Eosinophilia Induced by Blocking the IL-4/IL-13 Pathway: Potential Mechanisms and Clinical Outcomes

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Abstract

Five biological drugs are currently marketed for treatment of uncontrolled severe asthma. They all block type 2 inflammatory pathways by targeting IgE (omalizumab), the IL-5 pathway (mepolizumab, reslizumab, benralizumab), or the IL-4/IL-13 pathway (dupilumab). Hypereosinophilia has been observed in 4%-25% of patients treated with dupilumab and is transient in most cases, although there have been reports of persistent cases of symptomatic hypereosinophilia consistent with eosinophilic granulomatosis with polyangiitis (EGPA), eosinophilic pneumonia, eosinophilic vasculitis, and sudden worsening of asthma symptoms. Cases of EGPA have been reported with all biologics, including anti–IL-5 agents, and with leukotriene receptor antagonists in publications or in the EudraVigilance database. In many cases, EGPA appears during tapering of systemic corticosteroids or after switching from an anti–IL-5 biologic to dupilumab, suggesting that systemic corticosteroids or the anti–IL-5 agent were masking vasculitis. This review investigates plausible mechanisms of dupilumab-induced hypereosinophilia and review cases of symptomatic hypereosinophilia associated with dupilumab. Blockade of the IL-4/IL-13 pathway reduces eosinophil migration and accumulation of blood by inhibiting eotaxin-3, VCAM-1, and TARC without simultaneously inhibiting eosinophilopoiesis in bone marrow. When choosing the optimal biologic, it seems necessary to consider the presence of hypereosinophilia (>1500/μL), in which case an anti–IL-5/IL-5R agent is preferable. Furthermore, when switching from an anti–IL-5/5R to an anti–IL-4/13R agent, blood eosinophils and clinical progress should be closely monitored. Nevertheless, dual therapy with anti–IL-5/5R and anti–IL4/IL-13R agents may be needed for optimal control, since both the IL-5 and the IL-4/IL-13 pathways can simultaneously contribute to airway inflammation. This approach can prevent the development of EGPA and other types of symptomatic hypereosinophilia while maintaining control of nasal polyposis. In the near future, it will be possible to use a new generation of biological therapies for the treatment of severe asthma. These act at a higher level of the inflammatory cascade, as is the case of the antialarmins tezepelumab and itepekimab.

Key words: Asthma. Biologic treatment. Eosinophil granulomatosis with polyangiitis. Eosinophilia.

Resumen

Actualmente se dispone de cinco fármacos biológicos para el tratamiento del asma grave no controlada de tipo T2. Todos ellos, bloquean las vías inflamatorias de tipo 2, ya sea dirigiéndose a la vía de la IgE (omalizumab), la vía de la IL-5 (mepolizumab, reslizumab, benralizumab) o la vía de la IL-4/13 (dupilumab). Se ha descrito que, entre el 4% y el 25% de los pacientes tratados con dupilumab desarrollan hipereosinofilia, la cual es benigna y transitoria en la mayoría de los casos, aunque una minoría de pacientes presentan una hipereosinofilia persistente y acompañada de sintomatología clínica que varía desde la granulomatosis eosinofílica con poliangitis (GEPA), a la neumonía eosinofílica, la vasculitis eosinofílica o el empeoramiento repentino de los síntomas del asma. Se han comunicado casos de GEPA con todos los productos biológicos, incluidos los anti-IL-5, y con antagonistas de los receptores de leucotrienos, bien en forma de casos clínicos o pequeñas series publicadas, o bien en la base de datos de farmacovigilancia de la Agencia Europea del Medicamento (EMA) EudraVigilance. En muchos de estos pacientes, la GEPA aparece durante la reducción gradual de los esteroides sistémicos o después de cambiar de un biológico anti-IL-5 a dupilumab, por fallo terapéutico, lo cual sugiere que los esteroides sistémicos o los anti-IL-5 estaban enmascarando la vasculitis. Sin embargo, otros casos no pueden explicarse por la interpretación anterior, pudiendo deducirse como una consecuencia directa del uso del biológico. Esta revisión tiene como objetivo corroborar los mecanismos plausibles de la hipereosinofilia inducida por dupilumab y revisar la GEPA y otros casos de hipereosinofilia sintomática presumiblemente asociados con la terapia con dupilumab. El bloqueo de la vía IL-4/IL-13 puede causar una reducción de la migración de eosinófilos y su acumulación en sangre periférica, al inhibir la eotaxina-3, VCAM-1 y TARC sin inhibir simultáneamente la eosinofilopoyesis en la médula ósea. A

la hora de decidir el tratamiento biológico óptimo en asmáticos, parece necesario considerar la presencia de hipereosinofilia (>1.500/µL), donde es preferible utilizar un anti-IL-5/IL-5R, como recomiendan las guías y algoritmos de tratamiento de asma grave. También hay que tener presente esta posibilidad al cambiar de un anti-IL-5/5R a un anti-IL-4/13R. En todas estas situaciones está justificada una estrecha monitorización de los eosinófilos en sangre y de la evolución clínica. En algunos casos, la terapia dual con anti-IL-5/5R y anti-IL4/IL-13R pudiera ser necesaria para un control óptimo del asma, ya que tanto la vía de la IL-5 como la de la IL-4/13 estarían contribuyendo de forma simultánea a la inflamación de las vías respiratorias. Este enfoque puede evitar el desarrollo de EGPA u otras hipereosinofilias sintomáticas y, paralelamente, mantener el control de la poliposis nasal. En un futuro próximo, se podrá utilizar una nueva generación de terapias biológicas para el tratamiento del asma grave que actúen a un nivel superior de la cascada inflamatoria, como es el caso de los anti-alarminas como tezepelumab o itepekimab.

Palabras clave: Asma. Tratamiento biológico. Granulomatosis eosinófila con poliangitis. Eosinofilia.

Introduction

Severe uncontrolled asthma is characterized by persistent exacerbations or poor functional and clinical control despite treatment with high doses of inhaled corticosteroids and another controller drug. In addition, we must rule out the possibility that this poor control is genuinely related to the disease and not to the presence of comorbidities, lack of adherence to treatment, or exposures to allergens or other environmental factors such as smoking [1]. Five biological drugs are currently available for the treatment of uncontrolled severe asthma. They all block type 2 inflammatory pathways by targeting IgE (omalizumab), the IL-5 pathway (mepolizumab, reslizumab, benralizumab), or the IL-4/13 pathway (dupilumab) [2]. They are all particularly effective in reducing the frequency of asthma exacerbations, and significant corticosteroid-sparing events have been observed with dupilumab, mepolizumab, and benralizumab [3]. In both phase 2 and phase 3 studies, dupilumab has significantly improved lung function, specifically in baseline prebronchodilator FEV_1 [4]. These biologics have radically different effects on the clinical biomarkers generally used in the phenotyping and follow-up of patients with severe asthma. Although anti–IL-5 agents significantly decrease blood eosinophil levels and do not modify the fraction of exhaled nitric oxide (FeNO) or serum IgE values, dupilumab initially increases blood eosinophils and decreases serum FeNO and IgE values. Hypereosinophilia (≥1500/µL) has been observed in 4% to 25% of patients treated with dupilumab, and although it is very often transient, it can persist for more than 6 months in up to 14% of affected patients [4,5]. Furthermore, cases of symptomatic hypereosinophilia consistent with eosinophilic granulomatosis with polyangiitis (EGPA), eosinophilic pneumonia, eosinophilic vasculitis, and sudden worsening of asthma symptoms have been described.

This review investigates plausible mechanisms of dupilumab-induced hypereosinophilia and reviews EGPA and other cases of symptomatic hypereosinophilia presumably associated with dupilumab. Finally, recommendations are presented to improve patient safety when changing treatment from anti–IL-5 therapy to dupilumab and to justify the need for combined therapy with anti–IL-5 agents and dupilumab in specific cases.

Immunologic and Biologic Characteristics of Eosinophils as Drivers of Disease

Eosinophils enable cell malfunction in the pathophysiology of eosinophilic diseases through 3 key characteristics, namely, migratory capacity, proinflammatory activity, and enzymatic arsenal [6].

Eosinophils are derived from bone marrow progenitors and mature with the synergistic action of IL-3, IL-5, and GM-CSF [7]. Of these cytokines, IL-5, together with eotaxins, plays a critical role in recruiting eosinophils to the bloodstream [8]. Specifically, eotaxin-1 (CCL-11), eotaxin-2 (CCL-24), and eotaxin-3 (CCL-26) are key chemokine molecules that trigger migration of eosinophils to sites of inflammation, such as the lung and the intestine [9].

In the target tissues, eosinophils secrete a wide variety of proinflammatory cytokines and granule proteins that are the main cause of disease processes. These specific granules contain basic proteins, including major binding protein (MBP) 1 and 2, eosinophil-derived neurotoxin (EDN [RNAse-2]), eosinophil cationic protein (ECP [RNase-3]), and eosinophil peroxidase. Given the toxicity and enzymatic activity of these proteins, the defence mechanisms that protect eosinophils against pathogens are a double-edged sword, that is, they can also damage tissues, as described in several eosinophilic diseases [10,11].

Role of Eosinophils in the Pathophysiology of Asthma

Asthma is a very heterogeneous disease in terms of the pathways and immune mechanisms involved. Nonetheless, asthmatic patients are characterized by a series of major features, including airway hyperresponsiveness, bronchial damage, tissue remodelling, and mucus hypersecretion. These features cause the symptoms of the disease, namely, shortness of breath, cough, wheezing, and chest pain [12]. Although asthma is a heterogeneous disease, we can identify a major phenotype/endotype, namely, eosinophilic asthma, also known as type 2 asthma owing to the implication of type 2 immune mechanisms, which may be both innate and adaptative [13].

In type 2 asthma, type 2 innate lymphoid cells (ILC2s) can induce eosinophilia in the lung owing to the high number of IL-5$^+$, IL-13$^+$, and ILC2s in asthmatic sputum [14]. Together with T cells, they are the main source of type 2 cytokines that drive eosinophilia in the asthmatic lung [14]. Previous studies have shown a good correlation between eosinophilia and the severity of asthma and the number of exacerbations [15]. In fact, severe eosinophilic asthma has been defined as a unique entity requiring a more specific therapeutic approach, thus leading to an improvement in management [16]. Indeed, the high prevalence of the eosinophilic phenotype in severe asthmatics has been validated by a multicomponent, consensus-driven, evidence-based eosinophil gradient algorithm, which can identify up to 83% of severe asthmatics by eosinophilic phenotype [17].

Recruitment of eosinophils to the lungs is mediated mainly by chemoattractants, of which the eotaxins CCL11, CCL24, and CCL26 are the most active [18]. Among type 2 cytokines, IL-5 is the most capable of inducing maturation, proliferation, survival, activation, adhesion, and migration, thus favoring interactions between eosinophils and periostin [19]. This matricellular protein is involved in eosinophil transmigration and trafficking toward the bronchi [19]. The numerous other molecules that can induce eosinophil migration include 5-oxo-eicosatetraenoic acid (5-KETE), leukotrienes (LTD_4, LTC_4, and LTE_4), and prostaglandins (DP_2/CRT_H2) [20-22].

Eosinophils slide from vessels to lung tissue. Adhesion molecules play a key role in this process, with P-selectin and integrin VLA-4 binding to their molecular counterparts in endothelial cells and facilitating the rolling, activation, and extravasation to tissue that is characteristic of these granulocytes [23].

Finally, tissue damage and remodelling are the most prominent features of asthma induced by the direct effect of the eosinophil population once at the site of inflammation. Eosinophilic granule proteins have marked cytotoxic capacity owing to their nuclease activity. ECP is a ribonuclease capable of inducing epithelial and smooth muscle cell death and tissue remodelling through collagen deposition, thus increasing the activity of fibroblasts [24]. Similar effects have been described for MBP, which is secreted though cellular vesicles and is closely associated with asthma cytotoxicity [25]. EDN has been shown to enhance airway remodelling in chronic eosinophilic rhinosinusitis through dysregulation of the MMP-9 pathway and also has potential as a disease marker for asthma monitoring [26,27]. When it comes to the presence of airway hyperresponsiveness, this disease mechanism is driven by eosinophilic enzymes such as MBP, which causes hyperresponsiveness directly through interaction with the airway wall and the epithelium [28] and through indirect activation of mast cells and release of histamine [29]. Moreover, regarding fibrosis, the main event

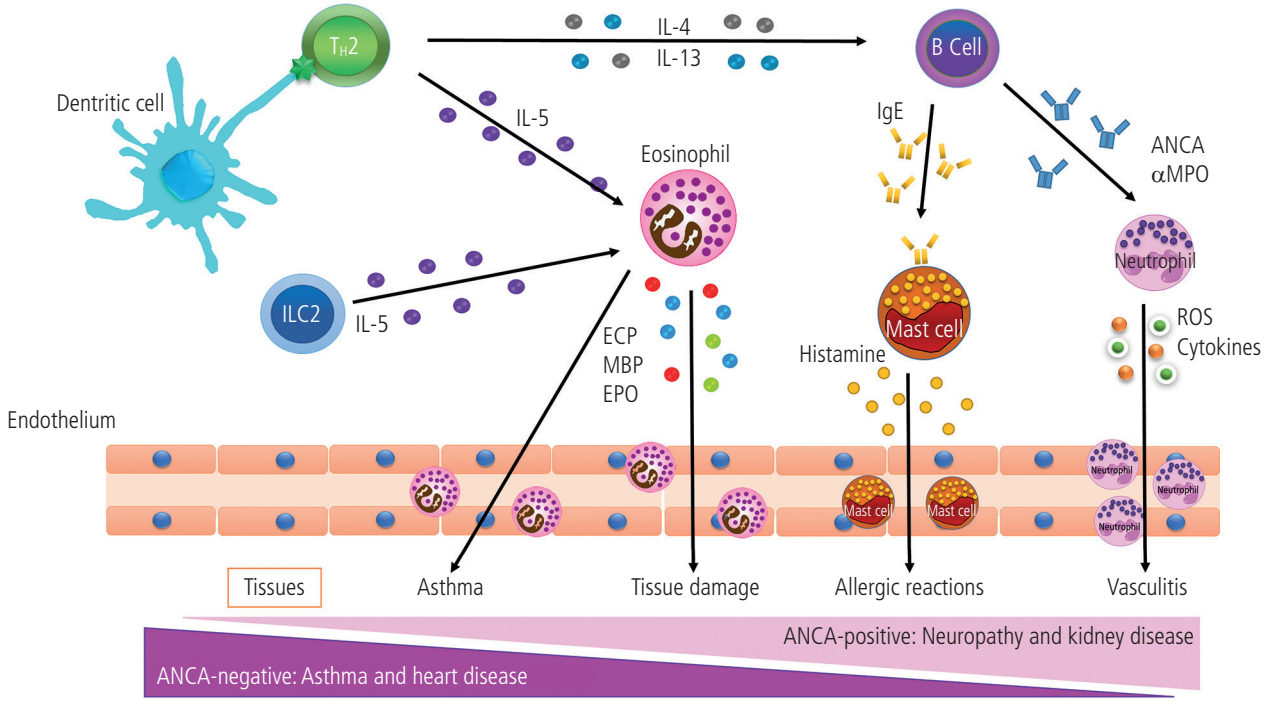

Figure 1. Pathophysiology of eosinophilic granulomatosis with polyangiitis (EGPA). EGPA is characterized by 2 different mechanisms. In the first (ANCA-negative), eosinophils driven by type 2 cytokines, such as IL-4/13 and IL-5 migrate to the tissues, where they release cytotoxic proteins causing mainly cardiac and vascular damage, while also inducing asthma in the lungs. The ANCA-positive mechanism is characterized by the presence of antimyeloperoxidase (MPO) antibodies produced by B lymphocytes stimulated with IL-4 and IL-13. These antibodies stimulate neutrophils, which release enzymes and oxygen reactive species (ROS), thus causing vasculitis, kidney disease, and neuropathy accompanied by IgE-mastocyte binding and allergic reactions. T_H indicates type 2 helper T cell; IL, interleukin; ILC, innate lymphoid cell; ECP, eosinophil cationic protein; MBP, major binding protein; ANCA, antineutrophil cytoplasmic antibody.

in remodeling, eosinophils release TGF-β, which induces fibroblast proliferation, collagen deposition, and inflammation. Therefore, eosinophils can induce tissue remodeling through multiple pathways [30].

Eosinophils as the Heart of EGPA Biology

EGPA is a rare multisystemic disease characterized by eosinophilic inflammation that causes systemic small vessel vasculitis. Formerly known as Churg-Strauss syndrome, EGPA has been reported in persons with asthma and peripheral/tissue eosinophilia [31]. Some patients develop specific antibodies against myeloperoxidase, ie, antineutrophil cytoplasm antibody (ANCA) [32]. Interestingly, studies have shown that ANCA-positive patients had increased renal, skin, and neuronal involvement [33]. In contrast, ANCA-negative patients present more cardiovascular and abdominal involvement, with cardiac involvement being one of the main predictors of poor outcome [34] (Figure 1). This classification into 2 possible phenotypes is supported by the genetic background, thus indicating that ANCA-positive EGPA is more an eosinophilic autoimmune disease with vasculitis and associated HLA-DQ [35]. At the same time, ANCA-negative EGPA seems to be associated with GP33 and IL-5/interferon regulatory factor (IRF) 1 and to originate in the mucosal barrier [35].

Eosinophilia is common to both phenotypes, and EGPA is widely described as an eosinophilic disorder. In common with other diseases, it leads to radicalized polarization of the immune response towards the type 2 axis through the presence of cytokines such as IL-5, IL-4, IL-10, and signal transducer and activator of transcription (STAT) 5A [36]. It is worth noting that EGPA seems to affect genetically predisposed individuals, thus accounting for type 2 immune polarization and eosinophilia. Variants in the *IRF1* and *IL5* genes have been associated with EGPA and with severe asthma, as previously mentioned [35]. Besides, promoter polymorphisms in the *IL10* gene have been associated with EGPA, specifically, the IL10.2 haplotype [37]. In contrast, variants in the *HLA-DR* gene, including HLA-DRB1*04 and *07 or HLA-DRB4, have been studied as key genetic determinants for this disease owing to their association with $CD4^+$ T-cell lymphocyte responses [38]. Moreover, variants in genes controlling apoptosis (*BCL2L11* and *MORRBID*) have been described in EGPA [35]. Some studies have found functional correspondence between the variability of these genes and reduced expression of proapoptotic genes such as *BCL2L13*, *CASP2*, and *CARD4* in EGPA [39].

Regarding the pathophysiology of EGPA, type 2 responses are the key element causing eosinophilia and symptoms. IL-5 is the most prominent type 2 cytokine, owing to its activity, which promotes maturation, survival, and proliferation of eosinophils, which are the key requisites for developing and maintaining eosinophilia [19]. $CD4^+$ T lymphocytes are the main source of IL-5 in the biology of EGPA. However, they are not the only secretors of this cytokine, as ILC2s can also secrete IL-5. These innate cells are increased in peripheral blood in active EGPA, also releasing IL-33, which accounts for active vasculitis [40]. Other cytokines such as IL-10, TNF-α, and IFN-γ are also important for the vasculitis phase of EGPA [41]. Interestingly, production of IL-4 and IL-13 by T cells is increased in this disease, showing that type 2 responses are the main cause of eosinophilia [42]. Finally, among eotaxins, CCL26 (eotaxin-3) is involved in EGPA and is the marker of disease activity [43].

In EGPA, the activity of eosinophils differs from their usual activity, as seen by the increase in CD69 and CD25 (related to apoptosis) and the secretion of IL-25, which is associated with a positive feedback loop in type 2 responses and eosinophilia [39,44]. When the eosinophil reaches the tissues, as in asthma, it releases several cytotoxic molecules, including ECP, the main enzyme related to cardiotoxicity through inhibition of cardiomyocyte membrane permeability and mitochondrial respiration [45]. Eosinophils also cause thrombosis through release of thrombin, and secretion of extracellular traps, ECP, MBP, and reactive oxygen species [46-48]. The mechanism underlying neuronal damage is not completely clear, although it seems to be associated with necrotizing vasculitis, where EDN might be of the utmost importance [49].

The key role of IL-5 in this disease has been that of opening the field for the use of biologics in the treatment of diseases of the IL-5/IL-5RA axis (eg, asthma), with reports of improvement in EGPA with mepolizumab and reduced need for prednisone [50].

Molecular Mechanism of Action of Dupilumab

Dupilumab consists of a human monoclonal IgG4 antibody that recognizes the IL-4Rα subunit of the IL-4 and IL-13 receptors [51]. The molecular basis of these receptors lies in the function of IL-4Rα subunit, which binds to other subunits of the receptor and translates intracellular signals upon recognition of the ligands IL-4 and IL-13 [52]. For recognition of IL-4, the IL-4Rα subunit pairs with the γc chain, conforming to the so-called type 1 IL-4R [53]. Conversely, for IL-13 binding, the IL-4Rα subunit interacts with IL-13Rα1, forming a high-affinity IL-13 receptor that is also able to recognize IL-4, namely, type 2 IL-4R [54]. Binding of IL-4 to IL-4Rα and IL-13 to IL-13Rα1 induces conformational triggering, which leads to recruitment of the second part of the receptor, recruitment of the other subunit, and initiation of signalling [55] (Figure 2).

After recruitment of the receptor subunits, the Janus family protein kinases associated with the specific subunit (JAK-1 for IL-4Rα, JAK-2/TYK-2 for IL-13Rα1, and JAK-3 for γc chain) are phosphorylated and activated, initiating a cascade of phosphorylation of IL-4Rα that ends with activation of transcription factors [52] (Figure 2). When IL-4 binds to IL-4Rα, recruitment and phosphorylation of STAT6 are initiated through the action of JAK-1. STAT6 then dimerizes and enters the nucleus, where it binds to DNA [56]. IL-13 binding to IL-13Rα1 phosphorylates STAT3 through phosphorylation of TYK-2. Both transcription factors (STAT6 and STAT3) bind

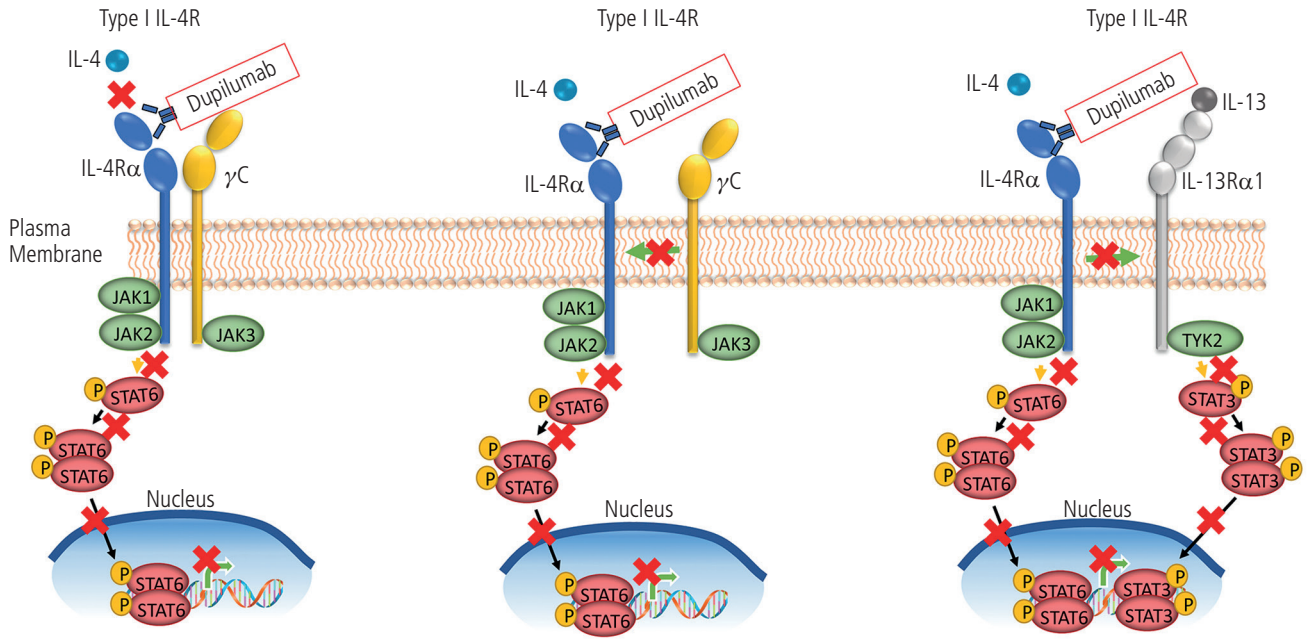

Figure 2. Mechanism by which dupilumab blocks signal transduction by IL-4/13R (receptor). There are 2 types of IL-4/13 receptor (type 1 and type 2). Type 1 IL-4R consists in the IL-4Rα and the γc subunits and recognizes IL-4, while type 2 consists in the IL-4Rα and IL-13Rα1 subunits and recognizes IL-13. In functional signalling, after ligand binding, JAK proteins are activated, resulting in STAT6 and/or STAT3 phosphorylation and activating a pathway that in turn activates specific gene expression at DNA level. Dupilumab is able to block receptor signaling by 3 mechanisms, as depicted in the figure (left). When dupilumab binds to IL-4Rα it impedes IL-4 binding and signal transduction. In addition, binding of dupilumab to IL-4Rα inhibits the coupling of the IL-4Rα chain with the γc subunit and blocks signal transduction (in the middle). Finally, dupilumab binds the IL-4Rα subunit and blocks it from coupling to the IL-13Rα1 subunit after binding to IL-13 and, consequently, suppressing intracellular signalling (right). IL indicates interleukin; JAK, Janus kinase; STAT, signal transducer and activator of transcription.

the regulatory gene promoters in IL-4 and IL-13, which are responsible for IgE synthesis, T_H2 polarization, and mucus secretion [56].

IL-4R plays an important role in T_H2 cell differentiation, mainly by activation of GATA-3 and production of type 2 cytokines [57]. Moreover, IL-4R is deeply involved in immunoregulation of IL-10, shifting the dominance of the T_H2 responses that are characteristic of signalling by IL-4 and IL-13 [58]. This polarization to type 2 immune responses relies not only on T lymphocytes, but also on ILC2s, which are a major source of IL-4 and IL-13 via innate immunity [59].

Given the importance of IL-4R in orchestrating type 2 immune responses, the action of dupilumab has been portrayed to be very beneficial for the control of type 2 diseases such as allergies and asthma [60]. As dupilumab can bind to IL-4Rα, it can shut down signaling through IL-4 and IL-13, thus inhibiting type 2 inflammation [60]. The specific mechanism of inhibition can act upon both type 1 and type 2 IL-4R. Regarding blockade of the type 1 receptor, when dupilumab binds to IL-4Rα, it impedes both binding of IL-4 to its receptor and recruitment of the γc subunit [61] (Figure 2). This same event is associated with the recruitment of IL-4Rα to the IL-13Rα1 subunit, thus inhibiting the type 2 IL-4R signaling that is seen in allergic diseases [62]. Blocking both type 1 and 2 IL-4R signaling provides a key advantage in treatment over blocking the individual ligands [62] (Figure 2).

Dupilumab as a Drug Targeting the IL-4/13 Pathway in Type 2 Diseases

IL-4 and IL-13 play an important role in type 2 immune responses, such as those where allergens act as the main antigen drivers. The aberrant expression and secretion of IL-4 and IL-13 have been associated with the pathophysiology of allergic diseases, including allergic asthma [63]. Both cytokines bind to their common IL-4/13 receptor, as previously mentioned. However, as they bind to different subunits of the receptor, they are able to perform both similar and differential roles, with IL-4 playing a critical role in antibody isotype switching of B cells to produce allergen-specific IgE [64].

Conventional T_H2 $CD4^+$ T cells and basophils are the main source of IL-4, which plays a key role in the migration of eosinophils toward eotaxin-1 [65]. IL-13 is secreted principally by T_H2 cells and ILC2s in tissue [66] and has a prominent role in airway hyperresponsiveness, mucus synthesis, smooth muscle alterations, and fibrosis induced by TGF-β; therefore, it is able to induce many asthma symptoms through a mechanism that does not seem to be dependent on IgE or eosinophils [67] (Figure 3).

The resourcefulness of a human biologic antibody targeting the IL-4/13 pathway, such as dupilumab, is of noticeable interest in biomedicine owing to the wide variety of diseases associated with this molecular axis [68]. Given the nature and possibilities of blocking a critical pathway of type 2 immune

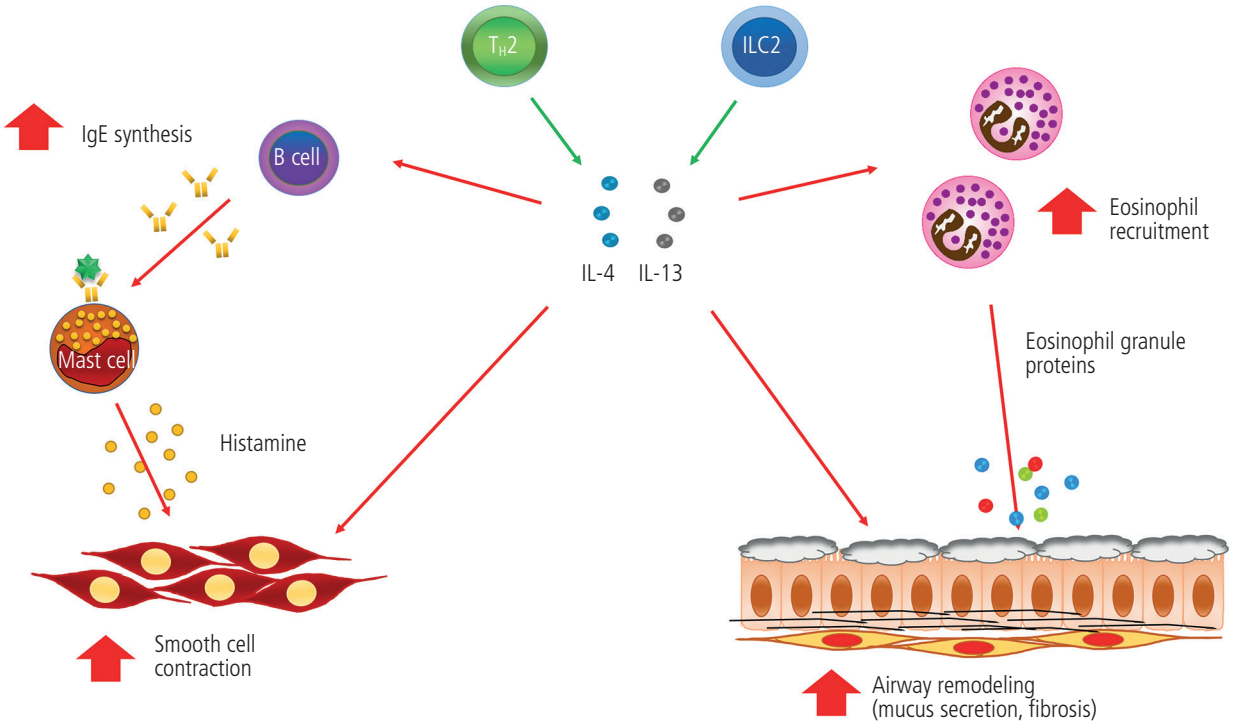

Figure 3. Roles of IL-4 and IL-13 in the pathophysiology of asthma. The main sources of IL-4 and IL-13 are type 2 lymphocytes and type 2 innate lymphoid cells (ILC2s). These cytokines induce many asthma hallmarks by B lymphocyte immunoglobulin isotype switching to IgE, which in turn recognizes an allergen and binds mast cells, leading to histamine release and smooth muscle contraction. IL-4 and IL-13 themselves are also capable of increasing muscle contraction. Il-4 and IL-13 induce migration of eosinophils to the lungs, where these cells release inflammatory mediators that, combined with IL-4/13, induce airway remodeling (increase in mucus deposition and subepithelial fibrosis). T_H indicates type 2 helper T cell; IL, interleukin.

responses, the indications for dupilumab are increasing. The drug is being explored as treatment for several diseases, including atopic dermatitis, allergic contact dermatitis, prurigo nodularis, chronic pruritus, chronic hand eczema, nummular eczema, chronic spontaneous urticaria, cholinergic urticaria, cold-inducible urticaria, bullous pemphigoid, localized scleroderma, alopecia areata, and Netherton syndrome [61]. In the field of respiratory diseases, dupilumab has been approved for treatment of severe asthma and chronic rhinosinusitis with nasal polyps (CRSwNP). Furthermore, it is in the development phase for treatment of allergic bronchopulmonary aspergillosis, allergic rhinitis, chronic eosinophilic pneumonia, and other type 2 diseases that affect other organs (eg, gastrointestinal disorders), with promising results in eosinophilic esophagitis and food allergy [61,69].

Symptomatic Hypereosinophilia Induced by Dupilumab: Case Reports and Cases of EGPA Described in Clinical Trials

As reported for most clinical trials on severe asthma, CRSwNP, and atopic dermatitis, a transient, clinically irrelevant increase in blood eosinophil counts was observed after treatment with dupilumab. However, real-life practice has highlighted these "not so rare" cases, some of which may progress to eosinophilic disease with clinical manifestations. Isolated cases of chronic eosinophilic pneumonia [70-72], eosinophilic gastritis, [73], and eosinophilic vasculitis [74] have been associated with dupilumab in patients with asthma. Therefore, dupilumab could be considered as being directly related to or unmasking previous vasculitis by discontinuation or reduction of corticosteroids in asthma patients, as reported by Ikeda et al [75] and Murag et al [76]. Importantly, cases reported by Eger et al [77] highlight the importance of considering the blood eosinophil count in asthmatics who switch from an anti–IL-5/5Ra agent to an anti–IL-4/13 agent. Hypereosinophilia with systemic clinical manifestations may develop if treatment does not focus on appropriate control of blood eosinophil levels, as anti–IL-5/5Ra agents and corticosteroids do [77-79]. These observations support the indication for dual therapy with anti–IL-5 agents and dupilumab in this clinical scenario, as suggested by Eger et al and supported by Descamps et al [74]. Table 1 summarizes clinical and demographic data for these case reports.

EGPA in Pivotal Studies on Asthma and CRSwNP Treated With Dupilumab

Table 2 summarizes data on patients with symptomatic eosinophilia from pivotal clinical trials on asthma. In Liberty Asthma Quest [80], 4.1% of patients (52 active patients)

Table 1. Case Reports of Symptomatic Eosinophilia, Including Eosinophil Granulomatosis With Polyangiitis (EGPA), Described in the Literature Associated With Dupilumab

Age	Sex	Diagnosis	CDA	Biological therapy	Days of therapy with dupilumab	Baseline eosinophils/μL	Peak eosinophils/μL or %	Acute clinical manifestation related to eosinophilia	Corrective treatment	Reference
59	F	CRS, severe asthma	+	Benralizumab switched to dupilumab	NR	100	5080	Asthma exacerbation, sinusitis, pulmonary infiltrates, neurologic, cardiovascular, fever, myalgia, EGPA	Prednisone	[77]
35	M	CRS, severe asthma	+	Reslizumab switched to dupilumab	NR	500	1050	Asthma exacerbation, sinusitis, pulmonary infiltrates, neurologic, cardiovascular, fever, myalgia, EGPA	Prednisone, reslizumab	[77]
47	F	CRS, severe asthma	+	Benralizumab switched to dupilumab	NR	90	5010	Asthma exacerbation	Benralizumab, Prednisone	[77]
63	F	Severe asthma	+	Mepolizumab switched to dupilumab	NR	60	3949	Pulmonary infiltrates, eosinophilic alveolitis, neurologic, EGPA	Prednisone, mepolizumab 300 mg/month	[77]
50	M	Polyposis, severe asthma	–	Dupilumab	120	390	3950	Asthma exacerbation, pulmonary infiltrates, cardiovascular, EGPA	Prednisone, benralizumab	[78]
24	F	Polyposis, EGPA ANCA-	+	Benralizumab switched to dupilumab	160	0	8100	Asthma exacerbation	Prednisone, benralizumab, dupilumab	[78]
56	F	Polyposis, severe asthma	–	Dupilumab	70	600	2800	Pulmonary infiltrates, eosinophilic alveolitis, fever	Prednisone	[70]
58	M	Severe asthma	+	Dupilumab	42	–	3200	Asthma exacerbation	Prednisone	[71]
56	M	Severe asthma, nasal polyposis	+	Dupilumab	730	NR	NR	Fever, cutaneous vasculitis, EGPA	Prednisone	[75]
55	F	Asthma, atopic dermatitis, bullous pemfigoid	–	Dupilumab	.NR	NR	1700	Fever, bilateral pulmonary infiltrates	Prednisone	[72]
41	M	Severe asthma, atopic dermatitis, food allergy	+	Dupilumab	180	100	1250	Fever, bilateral pulmonary infiltrates, EGPA	Mepolizumab	[76]

(*continued*)

Table 1. Case Reports of Symptomatic Eosinophilia, Including Eosinophil Granulomatosis With Polyangiitis (EGPA), Described in the Literature Associated With Dupilumab (*continue*)

Age	Sex	Diagnosis	CDA	Biological therapy	Days of therapy with dupilumab	Baseline eosinophils/μL	Peak eosinophils/μL or %	Acute clinical manifestation related to eosinophilia	Corrective treatment	Reference
63	M	Severe asthma	NR	Mepolizumab switched to dupilumab	180	NR	54%	Fever, myalgia, cutaneous vasculitis, eosinophilic myocarditis EGPA	Prednisone	[79]
77	M	Severe asthma	–	Dupilumab	16	152	860	Eosinophilic gastritis	Prednisone	[73]
61	F	Severe asthma, nasal polyposis	+	Dupilumab	150	2000	11500	Eosinophilic vasculitis, hypereosinophilic syndrome	Dupilumab, benralizumab	[74]

Abbreviations: Footnote: CDA, corticosteroid-dependent asthma; CRS, chronic rhinosinusitis; EGPA, eosinophil granulomatosis with polyangiitis; NR, not reported.

presented eosinophilia. Of these, 22 had hypereosinophila (>3000/μL). In 8 of these cases, treatment with dupilumab was discontinued. In 4 patients, eosinophilia was symptomatic. In the Venture study [81], which analyzed the oral corticosteroid–sparing effect of dupilumab in patients with severe corticosteroid-dependent asthma (210 included, 103 with active disease), 13% had hypereosinophilia >3000/μL. None were symptomatic. However, in the open-label extension study (Tranverse study) [82], and with all the patients included in the initial placebo group already in treatment with dupilumab, 2 out of 210 patients presented clinical manifestations consistent with EGPA. The Transverse study also includes the results of the long-term, open-label follow-up of the Liberty Quest study and a phase 2 dosing study. Three out of 2062 patients presented clinical manifestations consistent with EGPA (Table 2). Finally, in the pivotal dupilumab study in CRSwNP, 50% of patients included had comorbid asthma, and 3 patients with uncontrolled asthma had symptoms that were also consistent with EGPA [83]. In this study, 1 patient in the placebo group also developed EGPA. Of note, many of these patients experienced an intense relapse of their nasal symptoms after discontinuation of dupilumab, including those who were treated with an anti–IL-5 agent.

Given that EGPA can appear during tapering and discontinuation of systemic corticosteroids, systemic corticosteroids may be masking vasculitis [75,76,83].

EGPA Related to Other Antiasthma Drugs

Other drugs have been related to the development of EGPA. In a review by Bibby et al [84] based on the United States Food and Drug Administration pharmacovigilance database performed in 2010, before biologics were used for treatment of severe asthma, 90% of drug-related EGPA cases (181 of 190) involved montelukast or another leukotriene receptor antagonist, such as zafirlukast. The time between the introduction of treatment and the development of EGPA symptoms ranged from 3 to 180 days in most patients. In only 34% of patients, the onset of symptoms was also temporarily linked to a reduction in or withdrawal of systemic corticosteroids suggesting that EGPA was already present before introducing the leukotriene receptor antagonist. In their literature review, Nathani et al [85] reported that EGPA was related to the withdrawal of corticosteroids in 37% of patients. In the European study of control cases, the risk of developing EGPA with montelukast was established at 6.7 (95%CI, 1.3-34.1) [86].

Using the Novartis pharmacovigilance database in the biologics era, Wechsler et al [87] reported 13 cases of highly probable EGPA during treatment with omalizumab in patients with severe asthma. Eight of these 13 probable cases (62%) had EGPA symptoms prior to receiving omalizumab. In 6 of the 13 patients (46%), EGPA symptoms appeared immediately after tapering of systemic corticosteroids due to the introduction of omalizumab. The authors concluded that EGPA might develop in patients receiving asthma medications who have an underlying eosinophilic disorder unmasked by withdrawal of corticosteroids or in patients who delay specific therapy in favor of other medications. In their view, omalizumab may unmask EGPA owing to tapering of corticosteroids in some asthma patients or may delay corticosteroid treatment, thus enabling EGPA to manifest.

Two cases of EGPA have been described in patients with severe asthma a few months after starting treatment with benralizumab. Both patients presented a vasculitis-type EGPA phenotype accompanied by constitutional syndrome, fever, arthralgia, respiratory decompensation with pulmonary infiltrates, and skin lesions with necrosis. In both cases, the authors associated the onset of EGPA with the withdrawal of oral corticosteroids secondary to the introduction of benralizumab [88].

Table 2. Cases of Eosinophilic Granulomatosis With Polyangiitis (EGPA) Reported in Pivotal Trials

Age	Sex	Diagnosis	CDA	Biological therapy	Days of therapy with dupilumab	Baseline eosinophils/ μL	Peak eosinophils/ μL	Acute clinical manifestation related to eosinophilia	Corrective treatment	Reference
56	F	Allergic rhinitis, severe asthma	–	Dupilumab	445	NR	7680	Asthma exacerbation, pulmonary infiltrates, myalgia, fever	Rituximab, azathioprine, prednisone	[82]
30	F	CRS, severe asthma	–	Dupilumab	499	1390	11 000	Asthma exacerbation, neuropathy, gastritis	No	[82]
49	F	Polyposis, severe asthma	–	Dupilumab	406	NR	14 700	Asthma exacerbation, sinusitis, ANCA^{+}	Prednisone	[82]
38	F	Polyposis, severe asthma	+	Dupilumab	318	1800	11 400	Asthma exacerbation, pulmonary infiltrates, acute sinusitis	Prednisone	[82]
44	F	Severe asthma	+	Dupilumab	172	150	8500	Asthma exacerbation, pulmonary infiltrates, neurologic, cutaneous vasculitis	Azathioprine, prednisone	[82]
50	M	Severe asthma, polyposis	–	Dupilumab	16	570	10 280	Asthma exacerbation, myositis, fever	Prednisone	[80]
56	M	Severe asthma	–	Dupilumab	118	660	2700	Chronic eosinophilic pneumonia	Prednisone	[80]
28	F	Severe asthma	–	Dupilumab	114	690	8650	Asthma exacerbation, fever myalgia	Prednisone	[80]
52	F	Severe asthma	–	Dupilumab	127	1290	4920	Pulmonary infiltrates	Prednisone	[80]
NR	NR	Uncontrolled asthma	–	Dupilumab	NR	NR	NR	Bilateral pulmonary infiltrates, polyneuropathy, EGPA	Prednisone	[83]
NR	NR	Uncontrolled asthma	–	Dupilumab	NR	NR	NR	Fever, asthma exacerbation, arthralgia	Prednisone	[83]
NR	NR	Uncontrolled asthma	–	Dupilumab	NR	NR	NR	Bilateral pulmonary infiltrates, polyneuropathy EGPA	Prednisone	[83]

Abbreviations: ANCA, antineutrophil cytoplasmic antibody; CDA, corticosteroid-dependent asthma; CRS, chronic rhinosinusitis; EGPA, eosinophilic granulomatosis with polyangiitis; NR: not reported.

Table 3. Eosinophil Granulomatosis With Polyangiitis (EGPA) Cases Reported at the EudraVigilance (March 2022) Pharmacovigilance Database

Suspect drug	Total number of EGPA cases	Total number of all spontaneous case reports in EudraVigilance	%
Montelukast	776	14 090	5.51
Benralizumab	36	2720	1.32
Omalizumab	145	19 699	0.74
Mepolizumab	46	5725	0.80
Dupilumab	61	13 151	0.46
Reslizumab	0	184	NA

Abbreviation: NA, not available.

Analysis of the EudraVigilance Pharmacovigilance Public Database

EudraVigilance, the electronic pharmacovigilance database of the European Medical Agency (EMA), was accessed via its website, and all spontaneous EGPA case reports available up to 19/03/2022 were reviewed (level 1). Table 3 summarizes the total number of cases of EGPA reported with dupilumab in comparison to the total number of spontaneous case reports available in the database. The same comparison has been made with other biologics indicated for asthma and with montelukast. A total of 61 cases of EGPA were reported during treatment with dupilumab. This report includes the cases reported in the literature (Table 1). The indications for prescribing dupilumab were severe asthma and/or nasal polyposis. In addition, all cases but 2 had atopic dermatitis. Among the 61 patients who developed EGPA, 27 had recovered or were recovering at the time of notification, 1 patient recovered with sequelae, and 7 patients had not recovered at the last contact. The outcome is unknown for the remaining 26 case reports. The action taken with dupilumab after the onset of EGPA was withdrawal in 33 of 42 patients for whom this outcome was reported. In 1 of the 9 patients who continued dupilumab, an anti–IL-5 agent was added.

Compared with other biologic treatments, the rate of EGPA cases, and the total number of notifications reported, the highest rate was recorded for benralizumab (1.32), followed by mepolizumab (0.80) (Table 3). It is important to bear in mind the number of doses administered for each biologic, time on the market, and the number of indications.

Increased Eosinophil Levels With Dupilumab: The Story So Far

The presence of eosinophilia after treatment with dupilumab was first described in pivotal clinical trials. The initial hypothesis of why eosinophilia occurred was proposed by Castro et al [80] and Rabe et al [81]. The transient blood eosinophilia observed in these clinical trials could be related to inhibition of eosinophil migration and activation caused by inhibition of the IL-4/13 axis [80,81]. These authors argued that the blockade of IL-4 and IL-13 signaling mediates a reduction in serum eotaxin-3 levels and a decrease in vascular cell adhesion molecule 1 (VCAM-1), which inhibits migration of eosinophils to tissue (Figure 4) [89,90]. Moreover, STAT6, which acts as the downstream transcription factor of IL-4/13 binding to the receptor, is a regulator of eotaxin-1. Thus, inhibition of signalling through the IL-4/13R pathway may reduce migration of eosinophils and their accumulation in blood, in much the same way as with eotaxin-3 and VCAM-1 [91].

Accumulation in blood may also result from the action of thymus and activation-regulated chemokine (TARC/CCL17), a type 2 cytokine that binds to CCR4 and is involved in T_H2 cell trafficking in eosinophilic disorders [92]. TARC is synthesised by immune cells after stimulation of IL-4 following induction of STAT6, and this protein is also thought to be involved in eosinophil trafficking [93]. Increased TARC levels have been described in the bronchoalveolar lavage fluid of asthmatics and in EGPA lesions with eosinophilic infiltrations and CRT_H2^+ T cells [94,95].

With migration to the tissues blunted and eosinophilopoiesis not being inhibited (Figure 5A), eosinophils are generated continuously in bone marrow; therefore, they accumulate in the blood and cause hypereosinophilia [80,81]. However, in cases of eosinophilic pneumonia and EGPA associated with dupilumab, eosinophils migrate to other tissues (Figure 5B). Therefore, other mechanisms may be involved in the migration of eosinophils to local tissues and in the accumulation of eosinophils in blood, as eosinophilia can also be influenced by tapering corticosteroid doses after initiation of dupilumab (Figure 5C) [81].

Basic knockout mice models might play a role in clarifying this issue, as IL-$13^{-/-}$ mice develop blood and airway eosinophilia. In addition, while IL-4 antibody is able to reduce eosinophilic infiltration in the lung, IL-$13^{-/-}$ mice treated with ovalbumin and anti–IL-4 neutralizing antibody have more eosinophilic lung infiltrates than wild-type mice, with anti–IL-5 neutralizing antibody being the only mechanism capable of completely reducing airway eosinophils. The authors of this study propose that low levels of IL-13 may result in an increase in NF-κβ, which in turn increases synthesis of IL-5 via a mechanism that is independent of IL-4 regulation (Figure 5D), as observed in nonallergic asthmatics with high levels of IL-5 and eosinophils despite low IL-4 levels [96]. Regarding the source of IL-4/13 in airway eosinophilia, mice models with $CD4^+$ lymphocytes deficient in IL-4 and IL-13 are able to induce lung eosinophilia during allergic inflammation [97], thus highlighting the importance of ILC2s and other cells as sources of these cytokines [98]. Furthermore, ILC2s synthesize IL-4 and IL-13, although they are also major producers of IL-5. Since ILC2s do not require a previous IL-4– and IL-13–based $CD4^+$-dependent adaptive immune phase response, they might be able to produce high amounts of IL-5 only by the action of alarmins (IL-25, IL-33) released by the insulted airway epithelium (Figure 5D), similar to the mechanisms underlying eosinophilia after anti–IL-2 therapy [99]. In these cases, the trigger of the

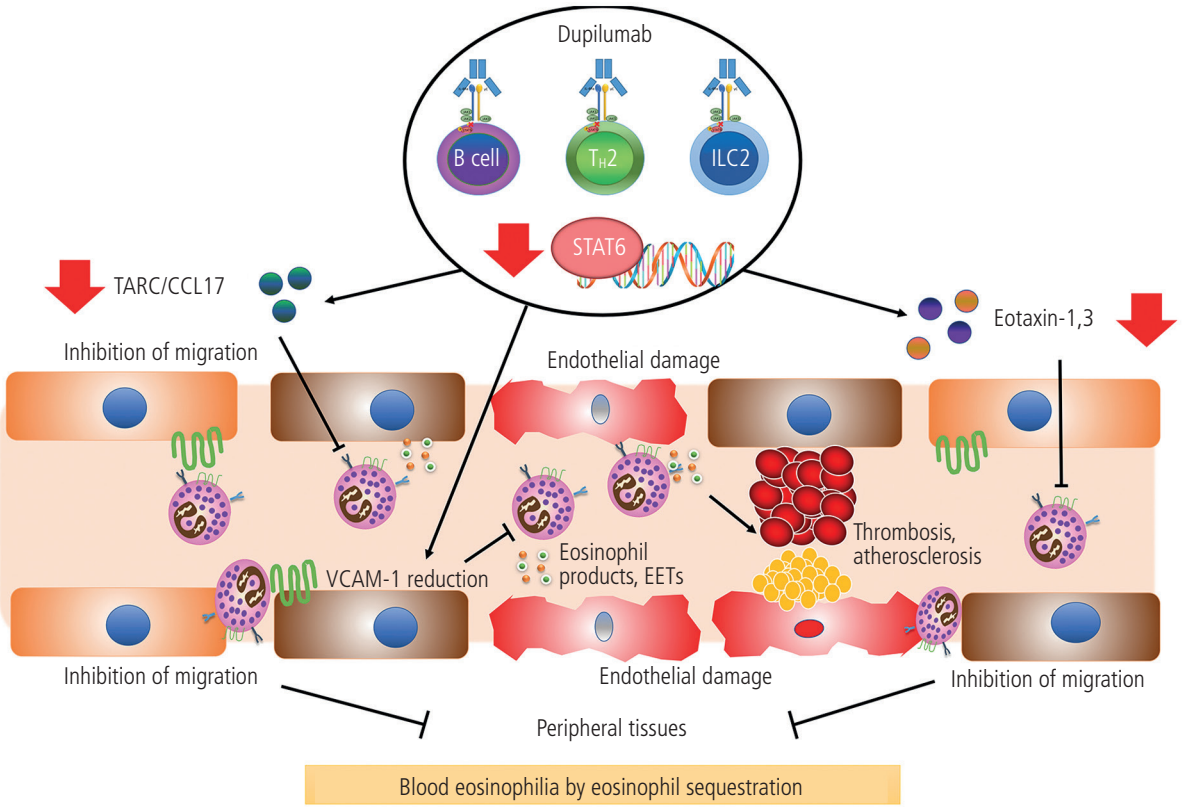

Figure 4. Possible mechanism of eosinophil sequestration in blood on initiation of dupilumab. Dupilumab binds to IL-4/13R in TH2 lymphocytes, type 2 innate lymphoid cells (ILC2s), and B lymphocytes. Inhibition of STAT6 transcription factor by dupilumab leads to inhibition of TARC/CCL17 and eotaxins 1-3, molecules that induce eosinophil migration. With reduced migratory molecules and VCAM-1 (adhesion molecule required for extravasation of eosinophils), eosinophils remain in the bloodstream and release their content inside the vessels, thus inducing endothelial damage, thrombosis, and atherosclerosis. T_H indicates type 2 helper T cell; ILC, innate lymphoid cell; JAK, Janus kinase; STAT, signal transducer and activator of transcription; VCAM, vascular cell adhesion molecule; EET, eosinophil extracellular trap.

innate immunity that activates ILC2s, for example, during viral infections in the airway epithelium, can lead ILC2s to release type 2 cytokines, including IL-4, IL-13, and IL-5, thus causing asthma exacerbations where both the IL-4/13 pathway, and the IL-5 pathway should be taken into account (Figure 5D) [100].

Migration of the eosinophil progenitor plays an important role, since IL-4 and IL-13 can control the priming and migration of hematopoietic progenitor cells derived from cord blood and peripheral blood ($CD34^+CD45^+$ cells) to stromal cell–derived factor 1α (SDF-1α). This chemokine activates leukocytes in inflammatory reactions. More importantly, IL-4 and IL-13 are not able to control migration of progenitors that have already committed to the eosinophil lineage ($CD34^+CD45^+IL5R\alpha^+$ cells) or migration of mature eosinophils to the SDF-1α chemoattractant (Figure 5C) [101]. Indeed, eosinophil progenitors that can migrate and that are not controlled by any treatment, such as a corticosteroid or anti–IL-5 therapy, will be able to accumulate both in the blood and in the lungs and, therefore, with the action of IL-5, will eventually evolve into mature eosinophils (in situ eosinophilopoiesis). These in turn can produce eosinophilic vasculitis, EGPA, and asthmatic symptoms when releasing their enzymatic granule content (Figure 5C) [102].

Conclusions and Recommendations

Eosinophils play a critical role in the pathogenesis of asthma, and several therapies target this cell to ameliorate symptoms.

The various biological drugs that are currently available for the treatment of severe uncontrolled type 2 asthma include anti-IgE, anti-IL-5, anti-IL-5Rα, and an anti-IL-4/IL-13. After years of use, all these agents have a good safety profile. However, cases of EGPA have been described during treatment. Other antiasthmatic drugs, such as montelukast and zafirlukast, have also been associated with this type of vasculitis. In many cases, vasculitis developed immediately after the new drug was introduced, although in others it appeared after months of therapy. At the same time, corticosteroid doses were being reduced after the switch from an anti–IL-5 agent, and patients presented symptoms that could be suggestive of EGPA before introducing the new therapy. These observations have led some authors to suggest that asthmatic patients whose disease is not controlled with standard medication had vasculitis masked by treatments such as systemic corticosteroids or anti–IL-5 therapy that was unmasked by the switch to another medication or when corticosteroid dosing was reduced. However, in other cases, no explanation for vasculitis is available.

Treatment with dupilumab can induce an increase in blood eosinophils, although this is usually transient and without clinical repercussions. The most plausible explanation for the effect is that blockade of the IL-4/13 pathway reduces migration of eosinophils and accumulation in blood by inhibiting eotaxin-3, VCAM-1, and TARC, but without simultaneously inhibiting eosinophilopoiesis in bone marrow.

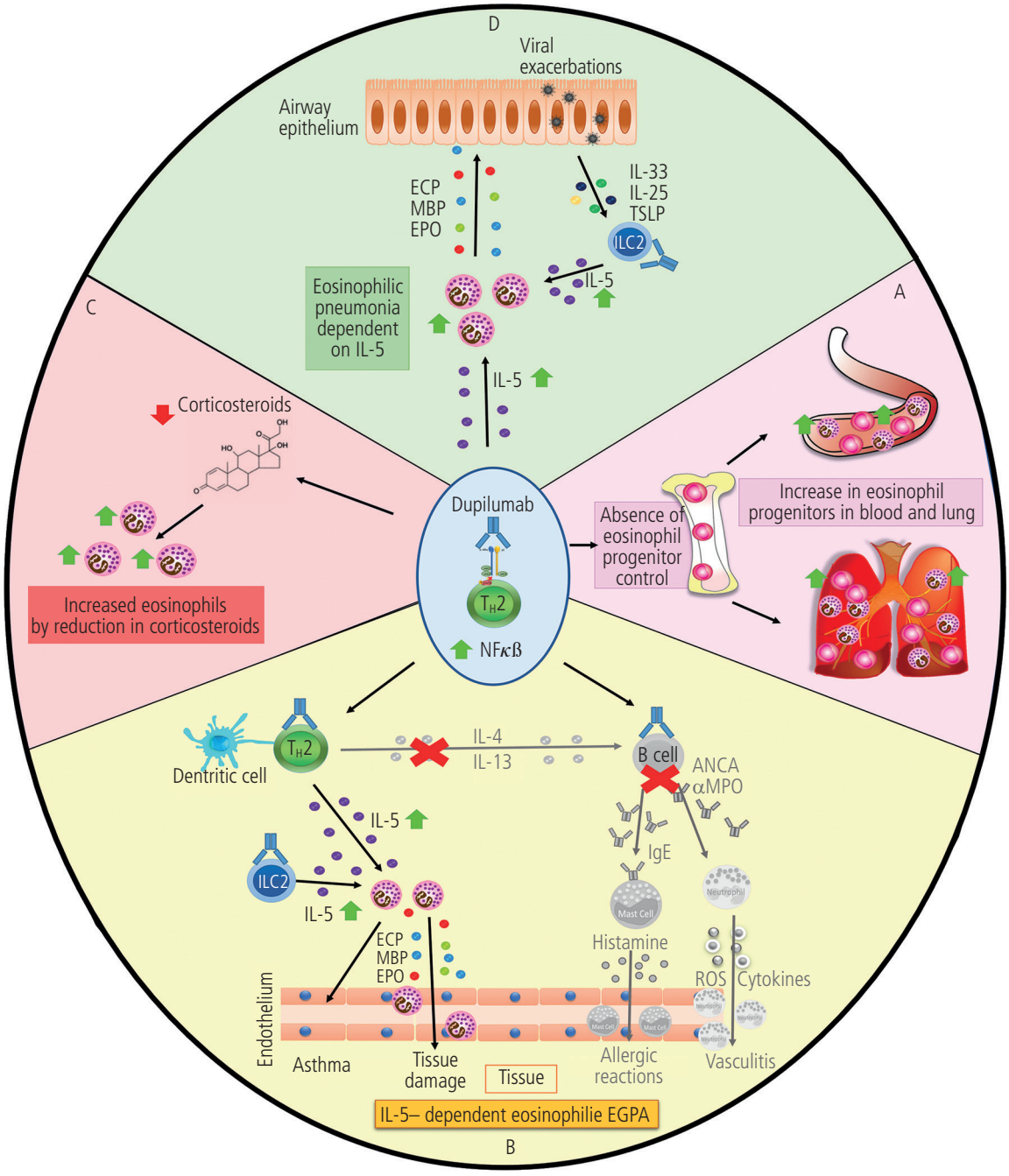

Figure 5. Pathways of eosinophilia increase as an adverse effect of dupilumab. A, Absence of eosinophil progenitor control in dupilumab treatment increases eosinophil progenitors in blood and lung. B, Eosinophilic granulomatosis with polyangiitis (EGPA) may occur after treatment with dupilumab owing to blockade of the IL-4/13 mechanisms in eosinophilia, albeit without affecting the IL-5 pathway. This induces eosinophilia, asthma, and tissue damage in an IL-5–dependent manner. C, Reducing the dosage of corticosteroids on initiation of dupilumab causes reversion of eosinophils to precorticosteroid levels. D, Accumulation and action of eosinophils in the lungs (eosinophilic pneumonia or asthma) may occur during treatment with dupilumab, as there is an increase in NF-κβ that may result from reduction in IL-13, which in turn translates into increased IL-5. Moreover, viral exacerbations induce the release of alarmins from the epithelium (IL-33, IL-25, TSLP). These molecules stimulate secretion of IL-5 by type 2 innate lymphoid cells (ILC2s), migration of eosinophils, and lung damage. IL indicates interleukin; ECP, eosinophil cationic protein; MBP, major binding protein; MPO, myeloperoxidase; T_H, type 2 helper T cell; ANCA, antineutrophil cytoplasmic antibody; ROS, reactive oxygen species.

The importance of selecting a biological treatment to target a specific molecule depends on the pathophysiology of the patient's asthma. Therefore, treatment should be personalized. Consequently, the effectiveness of the therapy selected could depend on the trigger of inflammation and the subsequent immune mechanisms involved. In this sense, it is important to take into account the presence of hypereosinophilia (>1500/μL) in asthmatics. In such cases, an anti–IL-5/IL-5R agent is preferable, as recommended in the EAACI guidelines on biologics for treatment of severe asthma [103]. Furthermore, dupilumab should not be used in severe asthma with hypereosinophilia, as this was an exclusion criterion in the pivotal clinical trials [80-82] and a recommendation in recent algorithms [5,104,105]. A similar approach should be adopted when considering changing from an anti–IL-5/5R agent to an anti–IL-4/13 agent. Close monitoring of blood eosinophils and clinical course seems justified in all these situations.

Finally, in some cases, dual therapy with anti–IL-5/5R and anti–IL4/IL-13 agents may be needed to optimize control, since both the IL-5 and the IL-4/13 pathways can simultaneously contribute to airway inflammation in patients with severe asthma. This approach may prevent serious complications, such as EGPA [74,77,78]. Otherwise, the worsening of nasal symptoms due to CRSwNP after discontinuation of dupilumab, even in patients whose therapy was switched to an anti–IL-5 agent, could reinforce the need for dual treatment.

In the near future, we may treat severe asthma with new-generation biologics, such as the antialarmins tezepelumab [106] and itepekimab [107], which act against the inflammatory cascade in the upper airway.

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Conflicts of Interest

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