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REVIEW

New anti-asthma therapies: suppression of the effect of interleukin (IL)-4 and IL-5

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New anti-asthma therapies: suppression of the effect of interleukin (IL)-4 and IL-5. J.C. Kips, K.G. Tournoy, R.A. Pauwels. ©ERS Journals Ltd 2001.

ABSTRACT: Asthma is currently defined as a chronic inflammatory disorder of the airways. The central role of allergen-specific Th2 cells in the regulation of this mucosal airway inflammation has been highlighted. Hence, there is large interest in the therapeutic potential of an anti-Th2 cell approach. One of the strategies which has been developed, is to inhibit the effect of interleukin (IL)-4 or IL-5, two main Th2 cell derived cytokines.

Interleukin-4 is pivotal in the pathogenesis of allergic disorders through its wide range of effects. An important observation, especially during secondary antigen exposure, is the possible redundancy with IL-13. Both cytokines share common elements in their receptor and intracellular signalling pathway. As a result, compounds can be developed that selectively inhibit the effect of either IL-4 or IL-13, or alternatively, by interfering with the common pathway, inhibit the effect of both cytokines.

Eosinophils are generally seen as a particularly harmful element in the allergic inflammation. The importance of IL-5 on eosinophil biology has clearly been established. Conversely, in man, the biological effects of IL-5 are largely limited to eosinophil function. Therefore, IL-5 antagonists offer the unique opportunity of selectively neutralizing the effect of eosinophils.

Several strategies have now been developed that successfully inhibit the biological effect of interleukin-4 or interleukin-5. Some of these compounds have proven to be biologically active in man. The challenge now is to establish their therapeutic role in asthma.

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Currently available treatment regimens are very effective at controlling asthma in the majority of patients. However, they do not cure the disease. The potential for future treatment approaches, lies in their ability to interfere more profoundly in the pathogenesis of asthma, so as to induce disease remissions. Th2 cells, like $CD4^+$ T-lymphocytes, are currently considered to largely orchestrate the chronic mucosal inflammation underlying atopy-related disorders such as asthma or rhinitis [1]. Hence selectively inhibiting allergen induced Th2 cell activation has raised interest as a novel form of therapy for these diseases. Several approaches are being developed. One of these consists of antagonizing Th2 cell derived cytokines. The therapeutic effect of steroids, the current mainstay of asthma treatment, has been largely attributed to an anti-cytokine effect, inhibiting the production of cytokines as well as the cytokine-induced intracellular signalling [2]. Biopsy studies confirm that in asthma, steroids reduce messenger ribonucleic acid (mRNA) expression of several, albeit not all, cytokines [3]. It can be hoped that a more selective and potent inhibition of key Th2 cytokines will offer even larger benefit. Within the range of cytokines produced by Th2 cells, interleukin-4 (IL-4) and interleukin-5 (IL-5) have received considerable interest to date. This review attempts to summarize current developments in this area.

Interleukin-4

Biological activities

IL-4 has a broad range of biological effects (table 1). In general terms it can be described as the main cytokine involved in the pathogenesis of allergic responses, at the same time downregulating acute inflammatory changes [4]. Additional effects that seem of particular importance for asthma include stimulation of mucus producing cells and fibroblasts, thus also implicating IL-4 in the pathogenesis of airway remodelling [5–7]. Inhalation of recombinant human IL-4 has been confirmed to induce airway eosinophilia and to cause some increase in the degree of bronchial hyperresponsiveness in atopic asthmatics [8]. In addition, bronchial biopsy studies have shown increased expression of IL-4, both at mRNA and protein level, in the airway mucosa of atopic and even nonatopic asthmatics, when compared to nonasthmatic controls [9–11]. IL-4 exerts its biological activities through binding with the IL-4 receptor which is expressed on the surface of diverse cell types. The IL-4 receptor is a heterodimer, consisting of the IL-4 binding IL-4Rα chain and a second chain which is either the γc chain (shared in common with the receptor for IL-2, IL-7, IL-9 and IL-15) or the IL-13Rα chain (for review see

Table 1. – Biological effects of interleukin-4 (IL-4), relevant to asthma

Isotype switching of B cells from immunoglobulins G to E synthesis
Differentiation/proliferation Th2 cells
Inhibition development Th1 cells
Upregulation Fc_eR_{II} and major histocompatibility complex Class II antigen expression on Antigen Presenting Cells
Upregulation endothelial vascular cell adhesion molecule-1 expression
Downregulation endothelial intercellular adhesion molecule-1 expression
Downregulation production of pro-inflammatory cytokines (tumour necrosis factor-α, interleukin-1β) and chemokines (regulated on activation, normal T cell expressed and secreted, interleukin-8)
Promoting growth of human basophils and eosinophils
Chemotaxis and activation of fibroblasts
Stimulation of mucus production

[12, 13]). As confirmed in bronchial biopsy studies, the epithelium and subepithelium of asthmatic airways shows increased expression of IL-4Rα mRNA and protein [14].

IL-4 antagonism

Based on these observations, it can be anticipated that targeting IL-4 has substantial effects in asthma. Several approaches can be considered (table 2). A first possibilty is to develop blocking antibodies. This strategy, however, has a number of potential disadvantages, such as possible neutralizing antibody formation, but especially the risk that, depending on the relative concentration of the antibody *versus* the cytokine, complex formation occurs that prolongs instead of inhibits the cytokine-mediated effects [15]. An alternative approach therefore consists of administering the soluble IL-4 receptor, which is a naturally occurring antagonist of IL-4 mediated effects. The soluble IL-4Rα contains only the extracellular portion of the Rα chain and lacks the transmembrane and intracellular domains. As a result, by binding IL-4 without transducing any cellular activitation, soluble IL-4 receptor prevents interaction of IL-4 with the transmembrane receptor, thus inhibiting IL-4 mediated effects. *In vivo* animal models have shown that the soluble IL-4Rα can block antigen induced immunoglobulin-E (IgE) production, but only when given from the period of primary sensitization onwards [16]. Therapeutically more revelant is the observation that, even when given in already sensitized animals, soluble IL-4 receptor can inhibit allergen induced airway eosinophil infiltration, vascular cell adhesion molecule-1 (VCAM-1) expression and mucus hypersecretion [17]. The human soluble IL-4Rα (sIL-4Rα) has now been cloned and produced in a mammalian expression system. Results from a phase I/II trial with sIL-4Rα were recently published. Inhalation of sIL-4Rα at two dose levels (0.5 and 1.5 mg) was compared to placebo in preventing clinical destabilization following abrupt withdrawal of inhaled steroids. A single inhalation of the highest dose was significantly more effective than placebo in inhibiting the decline in forced expired volume in one second (FEV_1) and deterioration of symptoms over the following two weeks. The compound was well tolerated [18]. Preliminary data confirm that more prolonged treatment, consisting of 12 weekly inhalations of 3.0 mg sIL-4Rα is more effective than placebo at preventing asthma deterioration when steroids are discontinued [19]. These data further illustrate the therapeutic potential of an anti-IL-4 strategy in asthma.

Table 2. – Interleukin-4 (IL-4) antagonism; possible targets

IL-4 neutralization
anti-IL-4 antibodies
soluble IL-4 receptor
IL-4 receptor α chain activation
anti-IL-4Rα antibodies
IL-4 mutant proteins (IL-4.Y124D, Bay 16-9996)
IL-4 receptor α chain signal transduction
inhibition of signal transducers and activator of transcription-6 (STAT6) (supressor of cytokine signalling-1 (SOCS-1))

Redundancy with IL-13

An important issue that needs to be considered is the redundancy between IL-4 and IL-13. As for IL-4, increased expression of IL-13 mRNA and protein has been demonstrated in asthmatic airways [20 – 22]. Both cytokines have very similar biological activities. This is reflected in the structure of their receptor (fig. 1). The IL-13 receptor consists of the IL-13$R\alpha_1$ or α_2 chain which binds IL-13, and again the IL-4Rα chain. The signal transduction pathways in common to the IL-4 and 13 receptor involve the intracytoplasmatic domain of both chains and are largely signal transducers and activator of transcription-6 (STAT6) dependent [23].

IL-4 can bind to both receptors through the IL-4Rα chain, IL-13 binds only to its own receptor. With the exception of T-cells, which do not carry functional IL-13 receptors [24], most cell types respond similarly to IL-4 and IL-13, indicating that they carry either the IL-13 receptor or both. Because of this large degree of redundancy, it is difficult to establish with certainty, the exact role of IL-4 relative to IL-13 in allergen induced airway changes, but it would appear that both are functionally active. It has been hypothesized that although IL-4 is crucial for the initial Th2 cell development during primary sensitization, it is not sufficient to cause all allergen induced airway changes [25]. In addition, during secondary antigen exposure, IL-13 release might prove more important [12]. This concept is illustrated in a number of *in vivo* animal models. The induction of allergen induced Th2 cell development and related phenomena such as IgE synthesis, airway eosinophilia and airway hyperresponsiveness has been shown to be totally abrogated in

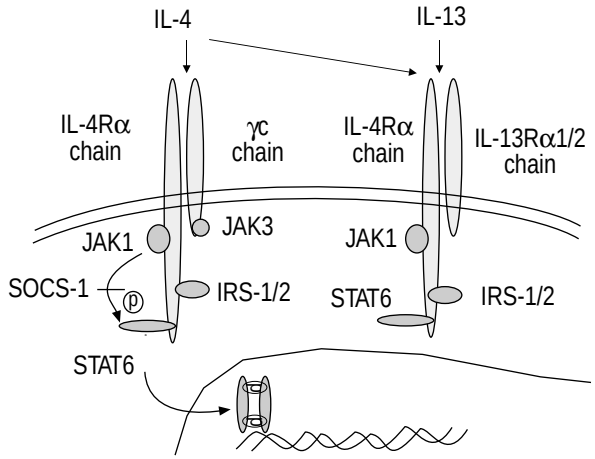

Fig. 1. – Schematic representation of interleukin (IL)-4/IL-13 receptor. STAT6: signal transducer and activator of transcription-6; SOCS-1: suppressor of cytokine signalling-1; JAK: Janus Kinase; IRS: insulin receptor substrate; P: phosphorylation.

STAT6 knock-out (-/-) [26, 27], IL-4Rα-/- [28] and IL-4-/- mice [29, 30], but not in IL-13-/- animals [31]. Administration of neutralizing anti-IL-4 antibodies during sensitization has similar inhibitory effects on Th2 cell development [30], thus confirming the importance of IL-4 during the early antigen response. However, when given only during secondary antigen presentation in already sensitized animals, anti-IL-4 is far less effective in reducing Th2 cytokine production, eosinophil influx and bronchial hyperresponsiveness, whereas the anti-IL-4 receptor maintains therapeutic effect [32]. This confirms *in vitro* data showing that once T-cells have been committed to a Th2 phenotype, they become IL-4 independent [33]. At the same time, this suggests that during secondary antigen exposure, IL-13 plays a more important role than IL-4. In line with these observations, neutralizing endogenously released IL-13 with an IL-13Rα2 Fc fusion protein during secondary antigen exposure, largely inhibits the characteristics of asthma in murine models [28, 34].

Based on these observations, it can be assumed that interfering with the common pathway between IL-4 and IL-13, namely the IL-4Rα activation or STAT6 induction, might be of greater therapeutic benefit than antagonizing either cytokine alone. As IL-13 does not bind directly to the IL-4Rα chain, sIL-4Rα cannot prevent interaction between IL-13 and the transmembrane receptor. In contrast, as the transmembrane IL-4Rα is essential for signal transduction, anti-IL-4Rα antibodies [32] will inhibit the effects of both IL-4 and IL-13 by sterically hindering binding of either cytokine to the receptor complex and/or inhibiting interaction between the two receptor chains. Another possibility that has been developed consists of mutant human IL-4 proteins that bind the IL-4Rα chain without inducing signal transduction, thus again acting as a competitive antagonist. One example is IL-4 Y124D in which tyrosine, in position 124, has been replaced by aspartic acid [35] and which has been shown to reduce both IL-4 and IL-13 induced IgE production *in vitro* or ongoing IgE production in severe combined immunodeficient-human (SCID-hu) mice [36]. Another example is the double mutant R121D/Y124D (Bay 19-9996), which in addition includes substitution of arginine at position 121 by aspartic acid and would seem to be an even more potent antagonist of human IL-4 and IL-13 [37]. Administration of this compound to sensitized primates, prior to secondary allergen exposure has been shown to inhibit the development of airway hyperresponsiveness and inflammation [38].

Another approach is to block the signal transduction pathway. Recent data indicate that this can be achieved through various mechanisms, including administration of SOCS-1 (suppressor of cytokine signalling-1) or decoy oligodeoxynucleotides (ODN), directed against STAT6 [39, 40]. To the best of our knowledge these various approaches have not yet been tested in man. It must be realised that profound interference in the common pathway shared by very broad acting cytokines such as IL-4 and IL-13 could have important side effects. Interference at this level could influence the Th1/Th2 balance in favour of Th1 cell development. *In vivo* animal models illustrate that despite the absence of eosinophils this can cause severe airway inflammation in its own right, accompanied by an important degree of bronchial hyperresponsiveness [41, 42]. This indicates that when developing an anti-Th2 approach care should be taken that this does not result in a Th1 overstimulation.

Interleukin-5

Biological activities

An alternative to the above mentioned broad immunomodulatory intervention therefore consists of a more selective therapeutic approach. Over the past years, the hypothesis has been generated that the eosinophil is the main effector cell in allergic inflammation, capable of causing most of the morphological and functional alterations observed in asthma. Several cytokines can effect eosinophils, but despite possible redundancy with IL-3 and granulocyte-macrophage colony stimulating factor (GM-CSF), IL-5 seems to be the primary cytokine involved *in vivo* in the production, differentiation, maturation and activation of the eosinophils [43]. This has been illustrated by several lines of investigation. Exogenous IL-5 administration has been shown to cause eosinophilia in a variety of *in vivo* models [44]. IL-5 transgene mice develop lifelong eosinophilia, whereas GM-CSF transgenes show increased numbers of mononuclear cells and neutrophils, but only a minimal increase in the number of eosinophils [45, 46]. Similar findings emerge from knock-out mice. For example, the eosinophil inflammatory response to thioglycollate is not abrogated in GM-CSF-/- mice [47]. IL-5-/- mice on the other hand have decreased numbers of circulating eosinophils and fail to mount a normal eosinophilic response to parasitic infections or to ovalbumin challenge [48, 49]. It should be pointed out that, even in IL-5-/- mice, a small number of morphologically normal eosinophils remain detectable in blood. A minor contribution of constitutively expressed IL-3 and GM-CSF to the

production of eosinophils can therefore not be excluded. However, it is of interest to note that in the absence of IL-5, local injection of CC chemokines such as eotaxin cannot induce tissue eosinophilia, even if donor eosinophils have been administered to restore or increase the circulating pool, a procedure which in wild type animals clearly enhances the tissue response to eotaxin [50]. This illustrates the importance of IL-5 even in the homing reaction to chemotactic agents of eosinophils. It has also been convincingly shown that IL-5, both at mRNA and protein level, is present in increased amounts in the mucosa of asthmatic airways. Expression of IL-5 mRNA has even been shown to correlate with clinical indices of disease severity [51, 52]. This corresponds with other studies, showing that the expression of IL-5 receptor in bronchial biopsies is more than 90% restricted to eosinophils, and that expression of the membrane form of the IL-5Rα chain inversely correlates with baseline FEV1, whereas expression of the soluble IL-5Rα which has IL-5 antagonistic properties, correlates positively with FEV1 [53]. In addition, inhalation of IL-5 has been shown to increase the percentage of eosinophils in induced sputum and to augment airway hyperresponsiveness in asthmatics [54].

Based on these various observations, antagonizing IL-5 could prove to be of substantial therapeutic benefit, especially as it would seem that effects of IL-5 in man are mainly focused on various aspects of eosinophil function, thus avoiding profound interference with the overall immune system (table 3). Limited data show that IL-5 can act as a terminal differentiation factor of human B cells, but only if these cells are specifically stimulated. Furthermore, IL-5 can augment the IL-2 dependent cytotoxic T cell generation and enhance mediator release from basophils, but these effects are minor in comparison to the effects on eosinophils [55]. Whether long term suppression of eosinophils in man has any deleterious effect is not known. Eosinophils typically increase in response to parasitic infections, but based on *in vivo* animal data, it remains unclear whether eosinophils actually protect against tissue dwelling parasites [56]. A recent study illustrates that toxocara canis infection in IL-5 knock-out mice does not result in a larger parasite burden than in wild type mice. On the contrary, in the IL-5-/- mice, less lung damage was observed than in wild type littermates [57]. Another area of concern is the unresolved role of eosinophils in tumour surveillance. Th2 cells have been shown in tumour vaccination studies to play an important role in the protective antitumour response. This was at least partly mediated through an IL-5 dependent eosinophil activation, as the protective effect was partly lost in similarly treated IL-5-/- mice [58]. This fits with observations in man, positively linking survival from gastric cancer to the number of eosinophils in the tumour [59]. Other studies however suggest that the matrix metalloproteinases (MMPs) produced by eosinophils facilitate spreading of the tumour [60]. This issue clearly needs to be further resolved.

Table 3. – Biological effects of interleukin-5 (IL-5) on human cells

Eosinophils
proliferation, differentiation, maturation, activation
enhanced survival
responsiveness to homing signals
chemoattraction at high concentrations
Basophils
enhanced mediator release (histamine, leukotriene C_4 (LTC_4))
B cells
terminal differentiation factor if appropiately stimulated
T cells
promotion of interleukin-2 (IL-2) dependent cytotoxic T cell proliferation
increased expression of IL-2Rα on T cells

IL-5 antagonism

On a theoretical basis, IL-5 antagonism can be achieved at various levels [61] (table 4). A first approach consists of interfering with IL-5 gene transcription. Control of IL-5 gene expression is regulated by transcriptional and postranslational mechanisms which appear to be different in mouse and man [62, 63]. The exact mechanisms involved, however, remain to be further identified. It has also been shown that IL-5 synthesis can be regulated independently from other cytokines such as IL-2 or IL-4 [64, 65], thus opening the perspective of selectively interfering with IL-5 production [66]. It was recently shown in mice that an IL-5 antisense oligonucleotide, that blocks IL-5 mRNA and protein production, inhibits antigen-induced eosinophilia and hyperresponsiveness [67].

Another approach consists of interfering with IL-5 induced signal transduction mechanisms. A strictly IL-5 specific transduction mechanism, similar to the IL-4 mediated STAT6 signalling pathway has not been identified at present. Instead, a variety of kinases can be activated, that would seem to regulate different aspects of eosinophil function [68]. Compounds interfering with these signal transduction pathways are currently being developed [69].

The approach which at present has been most extensively investigated, is blocking the interaction between IL-5 and its receptor on eosinophils. Again this has revealed to be more complex than initially anticipated. IL-5 is a homodimer glycoprotein, held together by two interchain disulfide bridges. The molecule can bind one receptor, which is a heterodimer comprising an IL-5 specific α chain and a β chain, shared in common with the IL-3 and GM-CSF receptor. The β chain thus does not directly bind IL-5 and increases the affinity of IL-5 binding only 2–4

Table 4. – Interleukin-5 (IL-5) antagonism; possible targets

IL-5 gene expression
antisense oligonucleotides, GATA-3 antagonists
IL-5/IL-5 receptor interaction
anti-hIL-5 antibodies (SCH55700, SB240563)
soluble IL-5α receptor
IL-5 mutant proteins (E12K, E13Q)
non-peptide molecules (isothiazolones)
IL-5 receptor signal transduction

fold, which is far less than the 20–50 fold increase in affinity for GM-CSF and the even 1000 fold shift for IL-3 binding. However, the β chain is crucial for receptor signal transduction, in collaboration with the short intracytoplasmatic domain of the membrane bound form of the α chain [43, 70, 71].

Several techniques can be adopted to block the interaction between IL-5 and its receptor. As for many cytokine receptors, it has been shown that alternative splicing can lead to the production of a nonmembrane bound soluble IL-5Rα chain which blocks the interaction between IL-5 and its receptor, thus constituting an ideal antagonist [72]. *In vitro* studies have shown that the soluble IL-5Rα derived from bacculovirus transfected insect cells inhibited IL-5 dependent cell proliferation, eosinophil survival and inflammatory mediator release. Experiments using other expression systems for the soluble IL-5Rα production have, however, failed to confirm these overall effects [73]. Another possibility to interfere with IL-5 receptor activation is by developing IL-5 mutant proteins that keep their affinity for the IL-5Rα chain of the receptor, but do not elicit signal transduction mechanisms, therefore acting as competitive antagonists of the native IL-5 protein. Point mutants at residue E12 and E13 have been shown not to reduce receptor binding but to largely diminish receptor activation [74, 75]. An interesting observation that has emerged from experiments with these compounds is their divergent effect on different aspects of eosinophil function. Some IL-5 point mutants have been shown to exert no proliferative activity, and to even antagonize proliferative activity of wild type IL-5, yet to fully enhance eosinophil survival, be it at a lower potency than wild type IL-5 [75]. This further illustrates that different signal transduction mechanisms are involved in various aspects of eosinophil activity.

An alternative approach that has been taken to interfere with IL-5 receptor activation, is the selection of small peptide and nonpeptide compounds through high throughput screening, in systems based on the soluble IL-5Rα. Although this has resulted in the identification of a number of promising molecules [76], some of them have proven to be too toxic for therapeutic applications or have been shown to also block IL-3 and GM-CSF mediated effects, again eliminating them as potential therapeutic agents [77, 78]. These various points illustrate the difficulty in properly antagonizing IL-5 receptor activation.

Anti-human IL-5 antibodies

Most of the data available to date on the inhibition of IL-5 receptor activation have obviously been derived with monoclonal antibodies directed against IL-5. Amongst the various animal studies, it was shown that one single administration of anti-IL-5 could protect against antigen induced bronchial hyperresponsiveness and eosinophilia for a period of 3–6 months [79, 80]. If confirmed in man, this could prove therapeutically very relevant. Two single dose trials with humanized antihuman IL-5 have now been presented. Administration of SB240563, at a dose of 10 $mg \cdot kg^{-1}$, in 8 subjects with mild allergic asthma, profoundly reduced circulating eosinophil counts for up to 16 weeks, but did not significantly inhibit the antigen-induced early or late asthmatic response [81]. The second study was primarily a safety assessment in patients with severe asthma, treated with oral or high doses of inhaled steroids. A single dose of 1 $mg \cdot kg^{-1}$ SCH55700 induced a similar reduction in circulating blood eosinophils, which persisted for 3 months. No significantly different effect on baseline FEV_1 was noted between 12 actively and 8 placebo treated patients [82]. Both compounds were well tolerated. Although these first results might appear disappointing, it needs to be remembered that both studies were conducted on a limited number of subjects and were not intended to evaluate clinical efficacy. Further clinical trials in a larger number of patients are now required to exactly position these compounds in the treatment of asthma, their prolonged biological activity potentially opening the prospect for longer term disease control and/or remission.

The availability of these compounds will also allow a number of concepts to be addressed, such as the precise relationship between eosinophils and airway hyperresponsiveness in asthma. Several animal studies clearly indicate that the relationship between eosinophils and altered airway behaviour is not always causally related [83, 84]. In one of these examples, it was shown that IL-5-/- mice, sensitization and exposure to parasite antigens, can induce a degree of airway hyperresponsiveness which is comparable to wild type animals, despite the absence of airway eosinophilia [85]. It also needs to be emphasized that in most of the models demonstrating a beneficial effect of anti-IL-5, the antibody was administered prior to antigen challenge. Once airway eosinophilia and hyperresponsiveness have already been established, a situation closer to the treatment of asthma, the antibody does not fully restore these alterations. In a model of Schistosoma sensitized and challenged mice, treatment with anti-IL-5 given between day 4 and 10 after allergen challenge, reduced eosinophils in bronchoalveolar lavage (BAL) to a similar extent as dexamethasone, but in contrast to the steroid did not restore mucoid cell hyperplasia or the already established hyperresponsiveness [86].

In addition, it has to be borne in mind that eosinophils not only produce a range of inflammatory and cytotoxic mediators, implicating the cell in acute airway inflammation and airway hyperresponsiveness. Eosinophils also release a variety of cytokines, attributing to them an important immunomodulatory function, and produce a number of growth factors, implicating them in airway remodelling [87]. The relative contribution of the eosinophil in each of these domains remains to be further elucidated.

Finally, clinical experiments with these compounds will also allow establishment of whether specific subgroups within the asthmatic population need to be targeted. It is likely that within asthma, hitherto unrecognized subgroups exist, driven by a specific cell or mediator. Anti-IL-5 offers even more than anti-IL-4, a very focused approach. This indicates that the response to these treatment modalities might vary substantially between different subgroups. It will be

interesting to see whether identifying genetic polymorphisms or recognizing phenotypic characteristics such as the degree of blood or sputum eosinophilia or circulating IgE levels will allow differentiation of responders from nonresponders.

Conclusions

Antagonism both of interleukin-4 and interleukin-5 represents a potentially important new therapeutic strategy in asthma. Biologically active compounds for human use have now been developed. These will resolve a number of remaining issues. Antagonizing interleukin-4 has been shown to have some therapeutic benefit in steroid treated asthmatics, but the safety of interfering in the Th1/Th2 cell balance with more profound interleukin-4 antagonists remains to be assessed. Single doses of anti-interleukin-5 induce a pronounced and prolonged reduction in circulating eosinophil counts, but the efficacy of repeated longer term administration in asthma remains to be demonstrated. Finally, what also needs to be addressed is whether specific patient populations can be identified that benefit particularly well from these novel, specifically targeted forms of asthma treatment.

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