



REVIEW

Biologicals targeting type 2 immunity: Lessons learned from asthma, chronic urticaria and atopic dermatitis

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During the last decades, progression of research has led to great achievements for treatment and therapy of several disabling disorders, particularly in the field of chronic inflammatory diseases. The increased knowledge of the molecular mechanisms operating in such diseases has represented the first step in this process, and the discovery of molecules able to interfere with the natural history of the diseases, has been the second. This review is focused on the effects of biologics on type 2 diseases such as asthma, chronic urticaria and atopic dermatitis, both biologics just approved for clinical application and also those that are currently undergoing clinical trials. We will also discuss aspects and emphasize clinical trials and recently published studies, as well as research that is currently in the progress, which will be highly relevant for basic immunologists. Likewise, we will cover aspects that are pertinent for clinical immunologists and highlight translational studies that are evaluating novel biologicals in animal models.

Keywords: atopic dermatitis · bronchial asthma · chronic urticaria · precision medicine

Introduction

The usage of biological drugs in clinical medicine represents the beginning of a new era of medicine, and two new terms have been coined to strengthen the importance of this concept, precision medicine and personalized medicine. Precision medicine indicates those treatments that target the mechanisms of the disease, based on the precise knowledge of the disease's endotype, whereas personalized medicine is used to indicate the individualized approach to single patient treatment. In other words precision medicine is probably the most important instrument to realize the personalized medicine, and biologicals represent surely the most important mean to this aim [1]. In the field of cancer, after several years of intense efforts, small molecules that can either reduce

immune suppression in the tumor milieu or enhance activation of cytotoxic lymphocyte responses to the tumor have now been conclusively demonstrated to be effective [2]. Nowadays biologicals are currently used also in the treatment of immune-rheumatologic disorders and allergic diseases. Among the latter, the use of biologic drugs is indicated in asthma, chronic spontaneous urticaria, and atopic dermatitis, that failed to respond or have an insufficient response to conventional treatments.

The type 2 response

Since human T helper (Th) lymphocytes show different functional features and are involved in the defence against different classes of pathogens, they are classified in several subsets, the most important of which are Th1, Th2 and Th17. Th2 lymphocytes protect from helminth infections, but, in allergic people, these cells are also responsible for acute and chronic inflammatory responses

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against allergens [3]. Even though it is well known since many years that Th2 polarization from naive CD4 T cell is mediated by the early production of IL-4 in the course of primary immune response [4], the source of IL-4 has not yet been identified. A recently discovered new subset of cells of the innate immunity may give a crucial contribution to this process, the group 2 innate lymphoid cells (ILC2) [5]. This cell subset belongs to the innate lymphoid cell (ILC) family, which also includes ILC1, ILC3, and NK cells, and differentiate in the bone marrow from a common ILC precursor. ILC2 reside in human respiratory and gastrointestinal submucosal tissues as well as in the derma, and share many functional similarities with Th2 lymphocytes, among which the ability to produce IL-4, IL-5, and IL-13 upon activation, is the most important. ILCs do not express rearranged antigen receptors, and are activated by several factors, whose knowledge is crucial to understand their physiologic and pathophysiologic activity. The epithelial cytokines IL-33, IL-25, and TSLP are the main source for ILC2 activation and this is the reason why they are considered a sort of a bridge between external environment and adaptive immunity. Nevertheless, also cytokines produced by T lymphocytes (IL-2, IL-4, IL-7, and IL-9), as well as lipid mediators (PGD₂, cysteinyl leukotriens), may activate ILC2, whereas other cytokines such as IL-27, IFN- γ , and IFN- α , and cell-to-cell interaction through ligation of E-cadherin with the receptor KLRG1 leads to their suppression. When activating signals overcome the inhibitory ones, ILC2 secrete IL-4, IL-13, the epidermal growth-factor-like molecule amphiregulin, and large amounts of IL-5, whose production is considered to be a hallmark of their function [6]. In this way, once activated, ILC2 may orchestrate the protective type 2 response that provokes helminth expulsion and tissue repair. On the other hand, when ILC2 are activated by environmental allergens, they work as initiators of allergic inflammation by stimulating both the innate and the adaptive immune response. Thanks to their ability to produce IL-5, ILC2 drive the recruitment and the activation of eosinophils in the lungs of patients with asthma and allergic airway diseases and their local production of toxic mediators, that are crucial in determining tissue damage and remodeling. The capacity to produce IL-4 and IL-13 has even more consequences, since these two cytokines allow ILC2 to interact with both specific and innate immunity cells. Indeed, it has been shown that human activated ILC2 express CD154 and are able in vitro to induce IgE production in B cells [7]. Moreover IL-13 is able to stimulate dendritic cells (DC) expressing Fc ϵ R1 to migrate into the lymph nodes to trigger Th2 responses. Finally, IL-4 has also trophic activity on basophils and mast cells. Basophils are short-lived circulating cells recruited to inflammatory sites under allergic conditions, while mast cells are long-lived tissue resident cells. These two lineages express an overlapping set of cytokines and biochemical compounds stored in their cytosolic granules, and many of these compounds are rapidly released after activation [8].

In this context, it is not surprising that ILC2 cells have been shown to accumulate together with Th2 lymphocytes at sites of allergic inflammation, such as the lungs of asthmatic patients, the nasal polyps and sinus mucosa of subjects with chronic rhinosinusitis, and the skin lesions in atopic dermatitis [6]. Thus, ILC2

and Th2 cells cooperate during type 2 immune responses, showing both a protective role against parasites and a pathogenic role in allergic inflammation (Fig. 1).

Asthma

Asthma is one of the most prevalent chronic diseases, affecting about 350 millions of patients worldwide. It is a multifactorial disease, characterized by both genetic predisposition and environmental exposure. However, despite the presence of common clinical manifestations, asthma is a heterogeneous disease. Based on the prevalence of distinct clinical manifestations, different phenotypes of asthma have been identified. Indeed, asthma phenotypes originate from the clusterization of visible properties that represent the disease expression of distinct groups of patients. These properties comprehend: clinical parameters, physiological properties, morphological features, clinically relevant outcomes, inflammatory parameters, and also treatment response. Different phenotypes of asthma are sustained by different pathogenetic mechanisms, commonly known as “endotypes” of asthma. The most common endotypes of asthma are “type 2” and “non-type 2”, which can be distinguished on the basis of prevalent infiltrating cells and soluble mediators. Despite the availability of effective inhaled therapies, asthma is sometimes poorly controlled in the community because of limited adherence to therapy or incorrect use of inhaler devices, and also because of an intrinsic resistance to therapy that is typical of the severe forms of the disease [6]. In these cases, despite optimized treatment, patients still experience exacerbations, inadequate symptoms control, lung-function impairment, or their combination [9]. For all these reasons, asthma is one of the diseases which has more benefited from the development of biologic drugs that target specific pathogenetic mechanisms. Several biologicals are already in clinical practice, while others are in course of evaluation. However, among the uncontrolled severe asthma patients, only those who suffer from an allergic phenotype may benefit from treatment with biologicals, to date [10].

Currently, the anti-IgE mAb omalizumab, and the IL-5 inhibitors mepolizumab, reslizumab, and benralizumab are the drugs approved for the treatment of uncontrolled asthma both in Europe and USA (Fig. 2). The FDA has recently approved the anti-IL-4R α mAb dupilumab for add-on maintenance treatment in patients with moderate to severe asthma with an eosinophilic phenotype or with oral corticosteroid-dependent asthma.

Omalizumab

Omalizumab, an anti-IgE mAb, was the first biological approved for treatment of uncontrolled severe asthma patients with perennial aeroallergens sensitization. Its efficacy as add-on treatment to inhaled therapy in uncontrolled severe asthma patients is established in terms of disease control, quality of life, reduction of

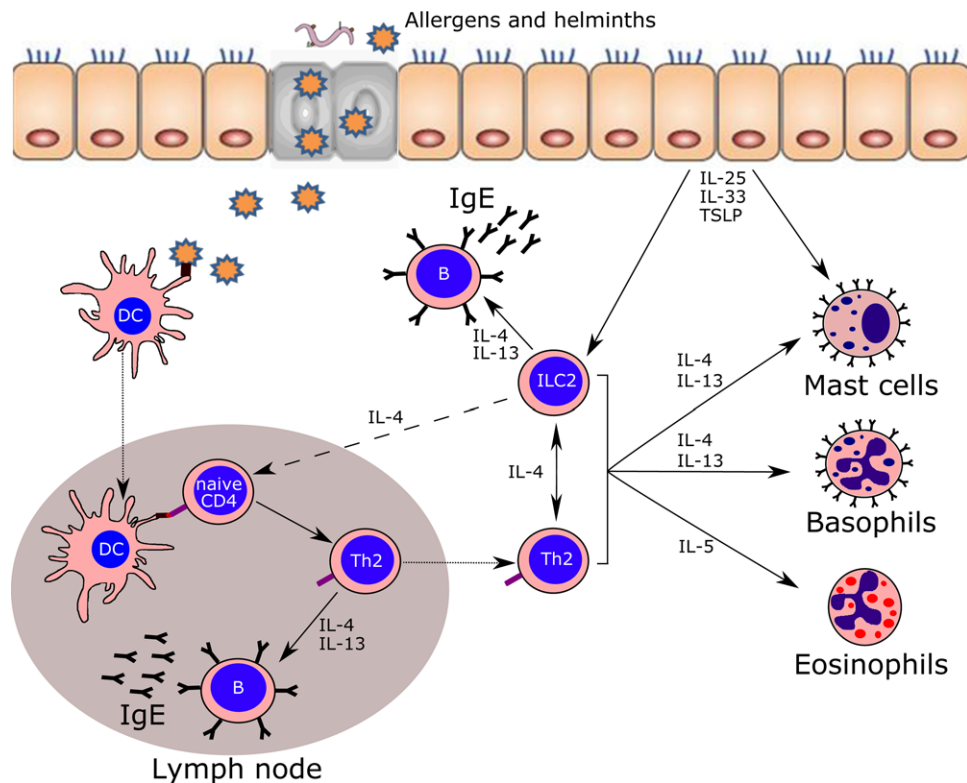


Figure 1. The type 2 immune response. Following epithelial barrier damage, allergens and/or helminths can invade the mucosa. Epithelial damage leads to the production of cytokines such as IL-25, TSLP, and the alarmin IL-33 that activate innate immunity components as ILC2, which then start producing type 2 effector cytokines. Simultaneously, DC loaded with foreign antigens migrate to draining lymph nodes where promote Th2 polarization of antigen-specific naïve T cells. ILC2 cells concur to the polarization process via the secretion of IL-4. Th2 and ILC2 cells cooperate in mastocytes and basophils activation via the production of IL-4 and IL-13, while IL-5 is important for eosinophil development and survival. ILC2 contribute to IgE production by B cells thanks to the production of IL-4 and IL-13.

serious asthma exacerbations rate, and also modulation of bronchial remodeling process [11, 12]. Omalizumab was introduced in clinical practice nearly twenty years ago and since then it has demonstrated a good safety profile. However, the optimal duration of this treatment has not yet been established [13]. The mechanisms by which omalizumab exerts its beneficial effects range from the neutralizing activity on soluble IgE to the capacity to downregulate FcεRI on basophils and mast cells. More recently it has also been demonstrated the ability of omalizumab to dissociate prebound IgE from the high affinity receptor on basophils, thus reducing basophils' allergen-dependent degranulation capacity [14]. Nevertheless, about one third of the potentially responder patients, fail to benefit from the treatment, and the reasons are not fully elucidated. It has been recently reported that most of patients affected by severe allergic asthma, who benefited from omalizumab, showed high levels of IL-33, IL-25 and TSLP in bronchial tissues, whereas a mixed eosinophilic and neutrophilic airway inflammation is predominantly found in non responders [15]. These findings strongly support the concept that an optimal characterization of both endotype and phenotype of the disease is mandatory to drive the choice of the right treatment.

IL-5 inhibitors for asthma therapy (mepolizumab, reslizumab and benralizumab)

More recently new molecules have been approved for the treatment of eosinophilic asthma, namely the IL-5 inhibitors. IL-5 plays a key role in eosinophilic inflammation promoting the differentiation of eosinophils from their precursors in the bone marrow and sustaining their survival of in the airways. Beyond some difference in the route and in the frequency of administrations to patients, the main difference among mepolizumab, reslizumab and benralizumab, concerns the mechanism of action. Indeed, these three mAbs belong to two different categories, the anti-IL-5, mepolizumab and reslizumab, and the anti-IL-5Rα, benralizumab. The mechanisms of action are quite different: both the anti-IL-5 mAbs block the main source of eosinophil activation, with no direct effect on eosinophils themselves, whereas benralizumab, binds to the IL-5Rα expressed on eosinophils, and promotes the antibody-dependent cell-mediated cytotoxicity [10, 16]. Despite their different mechanisms of action, the efficacy of these drugs in eosinophilic asthma are quite comparable. Mepolizumab has been proven to be able to increase the median time to exacerbation, to have a steroid-sparing effect and to decrease the number of

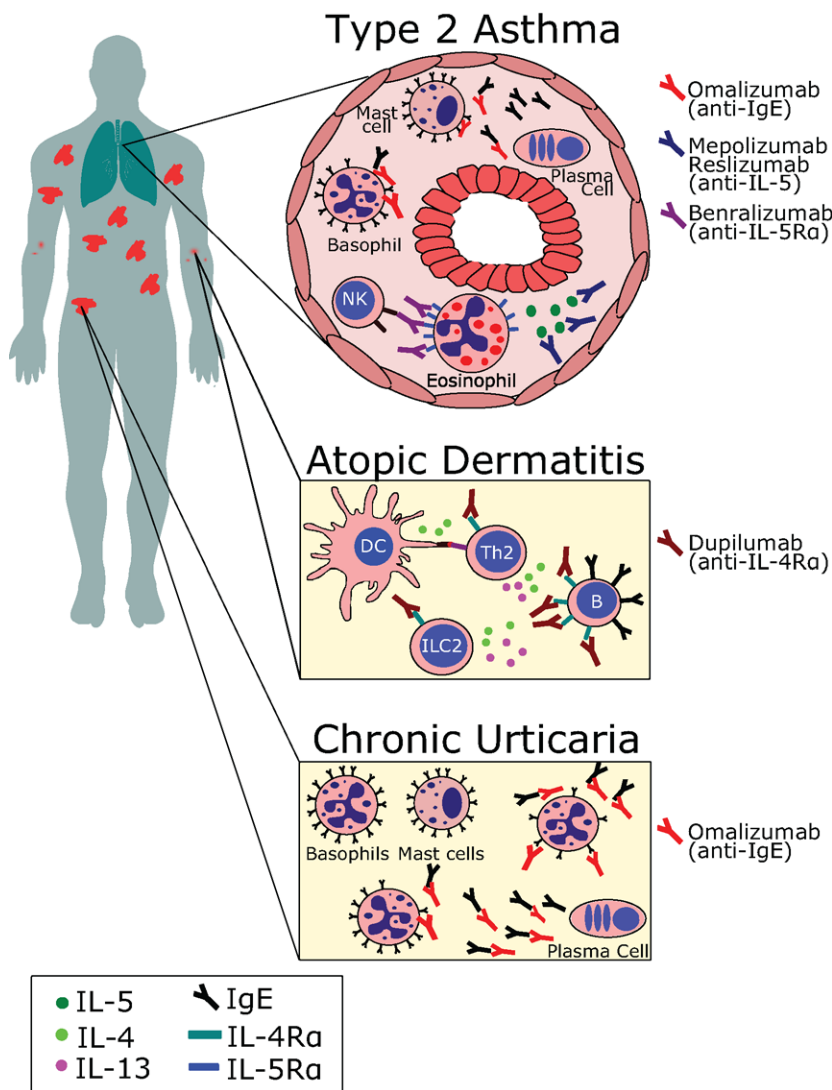


Figure 2. Biologicals in allergic diseases. Biologicals approved in severe asthma are Omalizumab, Mepolizumab, Reslizumab and Benralizumab. Omalizumab is an anti-IgE mAb that neutralizes free IgE, decreases FcεRI expression on the surface of basophils, mast cells and DC and also detaches FcεR-bound IgE from basophils. It is also approved in Chronic Urticaria. Mepolizumab and Reslizumab are anti-IL-5 mAbs that prevent eosinophils activation, their maturation in the bone marrow and survival in peripheral tissues. Benralizumab is an anti-IL-5Ra that prevents IL-5 signaling on IL-5Ra expressing cells and make these cells targets of ADCC. Dupilumab is an anti-IL-4Ra mAb that prevents both IL-4 and IL-13 signaling, approved for atopic dermatitis treatment.

sputum and blood eosinophils within normal limits [17, 18]. Despite these beneficial effects have been confirmed in several successive studies, and also improvement in asthma symptom scores have been found, only mild improvements in forced expired volume in 1 second (FEV1) have been reported with mepolizumab [19, 20]. The effectiveness of mepolizumab in eosinophilic granulomatosis with polyangiitis (also known as Churg–Strauss syndrome), has also been reported [21]. Similar to mepolizumab, reslizumab is a blocking antibody against IL-5, that reduces exacerbations rate in patients with poorly controlled asthma and blood eosinophils over 400 per microliter [22]. Also, benralizumab showed the same clinically relevant benefits on oral glucocorticoid use and exacerbation rates in eosinophilic asthma, without a sustained effect on the FEV1 [23]. Recently it has been performed an attempt to compare the effects of different IL-5 inhibitors in patients with severe eosinophilic asthma stratified for the numbers of circulating eosinophils [24]. Caution is needed in the interpretation of the results, due to the fact that the analyzed studies were performed in different groups of patients, different regions and

different periods, and within different health care delivery systems. Nevertheless, authors found that mepolizumab was associated with lower degree of clinically significant exacerbations and better asthma control than benralizumab in patients with similar blood eosinophil counts. These results suggest a speculative discussion on the homeostatic role of eosinophils. Indeed, benralizumab has a more rapid onset and a more pronounced effect than mepolizumab, in terms of eosinophils depletion, because of its mechanism of action. However, this phenomenon does not translate neither into an evident clinical advantage, nor in an evident reduction of asthma exacerbations. Thus, the question that raises is if eosinophils must be interpreted only as pathogenic cells rather than a cell subset with homeostatic properties. Indeed, Eosinophils have recently been described to cover different functions in diverse tissue sites, since they release cytokines that mediate a broad range of activities in the local environment [25]. Among these previously underappreciated activities, there is the ability to produce plasma cell survival factors such as APRIL, IL-6 and TGFβ1, that act on the maintenance of bone marrow plasma cells and

cooperate in the induction of mucosal IgA production [26]. Moreover, it must be considered that eosinophils belong to innate immunity and their role in defending the body from extracellular pathogens, is well known. Inhibition of IL-5 does not lead to complete inactivation of eosinophils, in fact, they maintain the ability to respond to further cytokines including IL-3 or GM-CSF, and to release their toxins [26]. In this view, the apparent paradox that a virtual complete eosinophils depletion in eosinophilic asthma patients treated with benralizumab is associated with more frequent asthma exacerbations compared to patients treated with an anti-IL-5, is not so surprising and further underlines the defensive role of these cells. Translating these observations in clinical practice, it is possible to speculate that benralizumab may be preferable in asthma with very high levels of circulating eosinophils, whereas mepolizumab and reslizumab may be used in conditions of less severe eosinophilia.

Dupilumab

Recently, the FDA has approved the IL-4R α mAb dupilumab for add-on maintenance treatment of patients older than 12 with moderate to severe asthma with an eosinophilic phenotype or with oral corticosteroid-dependent asthma. Since dupilumab inhibits the response to both IL-4 and IL-13 that are probably the milestone cytokines of the type 2 response, this drug became a candidate to be used in type 2 asthma. Asthmatic patients treated with dupilumab reported lower asthma exacerbation rates than those who received placebo, as well as an improvement of lung function and asthma control [27]. Moreover, patients with glucocorticoid-dependent severe asthma, treated with dupilumab, showed a reduction of oral glucocorticoid usage and of severe exacerbation rates, ameliorating the FEV₁ [28]. Intriguingly, transient hypereosinophilia was registered in some patients treated with dupilumab. This effect is not fully understood, but it is almost certainly an indirect effect, since eosinophils express very low levels of IL-4R α , and do not represent a target of dupilumab treatment.

The choice of the drug

One emerging problem for severe asthma patients that may benefit from biological therapies, is the right choice of the drug. Indeed allergic asthma and eosinophilic asthma represent two side of the same coin, since both IgE and eosinophils are milestones of type 2 inflammation. The consequence is that an overlap of eligibility for the treatment with IL-5 inhibitors and omalizumab, and in future also with dupilumab, exist [24, 29, 30]. To solve this problem, a careful clinical characterization of patients is mandatory. Early disease onset is typical of the allergic phenotype and is often associated with a prominent pathogenic role of IgE, whereas the eosinophilic phenotype is usually late onset, and in this view it is not surprising that IL-5 inhibition may work also in Chronic obstructive pulmonary disease (COPD) [31]. In any case, it is not possible to make the choice of the right biologic for the

right patient considering only a single parameter, and an important issue in this direction may be the identification of a panel of biomarkers associated with prevalent eosinophilic or allergic inflammation. To date, the more reliable biomarkers of allergic or eosinophilic inflammation are serum IgE and blood eosinophil count. Blood eosinophils in the majority of patients positively correlate with their numbers in lungs, even if some exceptions to this rule exist. Anyway, sputum induction is not so easily applicable, on all the patients [32].

Finally, considering the possibility that in the next future other biologics will be available for the treatment of the type 2 asthma, the physicians will have further opportunities to personalize treatment for their patients, but also further troubles in the choice of the best treatment. In this view, also the possibility to shift the treatment from one biologic to another, according to clinical and pathophysiological changes, should be considered [33].

Chronic spontaneous urticaria

Chronic spontaneous urticaria (CSU) is characterized by the presence of wheals (hives), angioedema or both, occurring for more than six weeks. CSU lesions are unpredictably, frequently present most days of the week, and can develop on virtually any part of the body, as consequence of mast cells activation. Several mediators released from activated mast cells, primarily histamine, but also leukotrienes, prostaglandins, cytokines and others, determine vasodilatation, sensory nerve activation, exudate formation and recruitment of further inflammatory cells to urticarial lesions [34]. The signals that activate the mast cells are not always identifiable and are often heterogeneous and diverse. The only recognized proof of a causative nature of a suspected agent in CSU, is the remission of symptoms following elimination and recurrence of symptoms following re-challenge in a double-blind provocation test [34]. Unfortunately, in the clinical practice the trigger factors of CSU remain frequently undefined, and in these conditions, once excluded differential diagnoses, the treatments are targeted to reduce symptoms. Therapy of CSU is based on anti-histamines that represent the first step and are efficacious in about half of patients. An add-on therapy must be considered in patients unresponsive to four-fold up-dosing of second-generation H1-antihistamines. Omalizumab has been approved for the treatment of CSU patients unresponsive to second-generation H1-antihistamines, and this approach, precedes cyclosporine, whose adverse effects in chronically exposed patients are well known [34].

Omalizumab treatment has been demonstrated to be efficacious and safe, and several studies have shown that a 12-week administration is effective in controlling urticaria manifestations for the subsequent 24 weeks. [35–37] (Fig. 2). However, the prevalence of urticaria relapse following omalizumab discontinuation is high, as in asthma, and the optimal duration of the treatment has not definitively been established, being different in each patient [13]. The biological reasons for omalizumab efficacy in CSU are not fully understood, since it is not an allergen-driven disease, but, for definition, with unknown causes. Among

the possible biomarkers of treatment response, total IgE levels were shown to predict the response to omalizumab, even if CSU is not considered an IgE-mediated disease [38]. CSU patients, unresponsive to omalizumab treatment have lower IgE levels and show less serum IgE increase after the beginning of treatment as compared to CSU patients benefit from omalizumab. Indeed, it is well known that omalizumab treatment, in responder patients, determines an increase of serum IgE, since standard IgE assays is able to detect both free and omalizumab-complexed IgE [38]. This increase in total IgE levels has been associated to the longer half-life of omalizumab-complexed IgE compared to free IgE [38], but probably also other mechanisms are involved in this paradoxical increase [14]. Indeed, beyond the well-known ability of omalizumab to capture free IgE preventing the activation of basophils, mast cells, and dendritic cells (DCs), and to reduce FcεRI expression on these cells, the capacity to dissociate prebound IgE from high-affinity Fcε receptor on basophils, has recently emerged as a novel mechanism of action [14]. This finding may help to explain why omalizumab works fine and very rapidly in CSU, even better than in asthma. Moreover, the ability of omalizumab to detach prebound IgE from their receptors opens the way to additional applications in those clinical conditions in which degranulation of basophils and mast cells must be limited to prevent anaphylaxis. In this direction, it is not surprising that omalizumab works fine in mastocytosis [39], allergen immunotherapy [40, 41], drug desensitization [42], and may be a promising option also for idiopathic nonhistaminergic acquired angioedema [43].

In conclusion, omalizumab improves patient health-related quality of life in H1-antihistamine refractory CSU patients. Its clinical efficacy in terms of symptom reduction (hives, itch and angioedema), is associated to a good profile of safety, even if some issues related to the management of patients with CSU remain challenging. Among these, treatment duration is probably the most important. Since omalizumab is not a disease-modifying agent, repeated treatment cycles will likely be required in several patients, and it is not possible to define an optimal duration of therapy, that has to be evaluated in each single case [44].

Atopic dermatitis

Atopic dermatitis (AD) is the main skin disease in terms of global burden measured by disability-adjusted life years [45]. AD is a chronic, relapsing inflammatory skin disease characterized by complex immune dysregulation, as well as skin barrier alterations that deeply affect the life-quality of patients and their families. Immune abnormalities in AD regard essentially the type 2 immune response and are present not only in the skin but also systemically. Although AD in infants and young children can spontaneously resolve, it is associated with an increased risk of sequential progression from AD to other atopic diseases, such as allergic asthma, allergic rhinitis and food allergy: the so called “atopic march” process [46]. Skin lesions can be present in a large body-surface area and are frequently associated to an intense and persistent pruritus, which is one of the main features of the disease. Patients

with moderate-to-severe grade of disease have often poor results with steroidal topical therapies, and systemic treatments are associated with toxic effects [47, 48]. The requirement of effective and safe long-term treatments for AD has prompted the development of novel therapeutic targets. Dupilumab, a fully human monoclonal antibody that binds the alpha chain subunit common to the interleukin-4 and interleukin-13 receptors, thereby inhibiting their signaling, has been approved by the FDA in March 2017 for the treatment of moderate-to-severe AD in adults (Fig. 2). Several cell types express the IL-4Rα chain, including both Th2 and B lymphocytes, ILC2, mast cells, fibroblasts, epithelial cells and mucus secreting cells, and their depletion may result in undesirable effects. However, since dupilumab is a human IgG4, an isotype that is well known to have reduced capacity to support ADCC, the drug has virtually no toxic effects. Accordingly, treatment with dupilumab is generally well tolerated and does not alter the immune responses to several vaccinations, supporting the concept that it does not induce any immunodeficiency. In conclusion, dupilumab represents an important new therapeutic approach for moderate-to-severe AD in adults, a clinical condition that is deficient of safe and effective long-term treatments [49].

Another biologic that is presently under evaluation for use in AD is nemolizumab, an anti-IL-31 receptor A mAb. IL-31 is a Th2-associated cytokine that in AD favors the alteration and damage of the physical skin barrier, and consequently the entrance of allergens and pathogens. IL-31 is also considered the main mediator of pruritus in these patients. Although the drug is not yet approved for clinical use, the results of clinical trials are promising. Treatment with nemolizumab resulted in clinical improvement with a reduction of pruritus and dermatitis, and no side effects [50]. Finally, also blocking IL-22, a Th-17 related cytokine that promotes epidermal hyperplasia and affects skin barrier protective function, has resulted to be promising in adults with moderate-to-severe AD poorly controlled by conventional treatments [51].

In conclusion, AD patients that fail to improve with conventional treatments, may benefit from biologic therapy, and the continuous progress in better understanding the immunopathology of the disease will expand the possible molecular targets of biologics.

Novel biologics

Although in recent years several biologics have been developed for use in allergic disease, the discovery of new cell subsets and new mediators contributing to type 2 immune response will unavoidably lead to further development of novel drugs. Many drugs are currently under evaluation for asthma treatment, especially for the type 2 endotype of the disease, both in animal models and clinical trials.

In particular, additional drugs targeting ILC2, whose contribution to allergic phlogosis is becoming clearer day by day [52], are developing. ILC2, owing to their strategical localization at the interface between the host and the environment, in the submucosa of the lung and intestine as well as in the derma, may

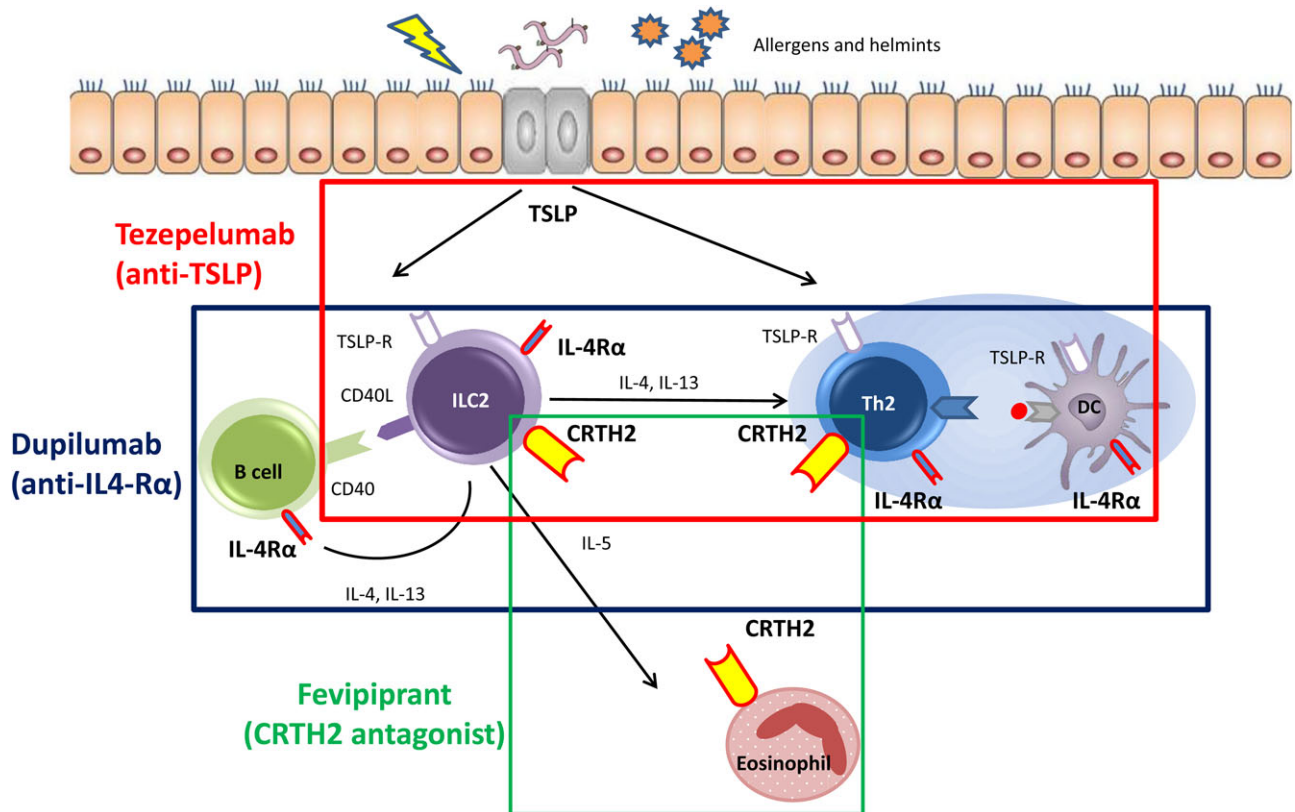


Figure 3. Novel small molecules in clinical development for the treatment of allergic asthma. Biologicals and reversible inhibitors currently under evaluation in clinical trials for their possible future use for the bronchial asthma treatment. All these drugs have a pleiotropic effect since both the receptors, IL-4Rα and CRTH2, are expressed by several inflammatory subsets of allergic phlogosis, and the chemokine TSLP is able to act on multiple targets. Indeed tezepelumab inhibits the effects of TSLP whose receptor is expressed on DC, Th2 cells and ILC2, and also mast cells. Dupilumab inhibits the signaling of both IL-4 and IL-13, by acting on IL-4Rα expressed on Th2 cells, ILC2, B cells, and DC. Fevipiprant avoid PGD2 binding to CRTH2, expressed by Th2 cells, ILC2 and eosinophils.

behave as initiators of type 2 immunity. Human ILC2s express the second receptor of PGD2 (DP2), also known as “chemokine receptor homologous molecule expressed on TH2 cells” (CRTH2, CD294), a molecule expressed also on human CD4⁺ Th2 cells and eosinophils [53]. Fevipiprant, an oral competitive antagonist of CRTH2, has been used in early-phase trials in patients with eosinophilic asthma; it resulted safe and able to induce an improvement in FEV1 and a reduction of eosinophils in peripheral blood [54]. CRTH2 inhibitors like fevipiprant, inhibit contemporary several crucial characters of type 2 inflammation, such as ILC2s, Th2 lymphocytes, and eosinophils, and this pleiotropic activity makes them as a very promising tool in dampening allergic inflammation [52].

Another interesting approach with biologics to treat uncontrolled asthma, is the thymic stromal lymphopoietin (TSLP)-specific mAb, tezepelumab [55, 56]. TSLP is a cytokine produced by epithelial cells in response to environmental stimuli and in proinflammatory conditions; its ability to activate ILC2, Th2 cells, dendritic cells, and B cells is crucial for the initiation of type 2 response. Consequently TSLP is expressed at higher levels in the airways of asthma patients than in healthy controls, and its levels correlate with Th2 cytokine serum levels, and with

disease severity [57]. Tezepelumab administration is associated with lower rates of clinically significant asthma exacerbations, compared to placebo, in patients treated with long-acting beta-agonists and medium-to-high doses of inhaled glucocorticoids [56]. The pleiotropic activity of these novel biologicals is summarized in Fig. 3.

The possibility to treat asthma interfering with ILC2 activation, is not limited to CRTH2 and TSLP. Targeting two additional epithelial cytokines, IL-25 and IL-33 is currently under evaluation in both mouse models and clinical trials. In particular, an anti-IL-33R monoclonal antibody is currently in phase I clinical development, for severe asthma treatment [58].

Finally, a novel and interesting approach for the treatment of type 2 asthma has recently been suggested, by using a bispecific antibody strategy that targets simultaneously two molecules or cytokines. Two mAbs neutralizing IL-4Rα and IL-5 were developed by using a llama-based antibody platform, and administered to C57BL/6J mice sensitized and challenged to house dust mite (HDM) extracts [59]. The simultaneous targeting of IL-4, IL-13, and IL-5 signaling pathways led to a synergistic effect that was associated to a reduction of lung eosinophilic inflammation and allergen-specific IgE production. Moreover, this treatment

prevented goblet cell metaplasia, and bronchial hyperreactivity, in response to HDM challenge. Beyond the demonstration of the possibility to develop bispecific mAbs targeting multiple cytokines, the results of this translational study suggest that targeting simultaneously the IL-4, IL-13, and IL-5 pathways may be a novel approach to treat patients with type 2 asthma. Since the monospecific antibodies neutralizing IL-4R α or IL-5 resulted to be less efficacious than the IL-4R α /IL-5-bispecific antibody, the lesson coming from this mouse model is that we should start thinking about therapies with more than one biologic in asthma patients [59]. In this regard, it is reported in literature that patients not responding to one biologic may be successfully shifted to another, but the concomitant usage in the same patient represents a new perspective [33].

Conclusion

Biological therapies in allergic diseases are an expanding world with very promising perspectives for many patients. The drugs currently used have shown their safety profiles in long term treated patients, as well as good clinical results, even if several discrepancies exist in terms of efficacy among different patients and diseases. An unmet need in this field is to identify predictive biomarkers and/or clinical markers that allow to distinguish between potential responders and not responders. To solve this problem, the understanding the patient's endotype together with a deep characterization of disease pathogenesis, represents probably the best way to run. An important contribution to prompt a further development in this field will come from mouse models, with the recent findings obtained by using a multitarget attempt to dampen type 2 inflammation [58]. This novel approach is a paradigmatic example of how translational medicine can contribute to realize the aims of both precision medicine and personalized medicine, in terms of treatments that specifically target the mechanisms of inflammation.

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