Eosinophilia induced by blocking the IL-4/IL-13 pathway. Potential mechanisms and clinical outcomes

Running title: Eosinophilia after IL-4/IL-13 blockade.

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ABSTRACT

Currently, five biological drugs for uncontrolled severe asthma treatment are marketed. They all block type 2 inflammatory pathways, either by targeting IgE (omalizumab), the IL-5 pathway (mepolizumab, reslizumab, benralizumab), or the IL-4/13 pathway (dupilumab). Hypereosinophilia has been observed in between 4% and 25% of patients treated with dupilumab, being transient in most cases, but persistent cases of symptomatic hypereosinophilia consistent with eosinophil granulomatosis with polyangiitis (EGPA), eosinophilic pneumonia, eosinophilic vasculitis or sudden worsening of asthma symptoms have been described. Cases of EGPA have been described with all biologics, including anti-IL-5, and with leukotriene receptors antagonists in publications or in the Eudravigilance database. In many cases of EGPA, it appears during systemic steroids tapering or after switching from an anti-IL-5 biologic to Dupilumab, suggesting that systemic steroids or the anti IL-5 were masking the vasculitis. This review aims to substantiate the plausible mechanisms of dupilumab-induced hypereosinophilia and review symptomatic hypereosinophilia cases associated with dupilumab therapy. Blockade of the IL-4/IL-13 pathway cause a reduction of eosinophil migration and blood accumulation by inhibiting eotaxin-3, VCAM-1, and TARC without simultaneously inhibiting eosinophilopoiesis in the bone marrow. When choosing the optimal biologic, it seems necessary to consider the presence of hypereosinophilia (>1,500/mL), where an anti-IL-5/IL-5R is preferable. Also, when changing from an anti-IL-5/5R to an anti-IL-4/13R. Close monitoring of blood eosinophils and clinical evolution seems justified in these situations. Nevertheless, dual therapy with anti-IL-5/5R and anti-IL4/IL-13R may be needed for optimal control since both IL-5, and IL-4/13 pathways can simultaneously contribute to airway inflammation.

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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, si bien una minoría de pacientes presentan una hipereosinofilia persistente y acompañada de sintomatología clínica que varía desde la de granulomatosis eosinofílica con poliangitis (GEPA), a la neumonía eosinofílica, lavasculitis eosinofílica o el empeoramiento repentino de los síntomas del asma. Se han comunicado casos de GEPA con 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 EGPA 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/mL), en 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-IL-4/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 o 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 itekepimab.

Introduction

Severe uncontrolled asthma is defined as asthma that continues to present exacerbations or poor functional clinical control despite treatment with high doses of inhaled corticosteroids and another controller drug. While also ruling out 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]. There are five biological drugs for treatment to uncontrolled severe asthma. They all block type 2 inflammatory pathways, either by targeting IgE (omalizumab), the IL-5 pathway (mepolizumab, reslizumab, benralizumab), or the IL-4/13 pathway (dupilumab) [2]. All of them have been shown to be particularly effective in reducing asthma exacerbations, and dupilumab, mepolizumab, and benralizumab have shown significant steroid-sparing effect [3]. In addition, dupilumab in both phase 2 and phase 3 studies can significantly improve lung function, specifically in baseline pre-bronchodilator FEV1 [4]. Their behaviour is radically different concerning the modifications to the clinical biomarkers usually used in the phenotyping and follow-up of patients with severe asthma. Although anti-IL5 significantly decreases blood eosinophil levels and does not modify FeNO (fraction of exhaled nitric oxide) or serum IgE values, dupilumab initially increases blood eosinophils and decreases serum FeNO and IgE values. This hypereosinophilia (blood eosinophils ≥1,500/μL) has been observed in between 4% and 25% of patients treated with dupilumab. Although in many cases, it is transient, hypereosinophilia can persist for more than six months in up to 14% of these patients [4,5] Furthermore, cases of symptomatic hypereosinophilia consistent with eosinophil granulomatosis with polyangiitis (EGPA), eosinophilic pneumonia, eosinophilic vasculitis or sudden worsening of asthma symptoms have been described.

This review aims to substantiate the plausible mechanisms of dupilumab-induced hypereosinophilia and to review EGPA and other symptomatic hypereosinophilia cases presumably associated with dupilumab therapy. Finally, some recommendations will be presented to improve patient safety when changing treatment from anti-IL5 to dupilumab and...
recommendations justifying the need, in certain patients, for combined therapy with anti-IL-5 and dupilumab.

**Immuno-biological characteristics of eosinophils as disease drivers**

The main myriad of eosinophil characteristics capable of causing these cells (mal)-functioning in the pathophysiology of eosinophilic diseases is sustained in three pillars: their migratory capacity, their proinflammatory activity and their enzymatic arsenal [6].

Eosinophils are derived from bone marrow progenitors, and are matured with the synergistic action of IL-3, IL-5 and GMCSF [7]. Of these cytokines, IL-5 together with eotaxins are critical 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 cause eosinophil migration to the sites of inflammation as lung and intestine [9].

In the target tissues, eosinophils are able to secrete a great variety of proinflammatory cytokines and granule proteins that are the main cause of the disease processes. These specific granules contain basic proteins, which include MBPs (major binding protein 1 and 2); EDN (eosinophil derived neurotoxin, RNase-2); ECP (eosinophil cationic protein, RNase-3) and EPX or EPO (eosinophil peroxidase). Due to this proteins’ toxicity and enzymatic activity, these defence mechanisms of eosinophils against pathogens may present a double-edge side, damaging tissues as described in several eosinophilic diseases [10,11].

**Eosinophil role in the pathophysiology of asthma**

Asthma is a very heterogeneous disease regarding the pathways and immune mechanisms involved. Nonetheless, major features characterize all the asthmatic patients, including airway hyperresponsiveness (AHR), bronchial damage, tissue remodelling and mucus hypersecretion. All are the culprits of causing the symptoms of the disease: shortness of breath, cough, wheezing and chest pain [12]. Among asthmatic heterogeneity, one major phenotype/endotype
arises inside asthma disease, eosinophilic asthma, also labeled as type 2 asthma due to the implication of type 2 immune mechanisms, either of innate and adaptative nature [13]. In T2 asthma, type 2 innate lymphoid cells (ILC2s) might be the local lung inductors of eosinophilia due to the highest number of IL-5+ IL-13+ ILC2s in asthmatic sputum [14]. Together with the T cells, they are the main source of type 2 cytokines that drive the eosinophilia in the asthmatic lungs [14]. Previous studies have shown the a good correlation between eosinophilia with asthma severity and the number of exacerbations [15]. Arrived at the point, severe eosinophilic asthma has been defined as a unique entity aiming a more specific therapeutic approach 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 has depicted 83% of the severe asthmatics as characterized by an eosinophilic phenotype [17].

Recruitment of eosinophils to the lungs is mediated mainly by chemoattractants, of which eotaxins are the most active, including CCL11, CCL24 and CCL26 as previously stated [18]. Among Type 2 cytokines IL-5 is the main cytokine capable of inducing maturation, proliferation, survival, activation adhesion and migration, favoring the interactions of eosinophils with peristin [19]. This matricellular protein is involved in eosinophils’ transmigration and trafficking toward the bronchi [19]. Moreover, there are other numerous molecules with the capacity to induce eosinophil migration, such as 5-Oxo-eicosatetraenoic acid (5-KETE), leukotrienes (LTD4, LTC4 and LTE4) and prostaglandins (DP2/CRTTH2) [20–22]. Eosinophils need to slide from the vessels to the lung tissue, and in this process, adhesion molecules are key factors, with P-selectin and integrin VLA-4 binding to their molecular counterparts in endothelial cells and facilitating the rolling, activation and extravasation to the tissue that is characteristic of these granulocytes [23].

Finally, tissue damage and remodelling are the most prominent asthma features developed by the eosinophil population direct effect once in the locus of inflammation. Eosinophilic granule proteins have great cytotoxic capacity due to their nuclease activity. ECP is a ribonuclease
capable of inducing epithelial and smooth muscle cell death and tissue remodelling through collagen deposition, increasing fibroblasts’ activity [24]. Similar effects have been described for MBP, which is secreted though cellular vesicles and has a deep connection with asthma cytotoxicity [25]. At the same time, EDN has been shown to enhance airway remodelling in chronic eosinophilic rhinosinusitis through MMP-9 pathway dysregulation and also has potential as a disease marker for asthma monitoring [26,27]. When it comes to the presence of ARH, this disease mechanism is driven by the eosinophil enzymes such as MBP, which cause hyperresponsiveness directly through their interaction with the airway wall and the epithelium [28], but also by indirect activation of mast cells and histamine release [29]. Moreover, regarding fibrosis, the main event in remodelling, eosinophils release TGF-β, which has been described as an inductor of fibroblast proliferation and collagen deposition, and inducing inflammation, showing how eosinophils can induce tissue remodelling through multiple pathways [30].

**Eosinophils as center of EGPA biology**

Eosinophil granulomatosis with polyangiitis (EGPA) is a rare multisystemic disease characterized by eosinophilic inflammation that causes systemic small vessel vasculitis. This disease was formerly called Churg-Strauss syndrome and is developed in subjects with asthma and peripheral-tissular eosinophilia [31]. Some patients develop specific antibodies against myeloperoxidase (MPO), called antineutrophil cytoplasm antibodies (ANCA) [32]. Interestingly, studies have shown that the positive ANCA patients had increased renal, skin and neuronal involvement [33]. In contrast, the ANCA negative patients present more cardiovascular and abdominal affectation, being cardiac involvement one of the most important predictors of the worst outcome [34] (Figure 1). This classification into two possible phenotypes has been supported by genetic background, showing that ANCA positive EGPA is more an eosinophilic autoimmune disease with vasculitis and HLA-DQ associated [35]. At the same
time, ANCA negative EGPA seems to be related to GP33 and IL5/IRF1 (interferon regulatory factor 1) and related to an origination at the mucosal barrier [35].

Despite the existence of two phenotypes, eosinophilia is common to both of them, and indeed, EGPA is widely described as an eosinophilic disorder that shares with other diseases a radicalized polarisation of the immune responses towards the type 2 axis by presence of cytokines as IL-5, IL-4, IL-10 and STAT5A [36]. It is worth noticing that EGPA seems to be genetically predisposed, which may account for the type 2 immune polarisation and the eosinophilia. It has been described that IRF1/IL5 genetic variants have been associated with the development of eosinophilic EGPA and with severe asthma as previously mentioned [35]. Besides, variants in IL10 gene promoter have been related with EGPA, specifically, the IL10.2 haplotype [37]. In contrast, variations in the HLA-DR gene including HLA-DRB1*04 and *07 or HLA-DRB4 have been studied as key genetic determinants for this disease due to their relation 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 of these gene variability’s with reduced expression of proapoptotic genes as BCL2L13, CASP2 and CARD4 in EGPA [39].

Regarding the EGPA disease pathophysiology model, type 2 responses are indeed the key element that causes eosinophilia and disease symptoms. Among the type 2 cytokines, IL-5 is the most prominent due to its activity promoting eosinophil maturation, survival and proliferation which are the key features required for developing and maintaining eosinophilia [19]. The main source of IL-5 in the biology of EGPA are CD4+ T lymphocytes. However, they are not the only secretors of this cytokine, as ILC2s can secrete IL-5. These innate cells have been described as increased in peripheral blood in active EGPA, also releasing IL-33 which accounts for the active vasculitis [40]. Other cytokines such as IL-10, TNFα and IFNγ are also important for the vasculitis phase of EGPA [41]. Interestingly, IL-4 and IL-13 production by T cells is also 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, being the marker of disease activity [43].

In EGPA, eosinophils display different activity compared to the usual, as seen by the increase of CD69, CD25 (related to apoptosis); and in IL-25 secretion, being this cytokine related to a positive feedback-loop in type 2 responses and eosinophilia [39,44]. When the eosinophil arrives at the tissues, as in asthma, it releases several cytotoxic molecules, including ECP, the main enzyme related to cardiotoxicity through inhibition of cardiomyocytes membrane permeability and mitochondrial respiration [45]. Eosinophils also cause thrombosis by thrombin release, secretion of extracellular traps (EETs), ECP, MPB and oxygen reactive species (ROS) [46–48]. As for the neuronal damage, the mechanism is not completely clear, but seems associated to necrotizing vasculitis, where eosinophil-derived neurotoxin (EDN) might be of foremost importance [49].

The key role of IL-5 in this disease has open the field for the use of biologicals treating the IL-5/IL-5RA axis, such as in asthma, with reports of the benefit of mepolizumab for EGPA improvement and prednisone treatment reduction [50].

**Dupilumab molecular mechanism of action**

Dupilumab consists on a monoclonal IgG4 human antibody that recognises the IL-4Rα subunit of the IL-4/13 receptor [51]. The molecular basis of the IL-4/13 receptor comes through the main functionality of IL-4Rα subunit, which is able to bind with other subunits of the receptor and translate intracellularly signals upon recognition of the ligands IL-4 or IL-13 [52]. For specific IL-4 recognition, the IL-4Rα subunit pairs with the γc chain, conforming to the named type I IL-4R [53]. Conversely, for IL-13 binding, IL-4Rα subunit interacts with IL-13Rα1, forming a high affinity IL-13 receptor that is also able to recognize IL-4, namely, the type II IL-4R [54]. The binding of IL-4 to IL-4Rα and IL-13 to IL-13Rα1 is capable of inducing a
conformational triggering which causes the recruitment of the second part of the receptor, the other subunit is recruited, and the signalling starts [55] (Figure 2).

After recruitment of the receptor subunits the Janus family protein kinases associated with the particular 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 IL-4Rα, signal transducer and activator of transcription 6 (STAT6) recruitment and phosphorylation occur by the action of JAK-1. And then, STAT6 dimerises and goes into the nucleus and binds the DNA [56]. On the other hand, IL-13 binding to IL-13Rα1 phosphorylates STAT3 through TYK-2 phosphorylation. Both transcription factors (STAT6 and STAT3) bind the IL-4 and IL-13 regulated gene promoters which are causative of IgE synthesis, Th2 polarisation and mucus secretion [56].

IL-4R is very important in Th2 cell differentiation, mainly by GATA-3 activation and production of type 2 cytokines [57]. Moreover, IL-4R is deeply involved in IL-10 immunoregulation, shifting the dominance of Th2 responses that are characteristic of the signalling by IL-4 and IL-13 [58]. This polarisation to type 2 immune responses does not only count on T lymphocytes, as ILC2s are also a major source of IL-4 and IL-13 via innate immunity [59].

Due to 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 T2 diseases such as allergies and asthma [60]. As dupilumab can bind to IL-4Rα it can shut down the signalling through IL-4 and IL-13, inhibiting the type 2 inflammation [60]. The specific mechanism of inhibition can act upon both types I and type II IL-4R. Regarding the type I receptor blockade, when dupilumab binds the IL-4Rα, it impedes both the binding of IL-4 to its receptor and the recruitment of the γc subunit [61] (Figure 2). This same event is associated with the recruitment of IL-4Rα to IL-13Rα1 subunit, thus inhibiting the type II IL-4R signalling that is seen in allergic diseases[62]. Overall, blocking both type I and II IL-4R signalling provide a key advantage in disease 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 II immune responses, such as those where allergens act as 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 their common receptor IL-4/13 as previously mentioned. However, as they can bind to different subunits of the receptor, they are able to perform both similar and differential roles, being IL-4 critical in the antibody isotype switch of B cells to produce allergen-specific IgE [64]. The main sources of IL-4 consist in the conventional Th2 CD4+ T cells, and basophils, being IL-4 important for eosinophils migration towards eotaxin-1 [65]. Regarding IL-13, this cytokine is secreted principally by tissue Th2 cells and ILC2s [66] and has a prominent role in the development of airway hyperresponsiveness, mucus synthesis, smooth muscle alterations and fibrosis induced by TGF-β, so this cytokine is able to induce many of the asthmatic symptoms through mechanism that does not seem so dependent on IgE and eosinophils [67] (Figure 3).

The resourcefulness of a human biological antibody targeting the IL-4/13 pathway, such as dupilumab, is of noticeable interest in biomedicine due to the wide variety of pathologies involved this molecular axis [68]. Given the nature and possibilities of blocking a critical pathway of T2 immune responses, the indications for this treatment are constantly increasing. It is being explored as a treatment for several diseases, including skin pathologies such as 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 to treat severe asthma and chronic rhinosinusitis with nasal polyps (CRSwNP). Furthermore, it is in the development phase for allergic bronchopulmonary aspergillosis, allergic rhinitis, chronic eosinophilic pneumonia, and other type 2 diseases that affect other organs such as gastrointestinal disorders with promising results in eosinophilic esophagitis and food allergies [61,69].
Symptomatic hypereosinophilia induced by dupilumab: Description of case reports and EGPA cases described in clinical trials

As described for most clinical trials of severe asthma, CRSwNP and atopic dermatitis, a transient increase in blood eosinophil counts and clinically irrelevant was observed after treatment with dupilumab. However, the real-life practice has spotlighted these “not so rare” cases that must be considered, as some of them can lead to an eosinophilic disease with clinical manifestations. Isolated cases of chronic eosinophilic pneumonia [70–72], eosinophilic gastritis [73] and eosinophilic vasculitis [74] have been described associated with dupilumab treatments in patients with asthma. In these cases of EGPA described in the literature, dupilumab treatment could be considered well as directly related to or unmasking a previous vasculitis by discontinuation or corticosteroid reduction in asthma patients, as described by Ikeda et al. and Murag et al. [75,76]. Importantly, cases reported by Eger et al. [77] have highlighted the importance of considering the blood eosinophil numbers in asthmatics who will be changed from an anti-IL-5/5Ra to an anti-IL-4/13. Hypereosinophilia with systemic clinical manifestations may be developed if the treatment does not focus on the correct control of blood eosinophil levels as anti-IL-5/5Ra or corticosteroids do [77–79]. These observations support the indication of dual therapy with anti IL5 and dupilumab in this clinical scenario as suggested by Eger et al. [77] and supported by the evolution of the case of Descamps et al. [74]. In Table 1 clinical and demographic data of all this case reports are summarized.

EGPA from Dupilumab asthma and CRSwNP pivotal studies.

Patients with symptomatic eosinophilia from pivotal asthma clinical trials are summarized in Table 2. In Liberty Asthma Quest [80], 4.1% of the patients (52 active patients) presented eosinophilia, 22 had more than 3000/μL of eosinophils. In 8 of these cases, treatment with dupilumab was definitively withdrawn. In four of these patients, eosinophilia was symptomatic. In the Venture study [81] that analyzed the oral corticosteroid-sparing effect of dupilumab and therefore, all patients had severe corticoid-dependent asthma (210 included, of which 103 were
active), 13% of patients had hypereosinophilia greater than 3000/mL, none of them symptomatic. However, in the open-label extension study (Tranverse study) [82] of the Venture study, 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. A total of 3 patients out of 2062 patients presented clinical manifestations consistent with EGPA (Table 2). Finally, in the pivotal dupilumab study in CRSwNP polyposis, 50% of patients included had comorbid asthma. 3 cases with uncontrolled asthma presented symptoms also consistent with EGPA [83]. In this study, one patient on placebo treatment also developed EGPA. Of note, many of these patients suffered an intense relapse of their nasal symptoms after discontinuation of dupilumab, including those that were treated with anti-IL-5.

In these cases of EGPA, dupilumab treatment could be related well as directly related or unmasking a previous vasculitis by discontinuation and or corticosteroid reduction in asthma patients [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], from the FDA pharmacovigilance database, in 2010, in the era before biologics treatment of severe asthma, 90% of drug-related EGPA cases (181 of 190), were implicated to montelukast or another leukotriene antagonist (LTRA) as zafirlukast. The time between the introduction of treatment and the development of EGPA symptoms in most patients ranged from 3 to 180 days. On the other hand, in only 34% of the patients, the onset of symptoms was also temporarily linked to a reduction or withdrawal of systemic corticosteroids suggesting a preexisting EGPA before introducing LTRA. In a review of the cases described in the literature, Nathani et al. [85] also describe that in 37% of patients, EGPA is related to the withdrawal of corticosteroids. In
the European study of control cases, the risk of developing EGPA with montelukast has been established at 6.7 (CI 1.3-34.1) [86].

At the time of biologics and using the Novartis pharmacovigilance database Wechsler et al. [87] notified, a total of 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 six of the 13 patients (46%), EGPA symptoms appeared just after tapering 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 therapy withdrawal with corticosteroids or in patients who delay specific therapy in favor of other medications. In their view, omalizumab treatment may unmask EGPA due to the weaning of corticosteroids in some asthma patients or may delay corticosteroid treatment allowing for 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, arthralgias, respiratory decompensation with pulmonary infiltrates and skin lesions with necrosis. In both cases, the authors relate the appearance of EGPA with the withdrawal of oral corticosteroids secondary to the introduction of benralizumab [88].

**Analysis of the Eudravigilance pharmacovigilance public database**

Eudravigilance, the electronic pharmacovigilance database from 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 III summarizes the total number of eosinophilic granulomatosis with polyangiitis (EGPA) cases 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 montelukast treatment. A total of 61 EGPA were reported during treatment with dupilumab. This report includes the cases reported
in the literature (Table I). Indications for prescribing dupilumab were severe asthma, or nasal polyposis in all but two cases suffered from atopic dermatitis. Among the 61 patients that developed EGPA, 27 patients recovered or were recovering at the time of case notification, one patient recovered with sequelae, and seven 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 drug withdrawal in 33 of 42 patients in which this outcome was notified. In one of the nine patients in which dupilumab therapy was continued, an anti-IL-5 was associated.

Compared with other biologic treatments, the rate of EGPA cases and the total number of notifications reported, benralizumab has the higher rate (1.32), followed by mepolizumab (0.80) (Table III). It must keep in mind the number of doses administered for each biologic, the time in the market and the number of indications.

**Eosinophil increases by dupilumab, the hypothesis so far**

The presence of eosinophilia after dupilumab treatment was firstly described in pivotal clinical trials. The first hypothesis of why eosinophilia occurred was proposed by Castro et al and Rabe et al [80,81]. The transient blood eosinophilia observed in these clinical trials could be related to the inhibition of eosinophil migration and activation caused by the inhibition of the IL-4/13 axis [80,81]. They argued that the blockade of IL-4 and IL-13 signalling mediates a reduction in eotaxin-3 levels (which they described in serum) and a decrease in vascular-cell adhesion molecule 1 (VCAM-1) that inhibit eosinophils migration to the 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, signalling inhibition trough the IL-4/13R may cause a reduction of eosinophil migration and blood accumulation, in a similar manner as for eotaxin-3 and VCAM-1 [91].
Another possible mechanism of blood accumulation is due to the action of Thymus and activation-regulated chemokine (TARC/CCL17), a type 2 cytokine that binds CCR4 and is involved in Th2 cells trafficking in eosinophilic disorders [92]. TARC is synthesised by immune cells after IL-4 stimulation after STAT6 induction, and it has been debated that this protein might also be involved in eosinophil trafficking [93]. Increased TARC levels have been described in bronchoalveolar lavage (BAL) of asthmatics and in EGPA lesions with eosinophilic infiltrations and CRTH2⁺ T cells [94,95].

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

Basic knockout mice models might have an answer for this subject as IL-13⁻/⁻ mice develop blood and airway eosinophilia, and while IL-4 antibody is able to reduce the lung eosinophil infiltration, IL-13⁻/⁻ mice treated with ovoalbumin and anti-IL-4 neutralizing antibody have more eosinophilic lung infiltrates than wild type mice, being anti-IL-5 neutralising antibody the only mechanism capable of completely reducing airway eosinophils. The authors of this study propose that in the low levels of IL-13 may result in the increase of NF-κβ, which in turn increases IL-5 synthesis in a mechanism independent of IL-4 regulation (Figure 5D), which might be similar as observed in nonallergic asthmatics with high levels of IL-5 and eosinophils despite low IL-4 [96]. Regarding the source of IL-4/13 in airway eosinophilia, it has been shown that mice models with CD4⁺ lymphocytes deficient in IL-4 and IL-13 are able to induce lung eosinophilia during allergic inflammation [97], which marks the importance of ILC2s and other cells as sources of these cytokines [98]. Furthermore, ILC2s synthesize IL-4 and IL-13, but they are also major producers of IL-5. They have no requirement of a previous IL-4 and IL-
13 CD4+ dependent adaptative immune phase response. So 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), something that could be similar to the eosinophilia mechanisms described after anti-IL-2 therapy [99]. In these cases, the trigger of the innate immunity that activates ILC2s, such as viral infections in the airway epithelium, can cause ILC2s to release type 2 cytokines including IL-4, IL-13 and IL-5, causing asthma exacerbations where IL-4/13 but also IL-5 pathways should be taken care of (Figure 5D) [100].

Moreover, the eosinophil progenitor migration should be also taken into account. Since it has been described that IL-4 and IL-13 can control the priming and migration of hematopoietic progenitor cells (HPC) derived from the 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 the migration of progenitors who have already committed to the eosinophil lineage (CD34+CD45+IL5Rα+ cells) and of mature eosinophils to the SDF-1α chemoattractant (Figure 5C) [101]. Indeed, eosinophil progenitors that are able to migrate and which are not controlled by any treatment, such as a corticosteroid or an anti-IL-5 therapy, will be able to accumulate both in the blood and in the lungs of patients, and therefore, with the action of IL-5 will eventually evolve into mature eosinophils (in-situ eosinophilopoiesis), who will be able to produce eosinophilic vasculitis, EGPA and asthmatic symptoms when releasing their enzymatic granule content (Figure 5C) [102].

CONCLUSIONS AND RECOMMENDATIONS

Eosinophils play a critical pathogenic role in many cases of asthma, and in fact, several therapies have been targeted to this cell to ameliorate symptoms.

We currently have several biological drugs to treat severe uncontrolled T2 asthma: anti-IgE, anti-IL-5, anti-IL-5Ra and an anti-IL-4/IL-13. After years of use, all have shown a good safety
profile. However, cases of EGPA have been described during its use. Other antiasthmatic drugs have also been associated with developing this type of vasculitis, such as montelukast and zafirlukast. The vasculitis developed immediately after introducing the new drug in many cases, but in others appeared after months of therapy. At the same time, the steroid doses were being reduced after switching from an anti-IL-5, the patients presented symptoms that could be suggestive of EGPA before introducing the new therapy. These facts have led some authors to suggest that some asthmatic patients, generally not controlled with the usual medication, had vasculitis masked by treatments such as systemic steroids or anti-IL-5 therapy that appears when changing to another medication and/or corticosteroids are reduced. However, in other cases, it has not been explained based on the previous considerations.

It is well known that treatment with dupilumab can induce an increase in blood eosinophils, which is usually transient and without clinical repercussions. The most plausible explanation for this effect is that blockade of the IL-4/IL-13 pathway may cause a reduction of eosinophil migration and blood accumulation by inhibiting eotaxin-3, VCAM-1, and TARC but without simultaneously inhibiting eosinophilopoiesis in the bone marrow.

The importance of selecting one biological treatment targeting a specific molecule relies on the pathophysiology of the subject’s asthma, and therefore, personalized medicine approaches should be applied. This fact implies that the effectiveness of the selected therapy could depend on the trigger of the inflammation and the subsequent immune mechanisms involved. In this sense, it seems necessary to highlight the importance of taking into account the presence of hypereosinophilia (>1,500/mL) in asthmatics; in this case, an anti-IL-5/IL-5R is preferable, as depicted in the European guidelines elaborated by the EAACI regarding biologicals treatment for severe asthma [103]. Furthermore in this scenario of severe asthma with hypereosinophilia (>1,500/mL), dupilumab should not be used as this was an exclusion criteria in the pivotal clinical trials [80–82], and recommended in recent algorithms [5,104,105]. However, also when considering changing from an anti-IL-5/5R to an anti-IL-4/13. Close monitoring of blood eosinophils and clinical evolutions seems justified in all these situations.
Finally, in some cases, dual therapy with anti-IL-5/R and anti IL-4/IL-13 may be needed for optimal control since both IL-5 and IL-4/13 pathways can simultaneously contribute to airway inflammation in patients with severe asthma. This approach may avoid some serious complications, such as the development of EGPA [74,77,78]. Otherwise, the worsening of nasal symptoms due to CRSwNP described after discontinuation of dupilumab, including those that were switched to an anti-IL-5, could reinforce the use of dual treatment.

In a near future, a new generation of biological therapies for severe asthma acting at an upper level of the inflammatory cascade, as in the case of anti-alarmins such as tezepelumab [106] or itekepimab [107], could be used.

**Conflicts of interest**

JMO: reports grants and personal fees from EVERSENS, grants form Sanofi, personal fees from GSK, personal fees from MUNDIPHARMA, Astra ZENECa, ALK, outside the submitted work. JMO is Editor in Chief of the J Investig Allergol Clin Immunol.

JS: reports grants and personal fees from SANOFI, personal fees from GSK, personal fees from NOVARTIS, MUNDIPHARMA, FAES FARMA, THERMOFISHER, LETI, ALK, outside the submitted work. JS is Associate Editor of the J Investig Allergol Clin Immunol.

JMRM: reports having been paid lecture fees by Astra Zeneca

VdP: reports having served as a consultant to Astra Zeneca and GSK; having been paid lecture fees by Astra Zeneca and GSK. VdP is Associate Editor of the J Investig Allergol Clin Immunol

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## TABLES

Table 1. Case reports of symptomatic eosinophilia, including eosinophil granulomatosis with polyangiitis (EGPA), described in the literature associated with dupilumab.

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>CDA</th>
<th>Biological therapy</th>
<th>Days of therapy with dupilumab</th>
<th>Baseline eosinophils (cel/µL)</th>
<th>Peak eos (cel/µL or %)</th>
<th>Acute clinical manifestation related to eosinophilia</th>
<th>Corrective treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>59</td>
<td>F</td>
<td>CRS, severe asthma</td>
<td>+</td>
<td>Benralizumab switch to dupilumab</td>
<td>NR</td>
<td>100</td>
<td>5080</td>
<td>Asthma exacerbation, sinusitis, pulmonary infiltrates, neurologic, cardiovascular, fever, myalgia, EGPA</td>
<td>Prednisone</td>
<td>[77]</td>
</tr>
</tbody>
</table>

<p>| 35  | M   | CRS, severe asthma | +   | Reslizumab switch to dupilumab  | NR                            | 500                          | 1050                        | Asthma exacerbation, sinusitis, pulmonary infiltrates, neurologic, cardiovascular, fever, myalgia, EGPA                       | Prednisone, Reslizumab | [77]      |</p>
<table>
<thead>
<tr>
<th>Age</th>
<th>Gender</th>
<th>Diagnosis</th>
<th>Mepolizumab to Dupilumab</th>
<th>Dose</th>
<th>Other Meds</th>
<th>Adverse Events</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>63</td>
<td>F</td>
<td>Severe asthma</td>
<td>NR</td>
<td>60</td>
<td>3949</td>
<td>Pulmonary infiltrates, eosinophilic alveolitis, neurologic, EGPA</td>
<td>Prednisone, Mepolizumab 300 mg/month [77]</td>
</tr>
<tr>
<td>50</td>
<td>M</td>
<td>Polyposis, severe asthma</td>
<td>Dupilumab</td>
<td>120</td>
<td>3950</td>
<td>Asthma exacerbation, pulmonary infiltrates, cardiovascular, EGPA</td>
<td>Prednisone, Benralizumab [78]</td>
</tr>
<tr>
<td>24</td>
<td>F</td>
<td>Polyposis, EGPA ANCA-</td>
<td>Benralizumab</td>
<td>160</td>
<td>8100</td>
<td>Asthma exacerbation</td>
<td>Prednisone, benralizumab, dupilumab [78]</td>
</tr>
<tr>
<td>56</td>
<td>F</td>
<td>Polyposis, severe asthma</td>
<td>Dupilumab</td>
<td>70</td>
<td>2800</td>
<td>Pulmonary infiltrates, eosinophilic alveolitis, fever</td>
<td>Prednisone [70]</td>
</tr>
<tr>
<td>58</td>
<td>M</td>
<td>Severe asthma</td>
<td>Dupilumab</td>
<td>42</td>
<td>3200</td>
<td>Asthma exacerbation</td>
<td>Prednisone [71]</td>
</tr>
<tr>
<td>56</td>
<td>M</td>
<td>Severe asthma, nasal polyposis</td>
<td>Dupilumab</td>
<td>730</td>
<td>NR</td>
<td>Fever, cutaneous vasculitis, EGPA</td>
<td>Prednisone [75]</td>
</tr>
<tr>
<td>55</td>
<td>F</td>
<td>Asthma, atopic</td>
<td>Dupilumab</td>
<td>.NR</td>
<td>1700</td>
<td>Fever, bilateral pulmonary infiltrates</td>
<td>Prednisone [72]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>---</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>41</strong></td>
<td><strong>M</strong></td>
<td>Severe asthma, atopic dermatitis, food allergy</td>
<td>+</td>
<td>Dupilumab</td>
<td>180</td>
<td>100</td>
<td>1250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe asthma, bullous pemfigoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>63</strong></td>
<td><strong>M</strong></td>
<td>Severe asthma</td>
<td>NR</td>
<td>Mepolizumab switch to dupilumab</td>
<td>180</td>
<td>NR</td>
<td>54%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>77</strong></td>
<td><strong>M</strong></td>
<td>Severe asthma</td>
<td>-</td>
<td>Dupilumab</td>
<td>16</td>
<td>152</td>
<td>860</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe asthma, nasal polyposis</td>
<td>+</td>
<td>Dupilumab</td>
<td>150</td>
<td>2000</td>
<td>11500</td>
</tr>
</tbody>
</table>

Footnote: ACD: Asthma Cortico Dependent. CRS: chronic rhinosinusitis, NR: not reported, EGPA: Eosinophil granulomatosis with polyangiitis
Table 2. Cases of eosinophil granulomatosis with polyangiitis (EGPA) reported in pivotal trials.

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>CDA</th>
<th>Biological therapy</th>
<th>Days of therapy with dupilumab</th>
<th>Baseline eosinophils (cel/μL)</th>
<th>Peak eosinophils (cel/μL)</th>
<th>Acute clinical manifestation related to eosinophilia</th>
<th>Corrective treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>F</td>
<td>Allergic rhinitis, severe asthma</td>
<td>-</td>
<td>Dupilumab</td>
<td>445</td>
<td>NR</td>
<td>7680</td>
<td>Asthma exacerbation, pulmonary infiltrates, myalgia, fever</td>
<td>Rituximab, Azathioprine, prednisone</td>
<td>[82]</td>
</tr>
<tr>
<td>30</td>
<td>F</td>
<td>CRS, severe asthma</td>
<td>-</td>
<td>Dupilumab</td>
<td>499</td>
<td>1390</td>
<td>11000</td>
<td>Asthma exacerbation, neuropathy, gastritis</td>
<td>No</td>
<td>[82]</td>
</tr>
<tr>
<td>49</td>
<td>F</td>
<td>Polyposis, severe asthma</td>
<td>-</td>
<td>Dupilumab</td>
<td>406</td>
<td>NR</td>
<td>14700</td>
<td>Asthma exacerbation, sinusitis, ANCA+</td>
<td>Prednisone</td>
<td>[82]</td>
</tr>
<tr>
<td>38</td>
<td>F</td>
<td>Polyposis, severe asthma</td>
<td>+</td>
<td>Dupilumab</td>
<td>318</td>
<td>1800</td>
<td>11400</td>
<td>Asthma exacerbation, pulmonary infiltrates, acute sinusitis</td>
<td>Prednisone</td>
<td>[82]</td>
</tr>
<tr>
<td>44</td>
<td>F</td>
<td>Severe asthma</td>
<td>+</td>
<td>Dupilumab</td>
<td>172</td>
<td>150</td>
<td>8500</td>
<td>Asthma exacerbation, pulmonary infiltrates, neurologic, cutaneous vasculitis</td>
<td>Azathioprine, prednisone</td>
<td>[82]</td>
</tr>
<tr>
<td>50</td>
<td>M</td>
<td>Severe asthma, polyposis</td>
<td>-</td>
<td>Dupilumab</td>
<td>16</td>
<td>570</td>
<td>10280</td>
<td>Asthma exacerbation, myositis, fever</td>
<td>Prednisone</td>
<td>[80]</td>
</tr>
<tr>
<td>56</td>
<td>M</td>
<td>Severe asthma</td>
<td>-</td>
<td>Dupilumab</td>
<td>118</td>
<td>660</td>
<td>2700</td>
<td>Chronic Eosinophilic pneumonia</td>
<td>Prednisone</td>
<td>[80]</td>
</tr>
<tr>
<td>28</td>
<td>F</td>
<td>Severe asthma</td>
<td>-</td>
<td>Dupilumab</td>
<td>114</td>
<td>690</td>
<td>8650</td>
<td>Asthma exacerbation, fever myalgia</td>
<td>Prednisone</td>
<td>[80]</td>
</tr>
<tr>
<td>52</td>
<td>F</td>
<td>Severe asthma</td>
<td>-</td>
<td>Dupilumab</td>
<td>127</td>
<td>1290</td>
<td>4920</td>
<td>Pulmonary infiltrates</td>
<td>Prednisone</td>
<td>[80]</td>
</tr>
<tr>
<td>NR</td>
<td>NR</td>
<td>Uncontrolled asthma</td>
<td>-</td>
<td>Dupilumab</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Bilateral Pulmonary infiltrates, polyneuropathie, EGPA</td>
<td>Prednisone</td>
<td>[83]</td>
</tr>
<tr>
<td>NR</td>
<td>NR</td>
<td>Uncontrolled asthma</td>
<td>-</td>
<td>Dupilumab</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Fever, asthma exacerbation, artralgias</td>
<td>Prednisone</td>
<td>[83]</td>
</tr>
<tr>
<td>NR</td>
<td>NR</td>
<td>Uncontrolled asthma</td>
<td>-</td>
<td>Dupilumab</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Bilateral Pulmonary infiltrates, polyneuropathie EGPA</td>
<td>Prednisone</td>
<td>[83]</td>
</tr>
</tbody>
</table>

Footnote: CDA: Cortico Dependent Asthma, CRS: chronic rhinosinusitis; NR: not reported, EGPA: Eosinophil granulomatosis with polyangiitis
Table 3. Eosinophil granulomatosis with polyangiitis (EGPA) cases reported at the Eudravigilance (March 2022) pharmacovigilance database.

<table>
<thead>
<tr>
<th>Suspect Drug</th>
<th>Total number of EGPA cases</th>
<th>Total number of all spontaneous case reports in Eudravigilance</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montelukast</td>
<td>776</td>
<td>14,090</td>
<td>5.51</td>
</tr>
<tr>
<td>Benralizumab</td>
<td>36</td>
<td>2,720</td>
<td>1.32</td>
</tr>
<tr>
<td>Omalizumab</td>
<td>145</td>
<td>19,699</td>
<td>0.74</td>
</tr>
<tr>
<td>Mepolizumab</td>
<td>46</td>
<td>5,725</td>
<td>0.80</td>
</tr>
<tr>
<td>Dupilumab</td>
<td>61</td>
<td>13,151</td>
<td>0.46</td>
</tr>
<tr>
<td>Reslizumab</td>
<td>0</td>
<td>184</td>
<td>N/A</td>
</tr>
</tbody>
</table>

EGPA: Eosinophil granulomatosis with polyangiitis, N/A: Non-available
FIGURE LEGENDS

Figure 1. Description of the pathophysiology of eosinophilic granulomatosis with polyangiitis (EGPA). EGPA is characterized by two different mechanisms of disease. In the first (ANCA negative), Eosinophils driven by type 2 cytokines as IL-4/13 and IL-5 migrate to the tissues where they release cytotoxic proteins causing mainly hearth and vascular damage, while also induce asthma in the lungs. In the ANCA positive mechanism there is existence of anti-mieloperoxidase (MPO) antibodies produced by B lymphocytes stimulated with IL-4 and IL-13. These antibodies are going to stimulate neutrophils, cells that release enzymes and oxygen reactive species (ROS) that cause vasculitis, kidney disease and neuropathy, accompanied by IgE-mastocyte binding and allergic reactions.
Figure 2. Mechanism of dupilumab blockade of signal transduction carried out by IL-4/13R (receptor). There are two IL-4/13 receptor types, namely the type I and the type II. Type I IL-4R consists in the IL-4Rα and the γc subunits and recognizes IL-4, while type II 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, activating a pathway that results in activation of specific-gene expression at DNA level. Dupilumab is able to block the receptor signalling by three mechanisms as depicted in the figure, on the left, when dupilumab binds IL-4Rα impedes IL-4 binding and signal transduction. Also, binding of dupilumab to IL-4Rα inhibits the coupling of IL-4Rα chain with γc subunit, and blocks signal transduction (in the middle). Finally, dupilumab binds the IL-4Rα and blocks it form coupling to the IL-13Rα1 subunits after IL-13 binding, and consequently suppressing intracellular signalling (in the right).
Figure 3. Roles of IL-4 and IL-13 in asthma pathophysiology. IL-4 and IL-13 main sources 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 that in turn recognizes an allergen and binds mast cell causing histamine release and smooth muscle contraction. IL-4 and IL-13 themselves are also capable of increasing muscle contraction. IL-4 and IL-13 are inducers of eosinophil migration to the lungs, where these cells release inflammatory mediators that combined with IL-4/13 induce airway remodeling (increase of mucus deposition and subepithelial fibrosis).
Figure 4. Possible mechanism of eosinophil sequestration in blood after dupilumab treatment. Dupilumab binds to IL-4/13R in Th2 lymphocytes, type 2 innate lymphoid cells (ILC2s) and B lymphocytes. Due to STAT6 transcription factor inhibition by dupilumab several genes are downregulated including TARC/CCL17 and eotaxins (1-3), molecules that induce eosinophil migration. With migratory molecules reduction, and VCAM-1 diminishment (an adhesion molecule required for eosinophil extravasation), eosinophils do not leave the bloodstream and release their content inside the vessels, inducing endothelial damage, thrombosis and atherosclerosis.
Figure 5. Pathways of eosinophilia increase as a dupilumab treatment adverse effect. A) Absence of eosinophil progenitor control in dupilumab treatment increases eosinophil progenitors in blood and lungs. B) Eosinophilic granulomatosis with polyangiitis (EGPA) occurs in some cases after dupilumab due to blockade of IL-4/13 mechanisms of eosinophilia, but without affecting the IL-5 pathway, which induces eosinophilia, asthma and tissue damage in an IL-5 dependent manner. C) Reduction of corticosteroids dosage after dupilumab treatment initiation causes reversion of eosinophils to pre-corticosteroid levels. D) Eosinophilic accumulation and action in the lungs (eosinophilic pneumonia or asthma) may occur while treatment with dupilumab, as there is an increase of NFκβ that might come from IL-13 reduction, which in turn traduces into IL-5 increase. Moreover, viral exacerbations induce alarmins release from the epithelium (IL-33, IL-25, TSLP), and these molecules stimulate IL-5 secretion by type 2 innate lymphoid cells (ILC2s), and eosinophil migration and lung damage.