COMMENTARY

With an antibody whose duty's double, a step towards ending asthma trouble?

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Asthma is a debilitating chronic inflammatory disease of the airways that results in shortness of breath, wheezing and coughing, often triggered by inhalation of common allergens. The prevalence and severity of asthma has increased dramatically over the last several decades to the point where several hundred million people worldwide are affected and each year tens of thousands of individuals die prematurely from asthma symptoms. The rise in disease prevalence is particularly evident in the developed world, where childhood exposure to bacteria and viruses has decreased dramatically, while the use of antibiotics, especially early in life, and births by Caesarean section have increased significantly; correlations that support the hygiene hypothesis. Central to the pathogenesis of asthma are the roles of T_H2-type cytokines, including IL (interleukin)-4, IL-5, IL-9 and IL-13, in stimulating and perpetuating an inflammatory cascade that drives detrimental changes in airway structure and function. IL-4 and IL-13, in particular, have been targets of therapeutic development because, together, they specifically promote nearly all of the clinical features of asthma. Their inflammatory functions are highly complementary, but not identical, in large part because they share cell-surface receptors and transcription factors through which they stimulate cell signalling. In this issue of the *Biochemical Journal*, Redpath et al. have taken advantage of the shared receptor nature of the IL-4- and IL-13-mediated inflammatory responses to generate a novel antibody to the third ectodomain (D3) of IL-13Rα1 (IL-13 receptor α1), which forms a requisite binding site for both interleukins. This antibody potently neutralizes both IL-4 and IL-13 activities *in vitro*. Using X-ray crystallography complemented by molecular interaction and functional analyses, they clearly define its mechanism of dual cytokine neutralization as one of molecular mimicry of the interleukin–receptor interaction. Considering the complexity of the cytokine-mediated inflammatory cascade that defines asthma pathogenesis, such therapeutics that can effectively and simultaneously neutralize the actions of multiple cytokines and may play a significantly expanded role in the treatment and prevention of asthma in the future.

Key words: antibody, asthma, interleukin 4 (IL-4), interleukin 13 (IL-13), X-ray crystallography.

Asthma is an inflammatory respiratory disorder that leads to airflow obstruction, AHR (airway hyperresponsiveness) and lung remodelling. These changes in airway structure and function result in afflicted individuals experiencing episodic difficulty in breathing, often combined with wheezing and coughing. Such asthma attacks are typically treated through the self-application of fast-acting β-adrenergic agonists using a meter-dose inhaler at the time of the attack; longer-term treatment is generally sought through the inhalation of corticosteroids. Notably, these treatments amount to disease management, not prevention. Asthma is increasing in both frequency and severity: more than 300 million people worldwide are now afflicted and some 25 000 asthma-related premature deaths occur annually. The need for preventative treatment options is acute.

The picture of asthma pathogenesis that has emerged over the last few decades is one in which cytokines orchestrate an inflammatory cascade [1]. Inhalation of allergens, such as those derived from house dust mites, cockroaches, animal dander and pollen, promotes the selective expansion of T-lymphocytes, particularly of the T_H2 type. These T_H2 cells then secrete numerous cytokines, including IL (interleukin)-4, IL-5, IL-9, IL-13 and GM-CSF (granulocyte/macrophage colony-stimulating factor), which induce many of the airway modifications associated with the disease. Together, these cytokines co-ordinate the cellular events, i.e. B-cell isotype switching to IgE synthesis, eosinophil, mast cell and basophil recruitment, differentiation, maturation and survival, that are the mechanistic hallmarks of asthma and other allergic responses. Not surprisingly, major efforts are underway to develop molecules that specifically inhibit the activity of T_H2 cytokines as preventative asthma therapeutics.

Of the T_H2 cytokines, IL-4 and IL-13 have received much of the attention and with good reason: they specifically promote most of the clinical features of asthma. These include AHR and IgE synthesis, as well as eosinophilia, goblet cell metaplasia, alternative macrophage and dendritic cell activation, smooth muscle remodelling and subepithelial fibrosis. Numerous studies in mice have contributed to ascribing the general functions of IL-4 and IL-13 in asthma: inhibition of IL-4 suppresses the development of the pulmonary T_H2 response and already established IgE synthesis, but does not affect established AHR, whereas inhibition of IL-13 suppresses AHR, but prevents IgE production significantly less.

The likely reasons for IL-4 and IL-13 having many overlapping, although not identical, functions in activating T_H2 cell-mediated inflammation is that they share receptors on the cell surface and activate cell signalling through the same transcription factor. IL-4

Abbreviations used: AHR, airway hyperresponsiveness; IL, interleukin; IL-4Rα, IL-4 receptor α; IL-13Rα1, IL-13 receptor α1; TSLP, thymic stromal lymphopoietin.

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first binds to IL-4Rα (IL-4 receptor α) and then recruits either the common γ chain receptor (γ_c) or IL-13Rα1 (IL-13 receptor α1) to form what are termed type I and type II receptor complexes respectively. IL-13, conversely, binds first to IL-13Rα1 and then to IL-4Rα; IL-13 only forms a type II receptor complex. Both type I and II complexes signal through the JAK (Janus kinase)/STAT (signal transducer and activator of transcription) cascade.

Molecules that specifically inhibit the formation of IL-4- and IL-13-dependent ternary receptor signalling have been tested in clinical trials with promising, but mixed, results. In an initial trial, administration of a soluble decoy IL-4Rα molecule, which can act as an IL-4 antagonist, exhibited increased asthma control [2]. This result, however, was not replicated in a larger follow-up study [3]. A clinical trial of lebrikizumab, a humanized IgG4 anti-IL-13 monoclonal antibody, indicated that its administration was associated with improved lung function [4].

Because IL-4 and IL-13 both contribute critically to asthma induction and progression by promoting inflammatory effects that are overlapping, but not entirely duplicative, efforts have been made to block these two cytokines in concert as a strategy for improving asthma therapeutics. Compared with therapies that block either IL-4 or IL-13 individually, such dual-acting approaches have demonstrated more impressive clinical outcomes. Pitrakinra is a human IL-4 variant that retains binding to IL-4Rα, but blocks the formation of productive ternary receptor complexes comprising IL-4 and IL-13, thereby inhibiting IL-4 and IL-13 cell signalling. In a Phase IIa trial, when pitrakinra was used as an inhalant and subjects were challenged with allergens from cat, grass and house dust mite, this protein-based therapeutic was associated with a statistically significant decreased allergen-induced reduction in forced expiratory volume in 1 s (FEV_1; a measure of lung capacity) [5]. Further clinical trials are ongoing for pitrakinra.

In this issue of the *Biochemical Journal*, Redpath et al. [6] have taken a riff on the same theme, but with a twist. Having originally cloned IL-13Rα1 and showed that it acted as a shared receptor for both IL-13 and IL-4 [7] nearly two decades ago, seminal work that helped to launch efforts to specifically inhibit these cytokines for therapeutic purposes, these investigators have now generated a potent anti-IL-13Rα1 antibody that blocks both IL-4 and IL-13 binding and cell signalling *in vitro*. In a remarkably comprehensive study, the authors have gone from start to finish to create a novel protein-based therapeutic and to define its molecular mechanism of action. They first immunized transgenic mice (into which human immunoglobulin genes had been incorporated in order to directly produce fully humanized antibodies) with the extracellular region of IL-13Rα1. They then evolved one of their lead candidate antibodies (10G5) by phage display to create an affinity-matured variant (10G5H6). Finally, having shown that the antibody inhibited the activities of both IL-4 and IL-13, they went on to determine its X-ray crystal structure alone and in complex with the third fibronectin ectodomain (D3) of IL-13Rα1, the portion of the receptor on which lies its epitope.

The structural basis of both IL-4 and IL-13 signalling through the type II IL-4Rα–IL-13Rα1 complex system had previously been elegantly elucidated using X-ray crystallography [8]. In these structures, the IL-4- and IL-13-mediated ternary signalling complexes are nearly superimposable, with the interleukins being encased by the two receptors through a number of protein–protein interfaces, including those between the interleukins and IL-4Rα (termed site I), between the interleukins and D3 closest to the cell membrane (site IIa) and the first ectodomain (D1) farthest from the cell surface (site III) of IL-13Rα1, as well as between the membrane-proximal residues of the two receptors themselves (site IIb). Site IIa is characterized by several loops of the IL-13Rα1 D3 fibronectin domain engaging the first (A) and fourth (D) helices of IL-4 or IL-13 with high shape complementarity and approximately 900 $Å^2$ (1 Å = 0.1 nm) of buried surface area. The contact residues in IL-4–IL-13Rα1(D3) and IL-13–IL-13Rα1(D3) are nearly all the same. Perhaps the most notable feature of the site IIa interface is a leucine residue located on the FG loop of IL-13Rα1(D3) that is inserted deeply within the cleft formed by the interleukin A and D helices. This critical IL-13Rα1 leucine residue makes intermolecular contacts with four residues from each of IL-4 and IL-13.

In comparing those crystal structures of the type II IL-4 and IL-13 signalling complexes with that of the 10G5H6 antibody–IL-13Rα1(D3) complex reported by Redpath et al. [6], the molecular mechanism of interleukin activity neutralization by the antibody becomes satisfyingly clear. 10G5H6 uses all of its CDR (complementarity-determining region) loops to form a large interface (∼2200 $Å^2$) with IL-13Rα1(D3) that includes those same fibronectin domain regions that are important for IL-4 and IL-13 engagement, namely the BC loop, C' strand and FG loop. The identical FG loop leucine residue that inserts between the A and D helices of IL-4 and IL-13 is effectively engulfed between the cleft formed by the heavy and light chains of the 10G5H6 antibody. Mutation of this leucine residue to alanine results in a 100-fold weaker binding affinity for the antibody. At the base of the cleft between heavy and light chains lies a methionine residue at position 100 of the antibody heavy chain. Tellingly, 10G5H6, which had been derived from 10G5 by phage display, contains a single amino acid change at this position from phenylalanine (10G5) to methionine (10G5H6). Molecular modelling of the 10G5–IL-13Rα1(D3) complex suggests that the phenylalanine residue in the wild-type antibody would sterically clash with the FG loop leucine residue, thereby reducing its relative affinity.

Redpath et al. [6] stopped short of testing their antibodies *in vivo*. However, the sub-nanomolar binding affinities of the wild-type and affinity-matured antibodies, which are several orders of magnitude higher than the IL-13Rα1-specific binding events of either the IL-4 recruitment (∼500 nM) or IL-13 initial engagement (30 nM) steps of type II receptor complex formation [8], suggest that they could be quite potent inhibitors of IL-4 and IL-13 signalling *in vivo*. With pitrakinra having established that, when it comes to asthma treatment in the clinic, it is advantageous to simultaneously antagonize the activities of these two interleukins, relative to inhibiting either one or the other, the similar dual action of 10G5H6 holds substantial clinical promise. That 10G5H6 functions by a distinct molecular mechanism from that of pitrakinra to achieve a similar result (i.e. blocking both IL-4 and IL-13 cell signalling) suggests that these two potential asthma therapeutics could well be highly complementary *in vivo*.

Whether such a strategy of the simultaneous inhibition of multiple key T_H2 cytokines will prove effective in treating asthma is an open question, the answer to which is still many years and clinical trials away. Considering the complexity of the chronic inflammatory condition induced by asthma, as well as the diversity of the disease (in terms of genetic polymorphisms, race- and ethnicity-related variation, clinical characteristics, etc.), it may be necessary to attack asthma from even more angles. One could certainly imagine future drug regimens that combine a multi-cytokine inhibitor like 10G5H6 with other multi- or single cytokine inhibitors of IL-5 and IL-9 to more comprehensively impair the T_H2 immune response. The flipside would be to take advantage of cytokines, such as IL-10 and IL-12, that are involved in suppressing asthma.

It may be that the current focus on T_H2 cytokines, although scientifically sound and already clinically promising, is a case

of coming late to the party. It could be reasonably argued that the self-perpetuating inflammatory cascades in asthma need to be stopped as close to the beginning of the cycle as possible. For that, we may need to swim upstream and attempt to inhibit the selective expansion of T_H2 cells, not their subsequent secretion of cytokines. After all, the allergens that trigger the asthmatic condition do not necessarily directly stimulate T_H2 cells, but more typically activate lung epithelial cells to produce TSLP (thymic stromal lymphopoietin), IL-25 and IL-33. These cytokines, in turn, are potent initiators of the T_H2 immune responses that results in IL-4, IL-5 and IL-13 production and the concomitant asthma-associated airway modifications. TSLP, IL-25 and IL-33 have all been shown to be important in animal models of asthma. Additionally, genome-wide association studies have identified IL-33 [9] and its exclusive receptor, ST2 [10], as being significantly associated with asthma. Perhaps future strategies for developing novel asthma therapeutics will involve inhibiting some or all of these upstream regulators of the T_H2 immune response.

Regardless of the ultimate path that leads to effective asthma therapeutics or cocktails thereof, the 10G5H6 antibody developed by Redpath et al. [6] has the potential to be an important weapon in the asthma treatment arsenal.

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