Firstly, Treg cells can be targeted on account of their known ability to suppress ILC2's inflammatory pathways (4).

Furthermore, recent studies showed the existence of regulatory ILC2s that produce IL-10. These IL-10-producing ILC2s can be obtained in vitro following stimulation of ILC2s with IL-2, IL-7, IL-33, and retinoic acid. These cells differ from Treg cells because they do not possess the transcription factor FoxP3, typical of Treg cells (4).

IL-10-producing ILCs are functional cells that are able to suppress Th2 cell responses. These regulatory ILC2s can be induced following subcutaneous and sublingual grass pollen immunotherapy, as well as house dust mite treatment in patients with asthma and/or allergic rhinitis (4).

A study regarding intranasal immunotherapy on mice revealed a suppression of IgE-mediated food allergy. This therapy induced the inhibition of allergen-specific Th2 cells. Furthermore, this intranasal immunotherapy increased the levels of IFN-γ, leading to a suppression of alarmin production by epithelial cells. In this context, IFN-γ may also be able to decrease the ILC2 number in the small intestine. Previous studies in mice revealed that INF-γ limited the recruitment and accumulation of ILC2s in the lung. The decreased number of ILC2s in mice exposed to intranasal immunotherapy is believed to be a crucial factor in reducing allergic reactivity (55).

In conclusion, all the observations mentioned above can be potential targets for the treatment of food allergy, using the ILC2 pathways that promote type 2 inflammation. However, the limited number of articles available about the role of ILC2s in food allergy treatment restricts the knowledge about this topic, which was included in this review. Therefore, further research in this area is necessary to fully understand the therapeutic potential of ILC2s.

9. Conclusion

The discovery of ILC2's functions was essential to better understand type 2 mediated inflammation, which occurs in food allergy. ILC2s have been identified in several human allergic conditions and have emerged as an important link between innate and adaptive immunity. These cells are activated by IL-25, IL-33, and TSLP released by damaged epithelium, and they mediate the development of allergic responses by secretion of cytokines (9).

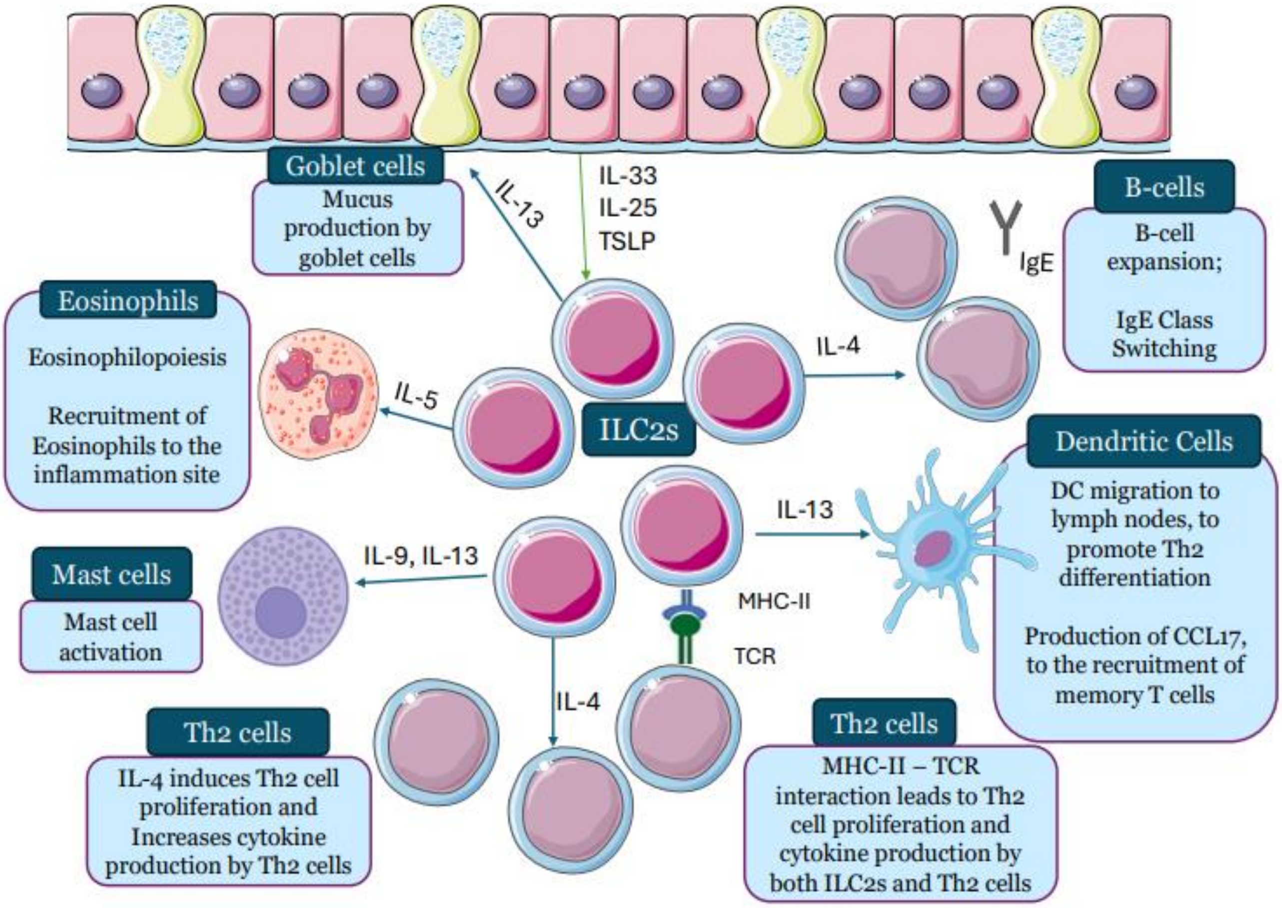

Figure 10: Functions of ILC2s and their effects on food allergy. Adapted from (4,30).

ILC2s can contribute to the development of food allergy through several mechanisms, as detailed in Figure 10. ILC2s produce IL-5, and IL-13 which exert specific functions regarding eosinophilopoiesis, B cell expansion, the induction of mucus production by goblet and Tuft cells, and also DC migration and memory T cell recruitment. IL-4 inhibits Treg cell activity, and increases mast cell activation, contributing to allergic sensitization. Moreover, ILC2s secrete IL-9 and simultaneously have an IL-9 receptor, which suggests an autocrine activation. Finally, ILC2s interact with T cells, promoting their proliferation and the production of type 2 cytokines (30).

Research on ILC2s in the past several years revealed new pathways and molecules involved in the activation and regulation of these cells, and knowledge about the immunobiology of ILC2s increased. Phenotypic and functional definitions of ILC2s also became more detailed and evolved to include ILC2's plasticity and heterogeneity (13). However, it is not clear if ILC2 plasticity exacerbates or ameliorates food allergy and if ILC2 heterogeneity affects food allergy development (33).

Some limitations persist. Most of the studies on the role of ILC2s in allergy are from murine models of these diseases. Therefore, the mechanisms that are known to explain how ILC2s are involved in the pathophysiology of allergic diseases were obtained from animal models too. On that account, it is uncertain how the findings in mice models translate to human disease, and therefore these observations need to be verified in humans. This is particularly important due to species differences in ILCs between humans and mice. (13).

Several questions remain. Compared to other allergic diseases such as asthma, fewer studies have been performed regarding the roles of ILC2s in food allergy and eosinophilic esophagitis Furthermore, it is unknown whether other types of ILC, such as ILC1, ILC3, and regulatory ILC2s promote or protect from allergic diseases (33).

In conclusion, one main goal of research in this area should be to understand how we could use our knowledge about the interactions between ILC2s and other mediators and cell types, to provide treatments for allergic diseases, such as food allergy, which should be effective, safe, and affordable (33).

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