EDITOR'S CHOICE

Interleukin 13 and its role in gut defence and inflammation

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ABSTRACT

Interleukin 13 (IL-13) is a cytokine of increasing interest to gastroenterologists because of its developing role in ulcerative colitis, eosinophilic oesophagitis (EO) and fibrosis. Recent data show that IL-13 may play an important role in a novel innate immune response since it can be released by signals from an injured or inflamed epithelium, of particular relevance to the gut. Animal models of IL-13-driven inflammation (from asthma to colitis and EO) are being translated to human disease and providing insight into potential strategies for new therapies. In fact, multiple clinical trials using anti-IL-13 drugs are underway in asthma and are being extended to gastrointestinal diseases. This review presents the current knowledge on IL-13 production and function in the gut, including the cells and receptor signalling pathways involved in mediating IL-13 effects, the proposed mechanisms of IL-13 induced gut disease and the many drugs currently being tested that target IL-13 related pathways.

INTRODUCTION

Interleukin 13 (IL-13) is a cytokine increasingly recognised for novel roles in allergic and other inflammatory conditions. Like many components of the immune response, IL-13 has a physiological role in fighting infection—that is, eliminating helminthic parasites from the intestine—but it also can exert pathological effects when excessively produced. IL-13 has been cited as a component of different types of mucosal inflammation, including allergic asthma, ulcerative colitis, eosinophilic oesophagitis (EO) and several diseases with an important component of fibrosis. Currently, there is a large effort to test anti-IL-13 strategies in the clinic, mostly driven by the proposed role of IL-13 in asthma but also by the discovery of its contribution to other diseases. This review will focus on the IL-13 cytokine and receptor pathways, the role of IL-13 in gut mucosal immune responses and inflammatory diseases, and highlight development and testing of drugs targeting IL-13 and the IL-13 receptor system.

IL-13 AND ITS RECEPTORS

IL-13 is a 33 amino acid peptide cytokine whose gene resides on human chromosome 5q31. It is located within a cluster of cytokine genes that includes IL-3, IL-4, IL-5 and granulocyte–macrophage colony stimulating factor (GM-CSF)[1]: it has only 25% homology with IL-4 but is structurally similar. IL-13 transcription is regulated by GATA3 (the classical Th2 cell transcription factor), the hedgehog pathway (in a murine model) and intergenic mechanisms involved with tissue specific expression.[2 3] A Th2 family cytokine (along with IL-4 and IL-5), IL-13 is produced by CD4 T cells that are the adaptive effector cells active in allergic asthma. However, in humans, innate immune cells such as eosinophils, basophils, mast cells, natural killer (NK) cells and NK T cells (see below, highlighted in ulcerative colitis), have also been reported to have a capacity to produce IL-13.[4–7] Recently, several novel IL-5- and IL-13 secreting cell types with innate immune function and negative for B and T cell markers were recovered from murine small bowel, gut associated lymphoid tissue and abdominal fat associated lymphoid tissue.[8] A potentially important connection between these IL-13 secreting innate lymphoid cells and the gut is that IL-13 production can be stimulated by gut epithelial derived signals such as IL-33, IL-25 (IL-17E) and thymic stromal lymphopoietin (TSLP) that are themselves released in response to inflammation and infection.[9–12] These innate lymphoid cells may be a source of IL-13 release in reaction to signals that do not require the presence of B or T cells.[13 14] These so-called innate helper cells or innate lymphoid cells have a human homologue that can be isolated from peripheral blood but has not yet been located in the gut.[15] So while IL-13 production in the human gut in response to helminth infection is assumed to be predominantly from Th2 type CD4 cells, different cells, like the NK T cell or even the innate lymphoid cell, may contribute significantly to the induction and maintenance of chronic idiopathic intestinal inflammation.

IL-13 binds to two cell surface receptors but predominantly signals through just one. The IL-13Rα1 receptor protein can bind IL-13 with low affinity, but on dimerising with IL-4Rα, it forms the type 1 IL-13 receptor, enhancing its affinity for IL-13 and transducing intracellular signals though phosphorylation of signal transducer and activator of transcription (STAT) 6 via Jak kinases (figure 1). The type 1 IL-13Rα1/IL-Rα receptor binds both IL-13 and IL-4 (although IL-4 can also signal through the IL-4Rα/common γ chain receptor). Although STAT6 seems to be the main signalling molecule in the type 1 IL-13R pathway, other second messenger molecule activation, including phosphatidylinositol 3-kinase (PI3K), STAT3 and mitogen activated protein kinase (MAPK), has been measured in different cell types (although these studies do not specify the types of IL-13 receptors expressed on these in vitro cell models;

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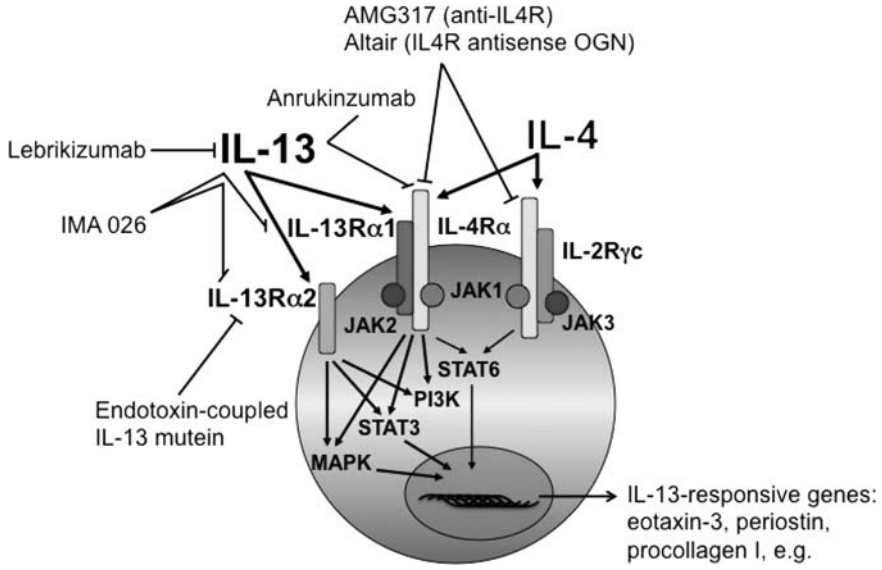


Figure 1 Interleukin (IL)-13 receptors and targets of anti-IL-13 and anti-IL-4 agents. The type I IL-13 receptor (IL-13rα1/IL-4Rα heterodimer) signals primarily through Jak2/signal transducer and activator of transcription (STAT) 6 after IL-13 or IL-4 binding. In certain cells, mitogen activated protein kinase (MAPK), STAT3 and phosphatidylinositol 3-kinase (PI3K) may be activated by this receptor. The type II IL-13 receptor (IL-13Rα2 monomer) binds only IL-13 and largely has no signalling although reports exist of MAPK,[26] PI3K[25] and STAT3[27] activation. Novel anti-IL-13 agents being tested for efficacy in IL-13 mediated diseases are shown with their targets: anti-IL-13 antibodies interact with IL-13 (lebrikizumab), either allowing it to interact with the receptors (anrukinzumab blocks IL-4Rα association with the IL-13/IL-13rα1 complex) or not (IMA-026); anti-IL-4Rα antibody blockade (AMG317 and knockdown of IL-4Rα protein (Altair)) disables the IL-13rα1/IL-4Rα heterodimer; and toxin linked mutated IL-13 binds with high affinity to IL-13Rα2, killing the cell once internalised (→, pathway activation; ⊣, pathway blockade).

in the case of HT-29 cells, it is likely that IL-13Rα1 is transducing a variety of downstream signals[16–19]).

By comparison, the type 2 IL-13 receptor, IL-13Rα2, exists largely as a monomer and has less well defined signalling properties. IL-13Rα2 binds IL-13 with higher affinity than the dimeric type 1 IL-13Rα1/IL-4Rα receptor. This difference in binding affinity is relevant because when interacting with the type 1 receptor, IL-13 first binds to the low affinity IL-13Rα1 monomer before dimerising with the IL-4Rα receptor, giving an added advantage to the type 2 receptor for capturing IL-13.[20] The IL-13Rα2 receptor appears to function largely as a 'sink' for IL-13, binding the cytokine and making it unavailable for activating the type I receptor, while not inducing its own intracellular signal (decoy receptor). Given the ability of IL-13Rα2 expressing cells to clear IL-13 from culture medium, it has also been considered an important scavenging system for IL-13 cytokine, limiting its activity.[21] Indeed, when the two receptors are co-expressed by a cell, IL-13 induced pSTAT6 production is generally inhibited.[22] The mechanism of this inhibition is attributed to the preferential binding of IL-13 to IL-13Rα2 since this inhibition can be overcome with excess IL-13 (exceeding the binding capacity of the IL-13Rα2 receptor). However, other data show that the IL-13Rα2 protein can inhibit IL-4 induced pSTAT6 (in the absence of IL-13; IL-13Rα2 does not bind IL-4) and IL-13Rα2 can physically interact with IL-4Rα,[23 24] suggesting that the IL-13Rα2 can mediate inhibition of type 1 receptor signalling by additional mechanisms beyond merely scavenging IL-13. While the IL-13Rα2 receptor has a short intracellular portion that lacks recognised binding sites for signalling molecules, there are some reports connecting IL-13Rα2 receptor binding to intracellular signals (STAT3, MAPK, PI3-K) but it is unknown whether this is a direct mechanism or through additional binding of scaffold proteins to the intracellular portion.[25–27] An additional curiosity about the IL-13Rα2 is that in mice, this monomeric protein can exist as a soluble protein capable of binding IL-13, the result of alternative splicing of the IL-13Rα2 gene.[28–30] However, a soluble form of this receptor protein does not appear to be active in humans (despite the fact that its extracellular protein structure makes it susceptible to release by cell surface proteases).

The IL-13 pathway is regulated at a number of points. Its transcription and production can be positively regulated by GATA3 and the hedgehog pathway, and negatively regulated by cytokines such as type 1 interferons.[31] Furthermore, the IL-13Rα2 receptor can be seen as a negative regulator of IL-13 effects since IL-13 exposure leads to its transcriptional upregulation and expression, the IL-13Rα2 receptor rapidly cycles from intracellular locations to capture and internalise IL-13 and IL-13Rα2 receptor expression is inversely related to IL-13 STAT6 activation.[22 32 33] Lastly, IL-13 signalling via the Jak/STAT pathway can induce suppressor of cytokine signalling proteins that actually attenuate the IL-13 signal itself.[34] Identifying the factors that result in dysregulation of IL-13, its receptors and their signal transduction will give great insight into the pathogenesis of allergic asthma and other IL-13 associated inflammatory diseases.

IL-13 IN THE GUT MUCOSAL IMMUNE RESPONSE

As a component of the gut mucosal immune response, IL-13 has been recognised primarily for its role in the inflammatory reaction to helminthic infections. IL-13 works at a number of levels to combat this infection, stimulating mucus production from goblet cells, inducing local eotaxin release to attract eosinophils and increasing IgE production (all responses resembling

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IL-13 induced airway pathology in experimental allergic asthma), as well as possibly contributing to increased gut motility and epithelial secretion (via disruption of the tight junction and possibly enhanced cystic fibrosis transmembrane conductance regulator mediated apical chloride secretion[35 36]). IL-13 is important to antihelminth immunity since IL-13 knockout mice (and IL-4Rα and STAT6 deficient, but not IL-4 deficient mice) have significantly longer parasite expulsion times after experimental intestinal nematode infection.[37 38]

Murine models show that in helminth infections, $CD4^+$ T cells are the source of gut mucosal IL-13. In fact, CD4 cells from *Trichuris muris* infected wild-type mice (harvested at the peak of Th2 cytokine production) can transfer resistance to infection in SCID (T and B cell deficient) mice.[39] In humans, a recent report documented the induction of Th2 cytokine production following experimental hookworm infection in naïve persons; subjects developed peripheral blood memory T cells that produced IL-13 in a helminth antigen specific manner.[40] No early innate IL-13 response to helminthic infection was observed in these naïve subjects but an adaptive IL-13 response clearly developed. It is not known whether innate responses specific to the gut (compared with peripheral blood) are active in helminth naïve or helminth experienced persons. Despite adaptive IL-13 responses and induced tissue eosinophilia however, humans infected with helminths usually do not eliminate the worms entirely.

IL-13 producing cells can be found in the healthy and non-inflamed human lamina propria, and IL-13Rα1 is expressed constitutively in gut epithelium, but it is unknown whether IL-13 plays an important homeostatic role or is only roused to action by infection or injury. On the one hand, human and mouse cells show that IL-13 can limit Th17 inflammation by blocking IL-17 (A) production by increasing IL-10 (in a murine model), suggesting perhaps a homeostatic role in the gut.[41–43] On the other hand, IL-13 may play a role in limiting evolving colitis injury: the IL-10 knockout mouse is susceptible to severe *T muris* gut infection but when the IL-13Rα2 decoy receptor is also deleted, effectively increasing the bioavailability of IL-13, there is significant protection from mortality and induction of colitis.[44] Interestingly, this protective effect of IL-13 is also associated with inhibition of IL-17 production. Th17 cells have been shown to express IL-13Rα1, and IL-13 can inhibit Th17 development from naïve T cells (not surprising perhaps, given the same effect of IL-4 previously described, possibly acting through the same type 1 receptor). So endogenous IL-13 may have a dual role of protecting against excess IL-17 effects in the gut and limiting colitogenic processes. IL-13 pretreatment is also effective at limiting ischaemia–reperfusion injury of the murine small bowel associated with inhibiting inflammatory cytokines, consistent with similar effects on inflammatory cytokine activity in the gut and periphery.[45–47] Conversely, when IL-13 is deliberately overexpressed in the murine gut, epithelial remodelling and dysfunction occurs. Linked to the fatty acid binding protein intestinal promoter, increased IL-13 expression by the small intestinal epithelium is associated with goblet cell hyperplasia (and mucus hypersecretion), villus shortening, epithelial cell hyperproliferation (without increased apoptosis) and cystic fibrosis transmembrane conductance regulator upregulation with increased apical chloride secretion.[35] Interestingly, this condition was not associated with apparent inflammation (B and T cell numbers and T cell activation markers were similar to wild-type mice). This lack of accompanying inflammation could be related to the facts that the epithelium (and not immune cells) was the source of the IL-13 and there was a concomitant increase in soluble IL-13Rα2 that could have controlled local activity of IL-13; it could also be due to enhanced Paneth cell function (not measured) and the relative lack of microbial load in the small intestinal lumen.

Little is known about the expression of IL-13 receptors in the gut, pointing out sites where IL-13 could exert effects. In the mouse gut, the IL-13Rα1 gene is expressed in the epithelium, myenteric plexi and smooth muscle layers of the small bowel and colon, whereas the IL-13Rα2 gene was solely and highly expressed in smooth muscle.[48] Expression of IL-13Rα1 is seen in epithelial cells isolated from non-inflamed human colon (IL-13 exposure induces phospho-STAT6 as well as transient increases in phospho-MAPK and phospho-p38MAPK) while IL-13Rα2 is expressed at very low levels if at all.[49 50] In ulcerative colitis, colonic epithelial cells can show an increased expression of both receptors,[49] and in Crohn's disease with fistula formation, IL-13Rα1 receptor protein has increased expression in myofibroblast-like and epithelial-like cells lining, and adjacent to, fistula tracts.[51] So there are sites where IL-13 can induce contractility, mucus production and increased secretion to fight helminth infection in the normal gut, but information on receptors on gut associated immune cells and tissue structures is lacking. One challenge to defining IL-13 and its role in gut mucosal immunity lies in identification of the cell phenotypes that provide the IL-13, whether a traditional Th2 T cell (making IL-4, IL-5 and/or IL-13), NK cells within the epithelium,[52] NK T cells in the lamina propria or the innate helper cell that can secrete high levels of IL-13 in response to epithelial derived cytokines. Another challenge involves delineating both the protective and injurious effects that IL-13 exerts in the gut wall and whether these thresholds are determined by acquired (during an infectious or inflammatory event) or genetic factors.

IL-13 AND HUMAN DISEASE

The prominent role of IL-13 in allergic asthma models and excess IL-13 production in human asthma are currently driving the development and testing of anti-IL-13 compounds in clinical trials. Animal models of allergic asthma clearly show that IL-13 unresponsive mice are resistant to allergen induced airway hyperresponsiveness and the ensuing inflammatory changes.[53 54] Now that early reports support the efficacy of anti-IL-13 antibodies for human asthma,[55 56] other conditions that implicate IL-13 in their pathogenesis may also benefit from this drug pipeline. For example, IL-13 may be an effector cytokine in fibrotic diseases, from idiopathic pulmonary arterial hypertension to progressive systemic sclerosis.[19 57–59] Below we review the association of IL-13 with several gastrointestinal inflammatory diseases, ulcerative colitis, EO and the fibrosis associated with Crohn's fistulous disease.

Ulcerative colitis

IL-13 has become the leading candidate effector cytokine driving inflammation in ulcerative colitis based on data from a murine model of ulcerative colitis, from subsequent confirmation of excess IL-13 production in human disease and from establishment of biologically plausible mechanisms of IL-13 induced gut injury using in vitro colonic epithelial (colon cancer) cell lines. In the oxazalone model of colitis, following skin sensitisation with the haptenating agent oxazolone, oxazolone is administered intrarectally in an alcohol vehicle; this induces an immune mediated inflammatory response to haptenated microbial antigens that access the lamina propria via an ethanol damaged epithelial barrier. The histopathological appearance closely resembles ulcerative colitis, particularly since

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the inflammation is limited to the mucosa.[60] After a short lived increase in IL-4, the hallmark cytokine of the Th2 response, there is a significant increase in IL-13 produced by lamina propria mononuclear cells. An innate immune cell, the NK T cell, was identified as the source of the IL-13 and found to be the major contributor to the inflammation since the lesion could be prevented by administration of an IL-13Rα2-Fc fusion protein (neutralising secreted IL-13) as well as by depleting mice of NK T cells or the cell surface molecule that presents specific antigens to the NK T cells (CD1 in mice, CD1d in humans).[61] Furthermore, when lamina propria mononuclear cells were stimulated with the invariant NK T cell specific antigen, α-galactosylceramide, the amount of IL-13 secreted was similar to polyclonal T cell receptor stimulation, suggesting that nearly all the IL-13 was derived from NK T cells. Interestingly, after intratracheal α-galactosylceramide analogue administration to mice, NK T cells directed lung dendritic cells to induce a Th2 inflammatory (IL-4, IL-5 and IL-13) T cell response to other antigens (although the NK T cell itself was not characterised as a source of IL-13), suggesting that NK T cells can be involved at different points in the induction of innate IL-13 responses.[62] More recently, investigators showed that not only was IL-25 (IL-17E) expression increased in colonic epithelium in oxazalone treated mice, but pretreatment blockade of IL-25 binding to lamina propria mononuclear cells (including T cells, NK T cells and a population of cells thought to contain non-T, non-B innate lymphoid cells) inhibited IL-13 production and prevented colitis.[63] These data suggest that while IL-13 might be a valuable target for novel therapy in ulcerative colitis, targeting the factors that regulate its production during inflammation may also be a useful strategy.

Observations in human disease show that patients with active ulcerative colitis produce significantly higher amounts of IL-13 from lamina propria mononuclear cells and increased epithelial pSTAT6 compared with active Crohn's patients and healthy controls.[4 64] Moreover, NK T cells appear to be the major source of IL-13 from the inflamed ulcerative colitis mucosa, but the cells are not of the majority type I invariant NK T cell population; instead they appear to be from the NK T cell type II that are non-invariant (ie, not sharing the Vα24Jα14 TCR chain and not stimulated by α-galactosylceramide) but respond to stimulation by CD1d expressing cells (presumably loaded with endogenous glycoprotein antigens).

The excessive production of IL-13 in ulcerative colitis is thought to lead to its deleterious effects, particularly as IL-13 has demonstrated toxic effects on colonic epithelial cells and the epithelial barrier. IL-13 activates the proapoptotic molecule caspase 3 in mouse colonic epithelial cells, a process involving the tumour necrosis factor superfamily cytokine TWEAK as well as tumour necrosis factor α itself, all proteins found to have increased expression in patients with active ulcerative colitis.[65] During acute exposure of gut epithelial cells modelled by the HT-29/B6 differentiated subclone of the well known colon cancer cell line, IL-13 can also induce epithelial cell apoptosis, inhibit movement of epithelial cells across a denuded area (interpreted as blocking restitution of the cell monolayers) and disrupt the tight junction by inducing a component, claudin 2, that interferes with the structured tight junction protein complex.[50] In fact, studies of the *T spiralis* nematode infection in mice directly linked IL-13 and IL-13 signalling through the type 1 receptor (produced by $CD3^-$ intraepithelial NK cells) to villus blunting and Goblet cell hyperplasia that can occur even in the absence of B and T cells.[52]

Overexpression of IL-13 in the inflamed mucosa of ulcerative colitis is a unique characteristic of this inflammatory bowel disease. What is less clear is how this inflammation begins. Is there an initial inflammation or injury that induces an innate IL-13 response that becomes uncontrolled or is there an induction of memory Th2 CD4 cells that contribute to ongoing and recurrent antigen specific responses? Studies of cytokine production in ulcerative colitis show that IL-13 is the predominant Th2 cytokine secreted along with IL-5 but without IL-4, consistent with an innate cell source (NK T or innate helper cell) versus a classical Th2 CD4 cell. However, it is still unclear whether there is dysregulation in the IL-13 cytokine and receptor pathways that predisposes to disease. Since IL-13Rα2 deficient mice have amplified responses to IL-13,[66] genetic defects in the IL-13 receptor system have been studied in asthma and fibrosis where excess IL-13 activity could mediate disease. One association with asthma and atopy is the IL-13 variant, Arg130Gln, that produces an IL-13 peptide that still activates the IL-13Rα1 receptor but has lower affinity for the IL-13Rα2 receptor[67] giving a phenotype that would be predicted to enhance IL-13 effects.[68–72] Furthermore, polymorphisms in the IL-13Rα2 gene that could affect the binding of transcription factors are significantly associated with systemic sclerosis patients[69] but there are no functional data to explain any of these effects on IL-13Rα2 expression. One small study in ulcerative colitis patients failed to show disease association with the Arg130Gln IL-13 variant.[72] However, in one study of infliximab treatment response (one or three infusions) in ulcerative colitis patients, IL-13Rα2 gene expression in colonic biopsies was found to be significantly higher in non-responders (defined as no endoscopic and histological healing) compared with responders.[73] Since tumour necrosis factor can induce IL-13Rα2 RNA,[74] this finding makes sense, but whether this subset of patients represents one with more intense IL-13 production (contributing to increased IL-13Rα2 gene expression) or dysfunction of the IL-13Rα2 receptor protein (higher expression should blunt IL-13 activity) is unknown. Finally, a pilot study looking at the effects of type I interferon treatment on ulcerative colitis showed that only treatment responders had significant decreases in IL-13 production; the non-responders showed no change in IL-13 production and also had significantly higher pretreatment production of IL-17.[75]

Eosinophilic oesophagitis

Eosinophilic oesophagitis (EO) is an increasingly recognised disease characterised by marked eosinophilic infiltration (≥15 eosinophils/high power field) of the oesophageal mucosa with clear links to allergic inflammatory states.[76] There are many examples from animal models of an IL-13 role in the induction of the typical lesion of EO. For instance, eosinophilia of the oesophagus followed nasal or tracheal administration of IL-13, and this outcome could be blocked in IL-5 deficient mice.[77] Following aeroallergen induction of an EO-like lesion, both IL-13 and STAT6 deficient mice were protected from developing the oesophageal inflammation.[78] Similarly, targeted overexpression of IL-13 in the lungs resulted in oesophageal eosinophil infiltration as well as oesophageal tissue remodelling (macroscopic increase in the oesophageal circumference, increased collagen deposition, epithelial cell layer hyperplasia and angiogenesis).[79] After incubation with IL-13, isolated murine oesophageal tissue produced potent, STAT6 dependent chemotactic factors for eosinophils (CCL11 (eotaxin-1) and CCL24 (eotaxin-3)), important in the development of EO.[80] In human

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disease, cytokine expression in EO shows significant upregulation of IL-13 and IL-5 expression as well as IL-13/STAT-6 induced chemokines such as eotaxin-3 and CCL23.[81] Significantly elevated IL-13 and IL-5 levels can be detected in the plasma of children with EO and food allergy.[82] These data link IL-13 to the development of EO in animal models and confirm similar cytokine disturbances in human disease. Further work will need to be done on the contribution of allergic, or IL-5 dependent, inflammation to EO and understanding what the hierarchy of cytokine contributions are in driving the eosinophilia and tissue remodelling which may occur by independent mechanisms (important for planning therapy and predicting non-response).

The pathogenesis of EO seems to be linked to allergen hypersensitivity, and given the familial association of EO, atopy and food allergy, a genetic component may be contributing to disease susceptibility. Several genes and gene loci have been identified as risk variants in EO using a candidate gene approach, including the 3′ untranslated region of eotaxin (*CCL26*), the transforming growth factor (TGF) β1 promoter, a filaggrin (*FLG*) exon and a TSLP intron and TSLP receptor (*CRLF2*) exon.[83] These associations make sense because eotaxin is excessively expressed in EO mucosa, FLG is a structural skin protein that helps to maintain barrier function (and is downregulated by IL-13) and TSLP has been shown to stimulate IL-13 production by innate helper cells in the lamina propria.[84] However, it is still unclear how these genetic polymorphisms translate to dysfunction of the IL-13 pathway in mediating susceptibility to EO.

Intestinal fibrosis

Tissue fibrosis is a recognised outcome of IL-13 exposure, including progressive systemic sclerosis,[85 86] hepatic fibrosis[87] and idiopathic pulmonary fibrosis.[88] IL-13 has been linked to the tissue remodelling seen in animal models of allergic asthma,[79 89] EO and bleomycin induced pulmonary fibrosis.[90] Interestingly, in the 2,4,6-trinitrobenzenesulfonic acid (TNBS) model of colitis in mice, typically a model of Crohn's disease in the early days after TNBS administration, chronic intrarectal administration of the haptenating agent progresses to an IL-13 dominant cytokine production profile. This increase in IL-13 production is accompanied by colonic fibrosis that is mediated by TGFβ, since blocking TGFβ abrogated the fibrosis. IL-13 itself had been previously recognised as inducing fibrosis via TGFβ following targeted overexpression of IL-13 in the lung.[91] So the chronic TNBS model demonstrates how fibrosis can occur following IL-13 production in the intestine, and while fibrostenotic complications of ulcerative colitis are generally low, fibrosis is recognised as a complication of longstanding ulcerative colitis and healing is associated with pronounced scarring.[92–94] On the other hand, where fibrostenotic complications are a hallmark of Crohn's disease, a role for IL-13 is emerging in the formation of fistulae. A recent report demonstrates that IL-13 is highly expressed in so-called transitional cells that line fistula tracks, and that IL-13 induces genes involved in cell invasion, presumably a mechanism of fistula formation through tissue[51]; interestingly, TGFβ was also highly expressed and induced IL-13 production by lamina propria fibroblasts. These observations support IL-13 as a target for antifibrotic therapies in certain settings.

Additional IL-13 effects in the GI tract

The role of IL-13 regulated intestinal contractility as a contributing mechanism for helminth expulsion has interested groups studying motility disorders. Whereas IL-13Rα2 is dominantly expressed in murine gut smooth muscle, it is observed that IL-13Rα2 deficient mice have hypercontractile responses to acetylcholine.[48] This hypercontractility is induced by *Nippostrongylus brasiliensis* infection where epithelial derived IL-25 drives the IL-13 and muscle responses.[9] Hypercontractility of the muscularis propria is seen in mouse gut after infection with parasites or after polyclonal T cell stimulation, suggesting that cytokines, including IL-13, could be contributing to the stimulation of gut smooth muscle.[95 96] Clearly this could be applied to hypotheses about the aetiology of post-infectious irritable bowel syndrome. It is not known whether IL-13 receptors are similarly expressed in human gut smooth muscle (although they are detected in the smooth muscle of the human pulmonary artery) but IL-13 was produced at significantly higher levels by stimulated peripheral blood lymphocytes isolated from patients with functional gastrointestinal disorders (irritable bowel syndrome, functional dyspepsia and non-cardiac chest pain) compared with normal controls.[97]

DEVELOPMENT OF ANTI-IL-13 DRUGS

In light of the central role IL-13 appears to be playing in allergic asthma, including lung eosinophilia, epithelial hypertrophy, mucus hyperproduction, inflammation, hyperresponsiveness and remodelling of the airway, many companies are developing and testing anti-IL-13 strategies as novel therapeutics, taking advantage of some of the pharmacological aspects of IL-13 and its receptor system (see table 1). The most common strategy is to develop an antibody against IL-13 that can bind to the soluble form of the cytokine and prevent it from binding to its primary signalling molecule IL-13Rα1 but also to IL-13Rα2. For instance, there are several antibodies directed against IL-13 that disrupt binding to the receptors, including anrukinzumab, lebrikinzumab and tralokinumab; in general, this strategy blocks all IL-13 signalling but will allow IL-4 to signal through the type 1 receptor as well as the alternate IL-4Rα/γ_c receptor.[98] There can be different mechanisms of action resulting from antibody design to the same protein however. For instance, IMA-026 and anrukinzumab are antibodies directed against non-shared epitopes of IL-13. Whereas IMA-026 blocks the binding of IL-13 to IL-13Rα1 and IL-13Rα2, anrukinzumab permits binding of IL-13 to IL-13Rα1 and IL-13Rα2 but inhibits formation of a IL-13Rα1 complex with IL-4Rα; both antibodies prevent activation through the IL-13Rα1/IL-4Ra receptor, but while IMA-026 potently interferes with IL-13 uptake and internalisation by the IL-13Rα2 receptor, anrukinzumab only does so partially (and the IL-13-anrukinzumab complex still can be internalised through the IL-13Ra2).[21 99] So use of the IMA-026 antibody can bind IL-13 but result in higher detectable circulating IL-13 (presumably antibody bound, biologically inactive IL-13) in treated patients.[56]

There is a strategy to block access to IL-4Rα, thereby blocking IL-13 and IL-4 engagement of the IL-13Rα1/IL-4α receptor complex (and IL2R-γ_c receptor). This strategy might enhance IL-13 binding to the IL-13Rα2 receptor whose decoy/scavenging activity could enhance anti-IL-13 effects or enhance IL-13 signals through IL-13Rα2, depending on the cell type and surface expression of IL-13Rα2. Another anti-IL-13 agent has been developed to target the IL-13Rα2 receptor specifically, since IL-13 plays a role as a growth factor for certain malignancies which express IL-13 receptors; in this case, agents are developed to destroy the IL-13Rα2 bearing cell, for instance using an endotoxin linked to a mutated IL-13 protein that preferentially binds to the IL-13Rα2 and can kill the cell on internalisation.[100]

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Table 1 Drugs in development targeting interleukin 13 or its receptors

Inhibitor	Company	Isotype	Mode of mechanism	Route	Clinical data
Anti-IL-13 MILR1444A Lebrikizumab	Roche/ Genentech	IgG4, κ, with stabilising point mutations	Binds to human IL-13 and inhibits IL-13 induced phosphorylation of STAT6 in TF-1 cells	sc	Positive phase 2 study in patients with adult asthma despite inhaled glucocorticoid therapy (NCT00930163). Monthly treatment with 250 mg improved FEV_1 at week 12. Reductions in serum Th2 chemokines (CCL13, CCL17) and IgE were observed.[55] Phase 3 studies in patients whose asthma is uncontrolled with inhaled corticosteroids and a second controller medication (LUTE, NCT01545440 and VERSE, NCT01545453) are ongoing, with primary end point being exacerbations during the 52 week placebo controlled period.
Anti-IL-13 CAT-354 Tralokinumab	Medimmune/ Astra Zeneca	IgG4, λ	Neutralises IL-13	sc	Phase 2 randomised, double blind, placebo controlled study in moderate to severe asthma (NCT00873860) showed improved FEV_1 at week 13, but no improvement in ACQ-6 score.[102] A phase IIa, randomised, double blind, placebo controlled, parallel arm, multicentre study to evaluate the efficacy and safety of tralokinumab (every 2 weeks for 12 weeks) as add on therapy, on clinical response in patients with active, moderate to severe, ulcerative colitis is ongoing (NCT01482884).
Anti-IL-13 QAX-576 Fully human	Novartis	IgG1, κ	Specific inhibitor of human IL-13 activity	iv	A double blind, placebo controlled study showed reduced IL-13 responses in intranasal grass pollen challenge model, with possible effect on total nasal symptom scores in subgroup with high late phase nasal IL-13 levels at screening (NCT00584584).[103] Phase 2 programmes are ongoing in moderate to severe asthma (NCT01130064) and idiopathic pulmonary fibrosis (NCT01266135). A phase 1 study on the sequential administration of a fixed dose of the anti-IL-4 antibody, VAK694, and single ascending doses of QAX576 in patients with well controlled mild to moderate asthma is completed (NCT01568762). In NCT01316601 the efficacy, safety and tolerability of QAX576 in the treatment of perianal fistulas in patients suffering from Crohn's disease is assessed. In patients with eosinophilic oesophagitis, the effects of a 12 week course of iv QAX576 6 mg/kg every 4 weeks is tested to reduce the number of eosinophils in the oesophagus (NCT01022970). The study has been completed, but results are pending
Anti-IL-13 ABT-308	Abbott		Blocks IL-13 interaction with IL-13Rα1/α2[104]	sc and iv	Phase 1 in patients with mild to moderate asthma is completed (NCT00986037)
Anti-IL-13 IMA-026 Humanised	Pfizer	IgG1, κ	Specific for IL-13 epitope that binds IL-13Rα1 and IL-13Rα2	sc	In phase 2 no significant effects on allergen induced late phase asthmatic response or sputum eosinophils could be observed (NCT00725582)[56]
Anti-IL-13 IMA-638 Anrukinzumab	Pfizer	IgG1, κ	Inhibits formation of a IL-13Rα1 complex with IL-4Rα	sc and iv	In phase 2 (NCT00410280), an attenuated early (19%) and late allergen induced asthmatic response (24%) was described in patients with mild atopic stable asthma.[56] A phase 2a study is recruiting patients with active ulcerative colitis and evaluates proof of mechanism of multiple iv doses of anrukinzumab by changes in mechanism based biomarker and pharmacodynamic biomarkers (NCT01284062)
Anti-IL-13 CNTO-5825 Fully human	Centocor	IgG1, κ	Neutralising IL-13	sc and iv	A randomised, placebo controlled, double blind phase I study to assess the safety, tolerability, immune response, pharmacokinetics and pharmacodynamics in healthy volunteers and healthy atopic volunteers is completed (NCT01081691)
IL-4 IL-13 targeted					
Anti-IL-4R SAR231893 (REGN668)	Sanofi/ Regeneron	IgG	Binds to IL-4R which blocks the function of IL-4 and IL-13	sc	A phase 2 study on once weekly injections for 12 weeks vs placebo on reducing the incidence of asthma exacerbations in patients with persistent moderate to severe eosinophilic asthma is ongoing
Anti-IL-4Rα antagonist AMG-317 Fully human	Amgen	IgG2a	Binds to IL-4Rα, which competitively blocks the function of IL-4 and IL-13	iv and sc	In a phase 2, randomised, double blind, placebo controlled study, patients received weekly sc injections of AMG 317 (75–300 mg) for 12 weeks. The primary end point, change from baseline at week 12 in ACQ symptom score, was not met in the total patient population. Patients with highest baseline ACQ were more likely to respond.[105] Significant IgE response in population pharmacokinetic model by fitting data from four early phase clinical trials[106]

Continued

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Table 1 Continued

Inhibitor	Company	Isotype	Mode of mechanism	Route	Clinical data
IL-4/IL-13 bispecific DvD-Ig SAR156597	Sanofi	Dvd-Ig (dual variable domain Ig)	Bispecific antibody which simultaneously binds both IL-4 and IL-13	sc	At Protein Engineering Summit May 2012, presentation on allergen induced airway hyperresponsiveness in monkeys, and on safety, tolerability and pharmacokinetics of single sc doses of SAR156597 in healthy human subjects (phase 1). Randomised, double blind, placebo controlled study of the safety, tolerability, pharmacokinetics and pharmacodynamics of ascending repeated doses in patients with idiopathic pulmonary fibrosis ongoing; orphan drug status granted by FDA
IL-4 mutein Pitrakinra	Aerovance/ Bayer	Similar to native IL-4 molecule, but with 2 amino acid changes (R121D/Y124D), thus competing for IL-4Rα	Binds to the IL-4Rα subunit and prevents the inflammation induced by IL-4 and IL-13	Inhaled (sc)	In phase 2a study with inhaled compound diminished late phase asthmatic response to allergen challenge in asthmatic patients.[107] Double blind, randomised, placebo controlled, multicentre trial in patients with uncontrolled, moderate to severe asthma did not reveal a difference in incidence of asthma exacerbations vs placebo after 12 week treatment period. Patients with blood eosinophilia > 350 cells/mm3 showed lower incidence of exacerbation.[108 109] Better activity in subjects with IL-4R polymorphism[110]
AIR-645	ISIS/Altair	IL-4Rα 2′-O-methoxyethyoligonucleotide antisense RNA	Targets the mRNA that encodes the α subunit of the human IL-4R	Inhaled	Positive safety profile, sputum half life ~ 5 days in sputum.[111] Following repeated inhalation, evidence of reductions in serum total IgE, sputum eosinophils in mild asthmatics.[112] Not enough activity in ph2 mild atopic asthma to warrant further development
(Anti IL-4/Anti IL-13 combo) QBX258	Novartis	Ab combo	Blocking IL-4 and IL-13	iv	Anti IL-4 Ab VAK694, in studies of allergic rhinitis. Anti IL-13 Ab QAX576 (see above)

ACQ, Asthma Control Questionnaire; FEV_1, forced expiratory volume in 1 s; IL, interleukin; iv, intravenous; sc, subcutaneous;

ANTI-IL-13 TRIALS

To date, most of the trials in humans using anti-IL-13 agents have been done in asthma with varying degrees of success. A phase 2 study of adult patients with active asthma despite inhaled glucocorticoid therapy treated with 250 mg of lebrikizumab subcutaneously each month showed significant improvement of forced expiratory volume in 1 s at week 12. This effect was more pronounced in a subgroup of patients with high periostin (an IL-13 inducible gene), indicating perhaps even more anti-inflammatory activity for lebrikizumab in patients with higher IL-13 activity. Table 1 lists published and ongoing studies with compounds targeting the IL-13 pathway.

Results from trials using anti-IL-13 agents in patients with inflammatory bowel disease are not yet available. The safety and efficacy of the anti-IL-13 antibody anrukinzumab (IMA 638, Pfizer) is being tested in a phase 2a study in patients with mild to moderate ulcerative colitis. Another phase 2a study is evaluating the efficacy and safety of tralokinumab (CAT-354, MedImmune/ Astra Zeneca), a fully human anti-IL-13 antibody in patients with moderate to severe ulcerative colitis. QAX576 (Novartis) is also a fully human anti-IL-13 antibody which is being studied in patients with perianal fistulas from Crohn's disease. Interferon β-1a (Avonex) has previously been used in ulcerative colitis based on the in vitro effects of type I interferons inhibiting IL-13 production from peripheral blood mononuclear cells[31] and IL-13 intracellular signalling in human monocytes.[34] In an open label pilot study that followed immune parameters before and after treatment, 16 patients with active ulcerative colitis were treated for 12 weeks with weekly interferon β-1a.[75] There was a clinical response rate of >65% at the end of treatment and all responders had a significant decrease in T cell receptor stimulated IL-13 production by lamina propria mononuclear cells (690 vs 297 pg/ml) compared with non-responders (542 vs 510 pg/ml). In a follow-on randomised, placebo controlled phase II study, interferon β-1a (Avonex) 30 μg subcutaneously twice a week for 12 weeks resulted in a similar significant clinical response at 8 (46% vs 68% placebo vs interferon β-1a; p=0.05) and 12 weeks (52% vs 75%; p=0.01) but no data on changes in cytokines are available.[101]

SUMMARY

IL-13 is a pleiotropic cytokine both in its cellular sources and in its observed actions. Preclinical and in vitro data point to the involvement of this cytokine in the pathogenesis of chronic inflammatory disorders of the gastrointestinal tract, particularly ulcerative colitis, EO and perianal fistula formation in Crohn's disease. Numerous compounds are available for neutralising IL-13 and activation of its receptor system, of which some have shown biological activity and efficacy in asthma. The study results from anti-IL-13 studies in ulcerative colitis, Crohn's disease and eventually EO are eagerly awaited to further understand the importance of IL-13 in supporting established inflammation of the gastrointestinal tract.

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