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Interleukin-16 and peptide derivatives as immunomodulatory therapy in allergic lung disease

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The therapeutic potential of interleukin (IL)-16 and derived peptides in allergic asthma is considered, focusing on key interactions with CD4 and associated chemokine receptors. IL-16 is a pleiotropic cytokine that has multiple effector functions with putative roles in varied T cell-mediated inflammatory diseases, such as asthma, inflammatory bowel disease and atopic dermatitis. Both *in vitro* and *in vivo*, IL-16 downregulates antigen-driven T cell activation, T helper 2 (Th2) cytokine production and allergic airway inflammation. Peptides derived from the C-terminal bioactive portion of IL-16 offer advantages related to their retained immunomodulatory properties and absence of signalling in and chemoattraction to T cells.

Keywords: asthma therapeutics, atopy, CD4, cytokines, immunomodulation, interleukin-16

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1. Introduction

1.1 Background – IL-16 as a CD4-binding T cell active cytokine

Interleukin (IL)-16, previously called lymphocyte chemoattractant factor (LCF), was identified in the early 1980s as a T cell chemoattractant in the supernatants of mitogen-stimulated peripheral blood mononuclear cells [1]. Thereafter, IL-16 has been identified in various immune and parenchymal cells, including $CD8^+$ and $CD4^+$ T cells, mast cells, eosinophils, fibroblasts and bronchial epithelial cells [2-6]. IL-16 has two major *in vitro* effects on $CD4^+$ T cells: chemoattraction, preferentially of T helper (Th)1 cells, and inhibition of CD3/T cell receptor-mediated activation, preferentially of Th2 cells. It is not surprising, therefore, that in allergic Th2 lung disease, accumulating evidence indicates an overall immunomodulatory effect for IL-16 and IL-16-derived peptides. As such, IL-16 and derivative peptides that map to its binding site on CD4 provide a more global therapeutic alternative to current therapies, which selectively target the inhibition of pro-inflammatory factors, such as Th2 cytokines, IL-4, IL-5 or IL-13, and mediators of the early phase of immediate-type hypersensitivity such as IgE.

The most studied and primary receptor for IL-16 is CD4, and its therapeutic potential in HIV infection has been investigated extensively and previously reviewed in this journal [7]. This review will focus on the therapeutic potential of IL-16 and derived compounds in $CD4^+$ T cell-mediated allergic lung disease.

1.2 Pulmonary inflammation

The mechanisms by which inflammatory cells are recruited to the lung are as varied as the insults that lead to recruitment. The non-resident cells of the innate immune system (e.g., neutrophils, monocytes) are primarily recruited by chemokines. By contrast, lymphocytes, playing a central role in the adaptive immune response, are recruited from the circulation to resident lymph nodes, activated by antigen via

contact with cognate antigen-presenting cells, and then to the lung parenchyma as effector cells. The effector phase of the inflammatory immune response is largely dependent on cytokine-mediated activation and recruitment signals. In allergic asthma, this phase is mediated by Th2 cytokines, including IL-4, IL-5 and IL-13 [8]. Perpetuation of the effector phase of the adaptive immune response depends on continued antigen-mediated T cell activation and ensuing cytokine release.

1.3 Allergic airway inflammation

The characterisation by Mosmann and colleagues in mice [9-11] (and later by Del Prete and Yamamura in humans [12,13]) of Th cell polarisation to Th1 and Th2 cells has markedly enhanced our understanding of $CD4^+$ T cell-mediated inflammatory processes. These seminal observations have been mechanistically expanded by the characterisation of the two mutually exclusive transcriptional control programs that regulate Th1 and Th2 cell differentiation. The development of interferon-gamma (IFN-γ)-producing Th1 cells, whose function is central to acquired host defence against single-cell infectious agents, is regulated by the T-box transcription factor T-bet [14]. Th2 cell development is regulated by GATA-3 and c-Maf [15,16]; these cells are responsible for humoral immunity and are defined by their release of IL-4, IL-5, IL-9 and IL-13. Although the proper function of both types of Th cells is important in the adaptive immune response, there are circumstances in which the inflammation that accompanies the immune response is deleterious. The atopic diatheses associated with allergic rhinitis, conjunctivitis, dermatitis and asthma are examples of Th2 immune responses that are destructive. Both Th2 cells and their products have been identified by several methods in the airways of human asthma. Similarly, the pulmonary overexpression of the Th2 cell-derived cytokines IL-9 and IL-13 in mice is sufficient to induce the key characteristics seen in human asthma [17,18]. These findings have led to several therapeutic strategies to control the dysregulated T cell response in asthma: developing compounds that antagonise the pro-inflammatory effect of overexpressed Th2 cytokines [19], treatment with bacterial extracts that downregulate Th2 cell differentiation by providing a Th1 'environment' [20] and development of compounds that alter the course of T cell activation [21]. This review will concentrate on the role of IL-16 and IL-16-derived peptides as immunomodulators of the allergic airway response through their inhibition of antigen-driven T cell activation and Th2 cytokine production.

2. IL-16 protein biology

2.1 IL-16 gene structure and expression

The IL-16 gene is a single copy gene located on human chromosome 15.26.1-3 and on chromosome 7 D2-D3 in the mouse [22,23]. The entire gene stretches over 20 kilobases and encodes for two forms of the precursor molecule, synthesised by alternative splicing. Larger 1322 and 1332 amino acid precursor forms (in mouse and human, respectively) are expressed in the brain and have an unknown number of exons [24,25]. The non-neuronal form of the human IL-16 gene contains 7 exons, of which the first is non-coding and results in a 2.6 kb mRNA species [23]. The promoter is TATA-less, yet contains two CAAT box-like motifs and three GA-binding protein transcription factor binding site consensus sequences that are functional in T cells, one of which lies in the 5' untranslated region [26].

Although the transcriptional regulation of IL-16 has not been fully characterised, the nature of IL-16 message production in different cell types has been examined in detail. $CD4^+$ and $CD8^+$ T cells [27], eosinophils [4], mast cells [3] and dendritic cells [28] constitutively generate IL-16 message. By contrast, non-immune cells, such as airway epithelial cells, must be induced to transcribe IL-16 message. With the notable exception of $CD8^+$ T cells, caspase-3 activation is a major regulator of IL-16 release in all cells that constitutively express mRNA and protein. $CD8^+$ T cells express constitutively active caspase-3 and store cleaved bioactive IL-16 that is released within hours following stimulation by histamine or serotonin via H2 or S2 receptors [2,29]. It is likely that the stimuli responsible for IL-16 release in non-T-cells result in new transcription and translation and post-translational cleavage by activated caspase-3. In the context of IL-16's proposed *in vivo* function (reviewed below), the finding that airway epithelium is a major source of IL-16 in allergic inflammation suggests that IL-16 may be a natural immunomodulator of the allergic airway response.

2.2 Cellular synthesis and functions of IL-16

The mechanisms that control IL-16 synthesis and secretion at the cellular level are an area of active investigation; the picture is incomplete.

IL-16 is synthesised as a 631 amino acid precursor that is cleaved into two fragments by caspase-3 [24,30]. Although only one functional caspase-3 cleavage site has been identified, further proteolytic processing has been suggested in dendritic cells [28]. In T cells, the ~ 60 kDa N-terminal product of caspase-3 cleavage contains two PDZ (disc-large homology) domains [31] and a dual phosphorylation-regulated nuclear localisation sequence [32], permitting translocation to the nucleus, where it has cell cycle regulatory properties whose therapeutic potential is beyond the scope of the present review [33,34]. The distal 16 amino acids of the 121 amino acid carboxy-terminal product of caspase-3 cleavage retains the key CD4 binding ability and consequent T cell chemotactic and immunomodulatory properties [35,36]. While of undetermined functional significance, the majority of this bioactive C-terminus peptide contains a third PDZ domain with a core GLGF sequence at Gly41 and represents the first description of a secreted PDZ-containing protein. There is low homology between the originally described PDZ domain-containing proteins and IL-16, and nuclear magnetic

resonance spectroscopy has revealed that the core GLGF region is partially occluded by a tryptophan residue [31]. While there is evidence that intracellular IL-16 autoaggregation into homotetramers is essential for bioactivity [36], the significance of this is likely to reside in its ability to crosslink CD4, which is required for signalling. Like IL-1, IL-16 does not contain a signal secretory peptide and work is ongoing to investigate the pathway for secretion.

There is a high degree of functional and sequence homology of IL-16 from all species (e.g., human, simian and murine) [23,37,38]. There is 82% homology between human and murine IL-16 and > 98% sequence homology between simian and human secreted IL-16. Both murine and simian IL-16 are chemotactic to human T cells, and monoclonal antibodies generated to secreted human IL-16 can be used to neutralise the bioactivity as well as to affinity purify murine IL-16. The conservation across species of protein structure and the biological actions of IL-16 imply a conserved evolutionary function, but more importantly bear on the determination of compounds assayed in different models as potential therapeutic targets.

3. Immunomodulatory functions of IL-16 and IL-16-derived peptides

3.1 Relationship between IL-16 and CD4

The ability of IL-16 to function as a chemotactic stimulus for $CD4^+$ T cells led to an investigation of the interaction between IL-16 and cell surface CD4. Although there are studies that suggest there may be alternate receptors for IL-16, these studies primarily address cells of the monocytic lineage [39]. All cells with a well described effector response to IL-16 express surface CD4, including T lymphocytes, eosinophils, dendritic cells and neuronal cells. IL-16 has a distinct binding site on CD4 from other CD4 ligands, including HIV gp120 and major histocompatibility class II antigens [40]. T cell migration to IL-16 can be inhibited by coincubation with monomeric Fab fragments directed against CD4, and murine T cell hybridomas transfected with mutated CD4 do not migrate to IL-16 [36]. It is worth noting that CD4 expression on $CD8^+$ T cells following anti-CD3/anti-CD28 costimulation confers IL-16 responsiveness, which is blocked by antibodies to CD4 [41]. While these experiments do not definitively rule out the possibility of a CD4-related alternative receptor for IL-16, evidence for a predominant IL-16–CD4 ligand–receptor relationship is compelling.

In response to IL-16 stimulation of $CD4^+$ T cells, there is a rise in intracellular calcium and inositol trisphosphate [42], activation of the CD4-associated *src*-related kinase $p56^{lck}$ via autophosphorylation [43] and translocation of protein kinase-C to the membrane [44]. Recent studies have also identified the involvement of phosphatidylinositol 3-kinase and AKT activation in the IL-16/CD4-induced migratory response of human T cells (T Ryan, unpublished observations). Although association of the cytoplasmic tail of CD4 with $p56^{lck}$ is required for IL-16-induced migration, this response does not depend on the catalytic activity of $p56^{lck}$ [43], suggesting that this protein may play an adaptor role for CD4's association with other signal transduction molecules.

The C-terminal 16 amino acids are essential for bioactivity of secreted IL-16. This has been demonstrated by the ability of peptides derived from this sequence to inhibit native IL-16/CD4 interaction and subsequent chemotactic activity [45]. In addition, monoclonal antibodies generated against the distal C-terminus fully inhibit IL-16's T cell attractant properties. Amino acid substitution analyses have determined four amino acids (^{106}RRKS^{109}) to be critical for bioactivity [45]. The precise binding sites on CD4 for IL-16 have been mapped by similar peptide inhibition and CD4 mutagenesis studies [35]. The IL-16 binding region is comprised of two 6 amino acid stretches that form a groove in the tertiary structure of the D4 region. This sequence of the D4 domain of CD4, closest to the cell membrane, is responsible for autoaggregation that facilitates activation of the TCR/CD3 antigen recognition complex [46,47]. The presence of C-C chemokine receptor (CCR)5, expressed primarily on Th1 cells, appears to contribute to IL-16 binding and is likely to account for the preferential migratory response of these cells [21]. The mechanism by which CCR5 increases IL-16 binding to CD4 has not as yet been identified. Mapping of the IL-16 and CD4 binding sites to their respective molecular sites has direct therapeutic implications for both IL-16 and IL-16-derived peptide-based therapies.

3.2 Effect of IL-16 on chemokine receptor desensitisation

Indications that the CD4/CCR5 receptor complex is more than a docking site for HIV-1 binding and internalisation were suggested by the observation that HIV-1 gp120 activates intracellular signalling of both CD4 and CCR5 [48]. This concept was furthered by observations that ligation of either CD4 or CCR5 by their natural ligands resulted in reciprocal receptor cross-desensitisation [49]. IL-16 treatment of $CD4^+$ T cells resulted in transient inhibition of macrophage inflammatory protein (MIP)-1β-induced chemoattraction, indicating that an IL-16–CD4 association desensitised CCR5 to stimulation by a natural CCR5 ligand. Reciprocal receptor cross-desensitisation was also observed: MIP-1β treatment of the same cells prevented IL-16-induced migration. This autoregulatory effect was not due to steric inhibition or changes in surface receptor expression; CCR5 desensitisation by IL-16 was dependent on intact signal transduction by CD4. The receptor cross-desensitisation phenomenon is not unique to CCR5. IL-16/CD4 signalling also desensitised C-X-C chemokine receptor 4 (CXCR4) response to stimulation with its ligand, stromal-derived factor (SDF)-1α [50]. IL-16/CD4 signalling had no effect on migration induced by macrophage chemoattractant protein-1/CCR2b, eotaxin/CCR3 or macrophage-derived chemokine/CCR4. It is intriguing to speculate that CD4/CCR5 exists as a heterodimeric receptor complex

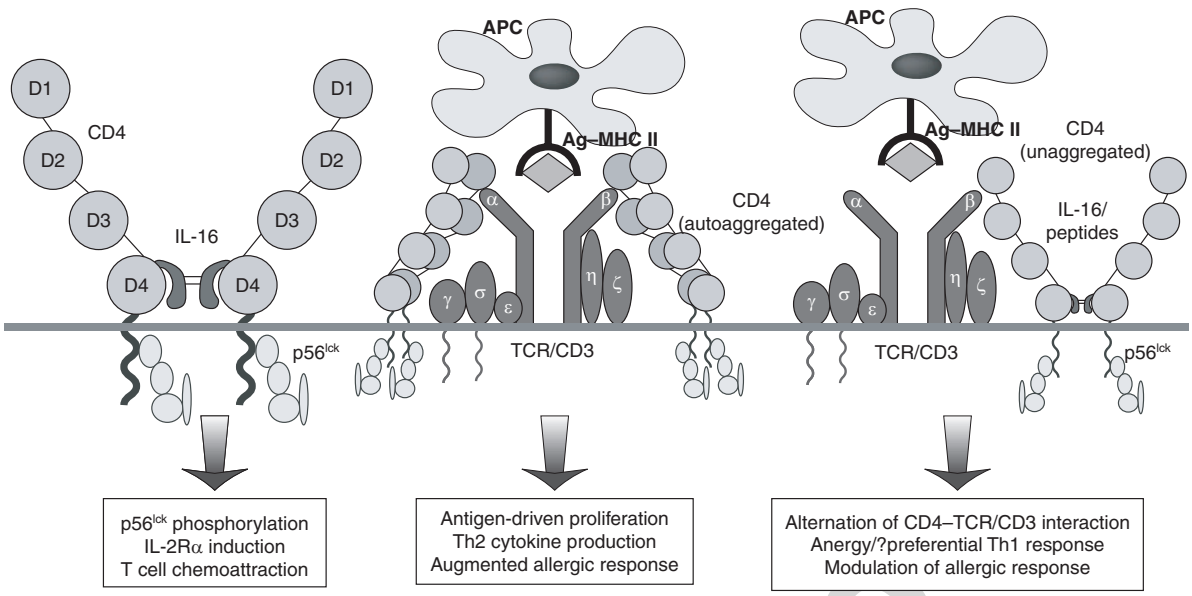

Figure 1. Proposed scheme of action of IL-16 and derived peptides in CD4-mediated pathways of antigen-mediated T lymphocyte activation. In resting T cells, IL-16 oligomers bind the D4 domain of CD4, causing a unique steric relationship that leads to coupling and autophosphorylation of $p56^{lck}$, (activation of PI-3 kinase, rises in intracellular Ca^{2+} and IP3; not shown) with subsequent induction of IL-2Rα and chemotaxis (left). This cascade, as depicted, does not occur upon stimulation with C-terminal 16-mer peptide. In the context of antigen presented by MHC II on an APC (centre), TCR/CD3-mediated cell activation is facilitated by D4 domain-medi ated autoaggregated CD4 of a different steric conformation than that bound by IL-16 or peptide, leading to antigen-driven proliferation and cytokine production that augments the allergic response. In the presence of IL-16 or peptide (right), CD4 autoaggregation and facilitation of TCR/CD3 activation by antigen/MHC II is altered, causing anergy, preferential migration of Th1 cells and modulation of antige n-driven Th2 responses.

APC: Antigen-presenting cell; IP3: Inositol trisphosphate; MHC: Major histocompatibility complex; PI: Phosphatidylinositol; TCR: T cell receptor; Th: T helper.

on the surface of Th1 cells with the capacity to regulate selected chemokine signalling.

Recent reports have described a direct cell surface interaction between CD4 and CCR5 in which MIP-1β binding affinity is decreased compared to CCR5 alone [51,52]. Despite this lower binding affinity, signalling induced by MIP-1β is enhanced. GTPase binding and activity, induced by MIP-1β–CCR5, is in fact greater in the presence of CD4. Along those lines, using $CD4^{-/-}$ T cells, it was determined that the presence of CD4 also augmented the migratory effect of MIP-1β [21]. This suggests that the presence of a CD4–CCR5 interaction augments MIP-1β-induced migration by causing CD4 signal transduction. This concept is supported by the finding that ligation of CCR5 can induce phosphorylation of $p56^{lck}$ [53].

Similarly, it has been determined that the presence of CCR5 contributes not only to IL-16/CD4 binding, but to CD4 signalling as well. T cells from $CCR5^{-/-}$ mice have a markedly diminished migratory response to IL-16 [21]. CCR5 signalling is probably contributory in this response, as pertussis toxin treatment of $CCR5^{+}$ cells reduces the migratory response to IL-16 to levels noted in $CCR5^{-/-}$ cells. The preferential migration of Th1 cells to IL-16 observed in this study has direct implications on the role of IL-16 in lymphocytic inflammatory disease, as the enhanced migration of Th1 cells over Th2 cells was not due to differences in surface CD4 expression, but due to the coreceptor function of CCR5.

3.3 IL-16 and peptide inhibition of T cell activation

The location of IL-16's binding site in the D4 region of CD4 bears directly on its ability to inhibit aspects of CD3–TCR-mediated T cell activation by antigen or that induced by a mixed lymphocyte reaction (MLR). In both humans and mice, 1 h recombinant IL-16 pretreatment of responder cells inhibits the MLR by 50%; this inhibition is reversible by co-treatment with either anti-IL-16 antibody or recombinant soluble CD4 [54]. These findings are consistent with the reports that activation of T cells by a specific antigen (tetanus toxoid) or by stimulation with immobilised anti-CD3 is similarly inhibited by IL-16 pretreatment [55]. Furthermore, treatment of antigen-specific murine T cells with IL-16 during antigen stimulation reduces production of the Th2 cytokines IL-4 and IL-5 without affecting release of IFN-γ or IL-10 [56]. Preliminary experiments have confirmed the functionality of an IL-16 derived peptide that maps to the CD4 binding region with respect to inhibition of T cell chemotaxis [57] and antigen-driven T cell activation (Cruikshank, unpublished observation). The mapping of the binding site of IL-16 to CD4 provides a molecular basis for further investigation of co-receptor molecules that may participate in IL-16 inhibition of antigen-driven T cell activation (**Figure 1**).

3.4 Modulation of allergic inflammation *in vivo* by IL-16 and derived peptides

The observation that IL-16 is a potent *in vitro* T cell chemoattractant led to investigations into the potential role of IL-16 as a pro-inflammatory cytokine in inflammatory disease. Although there is evidence that such a role exists in models of autoimmune inflammatory disease of the joints [58] and gastrointestinal tract [59], the weight of *in vitro* data in exogenous antigen-driven T cell activation suggests otherwise. Specifically, IL-16, through its direct interaction with CD4, is an important modulator of this response. These findings spawned two major areas of investigation – examining the role of IL-16 in humans with atopy and animal models of allergic lung disease, both with the focus on the development of potential peptide-based therapeutics for eventual human trials.

3.4.1 *Murine experimental allergic airway inflammation*

The immunomodulatory function of IL-16 on T cell activation observed *in vitro* has been matched with similar *in vivo* observations, targeted to understanding the nature and mechanism of immunomodulation in murine allergic airway inflammation. Several inbred mouse strains (e.g., BALB/c and C57BL/6) are susceptible to systemic sensitisation with purified chicken egg ovalbumin (OVA), resulting in high levels of antigen-specific IgE in the serum following two intraperitoneal injections 14 days apart. Thereafter, following a 5 – 7 day course of 20-minute airway challenges with aerosolised OVA, there is marked perivascular and peribronchial inflammation that is predominantly lymphocytic and eosinophilic. Both cell types can be isolated from saline bronchoalveolar lavage (BAL), as well as the prototypic Th2 cytokines, IL-4, IL-5 and IL-13 [60]. These features of allergic airway inflammation are essentially undetectable in OVA-sensitised, sham-challenged animals. In addition, these mice have a heightened bronchoconstrictor response to nonspecific, antigen-independent stimuli, such as systemic or aerosolised acetylcholine or methacholine. As these markers of allergic inflammation resolve within 8 weeks following challenge, this acute model differs from human asthma. Nevertheless, it remains a well-described and studied model of allergic airway inflammation, as it shares many features of human asthma.

Systemic treatment with IL-16 during the aerosol challenge phase of the OVA model has been shown to decrease histological and physiological markers of allergic airway inflammation [56]. Both histological markers of inflammation and airway hyper-reactivity were decreased in OVA-sensitised mice treated intraperitoneally with IL-16 immediately prior to sequential OVA aerosol challenge. This was accompanied by a log-fold decrease in BAL eosinophilia. Similar results are obtained when IL-16 or IL-16-derived peptide are administered by either intratracheal instillation or by aerosol (Little, unpublished observations). *Ex vivo* antigen-stimulated thoracic lymph node cells from IL-16-treated mice produced near baseline levels of IL-4 and IL-5. Related studies have shown that local airway treatment via intranasal instillation of a 16-mer peptide mapping to the CD4-binding region of IL-16 significantly decreased airway responsiveness to aerosolised methacholine [57]. This change was not noted in animals treated with two control peptides derived from IL-16 regions that are known not to be involved in intercellular signalling.

3.4.2 *Human studies of atopy*

The preferential migration of murine Th1 cells over Th2 cells towards IL-16 is paralleled with the observation that the function of human T cells with a Th2 phenotype is modulated by IL-16 treatment. In ragweed-allergic individuals, IL-5 release from *in vitro* allergen-stimulated peripheral blood mononuclear cells was reduced by IL-16 costimulation to levels comparable to unstimulated cells [61]. In this study, antigen-stimulated IFN-γ expression was not affected by costimulation with IL-16, indicating a selective effect on Th2 cells. The finding of IL-16 in the BAL of atopic asthmatics following segmental bronchoprovocation with allergen or histamine [62-64] and the identification of IL-16 in both bronchial epithelium and subepithelial T cells in these individuals [6] highlights the fact that both epithelial and T cells are potential functional sources of IL-16 in human asthma. This has direct relevance to the design strategy of IL-16 and peptide derivative delivery in clinical studies, by inhalation or systemically.

3.5 Summary

These preliminary studies in mice and humans provide *in vivo* confirmation of the well-described *in vitro* immunomodulatory effects of IL-16 and its derived peptides on allergic airway inflammation. In addition to decreasing inflammation in the lungs, a specific mechanism of preferential inhibition of Th2 cytokine synthesis is suggested, providing opportunities for further investigation and codifying the rationale for exploring the use of IL-16 and IL-16-derived compounds in the treatment of allergic airway inflammation.

4. Conclusion

IL-16 is detected in airway epithelial cells and T cells in the lungs of asthmatic individuals and is released into the airspace by segmental allergen or histamine bronchoprovocation. The *in vitro* role of IL-16 as a $CD4^+$ Th1 cell-selective chemoattractant, combined with its selective inhibitory properties on Th2 cell activation, suggests a potential role in therapeutics for allergic inflammation. We propose that IL-16 is a natural modulator of allergic airway inflammation by limiting Th2 cell recruitment and antigen-mediated T cell activation. Local delivery of IL-16 concentrations to the murine lung by aerosol inhalation, intratracheal or intranasal instillation results in a significant attenuation of allergic inflammation and airway hyper-reactivity. A similar effect is noted following treatment with a 16-amino acid C-terminal IL-16 peptide. Both protein and peptide modulate the allergic response in

sheep (Cruikshank, unpublished observation) as well as murine [56] models of asthma. The immunomodulatory function of IL-16 is likely to reside in:

- its inhibition of Th2 cell activation and cytokine production
- its preferential migration of CD4/CCR5$^+$ cells

Either alone or in combination, these effects would lead to downregulation of the allergic airway response by altering the ratio of Th1/Th2 cells and their cytokines at sites of allergic inflammation.

5. Expert opinion

Many studies indicate that asthmatic inflammation is driven and orchestrated by CD4$^+$ Th2 cells that have been recruited to the lung. Activation of these cells results in an increasingly expanding cascade of immune cell recruitment and cytokine production. For years, the predominant therapeutic approach to inhibiting inflammation associated with asthma has been the long-term use of inhaled corticosteriods. While effective, the potential of detrimental side effects makes this approach increasingly less attractive. In an attempt to circumvent the chronic use of steroid treatment, a number of different therapeutic modalities have been proposed and some are in the process of being tested in clinical trials.

There are four general approaches to inhibiting allergic airway inflammation. The first is the use of specific antibodies to neutralise pro-inflammatory cytokines. These efforts have centred mostly on inhibition of Th2 cytokines such as IL-4, IL-5, IL-13 and IL-9, but antibodies to TNF-α and IL-1 have also been addressed. The second approach is to use inhibitory cytokines. These cytokines act to skew the Th1/Th2 ratio towards Th1 by inhibiting Th2 cell development and activation and/or promoting Th1 cell development and activation. These cytokines include IL-1Rα, IL-10, IL-12, interferons and IL-18. The third approach is to inhibit cytokine synthesis. This approach includes general inhibitors, such as corticosteroids, but also includes specific signalling pathway inhibitors such as phosphodiesterase 4 inhibitors, nuclear factor-κB inhibitors and p38 mitogen-activated protein kinase inhibitors. The last group addresses inhibiting specific chemokines or chemokine receptors. The chemokine receptors CCR2, CCR3 and CCR4 have been shown in animal models to be involved in the recruitment and activation of eosinophils and T cells. Therefore, blocking these receptors or their cognate ligands, such as monocyte chemotactic protein-1, eotaxin and macrophage-derived chemokine, for CCR2, CCR3 and CCR4, respectively, would reduce immune cell recruitment and attenuate inflammation.

The ability of each of these therapeutic modalities to reduce inflammation has not as yet been determined. As human asthma is a complex disorder without a unique contributory pathway, marked clinical improvement is unlikely from and has not been systematically achieved by targeting a single pathogenic pathway in the dysregulated immune cascade [65,66]. Inhibition of several of the Th2 cytokines by blocking antibodies to receptor or ligand, such as IL-4 and IL-5, respectively, has resulted in partial attenuation of the clinical [67,68] and tissue responses [69,70]. Studies have not been completed addressing the efficacy of neutralising IL-13 protein. Similarly, the use of inhibitory cytokines or inhibition of single chemokines or chemokine receptors may not significantly reduce asthmatic inflammation. The use of signalling pathway inhibitors would affect a broader range of cells and consequently have an increased affect on inflammation. Such a broad range of transcriptional modulation is the mechanism of action of corticosteroids. This effect, however, is not limited to the inhibition of only those immune cells associated with the inflammatory process. In addition, corticosteroids affect non-immune cells with proven deleterious side effects, such as ocular cataracts and alterations in bone metabolism and growth. The challenge for immunomodulatory therapy in allergic lung disease is to selectively and locally target Th2 cell activation in the lung, which is likely to require a combination of several of the modalities mentioned.

Local delivery of IL-16 may provide such an immunomodulatory effect. By virtue of its direct interaction with CD4, IL-16 can induce CD4$^+$ T cell unresponsiveness to antigenic stimulation that predominates in Th2 cells. As noted earlier, IL-16 inhibits Th2 cytokine release with minimal associated change in Th1 cytokine production. In addition, there is an intimate regulatory relationship between IL-16/CD4 and certain chemokine receptors. Stimulation by ligands to CXCR4 and CCR5 responsiveness to their cognate ligands is transiently inhibited when the cells are prestimulated by IL-16. Although a role for the CXCR4 ligand, SDF-1α, has not been established in human asthma, blockade of CXCR4 attenuates the allergic airway response and pulmonary Th2 cytokine expression in a cockroach-induced murine asthma model [71]. There is also a direct reciprocal relationship between CD4 and CCR5, whereby the presence of CCR5 augments IL-16/CD4-induced migration and the presence of CD4 augments CCR5-induced migration. This suggests that the presence of either IL-16 or a CCR5 ligand, such as MIP-1β, would preferentially recruit CD4$^+$CCR5$^+$ T cells. These cells are classically defined as Th1 cells. Therefore, the presence of IL-16 in the lung would result in a decrease in antigen-induced Th2 cytokines, while simultaneously stimulating the recruitment of Th1 cells. Although the effects of IL-16 on Th1 and related cytokines, such as IFN-γ, IL-18 or tumour necrosis factor-α, have not been determined, IL-16 stimulation does not appear to alter IFN-γ production. We propose that by promoting Th1 cell recruitment in the setting of a Th_2-mediated disease such as asthma, IL-16 attenuates the allergic airway response. (**Figure 2**).

Although the potential exists for IL-16 therapeutics in asthma, there are a number of caveats. It is unclear how long-term exposure to IL-16 would alter T cell profiles in the lung and systemically. It is conceivable that chronic exposure to IL-16 would result in a systemic overdevelopment or activation of Th1 cells with the potential for Th2 immunosuppression.

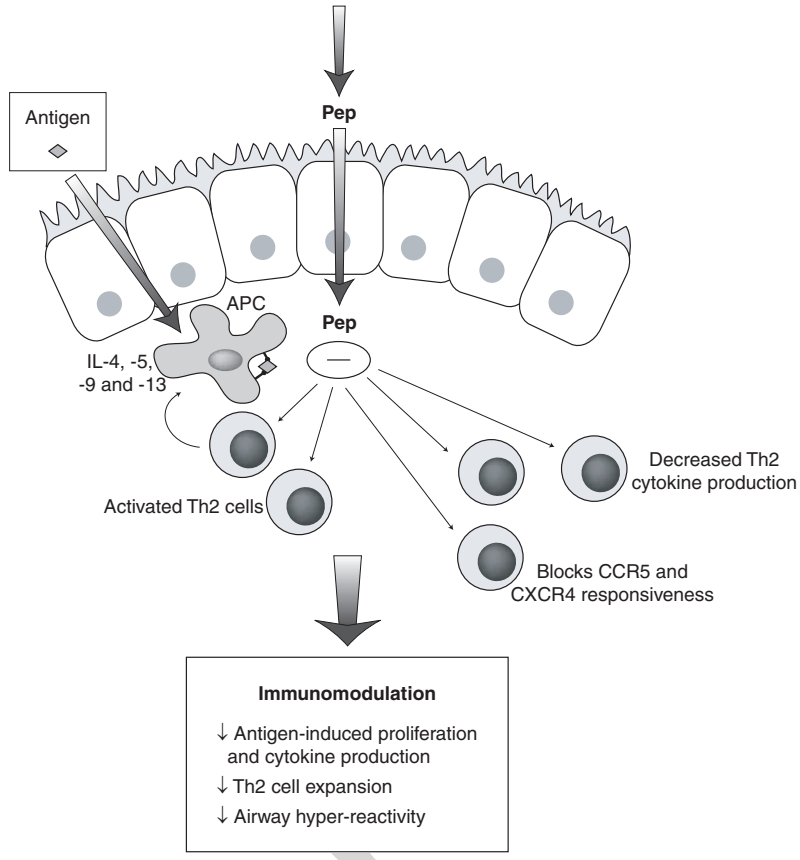

Figure 2. Model of the immunomoregulatory function of IL-16-derived peptides in allergic airway inflammation. Environmental allergenic antigen is processed and presented in the context of MHC II by epithelial dendritic cells, eliciting expansion of $CD4^+$ T lymphocytes in an atopic individual to the Th2 phenotype. Following further stimulation, Th2 cells release IL-4, -5, -9 and -13 and related chemokines. Locally delivered IL-16 peptide (Pep) causes T cell anergy, decreased cytokine production and chemokine receptor desensitisation. Unlike native IL-16 protein, peptide does not mediate T cell chemoattraction or signalling.
MHC: Major histocompatibility complex; Th: T helper.

Long-term intratracheal instillation of IL-16 in the mouse results in detectable inhibition of Th2 cytokines from cells obtained from the distal lymph nodes (unpublished data). It is also not clear what effect IL-16 administration would have on initiation or development of diseases in which a dysregulated Th1 response is pathologic, such as sarcoidosis, type I diabetes or rheumatoid arthritis. It is tempting to speculate that elevated levels of IL-16 for sustained periods of time might promote or exacerbate inflammation associated with these diseases.

As an alternative to IL-16 protein therapy, administration of the IL-16 peptide may be therapeutically more feasible. The C-terminal 16-mer peptide does not have any agonistic effects on T cell migration or activation. It has been demonstrated to be 70 – 80% as effective as IL-16 administration in the murine model of allergic asthma. This compound has a short half-life in serum of ~ 30 min (unpublished data). This short serum half-life is supported by studies demonstrating that peptide administration in the mouse results in antigenic unresponsiveness in $CD4^+$ T cells obtained from the lung and draining lymph node, whereas cells obtained from distal lymph nodes responded normally. As the peptide is generated from the native sequence of IL-16, it is not likely to be antigenic; rigorous studies analysing this supposition have not been performed. As the binding site for the IL-16 peptide has been identified, it would also be possible to design a peptidomimetic with increased affinity for CD4 and increased stability in the lung.

A strong case, therefore, can be made for the use of IL-16-derived peptides as inhaled therapeutics for asthmatic inflammation. This approach has been shown to be efficacious in both the sheep and murine models of allergic asthma, where it reduces airway hyper-reactivity and inflammation. By virtue of its interaction with CD4, the peptide has selective and differentially regulated effects on Th1 and Th2 $CD4^+$ cell recruitment and activation and, therefore, encompasses several different therapeutic modalities.

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