

Strategies targeting the IL-4/IL-13 axes in disease



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ABSTRACT

IL-4 and IL-13 are pleiotropic Th2 cytokines produced by a wide variety of different cell types and responsible for a broad range of biology and functions. Physiologically, Th2 cytokines are known to mediate host defense against parasites but they can also trigger disease if their activities are dysregulated. In this review we discuss the rationale for targeting the IL-4/IL-13 axes in asthma, atopic dermatitis, allergic rhinitis, COPD, cancer, inflammatory bowel disease, autoimmune disease and fibrotic disease as well as evaluating the associated clinical data derived from blocking IL-4, IL-13 or IL-4 and IL-13 together.

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1. Introduction

IL-4 was first cloned in 1986 from a mouse T cell line and was known as IgG1 induction factor [1]. In the same year the human IL-4 ortholog was cloned from a concanavalin A-activated human T-cell cDNA library and found to be a 153aa protein with a signal sequence that stimulated helper T cell and anti-IgM activated B cell proliferation [2]. Both human [3] and mouse [4] IL-4 were shown to induce IgE production from B cells and mouse [5] and human IL-4 [6] shown to be secreted by Th2 cells.

IL-13 was similarly cloned in 1989 from concanavalin A-activated mouse T-cells and was initially known as p600 [7], a protein secreted by Th2 cells. The human IL-13 ortholog was cloned by 2 teams [8,9] from activated human lymphocytes as a 132aa protein with a signal sequence that inhibited LPS-induced IL-6 production by peripheral blood mononuclear cells (PBMC) [8], stimulated PBMC MHC II and CD23 expression and induced B cell proliferation and immunoglobulin production [9]. IL-4 and IL-13 are 20–25% identical but with higher identity in the first and last alpha-helical regions [8] known to be key for IL-4 activity [10].

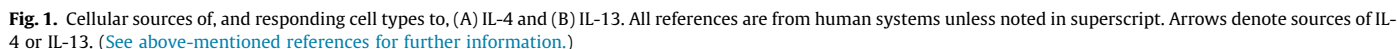
IL-4 mediates its biological effects by binding to IL-4R α ; subsequently either gamma c or IL-13 receptor alpha 1 (IL-13R α 1) are recruited to form a signaling complex [11]. IL-13 mediates its biological effects by binding to IL-13R α 1 and then recruiting IL-4R α to form a signaling complex [11,12]. IL-4:IL-4R α : γ c,

IL-4:IL-4R α :IL-13R α 1, IL-13:IL-13R α 1:IL-4R α all signal through STAT6 [13]. Studies on the activated IL-4/IL-4R α complex show that receptor components cluster densely on, and just beneath, the cell membrane in early sorting and recycling cortical endosomes trafficked via a constitutive internalization process [14]. Sources of IL-4 and IL-13 and cell types expressing the receptors are shown in Fig. 1.

Polymorphisms in the IL-4/IL-13 axis exist and modulate function positively and negatively. Q576R IL-4R α is a cytoplasmic-IL-4R α mutant associated with atopy [15]; I75V is an extracellular-IL-4R α mutant that when combined with Q576R mediates enhanced pharmacodynamic effects compared to WT IL-4R α and is associated with atopy [15] and atopic asthma [16]. R130Q IL-13 has been associated with asthma, atopy and increased serum IgE [17–19] and shown to activate the signaling of IL-13R α 1 and downstream functions more efficiently, and binding to the decoy receptor (IL-13R α 2) less efficiently, than WT IL-13 [20]. Additionally R130Q IL-13 is more stable in human plasma than WT IL-13; patients homozygous for R130Q have higher serum IL-13 levels than non-homozygotes [21]. Finally R130Q expression causes an elevated pharmacodynamic effect via Q576R I75V IL-4R α compared with WT IL-4R α [22]. On the other hand, IL-4 δ 2 is a polymorphic form of IL-4 generated by deletion of exon 2 from IL-4 mRNA [23] which acts as a functional antagonist of IL-4 at IL-4R α [24]. Other negative regulators include soluble IL-4R α , formed by a stop codon in exon 8 of the 12 exons present for the full length protein [25], and membrane IL-13R α 2 which lacks a significant cytoplasmic tail and is generally considered to be a decoy [26], involved in removing IL-13 by internalization [27],

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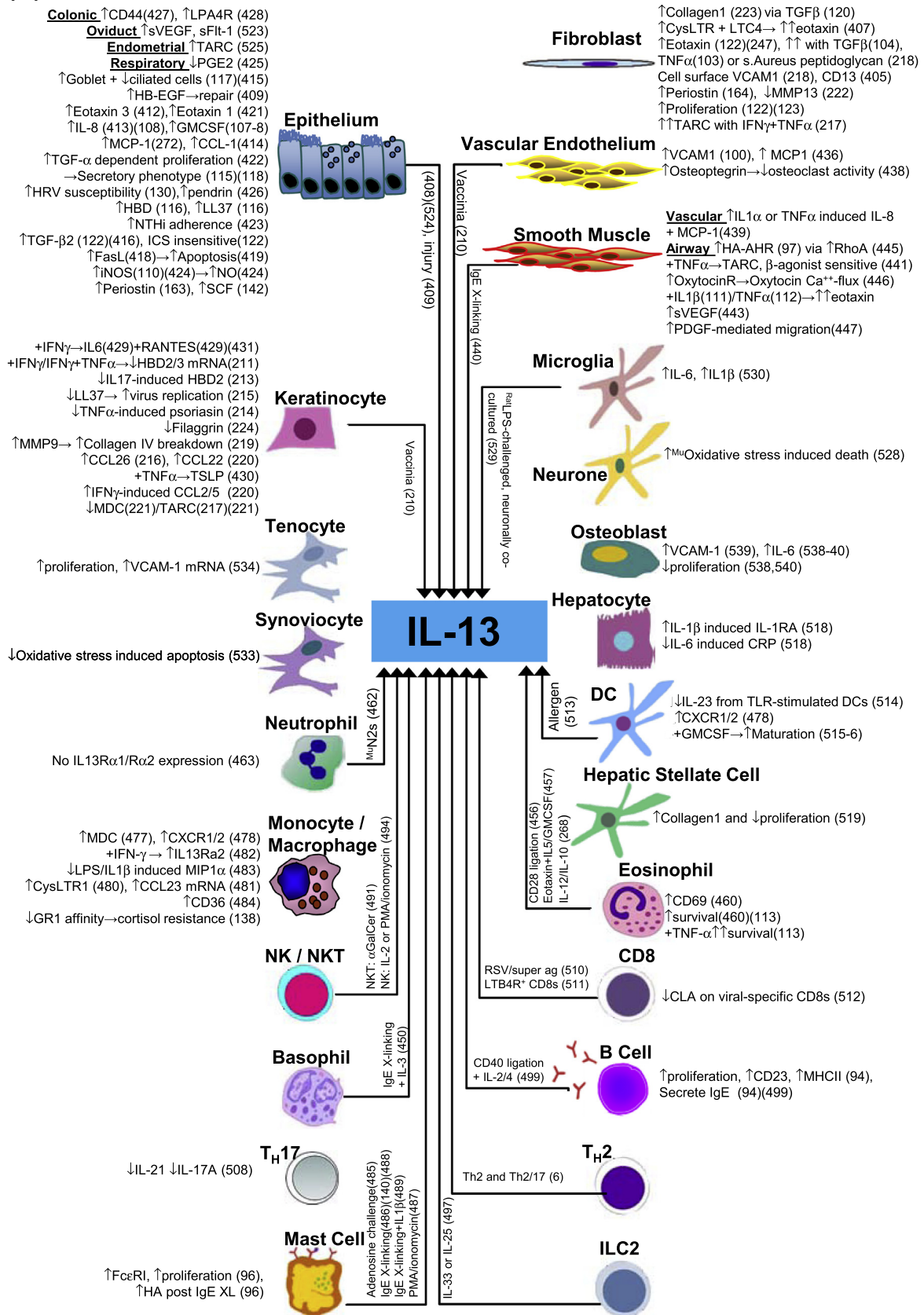


Fig. 1 (continued)

particularly in humans [28] where soluble IL-13R α 2 is not present due to lack of a differential splice site [29,30]. However there is an emerging literature associating IL-13R α 2 with fibrosis [31] and as a receptor for chitinase 3-like 1 [32]. STUB1, an intracellular ubiquitin ligase, has just been shown to interact with IL-4R α and shuttle it for degradation thereby decreasing IL-4/-13 signaling [33].

Both IL-4 and IL-13 reside on human chromosome 5q23-31 [34,35] in a cluster of allergy-related genes including GMCSF, IL-3 and IL-5 [36]. This cluster has been repeatedly linked with an increased risk of allergic-disease development (reviewed in [37]). The broad similarity in biological function, signal transduction, gene structure [38] and genomic localization of IL-4 and IL-13 have led to speculation that they may have arisen *via* gene duplication [39]. It is tempting to speculate that the natural advantage of having a highly active Th2 system is optimal extracellular parasite rejection [40]. In support of this genetic analysis suggests the 5q31 complex is under intense selection pressure in geographical regions with endemic parasites [41] and *Schistosoma mansoni* egg counts are lower in individuals with the gain of function Q130R IL-13 mutation [42].

Based on the broad biology of these 2 similar cytokines that has clear links to human disease, substantial efforts have been made to develop effective agents to block them. The types of approaches that have been tried and the rationale supporting them in the various disease areas will be covered in the following sections.

2. Asthma

2.1. Introduction

Asthma is a complex, persistent, heterogeneous inflammatory disease characterized by airways hyperresponsiveness (AHR) in association with airway inflammation. AHR drives reversible airway obstruction that is the archetypal feature of asthma. Mild asthma is treated with short-acting beta-2 agonists (SABA). As severity worsens to moderate or severe, treatment is correspondingly intensified with increasing doses of inhaled corticosteroids (ICS) and long-acting beta-2-adrenoceptor agonists (LABA) supplemented, in the worst cases, with oral corticosteroids (OCS). Therapy is focused on giving the patient 'control' or a relatively normal life not restricted by their asthma [43]. Although current therapy controls asthma for the majority of patients, it is recognized that a significant proportion of asthmatics are inadequately controlled even with maximal steroid treatment [44]. It is in this most severe space that biologics have utility; the only licensed biologic in 2015 is omalizumab (Xolair, Genentech, South San Francisco, CA, USA/Novartis, East Hanover, NJ, USA) which was approved in 2003 for the treatment of moderate-to-severe, persistent allergic asthma in patients whose symptoms are inadequately controlled by inhaled corticosteroids. Omalizumab acts primarily by blocking IgE in the serum from binding to the high affinity IgE receptor (Fc ϵ RI) on mast cells and basophils thus preventing their activation by allergens. As described below IL-4 and IL-13 are active in severe asthma. Intense investigations are being undertaken to understand whether neutralizing the IL-13 and/or IL-4 axes will bring clinical benefit to patients.

2.2. Disease expression

The preponderance of eosinophils [45] and Th2 cells [46] and the presence of heterogeneity, that not all asthmatics are eosinophilic, have long been recognized in severe asthma [47]. Multiple studies have explored the link between IL-4 and IL-13 and asthma. IL-4 mRNA and protein are increased in bronchial biopsies, bronchoalveolar lavage (BAL) fluid and exhaled breath condensate

(EBC) from asthmatics compared with control subjects [48–54]. IL-4 BAL expression is stimulated by allergen challenge in asthmatics [55,56]. Similarly IL-13 mRNA and protein are also increased in bronchial biopsies, sputum and BAL fluid from asthmatic compared with control subjects [49,57–59]. BAL IL-13 expression is also stimulated by allergen challenge in asthmatics [55,60]. Cigarette smoke-exposed pediatric asthmatics have been shown to have further elevations in IL-13 protein expression compared to non-smoke exposed pediatric asthmatics [61]. A variety of different cells can act as sources of IL-4 and IL-13 in the asthmatic lung (Fig. 1). A major source is likely to be Th2 cells; these cells are present in the asthmatic lung as demonstrated by the observation that IL-5 and IL-13 were secreted from allergen-challenged (DerP1), cultured bronchial biopsies in a CTLA4-Ig dependent fashion [62].

Lung IL-4 expression is associated with therapy utilization. In a pediatric population steroid-naïve asthmatics had higher IL-4 in EBC than healthy controls or ICS-treated asthmatics; IL-4 was measurable in all samples [53]. EBC IL-4 was also upregulated in mild adult asthmatics compared to healthy controls; ICS were shown to have a time-dependent suppressive effect on EBC IL-4 [54]. Oral prednisolone treatment of adult moderate asthmatics decreased the number of IL-4 mRNA positive cells in bronchial biopsies in the context of reduced AHR and improved lung function (increased forced expiratory volume in 1 s (FEV1)) [63]. IL-4 was rapidly consumed in *in vitro* culture [64] and hence likely drives more biology than its measured levels would suggest.

Lung IL-13 expression is correlated with both asthma severity and control. Mild steroid-naïve asthmatics and severe asthmatics who have poor control despite standard of care up to, and including, OCS had higher levels of sputum IL-13 than non-asthmatics [65]. IL-13 expression in moderate asthmatics, who are controlled by ICS, was equivalent to non-asthmatics [65]. Of note only 38% of the severe and 44% of the mild asthmatics had measurable IL-13 in their sputum [65].

Further light has been shed on the molecular, protein and cellular basis underlying the heterogeneity in asthma. Seminal work studying bronchial brushings from mild asthmatics demonstrated the presence of a clear dichotomy where patients were either Th2 high (IL-13, IL-5 gene and protein expression) or Th2 low (no Th2 mRNA or protein expression above control patients) [66]. Th2 high, but not Th2 low, patients responded to ICS with an improved FEV1 and Th2 high patients had more AHR, higher serum IgE, higher BAL and blood eosinophils than Th2 low patients [66]. Taken together it seems clear that IL-4 and IL-13 are closely associated with at least a proportion of human asthma.

2.3. Genetic data

As detailed in Table 1 individual, or pairs, of polymorphisms in IL-4, IL-13 and the associated receptor IL-4R α , but not IL-13R α 1, are associated with the risk of developing asthma and the severity of disease. A holistic genetic analysis of polymorphisms shown to cause functional change across the IL-4/-13 axis in 2240 German children showed that risk was cumulative; the maximum odds ratio of developing asthma for a single SNP was 1.6 (IL-4 C-589T), for two SNPs was 4.6 (STAT6 C2892T and IL-13 C-1112T) and for three SNPs was 27 (STAT6 C2892T, IL-13 C-1112T and IL-4 C-589T) [67]. More recent publications of genome-wide association studies (GWAS) have confirmed the importance of the Th2 axis, encompassing IL-4 and IL-13, in general. Analysis of GABRIEL containing 10,365 patients with asthma and 16,110 non-asthmatics showed highly significant associations of IL-33 (an epithelial cytokine associated with driving Th2 disease through, amongst other cytokines, IL-13 and IL-5) and significant associations of IL-13 with asthma [68]. A recent GWAS including 1173 and 2522 controls but focusing on severe pediatric asthma

Table 1

Genetic polymorphisms in the IL-4 and/or IL-13 axes and their disease association.

Gene and region	Mutation	Disease	Observed link
IL-4 promoter	C to T –285 A to G –81 C to T –589	– Mild asthma Asthma	Enhanced IL-4 transcription <i>via</i> tighter AP-1 interaction [376] TT allele associated with 4.5% lower FEV1 than CC allele in 682 white asthmatics [377] 15,164 person meta-analysis demonstrated increased odds ratio of asthma in the TT/TC vs CC haplotypes [378]
IL-4R α extracellular coding	Ile75Val ^a	– Atopy Asthma	Increased IL4R α receptor signaling [379] Increased odds ratio of Ile75 homozygote being atopic, having IgE > 400 IU/mL, being specific HDM IgE positive versus Val50 homozygote [379] Increased odds ratio of Ile75 homozygote being an atopic asthmatic in Japanese, not UK, population [17] Only V75 and R576 combination was associated with allergic asthma, V75 alone was not [16]
IL-4R α intracellular coding	S503P ^a G to A 1902 Q576R ^a Q576R ^a	Atopy Atopy Asthma Atopic dermatitis Smokers Asthma	P503 or R576 independently associated with lower serum IgE; combination of P503 and R576 most strongly associated [380] S503 associated with elevated serum IgE versus P503; combination of S503 and IL-13-1055 CT/TT has an odds-ratio of 5 for asthma development compared to the WT genes [381] 9.3x increase in relative risk of atopy for those with mutant (RR/RQ), versus wild-type (QQ), allele [15] Only V75 and R576 combination was associated with allergic asthma, R576 alone was not [16] Meta-analysis of 17 studies identified an odds-ratio of 1.8 for the R551 variant with atopic asthma [382] R576 allele associated with AD in patients up to 6 months of age who had not been given antibiotics [383] RR 576 allele associated with rapid decline (–4.1%/yr) in lung function; association became stronger in subjects with the –1055 TT IL-13 promoter haplotype or the IL-13 130RR haplotype [384] Presence of both minor alleles associated with clinical history of severe exacerbation and lower lung function [385]
IL-13R α 1 promoter	–281T > G	Asthma	No association of polymorphism with asthma [386]
IL-13R α 1 non-coding	A1398G	– Asthma	Increased odds ratio of high serum IgE [17] No association of polymorphism with asthma [386]
IL13R α 1 transmembrane region	C1050T L350L	–	Observed at 3% incidence in Japanese population [387]
IL-13 non-coding	–C1055T	Allergic asthma Asthma Atopy Atopic dermatitis Allergic rhinitis Smoking-associated rate of lung function decline COPD	TT genotype associated with allergic asthma and altered regulation of IL-13 production from T cells [388]. TT genotype associated with increased promoter activity in Th2 cells [389] TT genotype associated with allergic asthma with positive skin prick test, asthma and BHR [390] Associated with elevated serum IgE [391] Homo- or hetero-zygotes for 2282del4 <i>FLG</i> loss of function mutation with CT –1055 IL-13 genotype have a 6.4-odds ratio of developing AD compared with wild-type CC –1055 IL-13 genotype [392] T allele associated with risk of developing rhinitis [393] –C1055T not independently associated with lung function. CC/CT associated with 2.4% and TT associated with 8.2% reduction in mean% predicted FEV1 for each cumulative 20 pack year exposure [394] –1055T allele was more common in COPD patients than healthy controls or smokers with normal lung function. IL13 Q130R polymorphism was not associated [395]
	G4794A	Asthma	AA genotype associated with asthma with positive skin prick test, positive skin prick test and asthma [390]
IL-13 coding	R130Q	Asthma Reduced lung function Atopy Atopic dermatitis Allergic rhinitis	Q allele associated with increased odds ratio of atopic and non-atopic asthma and elevated serum IL-13 [17] Q allele associated with asthma [396] Q allele associated with elevated serum IL-13 in asthmatics and non-asthmatics [21] Asthmatic children expressing Q130 IL-13 had elevated eosinophils and serum IgE compared to asthmatic children expressing WT R130 IL-13 in 2 independent populations [397] Children with Q130 IL-13 had an increased odds ratio of developing subsequent wheezing compared to the WT R130 IL-13 [398] Q130 homo- or hetero-zygotes associated with 2.3 odds ratio for developing asthma [393] Children expressing the Q130 IL-13 variant had lower lung function (FEV1) than those expressing WT R130 [399] Q130 associated with elevated serum IgE [19,391,396] Q allele associated with increased odds ratio of high serum IgE and atopic dermatitis [18] Q allele more prevalent in AD patients than non-AD controls but Q allele not associated with blood eosinophil counts or serum IgE level [400] Children hetero- or homo-zygous for Q130 IL-13 and hetero- or homo-zygous for STAT6 (rs1059513) had a 1.52 or 2.01 [replication study] relative risk of developing AD compared to wild-type homozygotes for both genes [401] Adults hetero- or homo-zygous for Q130 IL-13 had an odds ratio of 3.6 or 4.3 of having AR compared to wild-type homozygotes [402] Adults hetero- or homo-zygous for Q130 IL-13 had an odds ratio of 1.24 of having AR compared to wild-type homozygotes [403]

^a Numbering includes 25aa signal peptide.

characterized by exacerbations also associated IL-33 with disease [69].

2.4. Animal model data

There are a vast number of *in vivo* studies implicating the IL-4 and IL-13 axes in animal models of allergic lung inflammation which mimic key aspects of a key subpopulation of human asthma. Transgenic overexpression of IL-4 in the mouse lung using a Clara cell 10 (CC10) promoter caused profound eosinophilic, lymphocytic and neutrophilic inflammation, epithelial hypertrophy associated with mucus hypersecretion, increased baseline lung resistance but no AHR [70]. IL-4 gene ablation or prophylactic antibody neutralization of IL-4, from initial sensitization through challenge, identified a crucial role for IL-4 in the initiation but not maintenance of the classic mouse allergen sensitization and challenge Th2 phenotype of lung eosinophilia [71] and AHR [72]. Similar effects in an allergic mouse model of reduced AHR and reduced serum total and specific IgE were seen if soluble IL-4 receptor was dosed prophylactically, either systemically or locally via nebulization [73]. Later mouse allergen sensitization and lung challenge studies demonstrated that locally, but not systemically, administered soluble IL-4 receptor could effectively ablate lung tissue and BAL eosinophilia and airway mucus occlusion but not AHR [74]. Interestingly 20 µg recombinant human IL-4 nebulized to mild allergic asthmatics caused increased AHR and elevated eosinophilia at 24 h [75].

Recombinant IL-13 administered to the airways of allergen-naïve mice caused AHR, airway inflammation and mucus production [76,77]. Transgenic overexpression of IL-13 in the mouse lung causes similar pathologies although chronic exposure results in the development of fibrosis [78]. The same 'asthmatic' lung phenotype is generated following lung allergen challenge in allergen-sensitized mice and is also associated with expression of IL-4 and IL-13 [72]. A potent IL-13 neutralizing agent, soluble IL-13R α 2-Fc, inhibits AHR, mucus hypersecretion and pulmonary inflammation associated with allergic mouse lung inflammation [76,77]. IL-13-deficient mice do not develop AHR in an allergic lung inflammation model [79]. In an analogous model IL-13R α 1-deficient mice do not develop AHR, elevations in serum IgE or mucus hypersecretion although BAL eosinophils are reduced by only approximately 30% [80]; a separate study replicated these data but found that elevations in serum IgE upon sensitization and challenge were not IL-13R α 1-dependent [81]. These observations have been extended into non-human primates (NHP) using some of the IL-13 neutralizing antibodies considered for human testing; IMA-638 reduced both BAL eosinophilia and BAL neutrophilia [82] and CAT-354 inhibited AHR and BAL eosinophilia [83] in cynomolgus monkey segmental allergen challenge models.

Neutralization of both mouse IL-4 and mouse IL-13 using an IL-4R α -blocking antibody reduced AHR and ablated BAL eosinophils but not BAL neutrophils or lymphocytes [84]. Similar effects were obtained with a truncated IL-4 that binds to, but does not activate, IL-4R α [85]. STAT6-deficient mice, unable to transduce IL-4 or IL-13 signals, did not develop AHR, mucus hypersecretion or elevations in serum IgE although BAL eosinophils were only partially diminished in a standard sensitization and lung challenge model [86]. STAT-6-deficient mice also had elevated levels of lung T regulatory cells numbers [87] suggesting that IL-4 and IL-13 normally suppress T regulatory cells. When STAT-6 was reconstituted only in the lung epithelia of IL-13-overexpressing mice AHR and mucus hypersecretion, but not BAL eosinophilia or fibrosis, phenotypes returned implicating the epithelium as a key IL-13 target cell type in the asthmatic phenotype [88]. Cynomolgus monkey testing of the IL-4 mutant pitrakinra demonstrated that local (inhaled) dosing delivered similar effects, of reduced AHR and a trend

toward decreased lung eosinophils, as systemic (subcutaneous) dosing [89]. This important study confirmed the notion that neutralization of IL-4 and IL-13 in the lung, rather than the periphery, is important for efficacy against AHR and eosinophilia in models mimicking key aspects of human asthma.

2.5. *In vitro* data

The biology of IL-4 and IL-13 is complex. IL-4 and IL-13 are produced by multiple cell types and act pleiotropically on a variety of responsive cell types (see Fig. 1) to mediate a variety of effects that taken together can be hypothesized to closely mimic the human asthma phenotype. The following studies are from human systems unless otherwise noted.

2.5.1. Atopy

IL-4 drives Th0 to Th2 polarization [90] although it should be noted that Th2 development can occur *in vivo* in IL-4-deficient mice through an unknown mechanism [91] which in mice may include T cell-activation derived IL-2 and innate lymphoid cell type II (ILC2) CD80 and CD86 [92]. Both IL-4 [93] and IL-13 [94] induce B cells to undergo isotype switching and produce IgE. IL-4 induces nasal mast cells to upregulate cell surface Fc ϵ RI [95]. IL-13 upregulates human lung mast cell (HLMC) Fc ϵ RI expression and increases histamine release following IgE/anti-IgE activation [96].

2.5.2. AHR

IL-13 directly modulates airway smooth muscle (ASM) contractility through potentiation of contractile agonist-induced calcium mobilization [97] at least in part via upregulation of cyclic ADP ribose hydrolase (CD38) [98]. IL-13 antagonizes smooth muscle relaxation induced by β 2-adrenoceptor agonists [99].

2.5.3. Inflammation

IL-4 and IL-13 upregulate VCAM-1 on the surface of vascular endothelium [100] which facilitates eosinophil adhesion via VLA-4 [101]. IL-4 [102] and IL-13 [103,104] can synergize with other cytokines found in the asthmatic lung, such as TNF- α [105] or TGF- β 1 [106], to induce fibroblasts to synthesize eosinophil chemoattractants such as eotaxin-1. IL-4 and IL-13 induce release of the granulocyte survival factor GM-CSF [107] and IL-8 [108] from airway epithelium. IL-4 potentiates IFN- γ induced [109], and IL-13 independently induces [110], iNOS production from primary bronchial epithelial cells. IL-4 and IL-13 cause eotaxin release from primary human airway smooth muscle cells (HASM); these responses synergize with IL-1 β [111] or TNF- α [112]. IL-4 and IL-13 upregulate eosinophil cell surface CD69 and prolong survival; these effects are synergistically increased by TNF- α [113].

2.5.4. Mucus production

IL-4 induces mucus production in the pulmonary mucopidermoid carcinoma cell line, NCI-H292 [114]. IL-4 causes resting HBECs to switch from an Na⁺-absorptive to an anion-secretory phenotype [115] and causes primary ALI cultures to increase the goblet cell-associated mRNAs MUC5AC and MUC2 [116]. IL-13 decreases the proportion of ciliated cells [117] and increases the proportion of goblet cells [117] and induces a switch to an epithelial secretory phenotype in primary epithelial cell culture [115,118].

2.5.5. Fibrosis

IL-4 induces α -smooth muscle actin and collagen 3 expression by normal human lung fibroblasts [119]. IL-13 indirectly causes asthmatic airway fibroblasts to secrete collagen I via MMP-2 released TGF- β 1 [120]. IL-13 also causes bronchoscopy-derived primary asthmatic lung fibroblasts to migrate through matrigel in a

TGF- β -dependent fashion [121]. IL-13 induces bronchial biopsy-derived primary lung epithelial cells to release TGF- β 2 in a corticosteroid-insensitive fashion [122]. IL-4 and IL-13 induce endobronchial biopsy-derived primary lung fibroblast proliferation from mild but not severe asthmatics [123].

2.6. Treatment hypothesis

Asthma standard of care seeks to improve airway patency and overcome reversible airway obstruction (SABA, LABA) or to suppress inflammation (ICS, OCS) or deliver both (theophylline). Theophylline suppresses IL-4 [124] and steroids suppress IL-4 [54,63] and IL-13 [65,66] in the human lung yet IL-13 is still detectable despite maximal therapy in the most severe patients [65]. Pollution can be a contributory factor; 2 ppm nitrogen dioxide (NO₂) upregulates IL-13 in bronchial epithelium [125] and diesel exhaust particles stimulate asthmatic PBMCs to produce more IL-4 than non-asthmatic PBMCs [126]. As IL-4 and IL-13 are present in symptomatic asthmatics, are downregulated by effective treatment but remain elevated in patients who remain uncontrolled despite maximal standard of care and drive pathology-relevant biology they would seem to be logical therapeutic targets.

Biologics are high-cost therapies and as such will tend to be used on the most severe patients and be expected to address the unmet medical need of that patient group. One of the key issues for this severe patient population is exacerbation which can result in deleterious consequences ranging from several weeks off work or school to hospitalization or even death. One of the key drivers of asthma exacerbations is viral infection which is present in up to 80% of children with an asthma exacerbation in a primary care setting [127] and 78% of patients admitted to hospital with acute asthma (83% of these viral infections were identified as human rhinovirus (HRV)) [128]. Viruses primarily infect airway epithelium; transfection of the viral dsRNA mimic polyIC into BEAS-2B epithelial cells upregulated IL-4R α and γ c which when stimulated with IL-4 resulted in increases in the eosinophil chemoattractant eotaxin 3 [129]. IL-13 increases the proportion of goblet cells in primary tracheal epithelial cell ALI culture; HRV preferentially infected goblet cells indicating that IL-13-treated epithelium was more susceptible to infection than untreated epithelium [130]. Rhinovirus has been shown to induce a type I (IL-12, IFN γ) response in non-asthmatic PBMCs but a mixed type I (lower than non-asthmatics) and II (IL-4, IL-10) response in atopic asthmatic PBMCs [131]. Viruses induce a CD8 response; CD8 cells from atopic asthmatics secrete IL-13 and IL-5 upon TCR engagement, both of which are enhanced by the presence of IL-4 [132]. Experimental rhinovirus infection of asthmatics and non-asthmatics shows that asthmatics develop an increase in BAL eosinophils, elevated nasal wash IL-4 and IL-13 and increased viral load compared to non-asthmatics [133].

Recent mouse data from a helminth model suggests that IL-4 and IL-13 activation of STAT-6 in macrophages skews toward an M2 phenotype via production of Ym1 and generally downregulates the host CD8 T cell response [134]. IL-4 and IL-13 signaling through STAT-6 downregulates T regulatory cell numbers [87]. Atopic patients also have the potential for a skewed immune response to common bacterial antigens. When T cells specific for purified protein derivative (PPD) were cultured with APCs and PPD antigen, T cells derived from atopics produced IL-4, IL-5, IL-2 and IFN- γ whereas those derived from normals produced IL-2 and IFN- γ [135]. Together these mechanisms could reduce anti-viral, and potentiate allergic, responses in the asthmatic lung.

IL-4 and IL-13 are associated with mechanisms of steroid resistance. The glucocorticoid (GC) dexamethasone drives apoptosis in T cell lines via downregulation of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (I κ B α); I κ B α

downregulation is functionally antagonized by IL-2 in Th1 cells and IL-4 in Th2 cells [136]. Asthmatics upregulate glucocorticoid receptor β (GR β) which binds GCs but antagonizes the transactivating activity of the active receptor GR α ; GR β can be upregulated in normal, or maintained in asthmatic, PBMCs by IL-4/IL-2 [137]. IL-13 alone reduced monocyte GR affinity to a similar extent as IL-2/IL-4 combination which could be demonstrated in decreased effect of hydrocortisone on LPS-induced IL-6 production [138].

Mast cells are key mediators of the allergic response and release multiple pro-asthmatic mediators, including IL-4 [139] and IL-13 [140], upon activation by cell membrane-bound IgE cross-linking. Mast cells in the lung smooth muscle bundle are a cardinal feature of asthma [141] and are similarly upregulated in the epithelium of Th2 high patients [142]. In addition to upregulating mast cell Fc ϵ RI expression to further increase allergen sensitivity [95,96], IL-13 induces epithelial cells to produce SCF [142] thereby providing a key survival signal for mast cells.

Taken together suppressing the IL-4 and IL-13 axes in asthma has the potential to reduce mucus hypersecretion, a key factor in fatal asthma due to airway obstruction (reviewed in [143]), reduce AHR, eosinophilia and fibrosis development, improve responsiveness to standard of care (LABA/ICS plus SABA), and rebalance the immune phenotype by decreasing Th2 polarization, reducing mast cell populations in the epithelium and smooth muscle, increasing T regulatory cells and the host response to viruses, resulting in improved symptoms and decreased exacerbation rate.

2.7. Clinical experience

Early asthma efficacy trials seek improvements in control, often via the asthma control questionnaire (ACQ) which includes measures of rescue medication (SABA) use, and increased airflow measured by increased FEV1. In later phase trials the focus is on exacerbation rate reduction. The minimally important differences (MID) are 0.5 in ACQ [144], 10% in FEV1 [145] and 0.8 puffs/day in SABA use [146]; there is no published MID for exacerbation rate reduction but it is notable that omalizumab (anti-IgE, Novartis) was approved by NICE for use by the UK's National Health Service (NHS) on the basis of data demonstrating a 45% reduction in severe exacerbations in a high risk subgroup (previous hospitalization) [147].

The first approach modulating the IL-4 or IL-13 axes tested was the IL-4 neutralizing recombinant soluble IL-4 receptor, altrakinept (sIL-4R α from Immunex/Amgen), which was initially studied in a 25-patient, single nebulized dose trial with a primary endpoint of safety [148]. Moderate ICS-taking atopic asthma patients stopped their ICS the day before being dosed with nebulized placebo, 500 μ g altrakinept or 1500 μ g altrakinept in a 2.5 mL volume. Altrakinept was well tolerated and had a serum half-life of 6–8 days after nebulized administration ($t_{0.5}$ as unpublished observation in [148]). As compared to placebo the 1500 μ g altrakinept group had improved FEV1 (day 4 change from baseline % predicted FEV1 of +5 sIL-4R α vs. –7 placebo), no ICS-withdrawal induced increase in symptom score (average day 1–15 total asthma symptom score of 2 for sIL-4R α vs. 5.5 placebo), decreased fractional exhaled nitric oxide (FeNO) (day 4 change in baseline ppb FeNO of –8 sIL-4R α vs. +9 placebo) and reduced requirement for rescue medication (change in mean puffs of SABA per day over the first 2 weeks of –0.6 sIL-4R α vs. +1.7 placebo). None of the 9 patients in the 1500 μ g altrakinept group experienced an asthma exacerbation in the 2 weeks post drug administration whereas 3 of 8 in the 500 μ g group and 2 of 8 in the placebo group experienced an exacerbation. Non-significant decreases in serum VCAM-1, ICAM-1 and ECP and increases in sCD23 were also seen in the 1500 μ g altrakinept group compared to placebo; no effects were seen on methacholine-induced AHR or blood eosinophils.

Following these encouraging single-dose data altrakinept was then studied in a 62-patient 12-week trial administering weekly 2.5 mL nebulized doses of 750 µg, 1500 µg or 3000 µg in ICS-dependent atopic asthmatics who had their ICS withdrawn with primary endpoints of safety and change in FEV1 [149]. Altrakinept was well tolerated; the proportion of the dose reaching the lung was 27% and the serum half-life was 5.3 days. By 4 weeks 33% of the 3000 µg group had withdrawn from study due to exacerbation compared to 50%, 47% and 50% in the 1500 µg, 750 µg and placebo groups, respectively. All doses of altrakinept protected patients from an increase in symptom score in the first month (0.1–0.15 in altrakinept groups vs. 1.4 placebo). At the 12 week endpoint the 3000 µg group had a –2% change in baseline FEV1 versus –12%, –6% and –13% in the 1500 µg, 750 µg and placebo groups, respectively. There were no effects on blood eosinophils or serum IgE.

Further studies were carried out with altrakinept but have not been published [150]. A 20-center study used 60-patient groups dosed either 0.9 mg or 1.8 mg sIL-4R nebulized weekly over 12 weeks and then followed patients for a further 8 weeks. During the first 12 weeks treated patients displayed no increase in clinic-measured FEV1 above placebo. However, home AM FEV1 measurements showed an almost significant ($p = 0.067$) increase in FEV1 with 1.8 mg. When patients were stratified into baseline FEV1 50–70% of expected a significant treatment effect of 1.8 mg sIL-4R was seen vs. placebo ($p = 0.007$). Interestingly, 0.9 mg sIL-4R prevented the placebo-induced NO increase ($p = 0.042$) whereas 1.8 mg sIL-4R decreased it below baseline ($p = 0.05$ vs 0.9 mg).

Two additional studies have been carried out with altrakinept; a 4-week dose ranging study with 17 patients receiving 25 mg nebulized weekly and 18 patients receiving 5 mg nebulized daily (5× per week); a 12 week ICS add on and taper study with 19 patients on nebulized weekly dosing. No significant treatment effects in the primary, or any of the secondary, efficacy variables were seen [150]. No further development of altrakinept has been reported. One challenge with this candidate may have been the modest affinity of sIL-4R for IL-4 which, unless sIL-4R is in great excess, could allow ongoing “leak” of free IL-4 to drive the biology; precedence has been reported *in vitro* [151].

The second approach taken to blocking IL-4 was GSK’s pascolizumab, a humanized IgG1 with an affinity (K_D) for IL-4 of 45 pM [152]. In 2001 GSK registered a trial examining the effects of pascolizumab in symptomatic steroid-naïve asthma (NCT00024544); no data have been reported. Although this trial is assumed to be negative, one issue that is not considered to be relevant is distribution of pascolizumab to the lung following systemic dosing. Preclinical studies in unchallenged cynomolgus monkeys demonstrated presence of antibody in bronchoalveolar lavage after either 10 mg/kg or 100 mg/kg i.v. dosing [152]. As of October 2014 pascolizumab was being studied in Singapore to test the hypothesis that neutralizing IL-4 will enhance host response to pulmonary tuberculosis (NCT01638520).

IL-13 neutralization has been evaluated in multiple studies using antibodies with 2 basic mechanisms of action: prevention of IL-13 binding to IL-13R α 1 and IL-13R α 2 (mechanism 1 [M1]) or prevention of IL-13:IL-13R α 1 complexes from recruiting IL-4R α but allowing IL-13 to interact with IL-13R α 2 (mechanism 2 [M2]). IL-13 mediates its biological effects by binding IL-13R α 1 on one side and then recruiting IL-4R α on the other side to form a signaling complex in which IL-13 is in the middle of the two receptors [11]. IL-13:IL-13R α 1 and IL-13R α 2 interactions both occur in helix D of IL-13 [153,154] which explains why M1 mAbs block binding of IL-13 to both IL-13 receptors and why M1 and M2 mAbs have distinct epitopes on IL-13 [155].

Wyeth have IMA-638 (M2 [156], also known as anrukinzumab [157]) and IMA-026 (M1 [156]) which are both humanized IgG1s with 14 pM and 92 pM affinity (K_D) for NHP IL-13 [158], respectively. IMA-638, dosed subcutaneously weekly for 2 weeks at 2 mg/kg, inhibited the week 3 early and late response to inhaled allergen challenge in a study of 27 stable mild atopic asthmatics without affecting AHR or sputum eosinophils [156]. In an identical study design evaluating 29 patients IMA-026 non-significantly inhibited the late phase response [156]. IMA-638 was then evaluated in a 12 week study of asthmatics uncontrolled despite ICS or ICS/LABA (NCT00425061) and did not demonstrate effects on symptoms, lung function, or time to exacerbation compared with placebo [156].

Genentech’s lebrikizumab (M2 [155]) is a humanized IgG4 with a single hinge mutation to increase stability [159] and an affinity (K_D) for IL-13 of less than 10 pM [155]. Lebrikizumab was evaluated in a study of whole lung allergen challenge in 29 mild asthmatics and found to non-significantly inhibit the late phase response by 48% and suppress serum IgE, CCL13 (MCP-4) and CCL17 (TARC) by 25% after 12 weeks of 5 mg/kg subcutaneous dosing every 4 weeks; AHR was not affected [160]. Exploratory analyses showed that elevated levels of peripheral blood eosinophils, serum IgE or serum periostin associated with elevated clinical response with periostin providing the best separation between response and no response [160].

A subsequent dose–response study in 212 steroid-naïve asthmatics tested 125 mg, 250 mg or 500 mg subcutaneous doses of lebrikizumab every 4 weeks for 12 weeks [161]. There were modest changes (5% increase over baseline) in FEV1 in the whole population and this response did not associate with serum periostin status. Lebrikizumab did not affect asthma control as measured by ACQ. All doses of lebrikizumab protected patients from treatment failure (need for ICS/OCS or intravenous steroids) although again this did not associate with serum periostin status. All doses of lebrikizumab also reduced FeNO by approximately 50% and serum IgE, serum CCL17 and serum CCL13 by approximately 10%. The biomarker and treatment failure data suggest that 125 mg Q4W was saturating in this mild patient population.

Lebrikizumab was next studied in 219 asthma patients who were uncontrolled despite medium–high doses of ICS [159]. 250 mg lebrikizumab given subcutaneously monthly improved FEV1 by 5.5% over placebo but not asthma control (as measured by ACQ5) and also suppressed serum CCL17, CCL13 and IgE. When patients were stratified by median serum periostin level, a surrogate of Th2 high or IL-13 positive lung status [66], low serum periostin patients had a 1.6% improvement in baseline FEV1 over placebo whereas high serum periostin patients had an 8.2% improvement. Lebrikizumab also reduced the rate of severe exacerbations in periostin-high patients by 67% versus placebo compared with 29% versus placebo in the periostin-low group.

Periostin is induced by IL-4 [162] and IL-13 [162,163] in airway epithelial cells and similarly by IL-4 and IL-13 in fibroblasts where it colocalizes with areas of fibrosis in human asthmatic lung [164]; periostin directly binds to matrix components including tenascin-C, fibronectin and collagen V [164]. IL-4 and IL-13 induced periostin production from fibroblasts, but not microvascular endothelial cells, is steroid sensitive [165]. As steroids are a key treatment for most inflammatory diseases and patient selection markers are increasingly relevant in heterogeneous diseases like asthma, observations around the relative steroid sensitivity of IL-13-induced periostin production by different cell types may yet have relevance for patient stratification in asthma and other diseases.

Two replicate trials of asthma patients uncontrolled despite ICS and a second controller, LUTE and VERSE, tested 4 weekly subcutaneous lebrikizumab doses of 37.5 mg, 125 mg and 250 mg. LUTE

and VERSE data were pooled and the 463 patient dataset recently reported (they were initially planned as phase 3 studies but converted to phase 2b due to a chemistry, manufacturing and controls (CMC) issue). As compared to placebo lebrikizumab reduced exacerbation rate in the all-comers (periostin high) groups by 62% (81%), 35% (77%) and 11% (22%) in the 37.5 mg, 125 mg and 250 mg dose groups, respectively [166].

Lebrikizumab is currently being studied in a number of different asthma trials as part of its registration package. First is a study of 300 SABA-only mild-to-moderate asthma patients examining FEV1 improvement (NCT02104674). Then there is a 120 patient study examining whether 12 weeks of lebrikizumab affects eosinophil-density in the lung mucosa of uncontrolled asthma patients who are taking ICS and another controller (NCT02099656). There are two 1050-asthma patient studies in those uncontrolled on ICS and a second controller who will be treated with 2 lebrikizumab dose regimens for 52 weeks and examining exacerbation rate (NCT01868061, NCT01867125). There is also a 225-patient study of severe asthma patients who depend on OCS examining reduction in OCS dose following 44 weeks of lebrikizumab (NCT01987492). Finally there is a 375-adolescent asthma patient study examining exacerbation rate in those uncontrolled on ICS and a second controller following treatment with 2 lebrikizumab dose regimens for 52 weeks (NCT01875003).

MedImmune's tralokinumab (M1 [167]) is a fully-human IgG4 with an affinity (K_D) of 165 pM for IL-13 [83]. The effect of tralokinumab on ACQ6, pre-bronchodilator lung function, SABA use and safety was evaluated in 194 moderate-to-severe uncontrolled ($ACQ6 \geq 1.5$) asthmatics when dosed subcutaneously at 150 mg, 300 mg or 600 mg every 2 weeks for 12 weeks; analysis was pre-specified as placebo versus all tralokinumab dose groups. Tralokinumab, as compared to placebo, failed to affect ACQ-6 (improvement of 0.15) but improved FEV1 by 150 mL and reduced SABA use by 0.5 puffs per day. The FEV1 response was dose-dependent. 29% of patients supplied a baseline sputum sample. When, as a post hoc analysis, the trial data were analyzed in terms of which patients were sputum IL-13 positive or negative at baseline there was an association between clinical effect and presence of baseline sputum IL-13 [168].

Tralokinumab was next tested in 452 patients with uncontrolled severe asthma to assess whether it could decrease exacerbation rate. Tralokinumab was dosed subcutaneously for 52 weeks at 300 mg every 2 weeks or 300 mg every 2 weeks for 12 weeks followed by 300 mg every 4 weeks; the latter dose regimen did not demonstrate efficacy. Three hundred milligrams fortnightly tralokinumab did not affect exacerbation rate in the whole population but demonstrated a 54% reduction in exacerbation rate in reversible, periostin-high patients [169].

Tralokinumab is also currently being studied in number of different asthma trials as part of its registration package. STRATOS 1 and STRATOS 2 are 1140- and 770-asthma patient studies in adults and adolescents uncontrolled on ICS/LABA, respectively. Both studies examine exacerbation rate after treatment with tralokinumab for 52 weeks (NCT02161757, NCT02194699). TROPOS is a 120-patient study of OCS-dependent adult and adolescent asthma patients examining reduction in OCS dose following 40 weeks of tralokinumab (NCT02281357).

GSK's mAb GSK679586 (M1) is a humanized IgG1 with an affinity (K_D) for IL-13 of 300–400 pM [170]. GSK679586 increased serum total IL-13 and reduced FeNO in 28 mild intermittent asthmatics versus placebo [170]. In a subsequent 12 week trial in 198 severe asthmatics who remained uncontrolled ($ACQ7 \geq 1.5$) despite being up-titrated to maximal doses of ICS ($\geq 1000 \mu\text{g}$ fluticasone per day), plus or minus LABA and OCS, GSK679586 was dosed intravenously at 10 mg/kg monthly. GSK679586 increased serum total IL-13 but did not improve FEV1 or ACQ and no

enhanced effect was seen in patients with increased blood eosinophils or elevated serum IgE [171].

Novartis's QAX576 (mechanism, isotype and affinity unreported) was evaluated in a 24 week study of 259 severe adult asthmatics uncontrolled despite ICS/LABA with a primary endpoint of ACQ [172]. QAX576, dosed intravenously at 6 mg/kg every 3 weeks, and placebo caused an ACQ improvement of 0.8 and 0.61, an FEV1 improvement of 59 mL and 8 mL, a reduction in SABA use per day of 1.5 and 1.1, and a mean number of exacerbations over the 24 weeks of 0.27 and 0.3, respectively. No patient stratification data for, nor further development of, QAX576 have been reported.

Other IL-13 neutralizing candidates have reported only early phase data. Centocor's CNTO-5825 (M1, isotype and affinity unreported) reduced serum IgE and CCL17 in a trial of 64 healthy atopic patients compared to placebo [173]. No additional development has been reported.

IL-4 and IL-13 neutralization has been evaluated with three different approaches, an inhaled IL-4 mutant protein (pitracinra) and two IL-4 receptor neutralizing antibodies, AMG-317 and dupilumab. Pitracinra is Aerovant's IL-4 double mutein (R121D/Y124D) that binds to but does not activate IL-4R α thereby acting as a functional antagonist of both IL-4 and IL-13. Pitracinra reduced the late-phase response to allergen challenge in 56 patients with mild atopic asthma after 4 weeks of either 60 mg twice-daily local (nebulized, inhaled) or 25 mg daily systemic (subcutaneous) dosing [174]. A subsequent 12-week trial in 534 patients with uncontrolled mild-moderate asthma examined the effect of 1, 3 or 10 mg DPI-delivered, inhaled pitracinra on exacerbations precipitated by withdrawal of LABA at 4 weeks and ICS from 6 weeks (in a tapered fashion) [175]. Pitracinra was not efficacious in the whole population but the 10 mg dose demonstrated a slower rate of treatment-withdrawal induced exacerbation than placebo in the high blood eosinophil subpopulation [175]. A number of IL-4R α polymorphisms were subsequently found to relate to positive clinical response in the pitracinra therapy-withdrawal induced exacerbation trial, suggesting a potential way forward via pharmacogenomic stratification for future trials [176]. No further development of pitracinra has been reported.

AMG-317 is Amgen's fully human IgG2 that binds IL-4R α with a K_D of 180 pM and in so doing prevents the biological activity of both IL-4 and IL-13 [177]. Two hundred and ninety-two symptomatic ($ACQ \geq 1.5$) moderate-severe (>200 to $<1000 \mu\text{g}/\text{d}$ ICS) asthmatics were dosed with 300 mg, 150 mg, 75 mg AMG-317 or placebo subcutaneously weekly for 12 weeks in a trial with a primary endpoint of change in ACQ [177]. In the whole trial population AMG-317 at any dose did not improve ACQ more than placebo nor any of the secondary endpoints (AQLQ, SABA use, AM or PM PEFR, pre-bronchodilator FEV1). There was a trend to effect on serum IgE with a median change in IgE of 0, -6, -13, -39 IU/mL from baseline in the placebo, 75 mg, 150 mg and 300 mg groups, respectively. A pre-planned efficacy analysis stratifying on tertiles of baseline ACQ revealed the clearest signals. The most severe patients, with baseline $ACQ \geq 2.86$, showed an ACQ improvement of -0.76, -1.09, -1.03, -1.28 in the placebo, 75, 150 and 300 mg groups, respectively. There were similar positive signals in FEV1; the difference between the 300 mg group and placebo was 0.34 L. Beta-agonist use was also improved with a reduction of 1.2 puffs per day in the 300 mg versus placebo group. Most interestingly there was also a trend in exacerbation reduction with the percentage of patients experiencing an exacerbation requiring OCS or doubling ICS being 28, 24, 8, 4 in the placebo, 75, 150 and 300 mg groups, respectively. No further development of AMG-317 has been reported.

Regeneron's dupilumab is a human antibody that binds to IL-4R α and like AMG-317 blocks IL-4 and IL-13 signaling [178]. A single dose of dupilumab (1, 3, 8 or 12 mg/kg intravenously or

150 mg or 300 mg subcutaneously) or placebo was dosed to 48 healthy volunteers; dupilumab caused suppression of both TARC and IgE [178]. Dupilumab was then evaluated in a steroid-withdrawal trial [179] very similar in design to the pitakinra trial [175] described above. One hundred and four patients with uncontrolled ($3 \geq \text{ACQ5} \geq 1.5$) moderate to severe asthma on moderate to high doses of ICS with LABA who also had eosinophilia, defined as blood eosinophils $\geq 300/\mu\text{L}$ or sputum eosinophils $\geq 3\%$, were recruited and treated weekly with subcutaneous placebo or subcutaneous 300 mg dupilumab for 12 weeks. At week 4 patients stopped LABAs and tapered ICS from week 6 with no ICS by week 9. If a patient exacerbated they were removed from study. In the initial 4 week period, where patients received drug or placebo and ICS/LABA, dupilumab caused a 210 mL improvement in FEV1 and 1.04 improvement in ACQ5 versus a 20 mL worsening in FEV1 and 0.66 improvement in ACQ5 for placebo; these differences between placebo- and dupilumab-treated patients were maintained even after LABA and ICS discontinuation. At week 4, the point of LABA cessation, there had also been decreases in eFeNO, serum TARC, and serum eotaxin 3 in the dupilumab-treated cohort compared to the placebo-treated cohort. By week 12 6% of the dupilumab cohort and 44% of the placebo cohort had experienced an exacerbation clearly demonstrating that dupilumab could prevent treatment-withdrawal induced exacerbations [179].

Regeneron put out a press release in November 2014 which covered the top line data from a 12-week interim analysis of an ongoing 24 week trial of 776 adult, mainly allergic, moderate-severe uncontrolled asthma patients where dupilumab was dosed at 300 mg fortnightly (Q2W), 200 mg Q2W, 300 mg monthly (Q4W) or 200 mg Q4W in addition to standard of care (ICS/LABA) [180]. In the whole (eosinophil high) population placebo improved FEV1 by 120 mL (180 mL), 300 mg dupilumab Q2W by 280 mL (390 mL) and 200 mg dupilumab Q2W by 310 mL (430 mL). Dupilumab reduced severe exacerbations by 64% in the whole treated population and 75% in the high eosinophil population.

Future therapeutic approaches in early preclinical development include bispecific antibodies targeting both IL-4 and IL-13 [181,182] which offer an alternative route from targeting IL-4Ra to achieve functional blockade of both IL-4 and IL-13. There is also an inhaled IL-13 antibody fragment [183,184] that could potentially offer the efficacy of systemically dosed mAbs *via* a more convenient route.

Taken together the clinical experience in asthma strongly suggests the IL-13 axis is active in a patient segment of human asthma that is characterized by being “Th2 high”. It also suggests that blocking IL-13 by mechanism M1 (prevention of binding to IL-13R α 1 and IL-13R α 2, e.g. tralokinumab) or mechanism M2 (prevention of IL-13:IL-13R α 1 complex recruiting IL-4R α , e.g. lebrikizumab) delivers similar effects of exacerbation rate reduction and lung function improvement although the mechanism by which IL-13 neutralization mediates these effects is not currently clear. Whether also blocking IL-4 as well as IL-13 offers additional clinical benefit over blocking IL-13 alone remains to be seen with the sort of larger trials that are currently underway. Additionally whether IL-4 and IL-13 directed approaches can deliver additional benefits that can be speculated from the preclinical literature such as reduction in mucus hypersecretion, immune system normalization and prevention of ongoing airway remodeling are also unknown. Whilst the field has come a long way there is still much work to do.

3. Atopic dermatitis

3.1. Introduction

Atopic dermatitis (AD) is a chronic, pruritic, inflammatory disease of the skin. AD patients are generally characterized by a defective skin barrier, atopy, elevated Th2 responses and a

predisposition to infection by viruses and bacteria such as herpes simplex and *Staphylococcus aureus* (reviewed in [185]). Some AD patients experience recurrent bacterial or disseminated viral skin infections such as eczema herpeticum (EH) which can be life threatening [186]. Standard therapies such as emollients and topical steroids are supplemented with calcineurin inhibitors in moderate-to-severe AD patients which are incompletely efficacious in this population [187,188] leaving a clear unmet medical need.

3.2. Disease expression

Early studies demonstrated that IL-13 mRNA expressing cells were more prevalent in skin from AD patients than skin from non-AD patients, that acute AD lesions had more IL-13 mRNA positive cells than chronic AD lesions and that conversely IL-12 mRNA positive cells were increased in chronic lesions versus acute lesions, uninvolved skin or skin from non-AD patients [189]. The presence of both IL-4 mRNA and IL-13 mRNA in lesional AD skin biopsies has been confirmed [190] although IL-13 mRNA is more commonly found than IL-4 mRNA [191]. Within lesions 43% T cells and 21% mast cells were found to express IL-13 protein whereas in non-lesional skin 3% T cells and 1% mast cells were IL-13 positive [192].

Similar numbers of IL-13 positive, but not IL-4 positive, cells were detected in the lesional skin of AD patients with both extrinsic AD (skin inflammation with high serum IgE, approximately 90% AD patients) and intrinsic AD dermatitis (normal serum IgE with no obvious sensitisation to allergen) although biopsy-recovered, CD3/CD28-activated T cells from intrinsic patients expressed much less IL-13 than their extrinsic counterparts and IL-4 was very modestly expressed in both disease types [193]. Also there were less eosinophils in the skin of intrinsic than extrinsic patients [193]. Recent data demonstrates that Th2 genes in general, including IL-4 and IL-13, are similarly expressed in intrinsic and extrinsic AD with the key differences between the disease subtypes being increased Th17 and Th22 in intrinsic patients; SCORAD clinical severity score correlated with Th2 gene expression in extrinsic AD and Th17 gene expression in intrinsic AD patients, respectively [194].

Cutaneous lymphocyte-associated Ag (CLA) is a skin-homing receptor present on effector and memory T cells. CLA⁺ T cells from AD patients were shown to be pre-activated compared to CLA⁺ T cells [195]. AD patient CLA⁺ T cells underwent spontaneous proliferation, induced B cell IgE production and were high IL-4, high IL-13 and low IFN- γ producers, whereas AD patient CLA⁺ T cells did not spontaneously proliferate, induced IgG4 from B cells and secreted very low levels of cytokines [195]. Both CD4⁺ and CD8⁺ cells from lesional and non-lesional biopsies express intracellular IL-4 and IL-13 with CD8⁺ cell distribution biased to the epidermis and CD4⁺ cell distribution biased to the dermis [196]. IL-4⁺ and IL-13⁺ iNKTs have been found in AD PBMCs with frequency of iNKTs in AD PBMCs and AD lesions correlated with disease severity [197]; IL-4⁺ NKTs have also been found in AD lesions [198].

IL-13 expression relates to disease severity and flares. The proportion of blood eosinophils and IL-13 expressing peripheral CD4 cells in acute paediatric AD is directly proportional to disease severity [199]. Following *ex vivo* DerP1-stimulation of PBMCs a higher proportion of IL-4 and IL-13 expressing CD4⁺ cells were found in PBMCs from severe adult AD patients compared with PBMCs from mild patients; mild patient PBMCs also contained more IL-4 and IL-13 expressing CD4⁺ cells than PBMCs from non-atopic control patients [200].

3.3. Genetic data

As detailed in Table 1 various IL-4/IL-13 axis polymorphisms are associated with the risk of developing AD. GWAS studies confirm

the importance of the IL-4/IL-13 axis in the context of a damaged barrier in the development of AD. Four loci were implicated in a study of 1563 AD cases and 4054 controls; epidermal differentiation complex, the region proximal to LRRC32, RAD50/IL-13 locus and the major histocompatibility complex (MHC) locus [201].

3.4. Animal model data

Transgenic overexpression of IL-4 directed only to mouse skin using a K14 keratinocyte promoter induced an AD-like phenotype including skin lesions, dry skin, conjunctivitis, pruritus, dermal inflammation (CD4⁺ T cells, mast cells, eosinophils and macrophages), *Staphylococcus aureus* infection and elevated serum IgE [202]. In a separate study repeated dermal injections of IL-4 reduced the cohesion of the stratum corneum by decreasing desmoglein 1 thereby reducing epidermal differentiation [203]. One potential reason for *S. aureus* infection in Th2 models was identified when it was shown that *S. aureus* preferentially bound to Th2 epidermis rather than Th1 epidermis through interactions with fibronectin and fibrinogen [204]. Transgenic overexpression of IL-13 in the mouse skin was achieved using a K5 keratinocyte promoter and induced an AD-like phenotype including skin lesions (with hair loss, dry skin, excoriation and crusting), pruritus, dermal inflammation (CD4⁺ T cells, mast cells, eosinophils, macrophages and Langerhans' cells), Th2-skewed lymph node lymphocytes, fibrosis, increased vasculature and elevated serum IgE [205]. Mice transgenic for a genomic locus containing mouse IL-4, IL-5 and IL-13 spontaneously developed an AD-skin like disease, like the IL-4 or IL-13 transgenic mice described above, as well as asthma-like lung inflammation including elevated lymphocytes, eosinophils and epithelial hypertrophy [206].

IL-13 was neutralized using antisense oligonucleotides in liposomes in an epicutaneous ovalbumin sensitisation and challenge mouse model. IL-13 antisense liposomes decreased skin thickness, reduced skin biopsy IL-4 and IL-5 and skin inflammation and reduced serum IgE in comparison to naked liposomes [207]. IL-4 and IL-13 were neutralized in a mouse ear model of repeated oxazolone ear exposure, which drives an AD like phenotype, using systemic exposure to a plasmid coding for an IL-4 double mutant after sensitization had developed (IL-4DM; blocks both IL-4 and IL-13 by competing for binding at IL-4R α). IL-4DM-treated mice did not develop ear-swelling, had reduced mast cell and eosinophil numbers in the ear, reduced serum IgE and plasma histamine compared to control plasmid exposed mice [208]. IL-4 and IL-13 also down-regulate genes in the epidermal differentiation complex (EDC) thereby decreasing barrier function; mice with constitutively active T-cell STAT-6 (STAT6VT) develop an AD-like disease associated with decreased barrier gene (filaggrin) expression. Antibody neutralization of IL-4 or IL-13 in the STAT6VT mice increased involucrin and filaggrin expression [209].

3.5. In vitro data

This section attempts to cover the biology of IL-4 and IL-13 over and above that for asthma (Section 2.5) as it speaks directly to AD pathology. The following studies are from human systems unless otherwise noted.

3.5.1. Host defense

Vaccinia viral infection induces keratinocytes and microvascular endothelial cells to secrete IL-13 but not IL-4 [210]. IL-4 and IL-13 suppress IFN- γ or IFN- γ /TNF- α -induced production of the anti-microbial proteins (AMP) human beta defensins (HBD) HBD-2 and HBD-3 mRNA from primary human keratinocytes [211]. HBD-3 suppression is via suppression of S100A11 [212]. IL-4 and IL-13 also suppress IL-17-induced production of HBD-2

from primary human keratinocytes [213]. IL-4 and IL-13 suppress TNF- α induced production of the AMP psoriasin by primary keratinocytes although psoriasin is dramatically (10 $\times$ fold) upregulated in AD versus normal skin [214]. IL-13 and IL-4 together facilitate vaccinia replication in keratinocyte culture via suppression of the AMP IL-37; these effects are reversed by IL-4 and IL-13 neutralization [215].

3.5.2. Inflammation

IL-4 induces CCL24 (eotaxin 2) and CCL26 (eotaxin 3) and IL-13 induces CCL26 production from the keratinocyte line HaCaT [216]. IL-4 and IL-13 synergize with IFN- γ and TNF- α in generating TARC from the NG1RGB skin fibroblast cell line [217]. IL-4 or IL-13 stimulation of corneal fibroblasts induces eotaxin release and upregulation of VCAM-1 which is synergistically enhanced by *S. aureus* peptidoglycan [218]. IL-13 stimulates MMP9 production from keratinocytes enabling basement membrane collagen IV dissolution and enabling lymphocyte trafficking [219]. IL-13 induces CCL22 (MDC) production which acts as a chemoattractant for CD4⁺CCR4⁺ Th2 cells (blocked by anti-CCL22) and supplements IFN- γ induced CCL2 (MCP-1) and CCL5 (RANTES) production [220].

3.5.3. Skin remodeling and barrier function

IL-4 and IL-13 decrease MDC and TARC production from the HaCaT keratinocyte cell line via decreased E-cadherin and hence decreased homophilic interactions whereas IFN- γ potently and efficiently stimulates MDC and TARC production [221]. IL-13 suppresses MMP-13 secretion from dermal fibroblasts, this may decrease collagen degradation and lead to dermal thickening [222]. IL-13 induces type I collagen production from primary dermal fibroblasts [223]. IL-4 and IL-13 together suppress filaggrin expression by primary human keratinocytes [224] via suppression of S100A11 [212].

Taken together the role of IL-4 and IL-13 in AD is to act on keratinocytes to generate T cell chemoattractants (recruiting more CLA⁺ T cells to secrete more IL-4 and IL-13), decrease differentiation and decrease production of host defense factors leading to a further decrease in barrier function (which is often already genetically compromised in AD patients). This results in an increased ability for pathogens such as *S. aureus* (and associated pro-inflammatory toxins including superantigens) and allergens to penetrate the skin and generate further Th2 responses. IL-4 and IL-13 also act directly on B cells to drive IgE production and thus load mast cells such that IgE-bearing mast cells are ready to trigger release of pruritic histamine, and more Th2 cytokines, once antigen is encountered.

3.6. Treatment hypothesis

A fascinating clinical study highlights the potential relevance of targeting IL-4 and IL-13 in AD. Three patients with co-occurring AD and psoriasis demonstrated a classic Th2 and Th1/17 response in AD and psoriatic lesions, respectively. The clinical course of their disease showed that when AD peaked, psoriasis troughed and vice versa [225]. In support of this psoriasis patients treated with IL-4 experienced clinical improvements [226]. Psoriatic patients challenged with epicutaneous allergen developed eczema lesions with Th2/Th22 infiltrates [225]. The authors conclude that psoriasis and AD are triggered by specific antigens driving specific lymphocytic reactions [225].

FoxP3 T regulatory cells, suppressors of Th2 effector cells, were notably absent from AD skin although TR1 cells (Th1 suppressors) were present [227]. In mice IL-4 and IL-13 signaling through STAT-6 has been shown to downregulate lung T regulatory cell numbers [87].

Successful therapy is associated with decreases in IL-13. Twelve weeks cyclosporine treatment supplemented only with emollients reduced SCORAD by 51% at 2 weeks and 72% by 12 weeks whilst reducing IL-13 mRNA and normalizing the transcriptome which at baseline had large differences between lesional (generally upregulated vs. non-lesional) and non-lesional skin but at week 12 was broadly comparable. Reductions in IL-13 mRNA and transcriptome normalization followed the kinetics of clinical improvement [228]. Six weeks of UVA1 phototherapy in AD patients reduced the SASSAD clinical score by 50% as well as decreasing skin biopsy IL-13, IL-31 and IL-5 mRNAs [229]. Three weeks of tacrolimus stand-alone therapy caused a 50% decrease in EASI score accompanied by decreased biopsy T cells, eosinophils, IL-13, IL-5 and IL-10 [230].

Given all of these data and the predominance of Th2 mechanisms in AD it is tempting to hypothesize that chronically removing IL-4 and IL-13, or either alone, could reduce clinical burden of disease and start to normalize the polarized skin immunology.

3.7. Clinical experience

The key readout in AD trials is clinical score. Flares (exacerbations) are a clinical issue, so are also followed, but there is little consensus on flare definition making implementation in trials challenging. Several clinical scores are utilized including severity scoring of atopic dermatitis (SCORAD), eczema area and severity index (EASI), patient-oriented eczema measure (POEM) and investigator's global assessment (IGA). SCORAD, EASI and POEM have been recommended for use in efficacy trials [231].

Pitrakinra was the first biologic targeting the IL-4/IL-13 axes to be assessed in AD. Twenty-five adults with uncontrolled, moderate-to-severe AD despite being on standard of care (topical corticosteroids and/or calcineurin inhibitors) received placebo or 30 mg subcutaneous pitrakinra twice a day in a one month trial. As compared to placebo pitrakinra caused a 65% reduction in disease flares, a 25% decrease in total IgE, a normalization of T-cell subset ratio (decreased CD4 and increased CD8) but only a numerical (non-significant) improvement in EASI [232].

Dupilumab has been profiled in a number of monotherapy trials (no other therapeutic agents permitted) as well as a trial in addition to topical corticosteroids; all the trials were reported together [233]. Two phase 1 dose-escalation trials were undertaken in 30 and 37 adult moderate-severe AD patients inadequately controlled by topical steroids and topical calcineurin inhibitors and evaluated the effect of weekly subcutaneous 75 mg, 150 mg, 300 mg or 150 mg and 300 mg dupilumab doses versus placebo, in the absence of any other controller, over 4 weeks; some patients provided optional skin biopsies and data were pooled for analysis. Dupilumab caused dose-dependent improvements in the proportion of patients achieving a 50% reduction in EASI score (EASI-50), improvements in weekly pruritus rating scale and reductions in serum TARC. By week 4 70%, 50%, 30% and 15% of patients had achieved EASI-50 in the 300 mg, 150 mg, 75 mg and placebo groups, respectively. Lesional skin biopsy transcriptomics demonstrated that 300 mg or 150 mg dupilumab improved the transcriptome (made it more normal) by 49% and 24%, respectively, whereas placebo treatment worsened the transcriptome by 21% [233,234].

An analogous trial examined the effect of 12 weeks of 300 mg weekly subcutaneous dupilumab, without any controller medication, on 109 adult moderate-severe atopic dermatitis patients inadequately controlled by topical steroids and topical calcineurin inhibitors [233]. Patients were stratified by serum IgE levels into intrinsic (<150 kU/L) and extrinsic ($\geq$ 150 kU/L) disease and then split equally between the 2 treatment groups. The treatment effect observed in the 4 week study was reproduced and enhanced with

longer exposure to dupilumab. By week 12 in the dupilumab and placebo cohorts EASI-50 was achieved by 85% and 35%, reduction in affected body surface area was 60% and 18% and pruritus numerical rating scale score was reduced by 56% and 15%, respectively. Serum TARC was also suppressed 80% by dupilumab versus 10% by placebo and this correlated with pruritus reduction. Dupilumab and placebo reduced serum IgE by 38% and -10%, respectively.

A fourth study examined the effect of 4 weeks of 300 mg weekly subcutaneous dupilumab, in addition to topical controller medication, in 31 inadequately controlled adult moderate-severe atopic dermatitis patients [233]. The dupilumab cohort used half the topical steroids of the placebo cohort. With this imbalance EASI-50 and EASI-75 was achieved by 50% and 40% of patients in the placebo cohort and 100% and 62% in the dupilumab cohort, respectively. Pruritus score was decreased by 25% by placebo and 71% by dupilumab.

Regeneron/Sanofi have initiated a phase 3 program for dupilumab in AD. SOLO 1 and SOLO 2 are duplicate studies, each of 600 chronic AD patients not on AD therapy with a baseline IGA $\geq$ 3, examining the effect of 2 dupilumab dosing regimens on the proportion of patients achieving an IGA between 0 and 1, as well as a 2 point reduction from baseline, at 16 weeks (NCT02277743, NCT02277769). There is also a study of 700 chronic AD patients on topical corticosteroid therapy examining the effect of 2 dupilumab dosing regimens on the proportion of patients achieving an IGA between 0 and 1, as well as a 2 point reduction from baseline, at 16 weeks (NCT02260986).

MedImmune has initiated a 12-week phase 2 study with tralokinumab in 184 moderate-to-severe AD patients examining the effect of 3 dose-regimens on EASI score (NCT02347176). Genentech has announced that 3 dose regimens of lebrikizumab will be studied in a 200-patient 12 week phase 2 study of patients with moderate-to-severe atopic dermatitis examining the proportion of patients achieving a 50% reduction in EASI score (NCT02340234).

Taken together the dupilumab data clearly show that the IL-4 and IL-13 axes are key players in the AD phenotype and offer great hope that there could be an effective biologic available to physicians and patients with unmet medical need in the next 3 years. Whether blocking IL-13 alone will be sufficient to deliver efficacy in AD remains to be seen.

4. Allergic rhinitis

4.1. Introduction

Allergic rhinitis (AR) is clinically defined as a disease of sneezing, airflow obstruction, nasal pruritus and a generally clear nasal discharge. The underlying disease mechanism is considered to be Th2-mediated allergy to common allergens such as pollens, molds and house dust mite (reviewed in [235]). Most asthma patients, atopic or non-atopic, have AR (reviewed in [236]). Current therapies include ICS, intranasal and oral anti-histamines and leukotriene receptor antagonists, and for severe patients allergen immunotherapy, but they do not provide disease control for all (reviewed in [235]).

4.2. Disease expression

IL-4 is elevated in the nasal lavage of 12–20 year olds with seasonal AR as compared to controls; treatment with budesonide decreased symptom scores and increased nasal lavage fluid sIL-4R [237]. IL-4 and IL-13 are upregulated in nasal mast cells from patients with perennial AR compared to nasal mast cells from

patients with chronic infective rhinitis [95]. IL-4 induces nasal mast cells to upregulate FcεRI mRNA and cell surface protein expression [95].

No difference was found in the number of IL-13 expressing cells from nasal biopsies between AR or control patients although allergen challenge of allergic rhinitis led to a dramatic increase in the number of IL-4 and IL-13 positive cells which could be ablated by nasal steroids [238]. Diesel exhaust particle (DEP) nasal challenge of a mixed population of atopic and non-atopic patients induced nasal lavage cell IL-4 mRNA and protein and IL-13 mRNA in all patients [239]. Grass pollen nasal allergen challenge in grass-pollen AR patients induced increased IL-13 and IL-5 in nasal lavage, in parallel with increases in symptoms and eosinophils [240]. Nasal steroid pretreatment ablated lavage IL-13 and IL-5 and blunted symptoms and nasal eosinophils [240].

AR has also been associated with *S. aureus* colonization. In a study of AR patients not using inhaled steroids 68% of allergic rhinitis and 22% control patients had *S. aureus* in nasal lavage. *S. aureus* + AR patients had higher symptom scores, a higher IL-13:IFN-γ ratio and higher cell activation markers in nasal lavage than *S. aureus* – AR patients [241].

AR can develop into chronic rhinosinusitis with nasal polyps (CRSwNP) which is also Th2 biased with elevated eosinophils and IL-5, tissue IgE and tissue eotaxin. CRSwNP is quite unlike chronic rhinosinusitis without nasal polyps (CRSSNP) which is Th1 biased [242]. CRSwNP mucosa contains more ILC2 cells than CRSSNP or normal mucosa and these cells secrete high levels of IL-13 upon stimulation with IL-33 which can be released by damage including that induced by *Alternaria fumigatus* fungal challenge [243].

4.3. Genetic data

As detailed in Table 1 various IL-4/IL-13 axis polymorphisms are associated with the risk of developing AR.

4.4. Animal model data

Nasal IL-13 installation was found to induce eosinophilia and goblet cell hypertrophy in the nasal mucosa of mice which could be decreased by approximately 60% by pretreatment with systemic TSLP-neutralising mAb [244]. *Ex vivo*, IL-13 was found to induce TSLP expression in mouse nasal explants in a STAT-6 dependent and mast cell independent fashion [244]. In a systemic sensitization and nasal challenge model of mouse nasal allergy IL-13 neutralization with soluble IL-13Rα2-Fc reduced nasal blockage (via the surrogate of respiratory rate as mice are obligate nasal breathers) and nasal resistance without affecting eosinophils in nasal tissue [245]. IL-4 and IL-13 signaling was prevented using STAT6 siRNA in a mouse systemic sensitization and nasal challenge model. When dosed during the nasal allergen challenge period STAT6 siRNA prevented allergen induced sneezing, nasal rubbing, ablated IL-4 and IL-5 production from *ex vivo* allergen challenged lymph node cells and reduced nasal mononuclear and eosinophil infiltration by approximately 50% [246].

4.5. In vitro data and treatment hypothesis

This section attempts to cover the biology of IL-4 and IL-13 over and above that for asthma (Section 2.5) as it speaks directly to AR pathology. The following studies are from human systems unless otherwise noted.

4.5.1. Inflammation

IL-4 and IL-13 stimulate nasal fibroblasts to induce eotaxin release which is synergistically enhanced by TNF-α [103] or LPS

[247]. IL-4 induces nasal mast cells to upregulate FcεRI cell surface protein expression [95].

Taken together with the asthma rationale data the scientific hypothesis would be that as part of an ongoing sensitization process DCs present antigen to Th2 cells which secrete significant amounts of IL-4 and IL-13. IgE generated by the Th2 skewing binds to the surface of nasal mast cells that upon exposure to allergen degranulate and thus release histamine as well as IL-4 and IL-13. During an infection nasal epithelial damage leads to IL-33 release and activation of nasal ILC2s causing significant additional IL-13 and IL-5 release [243]. IL-4 and IL-13 together act on fibroblasts and epithelial cells to generate significant levels of eosinophil chemoattractants and upregulate endothelial eosinophil homing molecules driving eosinophilia as well as promoting epithelial cell differentiation to induce mucus hypersecretion. Therefore inhibition of IL-4 and/or IL-13 could be an attractive approach to reduce these pathogenic effects and hence relieve symptoms.

4.6. Clinical experience

Only one study has been published investigating the potential of IL-13 blockade for allergic rhinitis. QAX-576 from Novartis was evaluated in a nasal allergen challenge study of 36 patients with grass pollen seasonal AR. Subjects were challenged with timothy grass pollen at baseline and then on 3 occasions after a single 6 mg/kg intravenous infusion of QAX-576. QAX-576 suppressed nasal lavage IL-13 but not IL-5, eosinophils or symptom scores. A post hoc analysis showed a trend toward a suppression of symptom scores in patients with the highest pre-dose IL-13 response to allergen challenge [248]. If allergen challenge activated ILC2s in this study then it is possible that ILC2-derived IL-5 could have driven the eosinophilia and maintained the symptom score. Whether chronic suppression of IL-13 could have benefits in AR remains to be seen.

Sanofi issued a press release on 30th September 2014 stating that 300 mg subcutaneous dupilumab dosed weekly for 16 weeks had delivered clinical benefit to patients with chronic rhinosinusitis with nasal polyps who were insensitive to ICSs. Nasal polyp score, a measure of polyp size, as well as measures of sinusitis were reduced. In patients who also had asthma (58% of trial cohort) there were improvements in asthma control [249].

5. COPD

5.1. Introduction

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the world. COPD is an obstructive lung disease primarily caused by decades of exposure to smoke from cigarettes or open fires and characterized by significant lung and systemic inflammation. Unlike asthma the airflow limitation in COPD is not reversible. In common with asthma COPD exacerbations represent a significant issue and exacerbation rate reduction is a key deliverable for any biologic agent. In COPD large airways are inflamed leading to chronic bronchitis in half of patients. Small airways are also inflamed causing a decreased lumen and increased resistance to airflow. Alveoli are destroyed resulting in enlarged airspaces, emphysema and reduced surface area for gas exchange. All airways are burdened with thick, sometimes infected, mucus. The prevailing mechanistic theory is that cigarette smoking causes inflammation mediated by neutrophils, macrophages and CD8⁺ T cells which persists even after smoking cessation. These inflammatory cells release elastase and other proteinases which mediate lung destruction (reviewed in [250]). Treatment recommendations are severity (symptoms and

breathlessness) and risk (spirometry and exacerbation history) based and employ different combinations of bronchodilators (short acting beta agonists, methylxanthines, long acting beta agonists, muscarinic antagonists) and anti-inflammatories (ICS, PDE4 inhibitors, OCS) [251]. Despite these treatment options there is enormous unmet medical need in COPD.

5.2. Disease expression

Evidence for IL-4 and IL-13 expression in COPD is contradictory. Submucosal gland cells expressing IL-4 mRNA were more numerous in chronic bronchitic patients than either COPD patients with chronic bronchitis or asymptomatic smokers; no CD8⁺ T cells expressed IL-4 mRNA [252]. Indeed stable COPD is considered a Th1 biased disease; lung resection lymphocytes derived from smokers with COPD demonstrated no expression of IL-4 or Th2 chemokine receptors such as CCR3/CCR4 but high levels of IFN- γ and Th1 chemokine receptors such as CXCR3 [253]. In contrast BAL lymphocytes have been reported to be of a mixed phenotype with the proportion of BAL, but not peripheral, IL-4⁺ CD4, IL-4⁺ CD8 and IL-13⁺ CD8 T cells from COPD patients greater than non-COPD smokers or controls [254].

Data on IL-13 expression in COPD is also mixed. IL-13 was measurable in 18% of induced sputum from COPD patients and none of the smoking controls with no difference in clinical characteristics between IL-13 positive and negative patients; in a second cohort very few IL-13 expressing cells could be detected immunohistochemically in proximal airway biopsies with no difference between COPD patients and controls [255]. In another contrasting study IL-13 was detected in 46% of induced sputum samples from COPD patients and 14% controls [256]. IL-13 mRNA has been detected in the lung tissue of very severe COPD patients undergoing transplant [257]. Plasma IL-13 expression has also been detected in COPD patients and was inversely correlated with both lung diffusion capacity test (DLCO) and % predicted FEV1 [258].

Even more so than for asthma, exacerbations are a key issue in COPD. Viruses in general and rhinovirus in particular can trigger exacerbations; 30% of patients admitted for an acute COPD exacerbation (AECOPD) had a respiratory virus and 77% of this 30% was rhinovirus [259]. Exacerbations were surprisingly of a mixed Th1 and Th2 phenotype in the lung and periphery; BAL IFN- γ from AECOPD was not universally expressed (25%) nor were levels different from stable COPD (36%) or control patients (28%); BAL IL-4 from AECOPD was universally expressed and upregulated versus stable COPD (61%) or control patients (32%); BAL IL-13 from AECOPD was not universally expressed (25%) nor were levels different from stable COPD (32%) or control patients (50%) [259]. A potential explanation for this finding was that HRV proteinase 2A could influence DCs to induce a mixed Th1/Th2 cell polarization [259]. Similarly IL-4 expressing CD8⁺ sputum T cells increase at the beginning of a mild AECOPD from the stable state and both the CD4 IFN- γ +/CD4 IL-4⁺ and CD8 IFN- γ +/CD8 IL-4⁺ ratios decrease [260]; sputum CD8 IFN- γ +/IL-4⁺ ratios also decreased at the onset of severe AECOPDs [261].

5.3. Genetic data

As detailed in Table 1 various IL-4/IL-13 axis polymorphisms are associated with the risk of accelerated lung function decline with smoking or the risk of developing COPD.

5.4. Animal model data

A key exposure underlying COPD is cigarette smoke. Rats exposed acutely (3 days) or chronically (8 or 16 weeks) to cigarette smoke developed AHR and airway remodeling (increased smooth

muscle actin) in association with elevations in lung IL-13 and lung TGF- β after chronic, but not acute, exposure [262]. Much of the interest in targeting IL-13 for COPD originated with an experiment in which IL-13 was overexpressed in the lungs of mice using a Clara cell promoter-driven transgene. IL-13 transgenic mice spontaneously developed emphysema in association with mucus metaplasia and macrophage, lymphocyte and eosinophil inflammation; much of the lung damage was driven through matrix metalloproteinases (MMPs) and cysteine proteinases [263].

In a mouse model mimicking key aspects of an acute COPD exacerbation mice were exposed to an elastase/LPS combination for a month which caused emphysema-like changes (increased chord length, increased total lung volume) in association with lung infiltration by macrophages and neutrophils, goblet cell metaplasia and airway obstruction. One week after elastase/LPS mice were also intranasally exposed to rhinovirus RV1B. Elastase/LPS/RV1B-treated mice had a much greater viral load, viral persistence, decreased anti-viral response (IFN- α and IFN- β mRNA), increased inflammatory response (IL-13, IL-5 and TNF- α mRNAs) and increased lung volume than PBS/RV1B-treated mice and AHR compared with PBS/RV1B- or elastase/LPS/PBS-treated mice [264]. A mouse model also identified a pathway whereby viral infection activated CD4⁺ NKT cells that recruited and activated macrophages to chronically produce IL-13 and drive inflammation, AHR and mucus production despite the virus being completely cleared [265]. Analogously COPD patients were shown to have more lung IL-13 mRNA, mucosal MUC5AC, CD4⁺ NKT and IL-13-positive macrophages than control patients [265].

5.5. In vitro data and treatment hypothesis

Two recent studies suggest that there is reason to strongly consider testing neutralization of the IL-4/IL-13 axis in COPD. The first built on observations in COPD where enhanced clinical benefit, in terms of a 62% reduction in exacerbations, could be achieved if patients were managed by titrating steroid doses to manage sputum eosinophil levels at or below 3% rather than by the British Thoracic Society (BTS) guidelines [266]. This first recent study evaluated the effects of benralizumab, an IL-5R directed aglycosyl IgG1 that blocks IL-5 function and also recruits enhanced cell-killing effector function thus effectively depleting eosinophils, in 101 patients with eosinophilic COPD. Benralizumab dosed over 48 weeks did not offer clinical benefit to all; in patients with blood eosinophils greater than 200 per μ L benralizumab reduced AECOPD and improved both FEV1 and symptom scores [267]. Eosinophils are significant sources of IL-4 and IL-13 [268]. The second recent study extends the Th2 paradigm from asthma [66] to COPD and identified a Th2-high cohort of COPD patients. The Th2 high COPD patients had no association with asthma history, airway reversibility, reduced hyperinflation following ICS treatment together with lower lung function, increased blood and lung wall eosinophils as compared to non-Th2 high COPD patients [269]. Asthma–COPD overlap syndrome (ACOS) is now well recognized as a disease with key aspects of both asthma and COPD [270,271].

There is little mechanistic rationale to consider IL-4 and/or IL-13 neutralization in “classic” macrophage/neutrophil COPD outside of the well-recognized effects on lung epithelium in driving excess mucus production [114,117] or GM-CSF/IL-8 [108] and MCP-1 [272] production as part of the drive for excess neutrophil and macrophage inflammation. In the context of an identifiable Th2-high population of COPD or ACOS patients IL-4/IL-13 neutralization seems much more obvious with predictable potential benefits of airflow improvement, reduced mucus production, reduced eosinophil mediated inflammation. Taken together the literature

demonstrates the potential involvement of an IL-4 and IL-13 axis in a subset of COPD and suggests that, with the appropriate personalized healthcare approach, the potential for clinical benefit could be assessed in a controlled study. This hypothesis has not yet been tested.

6. Cancer

Increased IL-4 and IL-13 activities have been closely associated with malignancy. They play significant roles in tumorigenesis and modulation of anti-tumor immune responses [273–276]. In Hodgkin's lymphoma (HL), the tumor cells (Reed-Sternberg) are rare (0.1–1%) in the tumor mass and the bulk of the remaining stroma is made up of infiltrating, reactive, cells including Th2 cells [277]. Co-expression of IL-13 and its receptor IL-13R α 1 is found in RS cells. IL-13 mediates autocrine stimulation of the proliferation of HL cells and inhibition of cell death [278,279]. STAT6 is phosphorylated upon activation of IL-4R and IL-13R by IL-4 and IL-13, respectively. Activation of STAT6, as well as STAT3, is important for HL cell growth [280,281]. Neutralizing antibodies to IL-13, IL-4 and their receptors IL-13R α 1 and IL-4R α or siRNAs against STAT6 had been shown to reduce proliferation [279,280,282,283] and induce cell death *via* inhibition of the expression of anti-apoptotic Bcl-xL *in vitro* [284]. Autocrine IL-4 suppresses immunosurveillance *in vivo*, thus facilitating pancreatic tumor growth and metastasis [285].

Besides IL-13 being an autocrine growth factor in HL, it has been shown to mediate cancer invasion and metastasis *via* IL-13R α 2 in an ERK/AP-1-dependent and MMP – mediated manner in a xenograft ovarian tumor model in mice [286]. In contrast to IL-13R α 1, IL-13R α 2 is not associated with IL-4R α for signaling [286]. Instead, IL-13 binds to IL-13R α 2 with high affinity and then either internalizes or mediates signaling transduction [31,287]. Over-expression of IL-13R α 2 increases cancer invasion and metastasis and shortens survival time of mice with human ovarian cancer [286]. Intraperitoneal injection of IL-13 further shortened the survival of IL-13R α 2-positive bearing mice compared to IL-13R α 2-negative mice [286]. Silencing of IL-13R α 2 in highly metastatic colorectal tumor cells led to a decrease in adhesion capacity *in vitro* and a reduction in liver homing *in vivo* [288]. NKT cells stimulated by tumor antigens produce IL-13 that then acts on Gr-1+ granulocytes to produce TGF- β thus suppressing CD8 $^{+}$ T cell activity in tumor surveillance [289]. Activities of TGF- β and IL-13 can result in intratumoral fibrosis which further dampens anti-tumor immune surveillance by preventing the recruitment of anti-tumor CD8 $^{+}$ cytotoxic T lymphocytes (CTL) and NK cells [290]. It has been reported that IL-13R α 2 is overexpressed in a variety of human tumors, including glioblastoma, head and neck, pancreatic, kidney, ovarian, breast, and Kaposi's sarcoma [291]. Therefore, IL-13R α 2 is recognized as a tumor associated antigen which can be used to target cancer cells for killing by immunotoxin conjugates [292,293]. A similar approach using IL-4-Pseudomonas exotoxin was also tested in patients with recurrent malignant glioma with an acceptable safety and toxicity profile [294].

Accumulation of tumor-associated macrophages (TAMs) is associated with poor prognosis of a wide range of solid tumors, such as lung cancer, pancreatic cancer, ovarian cancer, liver cancer, kidney cancer, brain tumor, and Hodgkin's lymphoma [295]. TAMs, also known as alternatively activated or M2 macrophages, are CD163 $^{+}$ and/or CD204 $^{+}$ [296,297]. They are closely linked to intra-tumor inflammation, angiogenesis, and tumor growth, invasiveness and metastasis. IL-4 produced by Th2 and tumor cells is crucial for the expansion of TAMs. Enhanced Th1 responses are associated with anti-tumor responses, whereas increased Th2

responses have the opposite effects. The imbalance and conversion of Th1 to Th2 cells are responsible for both the occurrence and progression of cancer. TAMs also produce immunosuppressive factors, including prostaglandin E2 (PGE2), indoleamine 2,3-dioxygenase, and IL-10 which contribute to the increase of regulatory CD4 $^{+}$ T cells and myeloid-derived suppressive cells (MDSC), resulting in the inhibition of anti-tumor cellular immunity [298]. TAMs also produce TGF- β . TAMs have been shown to mediate the production and activation of myofibroblasts *via* epithelial–mesenchymal transition (EMT), promote extracellular matrix modification by upregulation of MMPs and thus result in increased tumor invasiveness [299]. TAMs also induce the generation of cancer stem cells *via* EMT [300]. Therefore, IL-4 and IL-13 play direct and indirect roles in tumorigenesis. Blocking TAM differentiation through down-modulation of IL-4R α and IL-13R α 1 signaling is a potential strategy for mounting anti-tumor responses.

7. IBD

Th2 cytokines (IL-4, IL-5, and IL-13) have been recognized to play a primary role in the inflammatory reaction to helminthic infestation in the gut [301]. Among them, IL-13 works at a number of levels to combat the infestation by stimulating mucus production from goblet cells, inducing local eotaxin release to attract eosinophils, increasing IgE production, increasing gut motility and epithelial secretion (*via* disruption of tight junctions, and possibly enhancing cystic fibrosis transmembrane conductance regulator mediated apical chloride secretion) [302–304]. Intestinal epithelial cells produce thymic stromal lymphopoietin (TSLP) [305], which is necessary and sufficient for the initiation of Th2-mediated inflammation. IL-33 is also expressed early during parasitic infection and IL-33 binds to ST2 receptor on multiple cell types in lamina propria, such as mast cells, basophils and macrophages [306]. These cells produce Th2 cytokines and in particular IL-4 which promotes Th2 differentiation.

In spite of these beneficial effects of IL-13 on host defense against parasitic infestations in the gut, uncontrolled activation of IL-13 activities can cause serious inflammation. Inflammatory bowel diseases (IBDs) that include ulcerative colitis (UC) and Crohn's disease (CD) are chronic relapsing disorders of the gastrointestinal tract that are characterized pathologically by intestinal inflammation and epithelial injury. Several clinical and pathological features of UC and CD are distinct [307]. UC affects the colonic mucosa, generally involving the rectum and extending to part or the entire proximal colon. The inflammation in CD is often transmural, whereas in UC the inflammation is typically confined to the mucosa. CD can be associated with intestinal granulomas, strictures, and fistulas, but these are not typical findings in UC. The exact mechanisms of pathogenesis of IBDs are not fully understood. They involve the complex interactions of dysbiosis (imbalance of gut microbiome), dysregulated homeostasis of innate and adaptive immune response, diet, and genetic traits [308,309]. Increased IL-13 activity has been implicated in UC based on data from some mouse models and human intestinal epithelial cell *ex vivo* studies [310–314]. In an oxazolone-induced colitis model in mice which mimics key aspects of UC in humans, a marked IL-13 response from CD4 $^{+}$ NKT cells (not conventional CD4 $^{+}$ T cells) was observed [310]. Treatment to block IL-13 activity with IL-13R α 2-Fc or IL-13 neutralizing mAb or with specific mAbs to deplete NKT cells lead to the amelioration of intestinal inflammation and injury [310]. Mice with either CD1 or J α 281 (invariant TCR) deficiency which lack the ability to present antigens by NKT cells were also protected against disease in this model [310]. Stimulation of lamina propria T cells from UC patients with CD1-expressing antigen-presenting cells loaded with

α -galactosyl-ceramide, the nominal antigen of NKT, induced high levels of IL-13 production. CD4⁺ T cells isolated from lamina propria of UC patients produced high levels of IL-13, but not IL-4 or IFN γ , whereas cells from CD patients produced only slightly higher amounts of IL-13 than non-IBD controls [310]. Furthermore, CD4⁺ NKT cells isolated from lamina propria from UC patients were found to be cytotoxic to colonic epithelial cells *in vitro* [311]; the activity was IL-13 dependent. IL-13 (but not IL-4) reduced the electrical resistance of colonic epithelial cell monolayer by inducing apoptosis and increasing expression of pore-forming tight-junction protein claudin-2; claudin-2 upregulation is also found in UC patients [311]. Together, the data indicate that IL-13-producing CD4⁺ NKT cells mediate intestinal injury and damage epithelial cell integrity. A recent study provided conflicting data that IL-13 production by GI tract mucosal explants and activated lamina propria mononuclear cells did not differ between CD, UC and control subjects and was at least 1 log lower than IFN γ and IL-17A [312].

Intestinal fibrosis is a common and serious complication in IBD patients despite current anti-inflammatory therapies [315]. Interestingly, IL-13 signaling *via* IL-13R α 2 was shown to play an important role in the fibrosis developing in oxazolone colitis, trinitrobenzene sulfonic acid (TNBS)-induced colitis [289]. Fibrostenotic complication is common in Crohn's disease [316]. IL-13 is highly expressed in so-called transitional cells that line fistula tracks, and IL-13 induces genes involved in cell invasion, a potential mechanism of fistula formation through tissue [317].

In addition to the compelling data of the pathogenic roles of IL-13-expressing NKT cells from mouse models it is noteworthy that IL-13 can also be produced by conventional Th2 cells and innate lymphoid cells type 2 (ILC2) which are also present in the intestinal tract in mice and humans in particular during IBD [318]. ILC2s are activated by gut epithelial cytokines, such as IL-33, IL-25 and TSLP, released in response to epithelial damage and infection [305,306,319]. ILC2s also play a crucial role in eosinophilic colitis *via* IL-5 production and eosinophil recruitment [320].

Tralokinumab was tested for safety and efficacy in patients with moderate-to-severe UC (NCT01482884). The results indicate that add-on therapy with tralokinumab did not significantly improve clinical response vs. placebo in these patients. However, improvements in clinical remission rates and in mean partial Mayo scores with tralokinumab vs. placebo provide support for the importance of IL-13 in a subset of UC patients [321]. Another anti-IL-13 neutralizing mAb (anrukinzumab (IMA-638)) showed no clinical effect in active UC patients (NCT01284062) [157]. Together, the clinical data from these two clinical studies could indicate that IL-13 is not the predominant etiological driver of the disease. An alternative explanation is that patient stratification into those with and without an active IL-13 pathway is required to test the hypothesis that IL-13 is a key driver of UC.

8. Autoimmune disease

CD4⁺ T helper (Th) cells produce different cytokines to mediate diverse functions in response to environmental duress under the control of specific transcription factors [322,323]. Th1 cells (producing IFN γ and IL-2) mediate host defense against intracellular bacteria, whereas Th17 cells (producing IL-17A/F and IL-22) mediate host defense against extracellular bacteria and fungi. Th2 cells (producing IL-4, IL-13, IL-5, and IL-25) protect the host from parasitic infections. However, uncontrolled activities of Th1 and Th17 cells can cause inflammatory and autoimmune diseases, whereas Th2 cells are linked to atopic diseases such as allergy, asthma and atopic dermatitis (see sections above and [324]). The roles of Th2 cells in the pathogenesis of autoimmune disease are more

limited and less well studied as compared to Th1 and Th17 cells. Plasticity between Th1 and Th2 cells is regulated by specific transcription factors, Tbet/Stat4 and GATA3/Stat5, respectively [325]. Th2 to Th1 shift can augment autoimmune disease, whereas this reverses atopic diseases such as asthma. In insulin-dependent diabetes mellitus (IDDM) coexisting with IBD, Th1 cells are pathogenic and Th2 cells are protective. Consistent with these observations, helminth infections prevent autoimmune diseases in mice and humans by shifting Th1-mediated autoimmunity to a Th2 immune response [326].

The pathological involvement of IL-4 in autoimmune disease was recently demonstrated in lupus nephritis [327,328]. IL-4 and STAT6 gene polymorphisms are linked to systemic lupus erythematosus (SLE) [329]. Emerging data indicate the presence of autoreactive IgE in the sera of SLE patients with nephritis [327,328]. Tyrosine protein kinase Lyn is a critical negative regulator of the Th2 response [330]. Lyn^{-/-} mice develop a strong and constitutive Th2 skewing in early life and show exacerbated responses to Th2 challenge. In late life Lyn^{-/-} mice develop an autoimmune disease that mimics the features of nephritis in human SLE. Besides acting as antigen-presenting cells [331], basophils activated by IgE immunocomplexes (IgE/IC) by cross-linking surface Fc ϵ R1 produce IL-4 which then further promotes Th2 cell differentiation and proliferation. Increased IL-4 production facilitates the production of autoreactive IgG1 and IgE from B cells *via* the upregulation of IL-6 and B-cell activating factor (BAFF) [332–334]. BAFF serves a fundamental role during the transition from immature T1 to T2 B cells [335]. In addition to its crucial role in the maintenance of peripheral B cell pool, BAFF has been found to be essential for marginal zone (MZ) B cell development [336]. Anti-BAFF mAb (belimumab from GSK) is being tested in LN patients (NCT01639339). Basophils are also being implicated in the pathogenesis of rheumatoid arthritis, wherein citrullinated proteins can activate basophils *via* IgE anti-citrullinated protein [337,338]. Together, these findings indicate that IL-4 is an important regulator of B-cell dependent autoimmunity and the pathological involvement of basophils should be further examined [339].

In systemic sclerosis (SSc), gene polymorphism of IL-13 is linked to skin fibrotic manifestation [340]. CD4/CD8 double-positive cells producing high levels of IL-4 were found in lesional skin of SSc patients [341]. However, no correlation with IL-4, IL-13 or their receptor gene polymorphisms was found in another study with a much larger patient cohort [342].

Together, the data on the pathological involvement of IL-4 and IL-13 in autoimmune disease is still very limited. The majority of research and clinical development efforts are focused on Th1 and Th17 cells.

9. Fibrosis

Fibrosis is involved in the pathogenesis of a wide range of diseases, including pulmonary fibrotic disorders (idiopathic pulmonary fibrosis (IPF), severe asthma and COPD), systemic sclerosis, renal disease, IBD, cancer, and liver fibrosis [343,344]. Nearly 45% of all deaths in the developed world are linked to some type of chronic fibroproliferative disease (reviewed in [345]). Fibrosis is a normal consequence of tissue injury and chronic inflammation, characterized by the accumulation and activation of excessive numbers of fibroblasts, deposition of extracellular matrix (ECM) proteins, such as collagen, and distortion of normal tissue architecture [346]. Although fibrosis typically begins as a part of wound healing responses, excessive accumulation of collagen and other ECM components during chronic inflammation can lead to the destruction of normal tissue architecture and loss of function.

The transcription of several genes known to be involved in the mechanisms of wound healing and fibrosis are upregulated by Th2 cytokines (reviewed in [347]). The regulation and function of a few of these genes, such as those that encode procollagen I, procollagen III, arginase, lysyl oxidase, matrix metalloproteinase (MMP2), MMP9 and tissue inhibitor of matrix metalloproteinase 1 (TIMP1) have been reported [348–353]. Although IL-4 and IL-13 share similar receptor signaling mechanisms and can induce fibrosis, studies in which IL-4 and IL-13 were inhibited independently identified IL-13 as the dominant effector cytokine of fibrosis in several models [347,353]. Overexpression of IL-13 in the lung induced pronounced subepithelial airway fibrosis in mice in the absence of any other inflammatory stimuli [78], whereas treatment of IL-13-specific antibodies markedly reduced collagen deposition in the lungs of animals that were challenged with bleomycin [354] or *Aspergillus fumigatus* conidia [355]. However, transgenic mice overexpressing IL-4 showed little evidence of subepithelial airway fibrosis, despite developing an intense inflammatory response in the lung [70]. Inhibition of IL-13 by a neutralizing mAb (tralokinumb) reduces lung fibrosis and epithelial damage in a humanized SCID idiopathic pulmonary fibrosis (IPF) mouse model [356]. IL-13 is upregulated in the lungs of IPF patients as compared to healthy individuals [28,356].

IL-13 is also involved in the fibrosis generated from other disparate diseases. Schistosomiasis is a chronic disease caused by infection with the helminth *S. mansoni* that results in a Th2-biased response which leads to hepatic fibrosis [357]. In nonalcoholic steatohepatitis (NASH) – the most common cause of liver fibrosis [358,359] driven by poor diet, high levels of IL-13R α 2 are present that colocalize with smooth muscle actin and in a rat model of the liver disease, an IL-13R α 2-targeted cytotoxin ameliorates fibrosis. IL-13 is also involved in the manifestation of fibrosis found in systemic sclerosis (SSc) patients [360]. It was found that CD8⁺ T cells but not CD4⁺ T cells are the primary source of IL-13 which is upregulated in SSc patients [361]. These IL-13-producing CD8⁺ T cells expressed high levels of GATA3 and possessed homing receptors to the skin of SSc patients [362]. Incubation of these cells with healthy dermal fibroblasts results in elevation of ECM from these cells [362].

The significant pro-fibrotic role of IL-13 *in vivo* over IL-4 could be attributed to the signaling mechanism of IL-13R α 2 and/or the involvement of ILC2 which produce IL-13 and IL-5 but not IL-4 [363–365]. Moreover, results from several disease models indicate that differences in ligand concentration might also explain the differential activities of IL-4 and IL-13. When the production of IL-4 and IL-13 are compared, the concentrations of IL-13 often exceed those of IL-4 by a factor of 10–100 [365]. Together, these findings support the notion that IL-13 plays a dominant role in fibrosis, whereas IL-4 is associated with immune modulation and Th2 polarization.

TGF- β 1 is an important pro-fibrotic cytokine [347,366]. Although it can be produced by many cell types, the macrophage is the primary source responsible for tissue fibrosis. TGF- β 1 is stored in the cell as an inactive form, a disulfide-bonded homodimer that is non-covalently bound to a latency-associated protein (LAP) [331]. Binding of the cytokine to its receptors requires dissociation of LAP, a process that is catalyzed *in vivo* by several agents, such as plasmin, cathepsins, calpain, thrombospondin, α_v integrins (β 1, 6 and 8) and MMPs [331]. IL-13 plays an intimate role in the activation of TGF- β 1. IL-13 induces the production of latent TGF- β 1 [363,364]. IL-13 also indirectly activates TGF- β 1 by upregulating LAP-TGF- β 1 complex [367,368]. IL-13 is a potent stimulator of MMP and cathepsin-based proteolytic pathways in the lung and liver. Therefore, IL-13-producing Th2 cells (probably also ILC2) stimulate macrophage production of TGF- β 1 [347] which then functions as the primary stimulus for fibroblast activation

and collagen deposition. In support of this notion, when TGF- β 1 activity was neutralized in the lungs of IL-13-transgenic mice, development of subepithelial fibrosis was markedly reduced [367]. Taken together, IL-13 can promote fibrosis directly or indirectly through TGF- β 1. Its profibrotic activity in human fibroblasts is solely mediated by IL-13R α 1/IL-4R α complex but not IL-13R α 2 based on genome-wide transcriptional analysis. IL-13R α 2 functions as a decoy to inhibit IL-13 activity [28].

Based on the compelling data on the role of IL-13 in fibrosis, several anti-IL-13 mAbs are in clinical development for IPF. MedImmune is evaluating tralokinumab in a 186 mild-moderate IPF patient study examining change in FVC at 72 weeks (NCT01629667). Genentech is evaluating lebrikizumab in a study of 250 IPF patients examining progression-free survival over a time period up to 2.5 years (NCT01872689). Results from these studies will provide data to validate the pathogenic role of IL-13 in this disease.

10. Evolving biology

The role of Th2 cytokines in regulating metabolism associated with obesity and glucose sensitivity have been recently recognized in the mouse literature. Eosinophil-derived IL-4/IL-13 has been shown to stimulate fibro/adipogenic progenitors cells for muscle regeneration [369]. IL-4 and STAT6 signaling control peripheral nutrient metabolism and insulin sensitivity [370]. Disruption of STAT6 function decreases insulin action and enhances a peroxisome proliferator-activated receptor alpha (PPAR α) – mediated oxidative metabolism [370]. IL-4 and Th2 polarization improve glucose homeostasis in mice on a high-fat diet [370]. Genetic ablation of IL-13 in mice (IL-13 $^{-/-}$) results in hyperglycemia, which progresses to hepatic insulin resistance and systemic metabolic dysfunction as a result of upregulation of enzymes involving in hepatic gluconeogenesis *via* a IL-13R α 1/Stat3 mechanism [371].

Eosinophils in visceral adipose tissue (VAT) have recently been shown to play an important function in metabolic homeostasis and maintenance of alternatively activated macrophages (AAMs) which produce IL-4 but not IL-13 [372,373]. IL-5 deficiency significantly impairs VAT eosinophil accumulation resulting in increased adiposity and insulin resistance in mice on a high-fat diet. ILC2s residing in VAT are responsible for the production of IL-5 and IL-13 [373]. IL-33, which activates ILC2s, mediates the increase in VAT eosinophils and AAMs [373,374]. The most recent studies indicate that ILC2s and IL-33 are important in mediating beiging of white adipose tissues [374,375]. Beige (or brown) fat has a higher rate of thermogenesis (or caloric expenditure) than white fat through the expression of the uncoupling protein 1 (UCP1) in mitochondria [374]. Loss- and gain-of-function studies show that ILC2- and eosinophil-derived type 2 cytokines stimulate signaling *via* the IL-4R α in PDGFR α adipocyte precursor cells to promote beige fat biogenesis [374]. Furthermore, ILC2s produce methionine-enkephalin peptides that act directly on adipocytes to upregulate UCP1 expression *in vitro* and that promote beiging in mice [375]. Together, these data indicate that innate type 2 immune regulation plays an important role in mouse metabolic homeostasis. Interestingly there have been no reports of frank metabolic changes in patients from any of the IL-4, IL-13 or IL-4/13 neutralisation clinical trials despite exposures of up to a year. It remains to be seen whether these rodent findings will extrapolate to man.

11. Conclusion and future directions

IL-4 and IL-13 can be produced by multiple cell types, their receptors are ubiquitously expressed (see Fig. 1) and they mediate

a broad range of functions which can potentially mediate multiple diseases when their activities are dysregulated. Current rationale for targeting IL-4 and/or IL-13 is most robust in allergic disease (asthma, AD, AR) with decreasing levels of validation in fibrosis (IPF), IBD (UC), COPD, cancer and autoimmune disease, respectively. This is borne out by recent positive clinical data obtained after inhibiting IL-13 (tralokinumab and lebrikizumab) or IL-4 and IL-13 (pitakinra, dupilumab) in asthma, inhibiting IL-4 and IL-13 in atopic dermatitis (dupilumab) and negative data from IL-13 neutralization in IBD (tralokinumab, anrakinzumab) with data from IL-13 neutralization pending in IPF and AD. The main current and future challenge is patient selection and stratification, clearly evidenced by the periostin data with lebrikizumab and tralokinumab in asthma although this may only be relevant in diseases with known heterogeneity such as asthma and COPD. Patient selection does not currently seem an issue in AD with data on UC phenotypic heterogeneity absent. It seems sensible to explore stratification hypotheses in every trial in every disease to ensure a negative result is a true negative.

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References

- [1] Y. Noma, P. Sideras, T. Naito, S. Bergstedt-Lindqvist, C. Azuma, E. Severinson, et al., Cloning of cDNA encoding the murine IgG1 induction factor by a novel strategy using SP6 promoter, *Nature* 319 (1986) 640–646.
- [2] T. Yokota, T. Otsuka, T. Mosmann, J. Banchereau, T. DeFrance, D. Blanchard, et al., Isolation and characterization of a human interleukin cDNA clone, homologous to mouse B-cell stimulatory factor 1, that expresses B-cell- and T-cell-stimulating activities, *Proc. Natl. Acad. Sci. USA* 83 (1986) 5894–5898.
- [3] G. Del Prete, E. Maggi, P. Parronchi, I. Chrétien, A. Tiri, D. Macchia, et al., IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants, *J. Immunol.* 140 (1988) 4193–4198.
- [4] F. Lee, T. Yokota, T. Otsuka, P. Meyerson, D. Villaret, R. Coffman, et al., Isolation and characterization of a mouse interleukin cDNA clone that expresses B-cell stimulatory factor 1 activities and T-cell and mast-cell-stimulating activities, *Proc. Natl. Acad. Sci. USA* 83 (1986) 2061–2065.
- [5] T.R. Mosmann, H. Chervinski, M.W. Bond, M.A. Giedlin, R.L. Coffman, Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins, *J. Immunol.* 136 (1986) 2348–2357.
- [6] L. Cosmi, L. Maggi, V. Santarlasci, M. Capone, E. Cardilicchia, F. Frosali, et al., Identification of a novel subset of human circulating memory CD4(+) T cells that produce both IL-17A and IL-4, *J. Allergy Clin. Immunol.* 125 (2010) 222–230.
- [7] K.D. Brown, S.M. Zurawski, T.R. Mosmann, G. Zurawski, A family of small inducible proteins secreted by leukocytes are members of a new superfamily that includes leukocyte and fibroblast-derived inflammatory agents, growth factors, and indicators of various activation processes, *J. Immunol.* 142 (1989) 679–687.
- [8] A. Minty, P. Chalou, J.M. Derocq, X. Dumont, J.C. Guillemot, M. Kaghad, et al., Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses, *Nature* 362 (1993) 248–250.
- [9] A.N.J. McKenzie, J.A. Culpepper, Malefyt R. De Waal, F. Briere, J. Punnonen, G. Aversa, et al., Interleukin 13, a T-cell-derived cytokine that regulates human monocyte and B-cell function, *Proc. Natl. Acad. Sci. USA* 90 (1993) 3735–3739.
- [10] B.W. Morrison, P. Leder, A receptor binding domain of mouse interleukin-4 defined by a solid-phase binding assay and in vitro mutagenesis, *J. Biol. Chem.* 267 (1992) 11957–11963.
- [11] S.L. LaPorte, Z.S. Juo, J. Vaclavikova, L.A. Colf, X. Qi, N.M. Heller, et al., Molecular and structural basis of cytokine receptor pleiotropy in the Interleukin-4/13 system, *Cell* 132 (2008) 259–272.
- [12] B. Miloux, P. Laurent, O. Bonnin, J. Lupker, D. Caput, N. Vita, et al., Cloning of the human IL-13R alpha1 chain and reconstitution with the IL4R alpha of a functional IL-4/IL-13 receptor complex, *FEBS Lett.* 401 (1997) 163–166.
- [13] K. Takeda, T. Tanaka, W. Shi, M. Matsumoto, M. Minami, S. Kashiwamura, et al., Essential role of Stat6 in IL-4 signalling, *Nature* 380 (1996) 627–630.
- [14] H. Gandhi, R. Worch, K. Kurgoonaite, M. Hintersteiner, P. Schwill, C. Bökel, et al., Dynamics and interaction of interleukin-4 receptor subunits in living cells, *Biophys. J.* 107 (2014) 2515–2527.
- [15] G.K. Hershey, M.F. Friedrich, L.A. Esswein, M.L. Thomas, T.A. Chatila, The association of alpha with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor, *N. Engl. J. Med.* 337 (1997) 1720–1725.
- [16] K.A. Risma, N. Wang, R.P. Andrews, C.M. Cunningham, M.B. Erickson, J.A. Bernstein, et al., V75 R576 IL-4 receptor is associated with allergic asthma and enhanced IL-4 receptor function, *J. Immunol.* 169 (2002) 1604–1610.
- [17] A. Heinzmann, X.Q. Mao, M. Akaiwa, R.T. Kreomer, P.S. Gao, K. Ohshima, et al., Genetic variants of IL-13 signalling and human asthma and atopy, *Hum. Mol. Genet.* 9 (2000) 549–559.
- [18] X. Liu, R. Nickel, K. Beyer, U. Wahn, E. Ehrlich, L.R. Freidhoff, et al., An IL13 coding region variant is associated with a high total serum IgE level and atopic dermatitis in the German multicenter atopy study (MAS-90), *J. Allergy Clin. Immunol.* 106 (2000) 167–170.
- [19] P. Kauppi, K. Lindblad-Toh, P. Sevón, H.T. Toivonen, J.D. Rioux, A. Villapakkam, et al., A second-generation association study of the 5q31 cytokine gene cluster and the interleukin-4 receptor in asthma, *Genomics* 77 (2001) 35–42.
- [20] F.D. Vladich, S.M. Brazille, D. Stern, M.L. Peck, R. Ghittoni, D. Vercelli, IL-13 R130Q, a common variant associated with allergy and asthma, enhances effector mechanisms essential for human allergic inflammation, *J. Clin. Invest.* 115 (2005) 747–754.
- [21] K. Arima, R. Umeshita-Suyama, Y. Sakata, M. Akaiwa, X.Q. Mao, T. Enomoto, et al., Upregulation of IL-13 concentration in vivo by the IL13 variant associated with bronchial asthma, *J. Allergy Clin. Immunol.* 109 (2002) 980–987.
- [22] W. Chen, M.B. Erickson, L.S. Levin, G.K.K. Hershey, Functional effect of the R110Q IL13 genetic variant alone and in combination with IL4RA genetic variants, *J. Allergy Clin. Immunol.* 114 (2004) 553–560.
- [23] R.V. Sorg, J. Enczmann, U.R. Sorg, E.M. Schneider, P. Wernet, Identification of an alternatively spliced transcript of human interleukin-4 lacking the sequence encoded by exon 2, *Exp. Hematol.* 21 (1993) 560–563.
- [24] S.P. Atamas, J. Choi, V.V. Yarovsky, B. White, An alternative splice variant of human IL-4, IL-4 δ 2, inhibits IL-4-stimulated T cell proliferation, *J. Immunol.* 156 (1996) 435–441.
- [25] S. Kruse, J. Forster, J. Kuehr, K.A. Deichmann, Characterization of the membrane-bound and a soluble form of human IL-4 receptor alpha produced by alternative splicing, *Int. Immunol.* 11 (1999) 1965–1970.
- [26] T. Zheng, W. Liu, S.Y. Oh, Z. Zhu, B. Hu, R.J. Homer, et al., IL-13 receptor alpha2 selectively inhibits IL-13-induced responses in the murine lung, *J. Immunol.* 180 (2008) 522–529.
- [27] M.T. Kasaian, D. Raible, K. Marquette, T.A. Cook, S. Zhou, X.Y. Tan, et al., IL-13 antibodies influence IL-13 clearance in humans by modulating scavenger activity of IL-13R α 2, *J. Immunol.* 187 (2011) 561–569.
- [28] S. Chandriani, D.J. DePianto, E.N. N'Diaye, A.R. Abbas, J. Jackman, J. Bevers 3rd, et al., Endogenously expressed IL-13R α 2 attenuates IL-13-mediated responses but does not activate signaling in human lung fibroblasts, *J. Immunol.* 193 (2014) 111–119.
- [29] M. O'Toole, H. Legault, R. Ramsey, T.A. Wynn, M.T. Kasaian, A novel and sensitive ELISA reveals that the soluble form of IL-13R-alpha2 is not expressed in plasma of healthy or asthmatic subjects, *Clin. Exp. Allergy* 38 (2008) 594–601.
- [30] W. Chen, U. Sivaprasad, Y. Tabata, A.M. Gibson, M.T. Stier, F.D. Finkelman, et al., IL-13Ralpha2 membrane and soluble isoforms differ in humans and mice, *J. Immunol.* 183 (2009) 7870–7876.
- [31] S. Fichtner-Feigl, W. Strober, K. Kawakami, R.K. Puri, A. Kitani, IL-13 signaling through the IL-13Ralpha2 receptor is involved in induction of TGF-beta1 production and fibrosis, *Nat. Med.* 12 (2006) 99–106.
- [32] C.H. He, C.G. Lee, C.S. Dela Cruz, C.M. Lee, Y. Zhou, F. Ahangari, et al., Chitinase 3-like 1 regulates cellular and tissue responses via IL-13 receptor α 2, *Cell Rep.* 4 (2013) 830–841.
- [33] Q. Wei, Y. Sha, A. Bhattacharya, E. Abdel Fattah, D. Bonilla, S.S. Jyothula, et al., Regulation of IL-4 receptor signaling by STUB1 in lung inflammation, *Am. J. Respir. Crit. Care Med.* 189 (2014) 16–29.
- [34] G. Dolganov, S. Bort, M. Lovett, J. Burr, L. Schubert, D. Short, et al., Coexpression of the interleukin-13 and interleukin-4 genes correlates with their physical linkage in the cytokine gene cluster on human chromosome 5q23-31, *Blood* 87 (1996) 3316–3326.
- [35] J.G. Morgan, G.M. Dolganov, S.E. Robbins, L.M. Hinton, M. Lovett, The selective isolation of novel cDNAs encoded by the regions surrounding the human interleukin 4 and 5 genes, *Nucleic Acids Res.* 20 (1992) 5173–5179.
- [36] B.H. van Leeuwen, M.E. Martinson, G.C. Webb, I.G. Young, Molecular organization of the cytokine gene cluster, involving the human IL-3, IL-4, IL-5, and GM-CSF genes, on human chromosome 5, *Blood* 73 (1989) 1142–1148.
- [37] D. Potaczek, M. Kabesch, Current concepts of IgE regulation and impact of genetic determinants, *Clin. Exp. Allergy* 42 (2012) 582–571.
- [38] D.V. Smirnov, M.G. Smirnova, V.G. Korobko, E.I. Frolova, Tandem arrangement of human genes for interleukin-4 and interleukin-13: resemblance in their organization, *Gene* 155 (1995) 277–281.
- [39] A.N.J. McKenzie, X. Li, D.A. Largaespada, A. Sato, A. Kaneda, S.M. Zurawski, et al., Structural comparison and chromosomal localisation of the human and mouse IL-13 genes, *J. Immunol.* 150 (1993) 5436–5444.
- [40] W.I. Khan, B.A. Vallance, P.A. Blennerhassett, Y. Deng, E.F. Verdu, K.I. Matthaei, et al., Critical role for signal transducer and activator of transcription factor 6 in mediating intestinal muscle hypercontractility and worm expulsion in *Trichinella spiralis*-infected mice, *Infect. Immun.* 69 (2001) 838–844.
- [41] A.A. Elhassan, A.A. Hussein, H.S. Mohamed, K. Rockett, D. Kwiatkowski, A.M. Elhassan, et al., The 5q31 region in two African populations as a facet of natural selection by infectious diseases, *Genetika* 49 (2013) 279–288.

- [42] A.V. Grant, M.I. Araujo, E.V. Ponte, R.R. Oliveira, P. Gao, A.A. Cruz, et al., Functional polymorphisms in IL13 are protective against high *Schistosoma mansoni* infection intensity in a Brazilian population, *PLoS One* 7 (2012) e35863.
- [43] GINA, Global Strategy for Asthma Management and Prevention 2010.
- [44] S.P. Peters, G. Ferguson, Y. Deniz, C. Reisner, Uncontrolled asthma: a review of the prevalence, disease burden and options for treatment, *Respir. Med.* 100 (2006) 1139–1151.
- [45] J. Bousquet, P. Chanez, J.Y. Lacoste, G. Barnéon, N. Ghavanian, I. Enander, et al., Eosinophilic inflammation in asthma, *N. Engl. J. Med.* 323 (1990) 1033–1039.
- [46] D.S. Robinson, Q. Hamid, S. Ying, A. Tscopoulos, J. Barkans, A.M. Bentley, et al., Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma, *N. Engl. J. Med.* 326 (1992) 298–304.
- [47] S.E. Wenzel, L.B. Schwartz, E.L. Langmack, J.L. Halliday, J.B. Trudeau, R.L. Gibbs, et al., Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics, *Am. J. Respir. Crit. Care Med.* 160 (1999) 1001–1008.
- [48] C. Walker, W. Bauer, R.K. Braun, G. Menz, P. Braun, F. Schwarz, et al., Activated T cells and cytokines in bronchoalveolar lavages from patients with various lung diseases associated with eosinophilia, *Am. J. Respir. Crit. Care Med.* 150 (1994) 1038–1048.
- [49] T.C. Kotsimbos, P. Ernst, Q.A. Hamid, Interleukin-13 and interleukin-4 are coexpressed in atopic asthma, *Proc. Assoc. Am. Phys.* 108 (1996) 368–373.
- [50] S. Ying, S.R. Durham, C.J. Corrigan, Q. Hamid, A.B. Kay, Phenotype of cells expressing mRNA for TH2-type (interleukin 4 and interleukin 5) and TH1-type (interleukin 2 and interferon gamma) cytokines in bronchoalveolar lavage and bronchial biopsies from atopic asthmatic and normal control subjects, *Am. J. Respir. Cell Mol. Biol.* 12 (1995) 477–487.
- [51] M. Humbert, S.R. Durham, S. Ying, P. Kimmitt, J. Barkans, B. Assoufi, et al., IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against “intrinsic” asthma being a distinct immunopathologic entity, *Am. J. Respir. Crit. Care Med.* 154 (1996) 1497–1504.
- [52] J.C. Virchow Jr., C. Kroegel, C. Walker, H. Matthys, Inflammatory determinants of asthma severity: mediator and cellular changes in bronchoalveolar lavage fluid of patients with severe asthma, *J. Allergy Clin. Immunol.* 98 (1996) S27–S33.
- [53] S.K. Shahid, S.A. Kharitonov, N.M. Wilson, A. Bush, P.J. Barnes, Increased interleukin-4 and decreased interferon-gamma in exhaled breath condensate of children with asthma, *Am. J. Respir. Crit. Care Med.* 165 (2002) 1290–1293.
- [54] G.E. Carpagnano, M.P. Foschino Barbaro, O. Resta, E. Gramiccioli, N.V. Valerio, P. Bracciale, et al., Exhaled markers in the monitoring of airways inflammation and its response to steroid's treatment in mild persistent asthma, *Eur. J. Pharmacol.* 519 (2005) 175–181.
- [55] C. Kroegel, P. Julius, H. Matthys, J.C. Virchow Jr., W. Luttmann, Endobronchial secretion of interleukin-13 following local allergen challenge in asthma: relationship to interleukin-4 and eosinophil counts, *Eur. Respir. J.* 9 (1996) 899–904.
- [56] J.G. Zangrilli, J.R. Shaver, R.A. Cirelli, S.K. Cho, C.G. Garlisi, A. Falcone, et al., SVCAM-1 levels after segmental antigen challenge correlate with eosinophil influx, IL-4 and IL-5 production, and the late phase response, *Am. J. Respir. Crit. Care Med.* 151 (1995) 1346–1353.
- [57] M. Humbert, S.R. Durham, P. Kimmitt, N. Powell, B. Assoufi, R. Pfister, et al., Elevated expression of messenger ribonucleic acid encoding IL-13 in the bronchial mucosa of atopic and nonatopic subjects with asthma, *J. Allergy Clin. Immunol.* 99 (1997) 657–665.
- [58] T. Naseer, E.M. Minshall, D.Y. Leung, S. Laberge, P. Ernst, R.J. Martin, et al., Expression of IL-12 and IL-13 mRNA in asthma and their modulation in response to steroid therapy, *Am. J. Respir. Crit. Care Med.* 155 (1997) 845–851.
- [59] M. Komai-Koma, A. McKay, L. Thomson, C. McSharry, G.W. Chalmers, F.Y. Liew, et al., Immuno-regulatory cytokines in asthma: IL-15 and IL-13 in induced sputum, *Clin. Exp. Allergy* 31 (2001) 1441–1448.
- [60] S.K. Huang, H.Q. Xiao, J. Kleine-Tebbe, G. Paciotti, D.G. Marsh, L.M. Lichtenstein, et al., IL-13 expression at the sites of allergen challenge in patients with asthma, *J. Immunol.* 155 (1995) 2688–2694.
- [61] W. Feleszko, A. Zawadzka-Krajewska, K. Matysiak, D. Lewandowska, J. Peradzynska, Q.T. Dinh, et al., Parental tobacco smoking is associated with augmented IL-13 secretion in children with allergic asthma, *J. Allergy Clin. Immunol.* 117 (2006) 97–102.
- [62] Z. Jaffar, K. Roberts, A. Pandit, P. Linsley, R. Djukanovic, S. Holgate, B7 costimulation is required for IL-5 and IL-13 secretion by bronchial biopsy tissue of atopic asthmatic subjects in response to allergen stimulation, *Am. J. Respir. Cell Mol. Biol.* 20 (1999) 153–162.
- [63] A.M. Bentley, Q. Hamid, D.S. Robinson, E. Schotman, Q. Meng, B. Assoufi, et al., Prednisolone treatment in asthma. Reduction in the numbers of eosinophils, T cells, tryptase-only positive mast cells, and modulation of IL-4, IL-5, and interferon-gamma cytokine gene expression within the bronchial mucosa, *Am. J. Respir. Crit. Care Med.* 153 (1996) 551–556.
- [64] D.M. Bullens, A. Kasran, K. Peng, K. Lorre, J.L. Ceuppens, Effects of anti-IL-4 receptor monoclonal antibody on in vitro T cell cytokine levels: IL-4 production by T cells from non-atopic donors, *Clin. Exp. Immunol.* 113 (1998) 320–326.
- [65] S.K. Saha, M.A. Berry, D. Parker, S. Siddiqui, A. Morgan, R. May, et al., Increased sputum and bronchial biopsy IL-13 expression in severe asthma, *J. Allergy Clin. Immunol.* 121 (2008) 685–691.
- [66] P.G. Woodruff, B. Modrek, D.F. Choy, G. Jia, A.R. Abbas, A. Ellwanger, et al., T-helper type 2-driven inflammation defines major subphenotypes of asthma, *Am. J. Respir. Crit. Care Med.* 180 (2009) 388–395.
- [67] M. Kabesch, M. Schedel, D. Carr, B. Woitsch, C. Fritzsche, S.K. Weiland, et al., IL-4/IL-13 pathway genetics strongly influence serum IgE levels and childhood asthma, *J. Allergy Clin. Immunol.* 117 (2006) 269–274.
- [68] M.F. Moffatt, I.G. Gut, F. Demenais, D.P. Strachan, E. Bouzigon, S. Heath, et al., A large-scale, consortium-based genomewide association study of asthma, *N. Engl. J. Med.* 363 (2010) 1211–1221.
- [69] K. Bonnelykke, P. Sleiman, K. Nielsen, E. Kreiner-Moller, J.M. Mercader, D. Belgrave, et al., A genome-wide association study identifies CDHR3 as a susceptibility locus for early childhood asthma with severe exacerbations, *Nat. Genet.* 46 (2014) 51–55.
- [70] J.A. Rankin, D.E. Picarella, G.P. Geba, U.A. Temann, B. Prasad, B. DiCosmo, et al., Phenotypic and physiologic characterization of transgenic mice expressing interleukin 4 in the lung: lymphocytic and eosinophilic inflammation without airway hyperreactivity, *Proc. Natl. Acad. Sci. USA* 93 (1996) 7821–7825.
- [71] A.J. Coyle, G. Le Gros, C. Bertrand, S. Tsuyuki, C.H. Heusser, M. Kopf, et al., Interleukin-4 is required for the induction of lung Th2 mucosal immunity, *Am. J. Respir. Cell Mol. Biol.* 13 (1995) 54–59.
- [72] D.B. Corry, H.G. Folkesson, M.L. Warnock, D.J. Erle, M.A. Matthay, J.P. Wiener-Kronish, et al., Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity, *J. Exp. Med.* 183 (1996) 109–117.
- [73] H. Renz, K. Bradley, K. Enssle, J.E. Loader, G.L. Larsen, E.W. Gelfand, Prevention of the development of immediate hypersensitivity and airway hyperresponsiveness following in vivo treatment with soluble IL-4 receptor, *Int. Arch. Allergy Immunol.* 109 (1996) 167–176.
- [74] W.R. Henderson Jr., E.Y. Chi, C.R. Maliszewski, Soluble IL-4 receptor inhibits airway inflammation following allergen challenge in a mouse model of asthma, *J. Immunol.* 164 (2000) 1086–1095.
- [75] H.Z. Shi, J.M. Deng, H. Xu, Z.X. Nong, C.Q. Xiao, Z.M. Liu, et al., Effect of inhaled interleukin-4 on airway hyperreactivity in asthmatics, *Am. J. Respir. Crit. Care Med.* 157 (1998) 1818–1821.
- [76] G. Grunig, M. Warnock, A.E. Wakil, R. Venkayya, F. Brombacher, D.M. Rennick, et al., Requirement for IL-13 independently of IL-4 in experimental asthma, *Science (New York, NY)* 282 (1998) 2261–2263.
- [77] M. Wills-Karp, J. Luyimbazi, X. Xu, B. Schofield, T.Y. Neben, C.L. Karp, et al., Interleukin-13: central mediator of allergic asthma, *Science (New York, NY)* 282 (1998) 2258–2261.
- [78] Z. Zhu, R.J. Homer, Z. Wang, G.P. Geba, J. Wang, et al., Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production, *J. Clin. Invest.* 103 (1999) 779–788.
- [79] D.M. Walter, J.J. McIntire, G. Berry, A.N. McKenzie, D.D. Donaldson, R.H. DeKruyff, et al., Critical role for IL-13 in the development of allergen-induced airway hyperactivity, *J. Immunol.* 167 (2001) 4668–4675.
- [80] T.R. Ramalingam, J.T. Pesce, F. Sheikh, A.W. Cheever, M.M. Mentink-Kane, M.S. Wilson, et al., Unique functions of the type II interleukin 4 receptor identified in mice lacking the interleukin 13 receptor alpha1 chain, *Nat. Immunol.* 9 (2008) 25–33.
- [81] A. Munitz, E.B. Brandt, M. Mingler, F.D. Finkelman, M.E. Rothenberg, Distinct roles for IL-13 and IL-4 via IL-13 receptor alpha1 and the type II IL-4 receptor in asthma pathogenesis, *Proc. Natl. Acad. Sci. USA* 105 (2008) 7240–7245.
- [82] A. Bree, F.J. Schlerman, M. Wadani, L. Tchistiakova, K. Marquette, X.Y. Tan, et al., IL-13 blockade reduces lung inflammation after *Ascaris suum* challenge in cynomolgus monkeys, *J. Allergy Clin. Immunol.* 119 (2007) 1251–1257.
- [83] R.D. May, P.D. Monk, E.S. Cohen, D. Manuel, F. Dempsey, N.H. Davis, et al., Preclinical development of CAT-354, an IL-13 neutralizing antibody, for the treatment of severe uncontrolled asthma, *Br. J. Pharmacol.* 166 (2012) 177–193.
- [84] S.H. Gavett, D.J. O'Hearn, C.L. Karp, E.A. Patel, B.H. Schofield, F.D. Finkelman, et al., Interleukin-4 receptor blockade prevents airway responses induced by antigen challenge in mice, *Am. J. Physiol.* 272 (1997) L253–L261.
- [85] A. Tomkinson, C. Duez, G. Cieslewicz, J.C. Pratt, A. Joetham, M.C. Shanafelt, et al., A murine IL-4 receptor antagonist that inhibits IL-4- and IL-13-induced responses prevents antigen-induced airway eosinophilia and airway hyperresponsiveness, *J. Immunol.* 166 (2001) 5792–5800.
- [86] D. Kuperman, B. Schofield, M. Wills-Karp, M.J. Grusby, Signal transducer and activator of transcription factor 6 (Stat6)-deficient mice are protected from antigen-induced airway hyperresponsiveness and mucus production, *J. Exp. Med.* 187 (1998) 939–948.
- [87] N.J. Dorsey, S.P. Chapoval, E.P. Smith, J. Skupsky, D.W. Scott, A.D. Keegan, STAT6 controls the number of regulatory T cells in vivo, thereby regulating allergic lung inflammation, *J. Immunol.* 191 (2013) 1517–1528.
- [88] D.A. Kuperman, X. Huang, L.L. Koth, G.H. Chang, G.M. Dolganov, Z. Zhu, et al., Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma, *Nat. Med.* 8 (2002) 885–889.
- [89] A. Tomkinson, J. Tepper, M. Morton, A. Bowden, L. Stevens, P. Harris, et al., Inhaled vs subcutaneous effects of a dual IL-4/IL-13 antagonist in a monkey model of asthma, *Allergy* 65 (2010) 69–77.
- [90] E. Maggi, P. Parronchi, R. Manetti, C. Simonelli, M.P. Piccinini, F.S. Rugiu, et al., Reciprocal regulatory effects of IFN-gamma and IL-4 on the in vitro

- development of human Th1 and Th2 clones, *J. Immunol.* 148 (1992) 2142–2147.
- [91] N. van Panhuys, S.C. Tang, M. Prout, M. Camberis, D. Scarlett, J. Roberts, et al., In vivo studies fail to reveal a role for IL-4 or STAT6 signaling in Th2 lymphocyte differentiation, *Proc. Natl. Acad. Sci. USA* 105 (2008) 12423–12428.
 - [92] C.J. Oliphant, Y.Y. Hwang, J.A. Walker, M. Salimi, S.H. Wong, J.M. Brewer, et al., MHCII-mediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion, *Immunity* 41 (2014) 283–295.
 - [93] Y. Yanagihara, K. Ikizawa, K. Kajiwar, T. Koshio, Y. Basaki, K. Akiyama, Functional significance of IL-4 receptor on B cells in IL-4-induced human IgE production, *J. Allergy Clin. Immunol.* 96 (1995) 1145–1151.
 - [94] T. Defrance, P. Carayon, G. Billian, J.C. Guillemot, A. Minty, D. Caput, et al., Interleukin 13 is a B cell stimulating factor, *J. Exp. Med.* 179 (1994) 135–143.
 - [95] R. Pawankar, M. Okuda, H. Yssel, K. Okumura, C. Ra, Nasal mast cells in perennial allergic rhinitis exhibit increased expression of the Fc epsilonRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells, *J. Clin. Invest.* 99 (1997) 1492–1499.
 - [96] D. Kaur, F. Hollins, L. Woodman, W. Yang, P. Monk, R. May, et al., Mast cells express IL-13R alpha 1: IL-13 promotes human lung mast cell proliferation and Fc epsilonRI expression, *Allergy* 61 (2006) 1047–1053.
 - [97] O. Tliba, D. Deshpande, H. Chen, C. Van Besien, M. Kannan, R.A. Panettieri Jr., et al., IL-13 enhances agonist-evoked calcium signals and contractile responses in airway smooth muscle, *Br. J. Pharmacol.* 140 (2003) 1159–1162.
 - [98] D.A. Deshpande, S. Dogan, T.F. Walseth, S.M. Miller, Y. Amrani, R.A. Panettieri, et al., Modulation of calcium signaling by interleukin-13 in human airway smooth muscle: role of CD38/cyclic adenosine diphosphate ribose pathway, *Am. J. Respir. Cell Mol. Biol.* 31 (2004) 36–42.
 - [99] J.C. Laporte, P.E. Moore, S. Baraldo, M.H. Jouvin, T.L. Church, I.N. Schwartzman, et al., Direct effects of interleukin-13 on signaling pathways for physiological responses in cultured human airway smooth muscle cells, *Am. J. Respir. Crit. Care Med.* 164 (2001) 141–148.
 - [100] B. Schnyder, S. Lugli, N. Feng, H. Etter, R.A. Lutz, B. Ryffel, et al., Interleukin-4 (IL-4) and IL-13 bind to a shared heterodimeric complex on endothelial cells mediating vascular cell adhesion molecule-1 induction in the absence of the common gamma chain, *Blood* 87 (1996) 4286–4295.
 - [101] G.Q. Jia, J.A. Gonzalo, A. Hidalgo, D. Wagner, M. Cybulsky, J.C. Gutierrez-Ramos, Selective eosinophil transendothelial migration triggered by eotaxin via modulation of Mac-1/ICAM-1 and VLA-4/VCAM-1 interactions, *Int. Immunol.* 11 (1999) 1–10.
 - [102] M. Mochizuki, J. Bartels, A.I. Mallet, E. Christophers, J.M. Schroder, IL-4 induces eotaxin: a possible mechanism of selective eosinophil recruitment in helminth infection and atopy, *J. Immunol.* 160 (1998) 60–68.
 - [103] N. Terada, N. Hamano, T. Nomura, T. Numata, K. Hirai, T. Nakajima, et al., Interleukin-13 and tumour necrosis factor-alpha synergistically induce eotaxin production in human nasal fibroblasts, *Clin. Exp. Allergy* 30 (2000) 348–355.
 - [104] S.E. Wenzel, J.B. Trudeau, S. Barnes, X. Zhou, M. Cundall, J.Y. Westcott, et al., TGF-beta and IL-13 synergistically increase eotaxin-1 production in human airway fibroblasts, *J. Immunol.* 169 (2002) 4613–4619.
 - [105] Y. Obase, T. Shimoda, K. Mitsuta, N. Matsuo, H. Matsuse, S. Kohno, Correlation between airway hyperresponsiveness and airway inflammation in a young adult population: eosinophil, ECP, and cytokine levels in induced sputum, *Ann. Allergy, Asthma Immunol.: Off. Publ. Am. Coll. Allergy, Asthma, Immunol.* 86 (2001) 304–310.
 - [106] H.W. Chu, J.B. Trudeau, S. Balzar, S.E. Wenzel, Peripheral blood and airway tissue expression of transforming growth factor beta by neutrophils in asthmatic subjects and normal control subjects, *J. Allergy Clin. Immunol.* 106 (2000) 1115–1123.
 - [107] Y. Nakamura, M. Azuma, Y. Okano, T. Sano, T. Takahashi, Y. Ohmoto, et al., Upregulatory effects of interleukin-4 and interleukin-13 but not interleukin-10 on granulocyte/macrophage colony-stimulating factor production by human bronchial epithelial cells, *Am. J. Respir. Cell Mol. Biol.* 15 (1996) 680–687.
 - [108] J.L. Lordan, F. Bucchieri, A. Richter, A. Konstantinidis, J.W. Holloway, M. Thorner, et al., Cooperative effects of Th2 cytokines and allergen on normal and asthmatic bronchial epithelial cells, *J. Immunol.* 169 (2002) 407–414.
 - [109] F.H. Guo, K. Uetani, S.J. Haque, B.R. Williams, R.A. Dweik, F.B. Thunnissen, et al., Interferon gamma and interleukin 4 stimulate prolonged expression of inducible nitric oxide synthase in human airway epithelium through synthesis of soluble mediators, *J. Clin. Invest.* 100 (1997) 829–838.
 - [110] K. Chibana, J.B. Trudeau, A.T. Mustovich, H. Hu, J. Zhao, S. Balzar, et al., IL-13 induced increases in nitrite levels are primarily driven by increases in inducible nitric oxide synthase as compared with effects on arginases in human primary bronchial epithelial cells, *Clin. Exp. Allergy* 38 (2008) 936–946.
 - [111] S.J. Hirst, M.P. Hallsworth, Q. Peng, T.H. Lee, Selective induction of eotaxin release by interleukin-13 or interleukin-4 in human airway smooth muscle cells is synergistic with interleukin-1beta and is mediated by the interleukin-4 receptor alpha-chain, *Am. J. Respir. Crit. Care Med.* 165 (2002) 1161–1171.
 - [112] P.E. Moore, T.L. Church, D.D. Chism, R.A. Panettieri Jr., S.A. Shore, IL-13 and IL-4 cause eotaxin release in human airway smooth muscle cells: a role for ERK, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 282 (2002) L847–L853.
 - [113] W. Luttmann, T. Matthiesen, H. Matthys, J.C. Virchow Jr., Synergistic effects of interleukin-4 or interleukin-13 and tumor necrosis factor-alpha on eosinophil activation in vitro, *Am. J. Respir. Cell Mol. Biol.* 20 (1999) 474–480.
 - [114] K. Dabbagh, K. Takeyama, H.M. Lee, I.F. Ueki, J.A. Lausier, J.A. Nadel, IL-4 induces mucin gene expression and goblet cell metaplasia in vitro and in vivo, *J. Immunol.* 162 (1999) 6233–6237.
 - [115] H. Danahay, H. Atherton, G. Jones, R.J. Bridges, C.T. Poll, Interleukin-13 induces a hypersecretory ion transport phenotype in human bronchial epithelial cells, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 282 (2002) L226–L236.
 - [116] S. Zuyderduyn, D.K. Ninaber, J.A. Schrumpf, M.A. van Sterkenburg, R.M. Verhoosel, F.A. Prins, et al., IL-4 and IL-13 exposure during mucociliary differentiation of bronchial epithelial cells increases antimicrobial activity and expression of antimicrobial peptides, *Respir. Res.* 12 (2011) 59.
 - [117] S. Thavagnanam, J.C. Parker, M.E. McBrien, G. Skibinski, L.G. Heaney, M.D. Shields, Effects of IL-13 on mucociliary differentiation of pediatric asthmatic bronchial epithelial cells, *Pediatr. Res.* 69 (2011) 95–100.
 - [118] H.C. Atherton, G. Jones, H. Danahay, IL-13-induced changes in the goblet cell density of human bronchial epithelial cell cultures: MAP kinase and phosphatidylinositol 3-kinase regulation, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 285 (2003) L730–L739.
 - [119] V. Batra, A.I. Musani, A.T. Hastie, S. Khurana, K.A. Carpenter, J.G. Zangrilli, et al., Bronchoalveolar lavage fluid concentrations of transforming growth factor (TGF)-beta1, TGF-beta2, interleukin (IL)-4 and IL-13 after segmental allergen challenge and their effects on alpha-smooth muscle actin and collagen III synthesis by primary human lung fibroblasts, *Clin. Exp. Allergy* 34 (2004) 437–444.
 - [120] R. Firszt, D. Francisco, T.D. Church, J.M. Thomas, J.L. Ingram, M. Kraft, Interleukin-13 induces collagen type-1 expression through matrix metalloproteinase-2 and transforming growth factor-beta1 in airway fibroblasts in asthma, *Eur. Respir. J.* 43 (2014) 464–473.
 - [121] J.L. Ingram, M.J. Huggins, T.D. Church, Y. Li, D.C. Francisco, S. Degan, et al., Airway fibroblasts in asthma manifest an invasive phenotype, *Am. J. Respir. Crit. Care Med.* 183 (2011) 1625–1632.
 - [122] A. Richter, S.M. Puddicombe, J.L. Lordan, F. Bucchieri, S.J. Wilson, R. Djukanovic, et al., The contribution of interleukin (IL)-4 and IL-13 to the epithelial-mesenchymal trophic unit in asthma, *Am. J. Respir. Cell Mol. Biol.* 25 (2001) 385–391.
 - [123] M. Kraft, C. Lewis, D. Pham, H.W. Chu, IL-4, IL-13, and dexamethasone augment fibroblast proliferation in asthma, *J. Allergy Clin. Immunol.* 107 (2001) 602–606.
 - [124] J.P. Finnerty, C. Lee, S. Wilson, J. Madden, R. Djukanovic, S.T. Holgate, Effects of theophylline on inflammatory cells and cytokines in asthmatic subjects: a placebo-controlled parallel group study, *Eur. Respir. J.* 9 (1996) 1672–1677.
 - [125] S. Pathmanathan, M.T. Krishna, A. Blomberg, R. Helleday, F.J. Kelly, T. Sandstrom, et al., Repeated daily exposure to 2 ppm nitrogen dioxide upregulates the expression of IL-5, IL-10, IL-13, and ICAM-1 in the bronchial epithelium of healthy human airways, *Occup. Environ. Med.* 60 (2003) 892–896.
 - [126] E. Mamesier, A. Nieves, D. Vervloet, A. Magnan, Diesel exhaust particles enhance T-cell activation in severe asthmatics, *Allergy* 61 (2006) 581–588.
 - [127] S.L. Johnston, P.K. Pattemore, G. Sanderson, S. Smith, F. Lampe, L. Josephs, et al., Community study of role of viral infections in exacerbations of asthma in 9–11 year old children, *Br. Med. J. (Clinical Research ed.)* 310 (1995) 1225–1229.
 - [128] T.V. Grissell, H. Powell, D.R. Shafren, M.J. Boyle, M.J. Hensley, P.D. Jones, et al., Interleukin-10 gene expression in acute virus-induced asthma, *Am. J. Respir. Crit. Care Med.* 172 (2005) 433–439.
 - [129] K. Tsuji, S. Yamamoto, W. Ou, N. Nishi, I. Kobayashi, M. Zaitzu, et al., DsRNA enhances eotaxin-3 production through interleukin-4 receptor upregulation in airway epithelial cells, *Eur. Respir. J.* 26 (2005) 795–803.
 - [130] M.E. Lachowicz-Scroggins, H.A. Boushey, W.E. Finkbeiner, J.H. Widdicombe, Interleukin-13-induced mucous metaplasia increases susceptibility of human airway epithelium to rhinovirus infection, *Am. J. Respir. Cell Mol. Biol.* 43 (2010) 652–661.
 - [131] N.G. Papadopoulos, L.A. Stanciu, A. Papi, S.T. Holgate, S.L. Johnston, A defective type 1 response to rhinovirus in atopic asthma, *Thorax* 57 (2002) 328–332.
 - [132] L.A. Stanciu, K. Roberts, N.G. Papadopoulos, S.H. Cho, S.T. Holgate, A.J. Coyle, et al., IL-4 increases type 2, but not type 1, cytokine production in CD8+ T cells from mild atopic asthmatics, *Respir. Res.* 6 (2005) 67.
 - [133] D.J. Jackson, H. Makrinioti, B.M. Rana, B.W. Shamji, M.B. Trujillo-Torralbo, J. Footitt, et al., IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo, *Am. J. Respir. Crit. Care Med.* 190 (2014) 1373–1382.
 - [134] L.C. Osborne, L.A. Monticelli, T.J. Nice, T.E. Sutherland, M.C. Siracusa, M.R. Hepworth, et al., Coinfection. Virus-helminth coinfection reveals a microbiota-independent mechanism of immunomodulation, *Science (New York, NY)* 345 (2014) 578–582.
 - [135] P. Parronchi, M. De Carli, R. Manetti, C. Simonelli, M.P. Piccinini, D. Macchia, et al., Aberrant interleukin (IL)-4 and IL-5 production in vitro by CD4+ helper T cells from atopic subjects, *Eur. J. Immunol.* 22 (1992) 1615–1620.
 - [136] H. Xie, R.J. Seward, B.T. Huber, Cytokine rescue from glucocorticoid induced apoptosis in T cells is mediated through inhibition of IkappaBalpha, *Mol. Immunol.* 34 (1997) 987–994.

- [137] D.Y. Leung, Q. Hamid, A. Vottero, S.J. Szefer, W. Surs, E. Minshall, et al., Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor beta, *J. Exp. Med.* 186 (1997) 1567–1574.
- [138] J.D. Spahn, S.J. Szefer, W. Surs, D.E. Doherty, S.R. Nimmagadda, D.Y. Leung, A novel action of IL-13: induction of diminished monocyte glucocorticoid receptor-binding affinity, *J. Immunol.* 157 (1996) 2654–2659.
- [139] P. Bradding, I.H. Feather, P.H. Howarth, R. Mueller, J.A. Roberts, K. Britten, et al., Interleukin 4 is localized to and released by human mast cells, *J. Exp. Med.* 176 (1992) 1381–1386.
- [140] H. Kobayashi, Y. Okayama, T. Ishizuka, R. Pawankar, C. Ra, M. Mori, Production of IL-13 by human lung mast cells in response to Fcεpsilon receptor cross-linkage, *Clin. Exp. Allergy* 28 (1998) 1219–1227.
- [141] C.E. Brightling, P. Bradding, F.A. Symon, S.T. Holgate, A.J. Wardlaw, I.D. Pavord, Mast-cell infiltration of airway smooth muscle in asthma, *N. Engl. J. Med.* 346 (2002) 1699–1705.
- [142] R.H. Dougherty, S.S. Sidhu, K. Raman, M. Solon, O.D. Solberg, G.H. Caughey, et al., Accumulation of intraepithelial mast cells with a unique protease phenotype in T(H)2-high asthma, *J. Allergy Clin. Immunol.* 125 (2010). 1046–1053 e8.
- [143] J.V. Fahy, Goblet cell and mucin gene abnormalities in asthma, *Chest* 122 (2003) 320S–326S.
- [144] E.F. Juniper, K. Svensson, A.C. Mork, E. Stahl, Measurement properties and interpretation of three shortened versions of the asthma control questionnaire, *Respir. Med.* 99 (2005) 553–558.
- [145] H.K. Reddel, D.R. Taylor, E.D. Bateman, L.P. Boulet, H.A. Boushey, W.W. Busse, et al., An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice, *Am. J. Respir. Crit. Care Med.* 180 (2009) 59–99.
- [146] N.C. Santanello, J. Zhang, B. Seidenberg, T.F. Reiss, B.L. Barber, What are minimal important changes for asthma measures in a clinical trial?, *Eur. Respir. J.* 14 (1999) 23–27.
- [147] NICE, (National Institute for Health and Care Excellence), Omalizumab for Severe Persistent Allergic Asthma [TA133], 2007.
- [148] L.C. Borish, H.S. Nelson, M.J. Lanz, L. Claussen, J.B. Whitmore, J.M. Agosti, et al., Interleukin-4 receptor in moderate atopic asthma. A phase I/II randomized, placebo-controlled trial, *Am. J. Respir. Crit. Care Med.* 160 (1999) 1816–1823.
- [149] L.C. Borish, H.S. Nelson, J. Corren, G. Bensch, W.W. Busse, J.B. Whitmore, et al., Efficacy of soluble IL-4 receptor for the treatment of adults with asthma, *J. Allergy Clin. Immunol.* 107 (2001) 963–970.
- [150] J. Tocker, The Role of IL-4R in Asthma: Preclinical and Clinical Experiences. Asthma Therapeutics, London, 2002.
- [151] T. Jung, K. Wagner, C. Neumann, C.H. Heusser, Enhancement of human IL-4 activity by soluble IL-4 receptors in vitro, *Eur. J. Immunol.* 29 (1999) 864–871.
- [152] T.K. Hart, M.N. Blackburn, M. Brigham-Burke, K. Dede, N. Al-Mahdi, P. Zia-Amirhosseini, et al., Preclinical efficacy and safety of pascolizumab (SB 240683): a humanized anti-interleukin-4 antibody with therapeutic potential in asthma, *Clin. Exp. Immunol.* 130 (2002) 93–100.
- [153] A.B. Madhankumar, A. Mintz, W. Debinski, Alanine-scanning mutagenesis of alpha-helix D segment of interleukin-13 reveals new functionally important residues of the cytokine, *J. Biol. Chem.* 277 (2002) 43194–43205.
- [154] P.J. Lupardus, M.E. Birnbaum, K.C. Garcia, Molecular basis for shared cytokine recognition revealed in the structure of an unusually high affinity complex between IL-13 and IL-13Rα2, *Structure (London, England: 1993)* 18 (2010) 332–342.
- [155] M. Ultsch, J. Bevers, G. Nakamura, R. Vandlen, R.F. Kelley, L.C. Wu, et al., Structural basis of signaling blockade by anti-IL-13 antibody Lebrikizumab, *J. Mol. Biol.* 425 (2013) 1330–1339.
- [156] G.M. Gauvreau, L.P. Boulet, D.W. Cockcroft, J.M. Fitzgerald, C. Carlsten, B.E. Davis, et al., Effects of interleukin-13 blockade on allergen-induced airway responses in mild atopic asthma, *Am. J. Respir. Crit. Care Med.* 183 (2011) 1007–1014.
- [157] W. Reinisch, J. Panes, S. Khurana, G. Toth, F. Hua, G.M. Comer, et al., Anrukizumab, an anti-interleukin 13 monoclonal antibody, in active UC: efficacy and safety from a phase IIa randomised multicentre study, *Gut* 64 (2015) 894–900.
- [158] M.T. Kasaian, X.Y. Tan, M. Jin, L. Fitz, K. Marquette, N. Wood, et al., Interleukin-13 neutralization by two distinct receptor blocking mechanisms reduces immunoglobulin E responses and lung inflammation in cynomolgus monkeys, *J. Pharmacol. Exp. Ther.* 325 (2008) 882–892.
- [159] J. Corren, R.F. Lemanske, N.A. Hanania, P.E. Korenblat, M.V. Parsey, J.R. Arron, et al., Lebrikizumab treatment in adults with asthma, *N. Engl. J. Med.* 365 (2011) 1088–1098.
- [160] H. Scheerens, J.R. Arron, Y. Zheng, W.S. Putnam, R.W. Erickson, D.F. Choy, et al., The effects of lebrikizumab in patients with mild asthma following whole lung allergen challenge, *Clin. Exp. Allergy* 44 (2014) 38–46.
- [161] M. Noonan, P. Korenblat, S. Mosesova, H. Scheerens, J.R. Arron, Y. Zheng, et al., Dose-ranging study of lebrikizumab in asthmatic patients not receiving inhaled steroids, *J. Allergy Clin. Immunol.* 132 (2013). 567–574 e12.
- [162] N. Yuyama, D.E. Davies, M. Akaiwa, Y. Matsui, Y. Hamasaki, Y. Suminami, et al., Analysis of novel disease-related genes in bronchial asthma, *Cytokine* 19 (2002) 287–296.
- [163] P.G. Woodruff, H.A. Boushey, G.M. Dolganov, C.S. Barker, Y.H. Yang, S. Donnelly, et al., Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids, *Proc. Natl. Acad. Sci. USA* 104 (2007) 15858–15863.
- [164] G. Takayama, K. Arima, T. Kanaji, S. Toda, H. Tanaka, S. Shoji, et al., Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals, *J. Allergy Clin. Immunol.* 118 (2006) 98–104.
- [165] T. Shoda, K. Futamura, F. Kobayashi, H. Saito, K. Matsumoto, A. Matsuda, Cell type-dependent effects of corticosteroid on periostin production by primary human tissue cells, *Allergy* 68 (2013) 1467–1470.
- [166] N.A. Hanania, M.J. Noonan, J. Corren, P. Korenblat, Y. Zheng, W. Putnam, et al., Efficacy and safety of lebrikizumab in severe uncontrolled asthma: results from the lute and verse phase II randomized, double-blind, placebo-controlled trials, *J. Allergy Clin. Immunol.* 133 (2014) AB402.
- [167] P.D. Monk, L. Jeremutis, R.R. Minter, C.P. Shorrock, Human Antibody Molecules for IL-13, 2005/007699 A2, 2005.
- [168] E. Piper, C. Brightling, R. Niven, C. Oh, R. Faggioni, K. Poon, et al., A phase II placebo-controlled study of tralokinumab in moderate-to-severe asthma, *Eur. Respir. J.* 41 (2013) 330–338.
- [169] C.E. Brightling, D. She, K. Ranade, E. Piper, Efficacy and safety of tralokinumab, an anti-IL-13 monoclonal antibody, in a phase 2b study of uncontrolled severe asthma, *Am. J. Respir. Crit. Care Med.* 189 (2014) A6670.
- [170] P. Hodsman, C. Ashman, A. Cahn, E. De Boever, N. Locantore, A. Serone, et al., A phase I, randomized, placebo-controlled, dose-escalation study of an anti-IL-13 monoclonal antibody in healthy subjects and mild asthmatics, *Br. J. Clin. Pharmacol.* 75 (2013) 118–128.
- [171] E.H. De Boever, C. Ashman, A.P. Cahn, N.W. Locantore, P. Overend, I.J. Pouliquen, et al., Efficacy and safety of an anti-IL-13 mAb in patients with severe asthma: a randomized trial, *J. Allergy Clin. Immunol.* 133 (2014) 989–996.
- [172] Novartis, A Multi-Center, Randomized, Double Blind, Placebo-controlled, 'add-on' study to investigate the efficacy and safety of 24 weeks intravenous treatment with QAX576 in patients (≥ 18 –75 years) with persistent asthma not adequately controlled with ICS/LABA, Novartis Clinical Trials Results Database, 2013.
- [173] B. van Hartingsveldt, I.P. Nnane, E. Bouman-Thio, M.J. Loza, A. Piantone, H.M. Davis, et al., Safety, tolerability and pharmacokinetics of a human anti-interleukin-13 monoclonal antibody (CNTO 5825) in an ascending single-dose first-in-human study, *Br. J. Clin. Pharmacol.* 75 (2013) 1289–1298.
- [174] S. Wenzel, D. Wilbraham, R. Fuller, E.B. Getz, M. Longphre, Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies, *Lancet (London, England)* 370 (2007) 1422–1431.
- [175] S.E. Wenzel, P.W. Ind, B.A. Otulana, E.R. Bleecker, P. Kuna, Y.P. Yen, Inhaled pitrakinra, an IL-4/IL-13 antagonist, reduced exacerbations in patients with eosinophilic asthma, *Eur. Respir. J.* 36 (2010) P3980.
- [176] R.E. Slager, B.A. Otulana, G.A. Hawkins, Y.P. Yen, S.P. Peters, S.E. Wenzel, et al., IL-4 receptor polymorphisms predict reduction in asthma exacerbations during response to an anti-IL-4 receptor alpha antagonist, *J. Allergy Clin. Immunol.* 130 (2012). 516–522 e4.
- [177] J. Corren, W. Busse, E.O. Meltzer, L. Mansfield, G. Bensch, J. Fahrenholz, et al., A randomized, controlled, phase 2 study of AMG 317, an IL-4Rα antagonist, in patients with asthma, *Am. J. Respir. Crit. Care Med.* 181 (2010) 788–796.
- [178] A. Radin, H. Ren, P. Papino-Wood, U. Chaudhry, J.D. Hamilton, First-in-human study of REGN668/SAR231893 (IL-4Rα mAb): safety, tolerability and biomarker results of a randomized, double-blind, placebo-controlled, single ascending dose study in healthy volunteers, *J. Allergy Clin. Immunol.* 131 (2013) AB158.
- [179] S. Wenzel, L. Ford, D. Pearlman, S. Spector, L. Sher, F. Skobieranda, et al., Dupilumab in persistent asthma with elevated eosinophil levels, *N. Engl. J. Med.* 368 (2013) 2455–2466.
- [180] Regeneron, News Release: Regeneron and Sanofi Announce Positive Results from Phase 2b Study of Dupilumab in Patients with Moderate-to-Severe Asthma, 11 November 2014.
- [181] M.T. Kasaian, K. Marquette, S. Fish, C. DeClercq, R. Agostinelli, T.A. Cook, et al., An IL-4/IL-13 dual antagonist reduces lung inflammation, airway hyperresponsiveness, and IgE production in mice, *Am. J. Respir. Cell Mol. Biol.* 49 (2013) 37–46.
- [182] C. Spiess, J. Bevers 3rd, J. Jackman, N. Chiang, G. Nakamura, M. Dillon, et al., Development of a human IgG4 bispecific antibody for dual targeting of interleukin-4 (IL-4) and interleukin-13 (IL-13) cytokines, *J. Biol. Chem.* 288 (2013) 26583–26593.
- [183] J. Hacha, K. Tomlinson, L. Maertens, G. Paulissen, N. Rocks, J.M. Foidart, et al., Nebulized anti-IL-13 monoclonal antibody Fab' fragment reduces allergen-induced asthma, *Am. J. Respir. Cell Mol. Biol.* 47 (2012) 709–717.
- [184] D. Lightwood, V. O'Dowd, B. Carrington, V. Veverka, M.D. Carr, M. Tservistas, et al., The discovery, engineering and characterisation of a highly potent anti-human IL-13 fab fragment designed for administration by inhalation, *J. Mol. Biol.* 425 (2013) 577–593.
- [185] M. Boguniewicz, D.Y. Leung, Atopic dermatitis: a disease of altered skin barrier and immune dysregulation, *Immunol. Rev.* 242 (2011) 233–246.
- [186] F.Y. Liaw, C.F. Huang, J.T. Hsueh, C.P. Chiang, Eczema herpeticum: a medical emergency, *Can. Family Phys. Med. Famille Can.* 58 (2012) 1358–1361.
- [187] J. Lubbe, S.F. Friedlander, B. Cribier, M.A. Morren, A. Garcia-Diez, C. Gelmetti, et al., Safety, efficacy, and dosage of 1% pimecrolimus cream for the treatment of atopic dermatitis in daily practice, *Am. J. Clin. Dermatol.* 7 (2006) 121–131.

- [188] H.C. Frankel, A.A. Qureshi, Comparative effectiveness of topical calcineurin inhibitors in adult patients with atopic dermatitis, *Am. J. Clin. Dermatol.* 13 (2012) 113–123.
- [189] Q. Hamid, T. Naseer, E.M. Minshall, Y.L. Song, M. Boguniewicz, D.Y. Leung, In vivo expression of IL-12 and IL-13 in atopic dermatitis, *J. Allergy Clin. Immunol.* 98 (1996) 225–231.
- [190] M.M. Neis, B. Peters, A. Dreuw, J. Wenzel, T. Bieber, C. Mauch, et al., Enhanced expression levels of IL-31 correlate with IL-4 and IL-13 in atopic and allergic contact dermatitis, *J. Allergy Clin. Immunol.* 118 (2006) 930–937.
- [191] T. Tazawa, H. Sugiura, Y. Sugiura, M. Uehara, Relative importance of IL-4 and IL-13 in lesional skin of atopic dermatitis, *Arch. Dermatol. Res.* 295 (2004) 459–464.
- [192] W. Obara, Y. Kawa, C. Ra, K. Nishioka, Y. Soma, M. Mizoguchi, T cells and mast cells as a major source of interleukin-13 in atopic dermatitis, *Dermatology (Basel, Switzerland)* 205 (2002) 11–17.
- [193] C.A. Akdis, M. Akdis, D. Simon, B. Dibbert, M. Weber, S. Gratzl, et al., T cells and T cell-derived cytokines as pathogenic factors in the nonallergic form of atopic dermatitis, *J. Invest. Dermatol.* 113 (1999) 628–634.
- [194] M. Suarez-Farinas, N. Dhingra, J. Gittler, A. Shemer, I. Cardinale, C. de Guzman Strong, et al., Intrinsic atopic dermatitis shows similar TH2 and higher TH17 immune activation compared with extrinsic atopic dermatitis, *J. Allergy Clin. Immunol.* 132 (2013) 361–370.
- [195] M. Akdis, C.A. Akdis, L. Weigl, R. Disch, K. Blaser, Skin-homing, CLA+ memory T cells are activated in atopic dermatitis and regulate IgE by an IL-13-dominated cytokine pattern: IgG4 counter-regulation by CLA-memory T cells, *J. Immunol.* 159 (1997) 4611–4619.
- [196] D. Hijnen, E.F. Knol, Y.Y. Gent, B. Giovannone, S.J. Beijin, T.S. Kupper, et al., CD8(+) T cells in the lesional skin of atopic dermatitis and psoriasis patients are an important source of IFN-gamma, IL-13, IL-17, and IL-22, *J. Invest. Dermatol.* 133 (2013) 973–979.
- [197] W.H. Wu, C.O. Park, S.H. Oh, H.J. Kim, Y.S. Kwon, B.G. Bae, et al., Thymic stromal lymphopoietin-activated invariant natural killer T cells trigger an innate allergic immune response in atopic dermatitis, *J. Allergy Clin. Immunol.* 126 (2010). 290–299, 9 e1–e4.
- [198] D. Simon, E. Kozlowski, H. Simon, Natural killer T cells expressing IFN-gamma and IL-4 in lesional skin of atopic eczema, *Allergy* 64 (2009) 1681–1684.
- [199] S. La Grutta, P. Richiusa, G. Pizzolanti, A. Mattina, G.B. Pajno, R. Citarrella, et al., CD4(+)IL-13(+) cells in peripheral blood well correlates with the severity of atopic dermatitis in children, *Allergy* 60 (2005) 391–395.
- [200] S.L. Seneviratne, L. Jones, A.S. Bailey, A.P. Black, G.S. Ogg, Severe atopic dermatitis is associated with a reduced frequency of IL-10 producing allergen-specific CD4+ T cells, *Clin. Exp. Dermatol.* 31 (2006) 689–694.
- [201] S. Weidinger, S.A. Willis-Owen, Y. Kamotani, H. Baurecht, N. Morar, L. Liang, et al., A genome-wide association study of atopic dermatitis identifies loci with overlapping effects on asthma and psoriasis, *Hum. Mol. Genet.* 22 (2013) 4841–4856.
- [202] L.S. Chan, N. Robinson, L. Xu, Expression of interleukin-4 in the epidermis of transgenic mice results in a pruritic inflammatory skin disease: an experimental animal model to study atopic dermatitis, *J. Invest. Dermatol.* 117 (2001) 977–983.
- [203] Y. Hatano, Y. Adachi, P.M. Elias, D. Crumrine, T. Sakai, R. Kurahashi, et al., The Th2 cytokine, interleukin-4, abrogates the cohesion of normal stratum corneum in mice: implications for pathogenesis of atopic dermatitis, *Exp. Dermatol.* 22 (2013) 30–35.
- [204] S.H. Cho, I. Strickland, A. Tomkinson, A.P. Fehrer, E.W. Gelfand, D.Y. Leung, Preferential binding of *Staphylococcus aureus* to skin sites of Th2-mediated inflammation in a murine model, *J. Invest. Dermatol.* 116 (2001) 658–663.
- [205] T. Zheng, M.H. Oh, S.Y. Oh, J.T. Schroeder, A.B. Glick, Z. Zhu, Transgenic expression of interleukin-13 in the skin induces a pruritic dermatitis and skin remodeling, *J. Invest. Dermatol.* 129 (2009) 742–751.
- [206] G.R. Lee, R.A. Flavell, Transgenic mice which overproduce Th2 cytokines develop spontaneous atopic dermatitis and asthma, *Int. Immunol.* 16 (2004) 1155–1160.
- [207] S.T. Kim, K.M. Lee, H.J. Park, S.E. Jin, W.S. Ahn, C.K. Kim, Topical delivery of interleukin-13 antisense oligonucleotides with cationic elastic liposome for the treatment of atopic dermatitis, *J. Gene Med.* 11 (2009) 26–37.
- [208] T. Morioka, K. Yamanaka, H. Mori, Y. Omoto, K. Tokime, M. Kakeda, et al., IL-4/IL-13 antagonist DNA vaccination successfully suppresses Th2 type chronic dermatitis, *Br. J. Dermatol.* 160 (2009) 1172–1179.
- [209] S. Sehra, Y. Yao, M.D. Howell, E.T. Nguyen, G.S. Kansas, D.Y. Leung, et al., IL-4 regulates skin homeostasis and the predisposition toward allergic skin inflammation, *J. Immunol.* 184 (2010) 3186–3190.
- [210] L. Liu, Z. Xu, R.C. Fuhlbrigge, V. Pena-Cruz, J. Lieberman, T.S. Kupper, Vaccinia virus induces strong immunoregulatory cytokine production in healthy human epidermal keratinocytes: a novel strategy for immune evasion, *J. Virol.* 79 (2005) 7363–7370.
- [211] I. Nomura, E. Goleva, M.D. Howell, Q.A. Hamid, P.Y. Ong, C.F. Hall, et al., Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes, *J. Immunol.* 171 (2003) 3262–3269.
- [212] M.D. Howell, H.R. Fairchild, B.E. Kim, L. Bin, M. Boguniewicz, J.S. Redzic, et al., Th2 cytokines act on S100A11 to downregulate keratinocyte differentiation, *J. Invest. Dermatol.* 128 (2008) 2248–2258.
- [213] K. Eyerich, D. Pennino, C. Scarponi, S. Foerster, F. Nasorri, H. Behrendt, et al., IL-17 in atopic eczema: linking allergen-specific adaptive and microbial-triggered innate immune response, *J. Allergy Clin. Immunol.* 123 (2009). 59–66 e4.
- [214] R. Glaser, U. Meyer-Hoffert, J. Harder, J. Cordes, M. Wittersheim, J. Kobiakov, et al., The antimicrobial protein psoriasin (S100A7) is upregulated in atopic dermatitis and after experimental skin barrier disruption, *J. Invest. Dermatol.* 129 (2009) 641–649.
- [215] M.D. Howell, R.L. Gallo, M. Boguniewicz, J.F. Jones, C. Wong, J.E. Streib, et al., Cytokine milieu of atopic dermatitis skin subverts the innate immune response to vaccinia virus, *Immunity* 24 (2006) 341–348.
- [216] S. Kagami, H. Saeki, M. Komine, T. Kakinuma, Y. Tsunemi, K. Nakamura, et al., Interleukin-4 and interleukin-13 enhance CCL26 production in a human keratinocyte cell line, HaCaT cells, *Clin. Exp. Immunol.* 141 (2005) 459–466.
- [217] B. Yu, T. Koga, K. Urabe, Y. Moroi, S. Maeda, Y. Yanagihara, et al., Differential regulation of thymus- and activation-regulated chemokine induced by IL-4, IL-13, TNF-alpha and IFN-gamma in human keratinocyte and fibroblast, *J. Dermatol. Sci.* 30 (2002) 29–36.
- [218] K. Fukuda, T. Nishida, A. Fukushima, Synergistic induction of eotaxin and VCAM-1 expression in human corneal fibroblasts by staphylococcal peptidoglycan and either IL-4 or IL-13, *Allergol. Int.: Off. J. Jpn. Soc. Allergol.* 60 (2011) 355–363.
- [219] R. Purwar, M. Kraus, T. Werfel, M. Wittmann, Modulation of keratinocyte-derived MMP-9 by IL-13: a possible role for the pathogenesis of epidermal inflammation, *J. Invest. Dermatol.* 128 (2008) 59–66.
- [220] R. Purwar, T. Werfel, M. Wittmann, IL-13-stimulated human keratinocytes preferentially attract CD4+CCR4+ T cells: possible role in atopic dermatitis, *J. Invest. Dermatol.* 126 (2006) 1043–1051.
- [221] S. Fujii-Maeda, K. Kajiura, K. Ikizawa, M. Shinazawa, B. Yu, T. Koga, et al., Reciprocal regulation of thymus and activation-regulated chemokine/macrophage-derived chemokine production by interleukin (IL)-4/IL-13 and interferon-gamma in HaCaT keratinocytes is mediated by alterations in E-cadherin distribution, *J. Invest. Dermatol.* 122 (2004) 20–28.
- [222] C. Moriya, M. Jinnin, K. Yamane, K. Maruo, F.C. Muchemwa, T. Igata, et al., Expression of matrix metalloproteinase-13 is controlled by IL-13 via PI3K/Akt3 and PKC-delta in normal human dermal fibroblasts, *J. Invest. Dermatol.* 131 (2011) 655–661.
- [223] M. Jinnin, I. Ihn, K. Yamane, K. Tamaki, Interleukin-13 stimulates the transcription of the human alpha2(I) collagen gene in human dermal fibroblasts, *J. Biol. Chem.* 279 (2004) 41783–41791.
- [224] M.D. Howell, B.E. Kim, P. Gao, A.V. Grant, M. Boguniewicz, A. DeBenedetto, et al., Cytokine modulation of atopic dermatitis filaggrin skin expression, *J. Allergy Clin. Immunol.* 124 (2009) R7–R12.
- [225] S. Eyerich, A.T. Onken, S. Weidinger, A. Franke, F. Nasorri, D. Pennino, et al., Mutual antagonism of T cells causing psoriasis and atopic eczema, *N. Engl. J. Med.* 365 (2011) 231–238.
- [226] K. Ghoreschi, P. Thomas, S. Breit, M. Dugas, R. Mailhammer, W. van Eden, et al., Interleukin-4 therapy of psoriasis induces Th2 responses and improves human autoimmune disease, *Nat. Med.* 9 (2003) 40–46.
- [227] J. Verhagen, M. Akdis, C. Traidl-Hoffmann, P. Schmid-Grendelmeier, D. Hijnen, E.F. Knol, et al., Absence of T-regulatory cell expression and function in atopic dermatitis skin, *J. Allergy Clin. Immunol.* 117 (2006) 176–183.
- [228] S. Khattri, A. Shemer, M. Rozenblit, N. Dhingra, T. Czarnowicki, R. Finney, et al., Cyclosporine in patients with atopic dermatitis modulates activated inflammatory pathways and reverses epidermal pathology, *J. Allergy Clin. Immunol.* 133 (2014) 1626–1634.
- [229] T. Gambichler, A. Kreuter, N.S. Tomi, N. Othlinghaus, P. Altmeyer, M. Skrygan, Gene expression of cytokines in atopic eczema before and after ultraviolet A phototherapy, *Br. J. Dermatol.* 158 (2008) 1117–1120.
- [230] D. Simon, E. Vassina, S. Yousefi, E. Kozlowski, L.R. Braathen, H.U. Simon, Reduced dermal infiltration of cytokine-expressing inflammatory cells in atopic dermatitis after short-term topical tacrolimus treatment, *J. Allergy Clin. Immunol.* 114 (2004) 887–895.
- [231] J. Schmitt, S. Langan, H.C. Williams, European Dermato-Epidemiology N. What are the best outcome measurements for atopic eczema? A systematic review, *J. Allergy Clin. Immunol.* 120 (2007) 1389–1398.
- [232] R.W. Groves, D. Wilbraham, R. Fuller, M. Longphre, Inhibition of IL-4 and IL-13 with an IL-4 mutein (aeroderm) protects against flares in atopic eczema, *J. Invest. Dermatol.* 127 (2007) S54.
- [233] L.A. Beck, D. Thaci, J.D. Hamilton, N.M. Graham, T. Bieber, R. Rocklin, et al., Dupilumab treatment in adults with moderate-to-severe atopic dermatitis, *N. Engl. J. Med.* 371 (2014) 130–139.
- [234] J.D. Hamilton, M. Suarez-Farinas, N. Dhingra, I. Cardinale, X. Li, A. Kostic, et al., Dupilumab improves the molecular signature in skin of patients with moderate-to-severe atopic dermatitis, *J. Allergy Clin. Immunol.* 134 (2014) 1293–1300.
- [235] L.M. Wheatley, A. Togias, Clinical practice. Allergic rhinitis, *N. Engl. J. Med.* 372 (2015) 456–463.
- [236] A.A. Cruz, T. Popov, R. Pawankar, I. Annesi-Maesano, W. Fokkens, J. Kemp, et al., Common characteristics of upper and lower airways in rhinitis and asthma: ARIA update, in collaboration with GA(2)LEN, *Allergy* 62 (Suppl. 84) (2007) 1–41.
- [237] M. Benson, I.L. Strannegard, G. Wennergren, O. Strannegard, Cytokines in nasal fluids from school children with seasonal allergic rhinitis, *Pediatr. Allergy Immunol.: Off. Publ. Eur. Soc. Pediatr. Allergy Immunol.* 8 (1997) 143–149.
- [238] O. Ghaffar, S. Laberge, M.R. Jacobson, O. Lowhagen, S. Rak, S.R. Durham, et al., IL-13 mRNA and immunoreactivity in allergen-induced rhinitis: comparison

- with IL-4 expression and modulation by topical glucocorticoid therapy, *Am. J. Respir. Cell Mol. Biol.* 17 (1997) 17–24.
- [239] D. Diaz-Sanchez, A. Tsien, A. Casillas, A.R. Dotson, A. Saxon, Enhanced nasal cytokine production in human beings after in vivo challenge with diesel exhaust particles, *J. Allergy Clin. Immunol.* 98 (1996) 114–123.
- [240] E.M. Erin, B.R. Leaker, A.S. Zachariasiewicz, L.A. Higgins, T.J. Williams, M.J. Boyce, et al., Single dose topical corticosteroid inhibits IL-5 and IL-13 in nasal lavage following grass pollen challenge, *Allergy* 60 (2005) 1524–1529.
- [241] H. Riechmann, A. Essig, T. Deuschle, A. Rau, B. Rothermel, M. Weschta, Nasal carriage of *Staphylococcus aureus* in house dust mite allergic patients and healthy controls, *Allergy* 60 (2005) 1418–1423.
- [242] T. Van Zele, S. Claeys, P. Gevaert, G. Van Maele, G. Holtappels, P. Van Cauwenberge, et al., Differentiation of chronic sinus diseases by measurement of inflammatory mediators, *Allergy* 61 (2006) 1280–1289.
- [243] J.L. Shaw, S. Fakhri, M.J. Citardi, P.C. Porter, D.B. Corry, F. Kheradmand, et al., IL-33-responsive innate lymphoid cells are an important source of IL-13 in chronic rhinosinusitis with nasal polyps, *Am. J. Respir. Crit. Care Med.* 188 (2013) 432–439.
- [244] M. Miyata, Y. Nakamura, N. Shimokawa, Y. Ohnuma, R. Katoh, S. Matsuoka, et al., Thymic stromal lymphopoietin is a critical mediator of IL-13-driven allergic inflammation, *Eur. J. Immunol.* 39 (2009) 3078–3083.
- [245] S. Miyahara, N. Miyahara, S. Matsubara, K. Takeda, T. Koya, E.W. Gelfand, IL-13 is essential to the late-phase response in allergic rhinitis, *J. Allergy Clin. Immunol.* 118 (2006) 1110–1116.
- [246] K. Hosoya, T. Satoh, Y. Yamamoto, K. Saeki, K. Igawa, M. Okano, et al., Gene silencing of STAT6 with siRNA ameliorates contact hypersensitivity and allergic rhinitis, *Allergy* 66 (2011) 124–131.
- [247] M. Nonaka, R. Pawankar, A. Fukumoto, N. Ogihara, A. Sakanushi, T. Yagi, Induction of eotaxin production by interleukin-4, interleukin-13 and lipopolysaccharide by nasal fibroblasts, *Clin. Exp. Allergy* 34 (2004) 804–811.
- [248] G.C. Nicholson, H.H. Kariyawasam, A.J. Tan, J.M. Hohlfeld, D. Quinn, C. Walker, et al., The effects of an anti-IL-13 mAb on cytokine levels and nasal symptoms following nasal allergen challenge, *J. Allergy Clin. Immunol.* 128 (2011) 800–807 e9.
- [249] Regeneron, News Release: Regeneron and Sanofi Announce Positive Phase 2 Top-line Dupilumab Results in Patients with Chronic Sinusitis with Nasal Polyps, 2014.
- [250] M.G. Cosio, M. Saetta, A. Agusti, Immunologic aspects of chronic obstructive pulmonary disease, *N. Engl. J. Med.* 360 (2009) 2445–2454.
- [251] GOLD, Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease, 2015.
- [252] J. Zhu, S. Majumdar, Y. Qiu, T. Ansari, A. Oliva, J.C. Kips, et al., Interleukin-4 and interleukin-5 gene expression and inflammation in the mucus-secreting glands and subepithelial tissue of smokers with chronic bronchitis. Lack of relationship with CD8(+) cells, *Am. J. Respir. Crit. Care Med.* 164 (2001) 2220–2228.
- [253] S. Grumelli, D.B. Corry, L.Z. Song, L. Green, J. Huh, et al., An immune basis for lung parenchymal destruction in chronic obstructive pulmonary disease and emphysema, *PLoS Med.* 1 (2004) e8.
- [254] B. Barcelo, J. Pons, A. Fuster, J. Saulea, A. Noguera, J.M. Ferrer, et al., Intracellular cytokine profile of T lymphocytes in patients with chronic obstructive pulmonary disease, *Clin. Exp. Immunol.* 145 (2006) 474–479.
- [255] S. Saha, V. Mistry, R. Siva, D. Parker, R. May, P. Bradding, et al., Induced sputum and bronchial mucosal expression of interleukin-13 is not increased in chronic obstructive pulmonary disease, *Allergy* 63 (2008) 1239–1243.
- [256] O. Eickmeier, M. Huebner, E. Herrmann, U. Zissler, M. Rosewich, P.C. Baer, et al., Sputum biomarker profiles in cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD) and association between pulmonary function, *Cytokine* 50 (2010) 152–157.
- [257] Y.G. Alevy, A.C. Patel, A.G. Romero, D.A. Patel, J. Tucker, W.T. Roswit, et al., IL-13-induced airway mucus production is attenuated by MAPK13 inhibition, *J. Clin. Invest.* 122 (2012) 4555–4568.
- [258] J.S. Lee, M.R. Rosengart, V. Kondragunta, Y. Zhang, J. McMurray, R.A. Branch, et al., Inverse association of plasma IL-13 and inflammatory chemokines with lung function impairment in stable COPD: a cross-sectional cohort study, *Respir. Res.* 8 (2007) 64.
- [259] M. Singh, S.H. Lee, P. Porter, C. Xu, A. Ohno, R.L. Atmar, et al., Human rhinovirus proteinase 2A induces TH1 and TH2 immunity in patients with chronic obstructive pulmonary disease, *J. Allergy Clin. Immunol.* 125 (2010) 1369–1378 e2.
- [260] D. Makris, S. Lazarou, M. Alexandrakakis, T.V. Kourelis, N. Tzanakis, D. Kyriakou, et al., Tc2 response at the onset of COPD exacerbations, *Chest* 134 (2008) 483–488.
- [261] M. Tsoumakidou, N. Tzanakis, G. Chrysafakis, D. Kyriakou, N.M. Siafakas, Changes in sputum T-lymphocyte subpopulations at the onset of severe exacerbations of chronic obstructive pulmonary disease, *Respir. Med.* 99 (2005) 572–579.
- [262] P.R. Cooper, C.T. Poll, P.J. Barnes, R.G. Sturton, Involvement of IL-13 in tobacco smoke-induced changes in the structure and function of rat intrapulmonary airways, *Am. J. Respir. Cell Mol. Biol.* 43 (2010) 220–226.
- [263] T. Zheng, Z. Zhu, Z. Wang, R.J. Homer, B. Ma, R.J. Riese Jr., et al., Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema, *J. Clin. Invest.* 106 (2000) 1081–1093.
- [264] U. Sajjan, S. Ganesan, A.T. Comstock, J. Shim, Q. Wang, D.R. Nagarkar, et al., Elastase- and LPS-exposed mice display altered responses to rhinovirus infection, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 297 (2009) L931–L944.
- [265] E.Y. Kim, J.T. Battaile, A.C. Patel, Y. You, E. Agapov, M.H. Grayson, et al., Persistent activation of an innate immune response translates respiratory viral infection into chronic lung disease, *Nat. Med.* 14 (2008) 633–640.
- [266] R. Siva, R.H. Green, C.E. Brightling, M. Shelley, B. Hargadon, S. McKenna, et al., Eosinophilic airway inflammation and exacerbations of COPD: a randomised controlled trial, *Eur. Respir. J.* 29 (2007) 906–913.
- [267] C.E. Brightling, E.R. Bleeker, R.A. Panettieri Jr., M. Bafadhel, D. She, C.K. Ward, et al., Benralizumab for chronic obstructive pulmonary disease and sputum eosinophilia: a randomised, double-blind, placebo-controlled, phase 2a study, *Lancet Respir. Med.* 2 (2014) 891–901.
- [268] L.A. Spencer, C.T. Szela, S.A. Perez, C.L. Kirchhoffer, J.S. Neves, A.L. Radke, et al., Human eosinophils constitutively express multiple Th1, Th2, and immunoregulatory cytokines that are secreted rapidly and differentially, *J. Leukoc. Biol.* 85 (2009) 117–123.
- [269] S.A. Christenson, K. Steiling, M. van den Berge, K. Hijazi, P.S. Hiemstra, D.S. Postma, et al., Asthma-COPD overlap. Clinical relevance of genomic signatures of type 2 inflammation in chronic obstructive pulmonary disease, *Am. J. Respir. Crit. Care Med.* 191 (2015) 758–766.
- [270] M.A. Ghebre, M. Bafadhel, D. Desai, S.E. Cohen, P. Newbold, L. Rapley, et al., Biological clustering supports both “Dutch” and “British” hypotheses of asthma and chronic obstructive pulmonary disease, *J. Allergy Clin. Immunol.* 135 (2015) 63–72.
- [271] GOLD, GINA, Asthma, COPD and Asthma-COPD Overlap Syndrome (ACOS), 2014.
- [272] W.K. Ip, C.K. Wong, C.W. Lam, Interleukin (IL)-4 and IL-13 up-regulate monocyte chemoattractant protein-1 expression in human bronchial epithelial cells: involvement of p38 mitogen-activated protein kinase, extracellular signal-regulated kinase 1/2 and Janus kinase-2 but not c-Jun NH2-terminal kinase 1/2 signalling pathways, *Clin. Exp. Immunol.* 145 (2006) 162–172.
- [273] M. Kobayashi, H. Kobayashi, R.B. Pollard, F. Suzuki, A pathogenic role of Th2 cells and their cytokine products on the pulmonary metastasis of murine B16 melanoma, *J. Immunol.* 160 (1998) 5869–5873.
- [274] M. Terabe, S. Matsui, N. Noben-Trauth, H. Chen, C. Watson, D.D. Donaldson, et al., NKT cell-mediated repression of tumor immunosurveillance by IL-13 and the IL-4R-STAT6 pathway, *Nat. Immunol.* 1 (2000) 515–520.
- [275] S. Ostrand-Rosenberg, M.J. Grusby, V.K. Clements, Cutting edge: STAT6-deficient mice have enhanced tumor immunity to primary and metastatic mammary carcinoma, *J. Immunol.* 165 (2000) 6015–6019.
- [276] L. De Monte, M. Reni, E. Tassi, D. Clavenna, I. Papa, H. Recalde, et al., Intratumor T helper type 2 cell infiltrate correlates with cancer-associated fibroblast thymic stromal lymphopoietin production and reduced survival in pancreatic cancer, *J. Exp. Med.* 208 (2011) 469–478.
- [277] B.F. Skinnider, T.W. Mak, The role of cytokines in classical Hodgkin lymphoma, *Blood* 99 (2002) 4283–4297.
- [278] B.F. Skinnider, A.J. Elia, R.D. Gascoyne, L.H. Trumper, F. von Bonin, U. Kapp, et al., Interleukin 13 and interleukin 13 receptor are frequently expressed by Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma, *Blood* 97 (2001) 250–255.
- [279] Y. Trieu, X.Y. Wen, B.F. Skinnider, M.R. Bray, Z. Li, J.O. Claudio, et al., Soluble interleukin-13Ralpha2 decoy receptor inhibits Hodgkin's lymphoma growth in vitro and in vivo, *Cancer Res.* 64 (2004) 3271–3275.
- [280] D. Buglio, G.V. Georgakis, S. Hanabuchi, K. Arima, N.M. Khaskhely, Y.J. Liu, et al., Vorinostat inhibits STAT6-mediated TH2 cytokine and TARC production and induces cell death in Hodgkin lymphoma cell lines, *Blood* 112 (2008) 1424–1433.
- [281] U. Holtick, M. Vockerodt, D. Pinkert, N. Schoof, B. Sturzenhofecker, N. Kussebi, et al., STAT3 is essential for Hodgkin lymphoma cell proliferation and is a target of tyrosine kinase AG17 which confers sensitization for apoptosis, *Leukemia* 19 (2005) 936–944.
- [282] B.F. Skinnider, U. Kapp, T.W. Mak, The role of interleukin 13 in classical Hodgkin lymphoma, *Leukemia Lymphoma* 43 (2002) 1203–1210.
- [283] T. Diaz, A. Navarro, G. Ferrer, B. Gel, A. Gaya, R. Artells, et al., Lestaurtinib inhibition of the Jak/STAT signaling pathway in Hodgkin lymphoma inhibits proliferation and induces apoptosis, *PLoS One* 6 (2011) e18856.
- [284] A. Natoli, R. Lupertz, C. Merz, W.W. Muller, R. Kohler, P.H. Krammer, et al., Targeting the IL-4/IL-13 signaling pathway sensitizes Hodgkin lymphoma cells to chemotherapeutic drugs, *Int. J. Cancer* 133 (2013) 1945–1954.
- [285] O. Prokopcuk, Y. Liu, D. Henne-Bruns, M. Kormann, Interleukin-4 enhances proliferation of human pancreatic cancer cells: evidence for autocrine and paracrine actions, *Br. J. Cancer* 92 (2005) 921–928.
- [286] T. Fujisawa, B.H. Joshi, R.K. Puri, IL-13 regulates cancer invasion and metastasis through IL-13Ralpha2 via ERK/AP-1 pathway in mouse model of human ovarian cancer, *Int. J. Cancer* 131 (2012) 344–356.
- [287] K. Kawakami, J. Taguchi, T. Murata, R.K. Puri, The interleukin-13 receptor alpha2 chain: an essential component for binding and internalization but not for interleukin-13-induced signal transduction through the STAT6 pathway, *Blood* 97 (2001) 2673–2679.
- [288] R. Barderas, R.A. Bartolome, M.J. Fernandez-Acenero, S. Torres, J.I. Casal, High expression of IL-13 receptor alpha2 in colorectal cancer is associated with invasion, liver metastasis, and poor prognosis, *Cancer Res.* 72 (2012) 2780–2790.

- [289] W. Strober, A. Kitani, S. Fichtner-Feigl, I.J. Fuss, The signaling function of the IL-13Ralpha2 receptor in the development of gastrointestinal fibrosis and cancer surveillance, *Curr. Mol. Med.* 9 (2009) 740–750.
- [290] J. Harper, R.C. Sainson, Regulation of the anti-tumour immune response by cancer-associated fibroblasts, *Semin. Cancer Biol.* 25 (2014) 69–77.
- [291] H. Nakashima, S.R. Husain, R.K. Puri, IL-13 receptor-directed cancer vaccines and immunotherapy, *Immunotherapy* 4 (2012) 443–451.
- [292] H. Nakashima, M. Terabe, J.A. Berzofsky, S.R. Husain, R.K. Puri, A novel combination immunotherapy for cancer by IL-13Ralpha2-targeted DNA vaccine and immunotoxin in murine tumor models, *J. Immunol.* 187 (2011) 4935–4946.
- [293] K. Kawakami, M. Terabe, M. Kioi, J.A. Berzofsky, R.K. Puri, Intratumoral therapy with IL13-PE38 results in effective CTL-mediated suppression of IL-13Ralpha2-expressing contralateral tumors, *Clin. Cancer Res.: Off. J. Am. Assoc. Cancer Res.* 12 (2006) 4678–4686.
- [294] F. Weber, A. Asher, R. Bucholz, M. Berger, M. Prados, S. Chang, et al., Safety, tolerability, and tumor response of IL4-Pseudomonas exotoxin (NBI-3001) in patients with recurrent malignant glioma, *J. Neurooncol.* 64 (2003) 125–137.
- [295] R. Noy, J.W. Pollard, Tumor-associated macrophages: from mechanisms to therapy, *Immunity* 41 (2014) 49–61.
- [296] Y. Komohara, M. Jinushi, M. Takeya, Clinical significance of macrophage heterogeneity in human malignant tumors, *Cancer Sci.* 105 (2014) 1–8.
- [297] G. Solinas, G. Germano, A. Mantovani, P. Allavena, Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation, *J. Leukoc. Biol.* 86 (2009) 1065–1073.
- [298] M.R. Galdiero, C. Garlanda, S. Jaillon, G. Marone, A. Mantovani, Tumor associated macrophages and neutrophils in tumor progression, *J. Cell. Physiol.* 228 (2013) 1404–1412.
- [299] D.C. Radisky, P.A. Kenny, M.J. Bissell, Fibrosis and cancer: do myofibroblasts come also from epithelial cells via EMT?, *J. Cell. Biochem.* 101 (2007) 830–839.
- [300] Q.M. Fan, Y.Y. Jing, G.F. Yu, X.R. Kou, F. Ye, L. Gao, et al., Tumor-associated macrophages promote cancer stem cell-like properties via transforming growth factor-beta1-induced epithelial-mesenchymal transition in hepatocellular carcinoma, *Cancer Lett.* 352 (2014) 160–168.
- [301] L. Hussaerts, M. Yazdanbakhsh, B. Guigas, Priming dendritic cells for Th2 polarization: lessons learned from helminths and implications for metabolic disorders, *Front. Immunol.* 5 (2014) 499.
- [302] F.D. Finkelman, T. Shea-Donohue, S.C. Morris, L. Gildea, R. Strait, K.B. Madden, et al., Interleukin-4- and interleukin-13-mediated host protection against intestinal nematode parasites, *Immunol. Rev.* 201 (2004) 139–155.
- [303] D. Wu, R. Ahrens, H. Osterfeld, T.K. Noah, K. Groschwitz, P.S. Foster, et al., Interleukin-13 (IL-13)/IL-13 receptor alpha1 (IL-13Ralpha1) signaling regulates intestinal epithelial cystic fibrosis transmembrane conductance regulator channel-dependent Cl-secretion, *J. Biol. Chem.* 286 (2011) 13357–13369.
- [304] N.A. Hering, M. Fromm, J.D. Schulzke, Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics, *J. Physiol.* 590 (2012) 1035–1044.
- [305] J. Tanaka, K. Saga, M. Kido, H. Nishiura, T. Akamatsu, T. Chiba, et al., Proinflammatory Th2 cytokines induce production of thymic stromal lymphopoietin in human colonic epithelial cells, *Dig. Dis. Sci.* 55 (2010) 1896–1904.
- [306] L. Pastorelli, R.R. Garg, S.B. Hoang, L. Spina, B. Mattioli, M. Scarpa, et al., Epithelial-derived IL-33 and its receptor ST2 are dysregulated in ulcerative colitis and in experimental Th1/Th2 driven enteritis, *Proc. Natl. Acad. Sci. USA* 107 (2010) 8017–8022.
- [307] C. Abraham, J.H. Cho, Inflammatory bowel disease, *N. Engl. J. Med.* 361 (2009) 2066–2078.
- [308] N. Kamada, S.U. Seo, G.Y. Chen, G. Nunez, Role of the gut microbiota in immunity and inflammatory disease, *Nat. Rev. Immunol.* 13 (2013) 321–335.
- [309] K.L. Wallace, L.B. Zheng, Y. Kanazawa, D.Q. Shih, Immunopathology of inflammatory bowel disease, *World J. Gastroenterol.* WJG 20 (2014) 6–21.
- [310] I.J. Fuss, F. Heller, M. Boirivant, F. Leon, M. Yoshida, S. Fichtner-Feigl, et al., Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis, *J. Clin. Invest.* 113 (2004) 1490–1497.
- [311] F. Heller, P. Florian, C. Bojarski, J. Richter, M. Christ, B. Hillenbrand, et al., Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution, *Gastroenterology* 129 (2005) 550–564.
- [312] P. Biancheri, A. Di Sabatino, F. Ammoscato, F. Facciotti, F. Caprioli, R. Curciarello, et al., Absence of a role for interleukin-13 in inflammatory bowel disease, *Eur. J. Immunol.* 44 (2014) 370–385.
- [313] F. Heller, A. Fromm, A.H. Gitter, J. Mankertz, J.D. Schulzke, Epithelial apoptosis is a prominent feature of the epithelial barrier disturbance in intestinal inflammation: effect of pro-inflammatory interleukin-13 on epithelial cell function, *Mucosal Immunol.* 1 (Suppl. 1) (2008) S58–S61.
- [314] I.J. Fuss, B. Joshi, Z. Yang, H. Degheidy, S. Fichtner-Feigl, H. de Souza, et al., IL-13Ralpha2-bearing, type II NKT cells reactive to sulfatide self-antigen populate the mucosa of ulcerative colitis, *Gut* 63 (2014) 1728–1736.
- [315] G. Latella, R. Sferri, S. Specia, A. Vetusch, E. Gaudio, Can we prevent, reduce or reverse intestinal fibrosis in IBD?, *Eur. Rev. Med. Pharmacol. Sci.* 17 (2013) 1283–1304.
- [316] M. Scharl, P. Frei, S.M. Frei, L. Biedermann, A. Weber, G. Rogler, Epithelial-to-mesenchymal transition in a fistula-associated anal adenocarcinoma in a patient with long-standing Crohn's disease, *Eur. J. Gastroenterol. Hepatol.* 26 (2014) 114–118.
- [317] M. Scharl, S. Frei, T. Pesch, S. Kellermeier, J. Arikkat, P. Frei, et al., Interleukin-13 and transforming growth factor beta synergise in the pathogenesis of human intestinal fistulae, *Gut* 62 (2013) 63–72.
- [318] L.W. Peterson, D. Artis, Intestinal epithelial cells: regulators of barrier function and immune homeostasis, *Nat. Rev. Immunol.* 14 (2014) 141–153.
- [319] A. Zhao, J.F. Urban Jr., R. Sun, J. Stiltz, M. Morimoto, L. Notari, et al., Critical role of IL-25 in nematode infection-induced alterations in intestinal function, *J. Immunol.* 185 (2010) 6921–6929.
- [320] L. Pastorelli, C. De Salvo, M. Vecchi, T.T. Pizarro, The role of IL-33 in gut mucosal inflammation, *Mediators Inflamm.* 2013 (2013) 608187.
- [321] S. Danese, J. Rudzinski, W. Brandt, J.L. Dupas, L. Peyrin-Biroulet, Y. Bouhnik, et al., Tralokinumab for moderate-to-severe UC: a randomised, double-blind, placebo-controlled, phase IIa study, *Gut* 64 (2015) 243–249.
- [322] P. Li, R. Spolski, W. Liao, W.J. Leonard, Complex interactions of transcription factors in mediating cytokine biology in T cells, *Immunol. Rev.* 261 (2014) 141–156.
- [323] H. Yamane, W.E. Paul, Cytokines of the gamma(c) family control CD4+ T cell differentiation and function, *Nat. Immunol.* 13 (2012) 1037–1044.
- [324] I. Raphael, S. Nalawade, T.N. Eagar, T.G. Forsthuber, T cell subsets and their signature cytokines in autoimmune and inflammatory diseases, *Cytokine* 74 (2015) 5–17.
- [325] J. Geginat, M. Paroni, S. Maglie, J.S. Alfen, I. Kastirr, P. Guarini, et al., Plasticity of human CD4 T cell subsets, *Front. Immunol.* 5 (2014) 630.
- [326] T. Bashi, G. Bizzaro, D. Ben-Ami Shor, M. Blank, Y. Shoenfeld, The mechanisms behind helminth's immunomodulation in autoimmunity, *Autoimmun. Rev.* 14 (2015) 98–104.
- [327] N. Charles, D. Hardwick, E. Daugas, G.G. Illei, J. Rivera, Basophils and the T helper 2 environment can promote the development of lupus nephritis, *Nat. Med.* 16 (2010) 701–707.
- [328] B. Dema, N. Charles, C. Pellefigues, T.K. Ricks, R. Suzuki, C. Jiang, et al., Immunoglobulin E plays an immunoregulatory role in lupus, *J. Exp. Med.* 211 (2014) 2159–2168.
- [329] H.H. Yu, P.H. Liu, Y.C. Lin, W.J. Chen, J.H. Lee, L.C. Wang, et al., Interleukin 4 and STAT6 gene polymorphisms are associated with systemic lupus erythematosus in Chinese patients, *Lupus* 19 (2010) 1219–1228.
- [330] S.J. Beavitt, K.W. Harder, J.M. Kemp, J. Jones, C. Quilici, F. Casagrande, et al., Lyn-deficient mice develop severe, persistent asthma: Lyn is a critical negative regulator of Th2 immunity, *J. Immunol.* 175 (2005) 1867–1875.
- [331] C.L. Sokol, N.Q. Chu, S. Yu, S.A. Nish, T.M. Laufer, R. Medzhitov, Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response, *Nat. Immunol.* 10 (2009) 713–720.
- [332] C.M. Snapper, F.D. Finkelman, W.E. Paul, Regulation of IgG1 and IgE production by interleukin 4, *Immunol. Rev.* 102 (1988) 51–75.
- [333] A. Denzel, U.A. Maus, M. Rodriguez Gomez, C. Moll, M. Niedermeier, C. Winter, et al., Basophils enhance immunological memory responses, *Nat. Immunol.* 9 (2008) 733–742.
- [334] A. Davidson, B. Diamond, Activated basophils give lupus a booster shot, *Nat. Med.* 16 (2010) 635–636.
- [335] S.L. Rowland, K.F. Leahy, R. Halverson, R.M. Torres, R. Pelanda, BAFF receptor signaling aids the differentiation of immature B cells into transitional B cells following tonic BCR signaling, *J. Immunol.* 185 (2010) 4570–4581.
- [336] I. Puga, M. Cols, C.M. Barra, B. He, L. Cassis, M. Gentile, et al., B cell-helper neutrophils stimulate the diversification and production of immunoglobulin in the marginal zone of the spleen, *Nat. Immunol.* 13 (2012) 170–180.
- [337] J.L. Cromheecke, K.T. Nguyen, D.P. Huston, Emerging role of human basophil biology in health and disease, *Curr. Allergy Asthma Rep.* 14 (2014) 408.
- [338] J. Suurmond, A.J. Schuerwegh, R.E. Toes, Anti-citrullinated protein antibodies in rheumatoid arthritis: a functional role for mast cells and basophils?, *Ann Rheum. Dis.* 70 (Suppl. 1) (2011) i55–i58.
- [339] M. Sharma, J. Bayry, Autoimmunity: basophils in autoimmune and inflammatory diseases, *Nat. Rev. Rheumatol.* 11 (2015) 129–131.
- [340] J.C. Broen, P. Dieude, M.C. Vonk, L. Beretta, F.D. Carmona, A. Herrick, et al., Polymorphisms in the interleukin 4, interleukin 13, and corresponding receptor genes are not associated with systemic sclerosis and do not influence gene expression, *J. Rheumatol.* 39 (2012) 112–118.
- [341] Y. Parel, M. Aurrand-Lions, A. Scheja, J.M. Dayer, E. Roosnek, C. Chizzolini, Presence of CD4+CD8+ double-positive T cells with very high interleukin-4 production potential in lesional skin of patients with systemic sclerosis, *Arthritis Rheum.* 56 (2007) 3459–3467.
- [342] B. Granel, C. Chevillard, Y. Allanore, V. Arnaud, S. Cabantous, S. Marquet, et al., Evaluation of interleukin 13 polymorphisms in systemic sclerosis, *Immunogenetics* 58 (2006) 693–699.
- [343] T.A. Wynn, T.R. Ramalingam, Mechanisms of fibrosis: therapeutic translation for fibrotic disease, *Nat. Med.* 18 (2012) 1028–1040.
- [344] S.L. Friedman, D. Sheppard, J.S. Duffield, S. Violette, Therapy for fibrotic diseases: nearing the starting line, *Sci. Transl. Med.* 5 (2013) 167sr1.
- [345] T.A. Wynn, Cellular and molecular mechanisms of fibrosis, *J. Pathol.* 214 (2008) 199–210.
- [346] T.A. Wynn, Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases, *J. Clin. Invest.* 117 (2007) 524–529.
- [347] T.A. Wynn, Fibrotic disease and the T(H)1/T(H)2 paradigm, *Nat. Rev. Immunol.* 4 (2004) 583–594.
- [348] M. Hesse, M. Modolell, A.C. La Flamme, M. Schito, J.M. Fuentes, A.W. Cheever, et al., Differential regulation of nitric oxide synthase-2 and arginase-1 by type

- 1/type 2 cytokines in vivo: granulomatous pathology is shaped by the pattern of L-arginine metabolism, *J. Immunol.* 167 (2001) 6533–6544.
- [349] M. Decitre, C. Gleyzal, M. Racourt, S. Peyrol, E. Aubert-Foucher, K. Csiszar, et al., Lysyl oxidase-like protein localizes to sites of de novo fibrogenesis in fibrosis and in the early stromal reaction of ductal breast carcinomas, *Lab. Invest.*, *J. Tech. Methods Pathol.* 78 (1998) 143–151.
- [350] S. Wang, R. Hirschberg, BMP7 antagonizes TGF-beta-dependent fibrogenesis in mesangial cells, *Am. J. Physiol. Renal Physiol.* 284 (2003) F1006–F1013.
- [351] D.C. Underwood, R.R. Osborn, S. Bochnowicz, E.F. Webb, D.J. Rieman, J.C. Lee, et al., SB 239063, a p38 MAPK inhibitor, reduces neutrophilia, inflammatory cytokines, MMP-9, and fibrosis in lung, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 279 (2000) L895–L902.
- [352] H. Kim, T. Oda, J. Lopez-Guisa, D. Wing, D.R. Edwards, P.D. Soloway, et al., TIMP-1 deficiency does not attenuate interstitial fibrosis in obstructive nephropathy, *J. Am. Soc. Nephrol.: JASN* 12 (2001) 736–748.
- [353] T.A. Wynn, IL-13 effector functions, *Annu. Rev. Immunol.* 21 (2003) 425–456.
- [354] J.A. Belperio, M. Dy, M.D. Burdick, Y.Y. Xue, K. Li, J.A. Elias, et al., Interaction of IL-13 and C10 in the pathogenesis of bleomycin-induced pulmonary fibrosis, *Am. J. Respir. Cell Mol. Biol.* 27 (2002) 419–427.
- [355] K. Blease, C. Jakubczik, J. Westwick, N. Lukacs, S.L. Kunkel, C.M. Hogaboam, Therapeutic effect of IL-13 immunoneutralization during chronic experimental fungal asthma, *J. Immunol.* 166 (2001) 5219–5224.
- [356] L.A. Murray, H. Zhang, S.R. Oak, A.L. Coelho, A. Herath, K.R. Flaherty, et al., Targeting interleukin-13 with talokinumab attenuates lung fibrosis and epithelial damage in a humanized SCID idiopathic pulmonary fibrosis model, *Am. J. Respir. Cell Mol. Biol.* 50 (2014) 985–994.
- [357] Y. Liu, S. Munker, R. Mullenbach, H.L. Weng, IL-13 signaling in liver fibrogenesis, *Front. Immunol.* 3 (2012) 116.
- [358] T. Shimamura, T. Fujisawa, S.R. Husain, M. Kioi, A. Nakajima, R.K. Puri, Novel role of IL-13 in fibrosis induced by nonalcoholic steatohepatitis and its amelioration by IL-13R-directed cytotoxin in a rat model, *J. Immunol.* 181 (2008) 4656–4665.
- [359] H.L. Weng, Y. Liu, J.L. Chen, T. Huang, L.J. Xu, P. Godoy, et al., The etiology of liver damage imparts cytokines transforming growth factor beta1 or interleukin-13 as driving forces in fibrogenesis, *Hepatology* (Baltimore, MD) 50 (2009) 230–243.
- [360] R.B. Christmann, A. Mathes, A.J. Affandi, C. Padilla, B. Nazari, A.M. Bujor, et al., Thymic stromal lymphopoietin is up-regulated in the skin of patients with systemic sclerosis and induces profibrotic genes and intracellular signaling that overlap with those induced by interleukin-13 and transforming growth factor beta, *Arthritis Rheum.* 65 (2013) 1335–1346.
- [361] P. Fuschioti, A.T. Larregina, J. Ho, C. Feghali-Bostwick, T.A. Medsger Jr., Interleukin-13-producing CD8⁺ T cells mediate dermal fibrosis in patients with systemic sclerosis, *Arthritis Rheum.* 65 (2013) 236–246.
- [362] T.A. Medsger Jr., D.E. Ivanco, L. Kardava, P.A. Morel, M.R. Lucas, P. Fuschioti, GATA-3 up-regulation in CD8⁺ T cells as a biomarker of immune dysfunction in systemic sclerosis, resulting in excessive interleukin-13 production, *Arthritis Rheum.* 63 (2011) 1738–1747.
- [363] S.M. Brunner, G. Schiechl, R. Kesselring, M. Martin, S. Balam, H.J. Schlitt, et al., IL-13 signaling via IL-13Ralpha2 triggers TGF-beta1-dependent allograft fibrosis, *Transplant. Res.* 2 (2013) 16.
- [364] M. Kaviratne, M. Hesse, M. Leusink, A.W. Cheever, S.J. Davies, J.H. McKerrow, et al., IL-13 activates a mechanism of tissue fibrosis that is completely TGF-beta independent, *J. Immunol.* 173 (2004) 4020–4029.
- [365] E. Hams, M.E. Armstrong, J.L. Barlow, S.P. Saunders, C. Schwartz, G. Cooke, et al., IL-25 and type 2 innate lymphoid cells induce pulmonary fibrosis, *Proc. Natl. Acad. Sci. USA* 111 (2014) 367–372.
- [366] S.L. Nishimura, Integrin-mediated transforming growth factor-beta activation, a potential therapeutic target in fibrogenic disorders, *Am. J. Pathol.* 175 (2009) 1362–1370.
- [367] C.G. Lee, R.J. Homer, Z. Zhu, S. Lanone, X. Wang, V. Koteliansky, et al., Interleukin-13 induces tissue fibrosis by selectively stimulating and activating transforming growth factor beta(1), *J. Exp. Med.* 194 (2001) 809–821.
- [368] S. Lanone, T. Zheng, Z. Zhu, W. Liu, C.G. Lee, B. Ma, et al., Overlapping and enzyme-specific contributions of matrix metalloproteinases-9 and -12 in IL-13-induced inflammation and remodeling, *J. Clin. Invest.* 110 (2002) 463–474.
- [369] J.E. Heredia, L. Mukundan, F.M. Chen, A.A. Mueller, R.C. Deo, R.M. Locksley, et al., Type 2 innate signals stimulate fibro/adipogenic progenitors to facilitate muscle regeneration, *Cell* 153 (2013) 376–388.
- [370] R.R. Ricardo-Gonzalez, A. Red Eagle, J.I. Odegaard, H. Jouihan, C.R. Morel, J.E. Heredia, et al., IL-4/STAT6 immune axis regulates peripheral nutrient metabolism and insulin sensitivity, *Proc. Natl. Acad. Sci. USA* 107 (2010) 22617–22622.
- [371] K.J. Stanya, D. Jacobi, S. Liu, P. Bhargava, L. Dai, M.R. Gangl, et al., Direct control of hepatic glucose production by interleukin-13 in mice, *J. Clin. Invest.* 123 (2013) 261–271.
- [372] D. Wu, A.B. Molofsky, H.E. Liang, R.R. Ricardo-Gonzalez, H.A. Jouihan, J.K. Bando, et al., Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis, *Science* (New York, NY) 332 (2011) 243–247.
- [373] A.B. Molofsky, J.C. Nussbaum, H.E. Liang, S.J. Van Dyken, L.E. Cheng, A. Mohapatra, et al., Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages, *J. Exp. Med.* 210 (2013) 535–549.
- [374] M.W. Lee, J.I. Odegaard, L. Mukundan, Y. Qiu, A.B. Molofsky, J.C. Nussbaum, et al., Activated type 2 innate lymphoid cells regulate beige fat biogenesis, *Cell* 160 (2015) 74–87.
- [375] J.R. Brestoff, B.S. Kim, S.A. Saenz, R.R. Stine, L.A. Monticelli, G.F. Sonnenberg, et al., Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity, *Nature* 519 (2015) 242–246.
- [376] Z. Song, V. Casolaro, R. Chen, S.N. Georas, D. Monos, S.J. Ono, Polymorphic nucleotides within the human IL-4 promoter that mediate overexpression of the gene, *J. Immunol.* 156 (1996) 424–429.
- [377] E.G. Burchard, E.K. Silverman, L.J. Rosenwasser, L. Borish, C. Yandava, A. Pillari, et al., Association between a sequence variant in the IL-4 gene promoter and FEV(1) in asthma, *Am. J. Respir. Crit. Care Med.* 160 (1999) 919–922.
- [378] W. Nie, Z. Zhu, X. Pan, Q. Xiu, The interleukin-4 –589C/T polymorphism and the risk of asthma: a meta-analysis including 7345 cases and 7819 controls, *Gene* 520 (2013) 22–29.
- [379] H. Mitsuyasu, K. Izuhara, X.Q. Mao, P.S. Gao, Y. Arinobu, T. Enomoto, et al., Ile50Val variant of IL4R alpha upregulates IgE synthesis and associates with atopic asthma, *Nat. Genet.* 19 (1998) 119–120.
- [380] S. Kruse, T. Japha, M. Tedner, S.H. Sparholt, J. Forster, J. Kuehr, et al., The polymorphisms S503P and Q576R in the interleukin-4 receptor alpha gene are associated with atopy and influence the signal transduction, *Immunology* 96 (1999) 365–371.
- [381] T.D. Howard, G.H. Koppelman, J. Xu, S.L. Zheng, D.S. Postma, D.A. Meyers, et al., Gene-gene interaction in asthma: IL4RA and IL13 in a Dutch population with asthma, *Am. J. Hum. Genet.* 70 (2002) 230–236.
- [382] M.J. Loza, B.L. Chang, Association between Q551R IL4R genetic variants and atopic asthma risk demonstrated by meta-analysis, *J. Allergy Clin. Immunol.* 120 (2007) 578–585.
- [383] R.E. Callard, R. Hamvas, C. Chatterton, C. Blanco, M. Pembrey, R. Jones, et al., An interaction between the IL-4Ralpha gene and infection is associated with atopic eczema in young children, *Clin. Exp. Allergy* 32 (2002) 990–993.
- [384] J.Q. He, J.E. Connett, N.R. Anthonisen, A.J. Sandford, Polymorphisms in the IL13, IL13RA1, and IL4RA genes and rate of decline in lung function in smokers, *Am. J. Respir. Cell Mol. Biol.* 28 (2003) 379–385.
- [385] S.E. Wenzel, S. Balzar, E. Ampleford, G.A. Hawkins, W.W. Busse, W.J. Calhoun, et al., IL4R alpha mutations are associated with asthma exacerbations and mast cell/IgE expression, *Am. J. Respir. Crit. Care Med.* 175 (2007) 570–576.
- [386] A.K. Konstantinidis, S.J. Barton, I. Sayers, I.A. Yang, J.L. Lordan, S. Rorke, et al., Genetic association studies of interleukin-13 receptor alpha1 subunit gene polymorphisms in asthma and atopy, *Eur. Respir. J.* 30 (2007) 40–47.
- [387] S. Ahmed, K. Ihara, Y. Sasaki, F. Nakao, S. Nishima, T. Fujino, et al., Novel polymorphism in the coding region of the IL-13 receptor alpha' gene: association study with atopic asthma in the Japanese population, *Exp. Clin. Immunogenet.* 17 (2000) 18–22.
- [388] T.C. van der Pouw Kraan, A. van Veen, L.C. Boeijs, S.A. van Tuyl, E.R. de Groot, S.O. Stapel, et al., An IL-13 promoter polymorphism associated with increased risk of allergic asthma, *Genes Immun.* 1 (1999) 61–65.
- [389] L. Cameron, R.B. Webster, J.M. Strempel, P. Kiesler, M. Kabesch, H. Ramchandran, et al., Th2 cell-selective enhancement of human IL13 transcription by IL13-1112C>T, a polymorphism associated with allergic inflammation, *J. Immunol.* 177 (2006) 8633–8642.
- [390] T.D. Howard, P.A. Whittaker, A.L. Zaiman, G.H. Koppelman, J. Xu, M.T. Hanley, et al., Identification and association of polymorphisms in the interleukin-13 gene with asthma and atopy in a Dutch population, *Am. J. Respir. Cell Mol. Biol.* 25 (2001) 377–384.
- [391] X. Liu, T.H. Beaty, P. Deindl, S.K. Huang, S. Lau, C. Sommerfeld, et al., Associations between total serum IgE levels and the 6 potentially functional variants within the genes IL4, IL13, and IL4RA in German children: the German Multicenter Atopy Study, *J. Allergy Clin. Immunol.* 112 (2003) 382–388.
- [392] A. Lesiak, P. Kuna, M. Zakrzewski, M. van Geel, R.S. Bladergroen, K. Przybylowska, et al., Combined occurrence of filaggrin mutations and IL-10 or IL-13 polymorphisms predisposes to atopic dermatitis, *Exp. Dermatol.* 20 (2011) 491–495.
- [393] R.W. Bottema, I.M. Nolte, T.D. Howard, G.H. Koppelman, A.E. Dubois, G. de Meer, et al., Interleukin 13 and interleukin 4 receptor-alpha polymorphisms in rhinitis and asthma, *Int. Arch. Allergy Immunol.* 153 (2010) 259–267.
- [394] A. Sadeghnejad, D.A. Meyers, M. Bottai, D.A. Sterling, E.R. Bleeker, J.A. Ohar, IL13 promoter polymorphism 1112C/T modulates the adverse effect of tobacco smoking on lung function, *Am. J. Respir. Crit. Care Med.* 176 (2007) 748–752.
- [395] T.C. van der Pouw Kraan, M. Kucukaycan, A.M. Bakker, J.M. Baggen, J.S. van der Zee, M.A. Dentener, et al., Chronic obstructive pulmonary disease is associated with the –1055 IL-13 promoter polymorphism, *Genes Immun.* 3 (2002) 436–439.
- [396] A. Heinzmann, S.P. Jerkic, K. Ganter, T. Kurz, S. Blattmann, L. Schuchmann, et al., Association study of the IL13 variant Arg110Gln in atopic diseases and juvenile idiopathic arthritis, *J. Allergy Clin. Immunol.* 112 (2003) 735–739.
- [397] G.M. Hunninghake, M.E. Soto-Quiros, L. Avila, J. Su, A. Murphy, D.L. Demeo, et al., Polymorphisms in IL13, total IgE, eosinophilia, and asthma exacerbations in childhood, *J. Allergy Clin. Immunol.* 120 (2007) 84–90.
- [398] M.J. Ermers, B. Hoebee, H.M. Hodemaekers, T.G. Kimman, J.L. Kimpen, L. Bont, IL-13 genetic polymorphism identifies children with late wheezing after respiratory syncytial virus infection, *J. Allergy Clin. Immunol.* 119 (2007) 1086–1091.

- [399] H.W. Park, J.E. Lee, S.H. Kim, Y.K. Kim, K.U. Min, Y.Y. Kim, et al., Genetic variation of IL13 as a risk factor of reduced lung function in children and adolescents: a cross-sectional population-based study in Korea, *Respir. Med.* 103 (2009) 284–288.
- [400] Y. Tsunemi, H. Saeki, K. Nakamura, T. Sekiya, K. Hirai, T. Kakinuma, et al., Interleukin-13 gene polymorphism G4257A is associated with atopic dermatitis in Japanese patients, *J. Dermatol. Sci.* 30 (2002) 100–107.
- [401] A.H. Ziyab, G.A. Davies, S. Ewart, J.M. Hopkin, E.M. Schaubberger, M. Wills-Karp, et al., Interactive effect of STAT6 and IL13 gene polymorphisms on eczema status: results from a longitudinal and a cross-sectional study, *BMC Med. Genet.* 14 (2013) 67.
- [402] A. Yadav, G.K. Govindasamy, R. Naidu, Polymorphic variants of interleukin-13 R130Q, interleukin-4 T589C, interleukin-4RA I50V, and interleukin-4RA Q576R in allergic rhinitis: a pilot study, *Allergy Rhinol. (Providence, RI)* 3 (2012) e35–e40.
- [403] A.K. Andiappan, O. Rotzschke, Y. Wang de, F.T. Chew, Association of interleukin-13 SNP rs20541 (Arg > Gln) to allergic rhinitis in an Asian population of ethnic Chinese in Singapore, *Gene* 529 (2013) 357–358.
- [404] M. Yoshikawa, T. Nakajima, T. Tsukidate, K. Matsumoto, M. Iida, N. Otori, et al., TNF-alpha and IL-4 regulate expression of IL-13 receptor alpha2 on human fibroblasts, *Biochem. Biophys. Res. Commun.* 312 (2003) 1248–1255.
- [405] R. Kunii, E. Nemoto, S. Kanaya, T. Tsubahara, H. Shimachi, Expression of CD13/aminopeptidase N on human gingival fibroblasts and up-regulation upon stimulation with interleukin-4 and interleukin-13, *J. Periodontol. Res.* 40 (2005) 138–146.
- [406] M. Higashino, T. Takabayashi, N. Takahashi, M. Okamoto, N. Narita, A. Kojima, et al., Interleukin-19 downregulates interleukin-4-induced eotaxin production in human nasal fibroblasts, *Allergol. Int.: Off. J. Jpn. Soc. Allergol.* 60 (2011) 449–457.
- [407] K. Chibana, Y. Ishii, T. Asakura, T. Fukuda, Up-regulation of cysteinyl leukotriene 1 receptor by IL-13 enables human lung fibroblasts to respond to leukotriene C4 and produce eotaxin, *J. Immunol.* 170 (2003) 4290–4295.
- [408] R.J. Freishtat, A.M. Watson, A.S. Benton, S.F. Iqbal, D.K. Pillai, M.C. Rose, et al., Asthmatic airway epithelium is intrinsically inflammatory and mitotically dysynchronous, *Am. J. Respir. Cell Mol. Biol.* 44 (2011) 863–869.
- [409] S. Allahverdian, N. Harada, G.K. Singhera, D.A. Knight, D.R. Dorscheid, Secretion of IL-13 by airway epithelial cells enhances epithelial repair via HB-EGF, *Am. J. Respir. Cell Mol. Biol.* 38 (2008) 153–160.
- [410] S.R. White, L.D. Martin, R. Stern, B. Laxman, B.A. Marroquin, Expression of IL-4/IL-13 receptors in differentiating human airway epithelial cells, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 299 (2010) L681–L693.
- [411] V.H. van der Velden, B.A. Naber, A.F. Wierenga-Wolf, R. Debets, H.F. Savelkoul, S.E. Overbeek, et al., Interleukin 4 receptors on human bronchial epithelial cells. An in vivo and in vitro analysis of expression and function, *Cytokine* 10 (1998) 803–813.
- [412] S. Yamamoto, I. Kobayashi, K. Tsuji, N. Nishi, E. Muro, M. Miyazaki, et al., Upregulation of interleukin-4 receptor by interferon-gamma: enhanced interleukin-4-induced eotaxin-3 production in airway epithelium, *Am. J. Respir. Cell Mol. Biol.* 31 (2004) 456–462.
- [413] I. Striz, T. Mio, Y. Adachi, R.A. Robbins, D.J. Romberger, S.I. Rennard, IL-4 and IL-13 stimulate human bronchial epithelial cells to release IL-8, *Inflammation* 23 (1999) 545–555.
- [414] R. Montes-Vizuet, A. Vega-Miranda, E. Valencia-Maqueda, M.C. Negrete-Garcia, J.R. Velasquez, L.M. Teran, CC chemokine ligand 1 is released into the airways of atopic asthmatics, *Eur. Respir. J.* 28 (2006) 59–67.
- [415] J. Laouikili, E. Perret, T. Willems, A. Minty, E. Parthoens, O. Houcine, et al., IL-13 alters mucociliary differentiation and ciliary beating of human respiratory epithelial cells, *J. Clin. Invest.* 108 (2001) 1817–1824.
- [416] F.Q. Wen, T. Kohyama, X. Liu, Y.K. Zhu, H. Wang, H.J. Kim, et al., Interleukin-4 and interleukin-13-enhanced transforming growth factor-beta2 production in cultured human bronchial epithelial cells is attenuated by interferon-gamma, *Am. J. Respir. Cell Mol. Biol.* 26 (2002) 484–490.
- [417] G. Candiano, M. Bruschi, N. Pedemonte, E. Caci, S. Liberatori, L. Bini, et al., Gelsolin secretion in interleukin-4-treated bronchial epithelia and in asthmatic airways, *Am. J. Respir. Crit. Care Med.* 172 (2005) 1090–1096.
- [418] S.J. Wadsworth, R. Atsuta, J.O. McIntyre, T.L. Hackett, G.K. Singhera, D.R. Dorscheid, IL-13 and TH2 cytokine exposure triggers matrix metalloproteinase 7-mediated Fas ligand cleavage from bronchial epithelial cells, *J. Allergy Clin. Immunol.* 126 (2010) 366–374, 74 e1–e8.
- [419] A. Borowski, M. Kuepper, U. Horn, U. Knupfer, G. Zissel, K. Hohne, et al., Interleukin-13 acts as an apoptotic effector on lung epithelial cells and induces pro-fibrotic gene expression in lung fibroblasts, *Clin. Exp. Allergy* 38 (2008) 619–628.
- [420] N. Pedemonte, E. Caci, E. Sondo, A. Caputo, K. Rhoden, U. Pfeffer, et al., Thiocyanate transport in resting and IL-4-stimulated human bronchial epithelial cells: role of pendrin and anion channels, *J. Immunol.* 178 (2007) 5144–5153.
- [421] S. Matsukura, C. Stellato, S.N. Georas, V. Casolaro, J.R. Plitt, K. Miura, et al., Interleukin-13 upregulates eotaxin expression in airway epithelial cells by a STAT6-dependent mechanism, *Am. J. Respir. Cell Mol. Biol.* 24 (2001) 755–761.
- [422] B.W. Booth, K.B. Adler, J.C. Bonner, F. Tournier, L.D. Martin, Interleukin-13 induces proliferation of human airway epithelial cells in vitro via a mechanism mediated by transforming growth factor-alpha, *Am. J. Respir. Cell Mol. Biol.* 25 (2001) 739–743.
- [423] G.C. Simon, R.J. Martin, S. Smith, J. Thaikootathil, R.P. Bowler, S.J. Barenkamp, et al., Up-regulation of MUC18 in airway epithelial cells by IL-13: implications in bacterial adherence, *Am. J. Respir. Cell Mol. Biol.* 44 (2011) 606–613.
- [424] V. Suresh, J.D. Mih, S.C. George, Measurement of IL-13-induced iNOS-derived gas phase nitric oxide in human bronchial epithelial cells, *Am. J. Respir. Cell Mol. Biol.* 37 (2007) 97–104.
- [425] J. Trudeau, H. Hu, K. Chibana, H.W. Chu, J.Y. Westcott, S.E. Wenzel, Selective downregulation of prostaglandin E2-related pathways by the Th2 cytokine IL-13, *J. Allergy Clin. Immunol.* 117 (2006) 1446–1454.
- [426] I. Nakao, S. Kanaji, S. Ohta, H. Matsushita, K. Arima, N. Yuyama, et al., Identification of pendrin as a common mediator for mucus production in bronchial asthma and chronic obstructive pulmonary disease, *J. Immunol.* 180 (2008) 6262–6269.
- [427] L.K. Trejdosiewicz, R. Morton, Y. Yang, R.E. Banks, P.J. Selby, J. Southgate, Interleukins 4 and 13 upregulate expression of cd44 in human colonic epithelial cell lines, *Cytokine* 10 (1998) 756–765.
- [428] K. Gronert, A. Gewirtz, J.L. Madara, C.N. Serhan, Identification of a human enterocyte lipoxin A4 receptor that is regulated by interleukin (IL)-13 and interferon gamma and inhibits tumor necrosis factor alpha-induced IL-8 release, *J. Exp. Med.* 187 (1998) 1285–1294.
- [429] J. Wongpiyabovorn, H. Suto, H. Ushio, K. Izuhara, K. Mitsuishi, S. Ikeda, et al., Up-regulation of interleukin-13 receptor alpha1 on human keratinocytes in the skin of psoriasis and atopic dermatitis, *J. Dermatol. Sci.* 33 (2003) 31–40.
- [430] S.I. Bogiatzi, I. Fernandez, J.C. Bichet, M.A. Marloie-Provoost, E. Volpe, X. Sastre, et al., Cutting Edge: proinflammatory and Th2 cytokines synergize to induce thymic stromal lymphopoietin production by human skin keratinocytes, *J. Immunol.* 178 (2007) 3373–3377.
- [431] M. Wakugawa, K. Nakamura, M. Akatsuka, H. Nakagawa, K. Tamaki, Interferon-gamma-induced RANTES production by human keratinocytes is enhanced by IL-1beta, TNF-alpha, IL-4 and IL-13 and is inhibited by dexamethasone and tacrolimus, *Dermatology (Basel, Switzerland)* 202 (2001) 239–245.
- [432] L. Bao, V.Y. Shi, L.S. Chan, IL-4 up-regulates epidermal chemotactic, angiogenic, and pro-inflammatory genes and down-regulates antimicrobial genes in vivo and in vitro: relevant in the pathogenesis of atopic dermatitis, *Cytokine* 61 (2013) 419–425.
- [433] R.L. Palmer-Crocker, J.S. Pober, IL-4 induction of VCAM-1 on endothelial cells involves activation of a protein tyrosine kinase, *J. Immunol.* 154 (1995) 2838–2845.
- [434] L. Yao, J. Pan, H. Setiadi, K.D. Patel, R.P. McEver, Interleukin 4 or oncostatin M induces a prolonged increase in P-selectin mRNA and protein in human endothelial cells, *J. Exp. Med.* 184 (1996) 81–92.
- [435] T. Wierzbicki, S.M. Iqbal, S.L. Cuvelier, G. Awong, L.A. Tibbles, K.D. Patel, IL-4 primes human endothelial cells for secondary responses to histamine, *J. Leukoc. Biol.* 74 (2003) 420–427.
- [436] M. Goebeler, B. Schnarr, A. Toksoy, M. Kunz, E.B. Brocker, A. Duschl, et al., Interleukin-13 selectively induces monocyte chemoattractant protein-1 synthesis and secretion by human endothelial cells. Involvement of IL-4R alpha and Stat6 phosphorylation, *Immunology* 91 (1997) 450–457.
- [437] Y.W. Lee, B. Hennig, M. Toborek, Redox-regulated mechanisms of IL-4-induced MCP-1 expression in human vascular endothelial cells, *Am. J. Physiol. Heart Circ. Physiol.* 284 (2003) H185–H192.
- [438] N.C. Stein, C. Kreutzmann, S.P. Zimmermann, U. Niebergall, L. Hellmeyer, C. Goettsch, et al., Interleukin-4 and interleukin-13 stimulate the osteoclast inhibitor osteoprotegerin by human endothelial cells through the STAT6 pathway, *J. Bone Miner. Res.: Off. J. Am. Soc. Bone Miner. Res.* 23 (2008) 750–758.
- [439] N.J. Jordan, M.L. Watson, R.J. Williams, A.G. Roach, T. Yoshimura, J. Westwick, Chemokine production by human vascular smooth muscle cells: modulation by IL-13, *Br. J. Pharmacol.* 122 (1997) 749–757.
- [440] A.S. Gounni, V. Wellemans, J. Yang, F. Bellesort, K. Kassiri, S. Gangloff, et al., Human airway smooth muscle cells express the high affinity receptor for IgE (Fc epsilon RI): a critical role of Fc epsilon RI in human airway smooth muscle cell function, *J. Immunol.* 175 (2005) 2613–2621.
- [441] D.S. Faffe, T. Whitehead, P.E. Moore, S. Baraldo, L. Flynt, K. Bourgeois, et al., IL-13 and IL-4 promote TARC release in human airway smooth muscle cells: role of IL-4 receptor genotype, *Am. J. Physiol. Lung Cell. Mol. Physiol.* 285 (2003) L907–L914.
- [442] G. Martin, R.J. O'Connell, A.Z. Pietrzykowski, S.N. Treisman, M.F. Ethier, J.M. Madison, Interleukin-4 activates large-conductance, calcium-activated potassium (BKCa) channels in human airway smooth muscle cells, *Exp. Physiol.* 93 (2008) 908–918.
- [443] D.S. Faffe, L. Flynt, K. Bourgeois, R.A. Panettieri Jr., S.A. Shore, Interleukin-13 and interleukin-4 induce vascular endothelial growth factor release from airway smooth muscle cells: role of vascular endothelial growth factor genotype, *Am. J. Respir. Cell Mol. Biol.* 34 (2006) 213–218.
- [444] D.S. Faffe, L. Flynt, M. Mellema, P.E. Moore, E.S. Silverman, V. Subramaniam, et al., Oncostatin M causes eotaxin-1 release from airway smooth muscle: synergy with IL-4 and IL-13, *J. Allergy Clin. Immunol.* 115 (2005) 514–520.
- [445] Y. Chiba, S. Nakazawa, M. Todoroki, K. Shinozaki, H. Sakai, M. Misawa, Interleukin-13 augments bronchial smooth muscle contractility with an up-regulation of RhoA protein, *Am. J. Respir. Cell Mol. Biol.* 40 (2009) 159–167.
- [446] Y. Amrani, F. Syed, C. Huang, K. Li, V. Liu, D. Jain, et al., Expression and activation of the oxytocin receptor in airway smooth muscle cells: regulation by TNFalpha and IL-13, *Respir. Res.* 11 (2010) 104.

- [447] K. Parameswaran, K. Radford, A. Fanat, J. Stephen, C. Bonnans, B.D. Levy, et al., Modulation of human airway smooth muscle migration by lipid mediators and Th-2 cytokines, *Am. J. Respir. Cell Mol. Biol.* 37 (2007) 240–247.
- [448] B. Ochensberger, S. Rihs, T. Brunner, C.A. Dahinden, IgE-independent interleukin-4 expression and induction of a late phase of leukotriene C4 formation in human blood basophils, *Blood* 86 (1995) 4039–4049.
- [449] F.H. Falcone, C.A. Dahinden, B.F. Gibbs, T. Noll, U. Amon, H. Hebestreit, et al., Human basophils release interleukin-4 after stimulation with *Schistosoma mansoni* egg antigen, *Eur. J. Immunol.* 26 (1996) 1147–1155.
- [450] H. Li, T.C. Sim, R. Alam, IL-13 released by and localized in human basophils, *J. Immunol.* 156 (1996) 4833–4838.
- [451] M. Nonaka, R. Nonaka, K. Woolley, E. Adelroth, K. Miura, Y. Okhawara, et al., Distinct immunohistochemical localization of IL-4 in human inflamed airway tissues. IL-4 is localized to eosinophils in vivo and is released by peripheral blood eosinophils, *J. Immunol.* 155 (1995) 3234–3244.
- [452] R. Moqbel, S. Ying, J. Barkans, T.M. Newman, P. Kimmitt, M. Wakelin, et al., Identification of messenger RNA for IL-4 in human eosinophils with granule localization and release of the translated product, *J. Immunol.* 155 (1995) 4939–4947.
- [453] C. Bandeira-Melo, K. Sugiyama, L.J. Woods, P.F. Weller, Cutting edge: eotaxin elicits rapid vesicular transport-mediated release of preformed IL-4 from human eosinophils, *J. Immunol.* 166 (2001) 4813–4817.
- [454] R.C. Melo, L.A. Spencer, S.A. Perez, I. Ghiran, A.M. Dvorak, P.F. Weller, Human eosinophils secrete preformed, granule-stored interleukin-4 through distinct vesicular compartments, *Traffic (Copenhagen, Denmark)* 6 (2005) 1047–1057.
- [455] L.A. Spencer, R.C. Melo, S.A. Perez, S.P. Bafford, A.M. Dvorak, P.F. Weller, Cytokine receptor-mediated trafficking of preformed IL-4 in eosinophils identifies an innate immune mechanism of cytokine secretion, *Proc. Natl. Acad. Sci. USA* 103 (2006) 3333–3338.
- [456] G. Woerly, P. Lacy, A.B. Younes, N. Roger, S. Loiseau, R. Moqbel, et al., Human eosinophils express and release IL-13 following CD28-dependent activation, *J. Leukoc. Biol.* 72 (2002) 769–779.
- [457] P. Schmid-Grendelmeier, F. Altnauer, B. Fischer, C. Bizer, A. Straumann, G. Menz, et al., Eosinophils express functional IL-13 in eosinophilic inflammatory diseases, *J. Immunol.* 169 (2002) 1021–1027.
- [458] A.E. Elovic, H. Ohyama, A. Sauty, J. McBride, T. Tsuji, M. Nagai, et al., IL-4-dependent regulation of TGF- α and TGF- β 1 expression in human eosinophils, *J. Immunol.* 160 (1998) 6121–6127.
- [459] G.R. Dubois, R.C. Schweizer, C. Versluis, C.A. Bruijnzeel-Koomen, P.L. Bruijnzeel, Human eosinophils constitutively express a functional interleukin-4 receptor: interleukin-4-induced priming of chemotactic responses and induction of PI-3 kinase activity, *Am. J. Respir. Cell Mol. Biol.* 19 (1998) 691–699.
- [460] W. Luttmann, B. Knoechel, M. Foerster, H. Matthys, J.C. Virchow Jr., C. Kroegel, Activation of human eosinophils by IL-13. Induction of CD69 surface antigen, its relationship to messenger RNA expression, and promotion of cellular viability, *J. Immunol.* 157 (1996) 1678–1683.
- [461] E. Brandt, G. Woerly, A.B. Younes, S. Loiseau, M. Capron, IL-4 production by human polymorphonuclear neutrophils, *J. Leukoc. Biol.* 68 (2000) 125–130.
- [462] F. Chen, W. Wu, A. Millman, J.F. Craft, E. Chen, N. Patel, et al., Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion, *Nat. Immunol.* 15 (2014) 938–946.
- [463] C. Ratthe, M. Pelletier, S. Chiasson, D. Girard, Molecular mechanisms involved in interleukin-4-induced human neutrophils: expression and regulation of suppressor of cytokine signaling, *J. Leukoc. Biol.* 81 (2007) 1287–1296.
- [464] J. Ennaciri, D. Girard, IL-4R(α), a new member that associates with Syk kinase: implication in IL-4-induced human neutrophil functions, *J. Immunol.* 183 (2009) 5261–5269.
- [465] D. Girard, R. Paquin, A.D. Beaulieu, Responsiveness of human neutrophils to interleukin-4: induction of cytoskeletal rearrangements, de novo protein synthesis and delay of apoptosis, *Biochem. J.* 325 (Pt 1) (1997) 147–153.
- [466] D. Vercelli, H.H. Jabara, B.W. Lee, N. Woodland, R.S. Geha, D.Y. Leung, Human recombinant interleukin 4 induces Fc ϵ 2/CD23 on normal human monocytes, *J. Exp. Med.* 167 (1988) 1406–1416.
- [467] A.A. te Velde, J.P. Klomp, B.A. Yard, J.E. de Vries, C.G. Figdor, Modulation of phenotypic and functional properties of human peripheral blood monocytes by IL-4, *J. Immunol.* 140 (1988) 1548–1554.
- [468] M. Wieser, R. Bonifer, W. Oster, A. Lindemann, R. Mertelsmann, F. Herrmann, Interleukin-4 induces secretion of CSF for granulocytes and CSF for macrophages by peripheral blood monocytes, *Blood* 73 (1989) 1105–1108.
- [469] P.H. Hart, G.F. Vitti, D.R. Burgess, G.A. Whitty, D.S. Piccoli, J.A. Hamilton, Potential antiinflammatory effects of interleukin 4: suppression of human monocyte tumor necrosis factor α , interleukin 1, and prostaglandin E2, *Proc. Natl. Acad. Sci. USA* 86 (1989) 3803–3807.
- [470] L. Weiss, N. Haeflner-Cavaillon, M. Laude, J.M. Cavaillon, M.D. Kazatchkine, Human T cells and interleukin 4 inhibit the release of interleukin 1 induced by lipopolysaccharide in serum-free cultures of autologous monocytes, *Eur. J. Immunol.* 19 (1989) 1347–1350.
- [471] S. Sone, H. Yanagawa, Y. Nishioka, E. Orino, G. Bhaskaran, A. Nii, et al., Interleukin-4 as a potent down-regulator for human alveolar macrophages capable of producing tumour necrosis factor- α and interleukin-1, *Eur. Respir. J.* 5 (1992) 174–181.
- [472] P. Chanez, A.M. Vignola, N. Paul-Eugene, B. Dugas, P. Godard, F.B. Michel, et al., Modulation by interleukin-4 of cytokine release from mononuclear phagocytes in asthma, *J. Allergy Clin. Immunol.* 94 (1994) 997–1005.
- [473] G. Bhaskaran, A. Nii, S. Sone, T. Ogura, Differential effects of interleukin-4 on superoxide anion production by human alveolar macrophages stimulated with lipopolysaccharide and interferon- γ , *J. Leukoc. Biol.* 52 (1992) 218–223.
- [474] S. Lacraz, L. Nicod, B. Galve-de Rochemonteix, C. Baumberger, J.M. Dayer, H.G. Welgus, Suppression of metalloproteinase biosynthesis in human alveolar macrophages by interleukin-4, *J. Clin. Invest.* 90 (1992) 382–388.
- [475] M.L. Corcoran, W.G. Stetler-Stevenson, P.D. Brown, L.M. Wahl, Interleukin 4 inhibition of prostaglandin E2 synthesis blocks interstitial collagenase and 92-kDa type IV collagenase/gelatinase production by human monocytes, *J. Biol. Chem.* 267 (1992) 515–519.
- [476] B. Galve-de Rochemonteix, L.P. Nicod, R. Chicheportiche, S. Lacraz, C. Baumberger, J.M. Dayer, Regulation of interleukin-1 α , interleukin-1 α , and interleukin-1 β production by human alveolar macrophages with phorbol myristate acetate, lipopolysaccharide, and interleukin-4, *Am. J. Respir. Cell Mol. Biol.* 8 (1993) 160–168.
- [477] D.P. Andrew, M.S. Chang, J. McNinch, S.T. Wathen, M. Rihane, J. Tseng, et al., STCP-1 (MDC) CC chemokine acts specifically on chronically activated Th2 lymphocytes and is produced by monocytes on stimulation with Th2 cytokines IL-4 and IL-13, *J. Immunol.* 161 (1998) 5027–5038.
- [478] R. Bonicchi, F. Facchetti, S. Dusi, W. Luini, D. Lissandrini, M. Simmelink, et al., Induction of functional IL-8 receptors by IL-4 and IL-13 in human monocytes, *J. Immunol.* 164 (2000) 3862–3869.
- [479] B. Chen, S. Tsui, W.E. Boeglin, R.S. Douglas, A.R. Brash, T.J. Smith, Interleukin-4 induces 15-lipoxygenase-1 expression in human orbital fibroblasts from patients with Graves disease. Evidence for anatomic site-selective actions of Th2 cytokines, *J. Biol. Chem.* 281 (2006) 18296–18306.
- [480] M. Thivierge, J. Stankova, M. Rola-Pleszczynski, IL-13 and IL-4 up-regulate cysteinyl leukotriene 1 receptor expression in human monocytes and macrophages, *J. Immunol.* 167 (2001) 2855–2860.
- [481] H. Novak, A. Muller, N. Harre, C. Gunther, J.M. Carballido, M. Woisetschlager, CCL23 expression is induced by IL-4 in a STAT6-dependent fashion, *J. Immunol.* 178 (2007) 4335–4341.
- [482] M.O. Daines, G.K. Hershey, A novel mechanism by which interferon- γ can regulate interleukin (IL)-13 responses. Evidence for intracellular stores of IL-13 receptor α -2 and their rapid mobilization by interferon- γ , *J. Biol. Chem.* 277 (2002) 10387–10393.
- [483] N. Berkman, M. John, G. Roeseams, P. Jose, P.J. Barnes, K.F. Chung, Interleukin 13 inhibits macrophage inflammatory protein-1 α production from human alveolar macrophages and monocytes, *Am. J. Respir. Cell Mol. Biol.* 15 (1996) 382–389.
- [484] A. Berry, P. Balard, A. Coste, D. Olanier, C. Lagane, H. Authier, et al., IL-13 induces expression of CD36 in human monocytes through PPAR γ activation, *Eur. J. Immunol.* 37 (2007) 1642–1652.
- [485] S. Ryzhov, A.E. Goldstein, A. Matafonov, D. Zeng, I. Biaggioni, I. Feoktistov, Adenosine-activated mast cells induce IgE synthesis by B lymphocytes: an A2B-mediated process involving Th2 cytokines IL-4 and IL-13 with implications for asthma, *J. Immunol.* 172 (2004) 7726–7733.
- [486] P.R. Burd, W.C. Thompson, E.E. Max, F.C. Mills, Activated mast cells produce interleukin 13, *J. Exp. Med.* 181 (1995) 1373–1380.
- [487] T. Fushimi, H. Okayama, S. Shimura, H. Saitoh, K. Shirato, Dexamethasone suppresses gene expression and production of IL-13 by human mast cell line and lung mast cells, *J. Allergy Clin. Immunol.* 102 (1998) 134–142.
- [488] H. Toru, R. Pawankar, C. Ra, J. Yata, T. Nakahata, Human mast cells produce IL-13 by high-affinity IgE receptor cross-linking: enhanced IL-13 production by IL-4-primed human mast cells, *J. Allergy Clin. Immunol.* 102 (1998) 491–502.
- [489] S.A. Lee, S.M. Fitzgerald, S.K. Huang, C. Li, D.S. Chi, D.M. Milhorn, et al., Molecular regulation of interleukin-13 and monocyte chemoattractant protein-1 expression in human mast cells by interleukin-1 β , *Am. J. Respir. Cell Mol. Biol.* 31 (2004) 283–291.
- [490] F. Thienemann, B.M. Henz, M. Babina, Regulation of mast cell characteristics by cytokines: divergent effects of interleukin-4 on immature mast cell lines versus mature human skin mast cells, *Arch. Dermatol. Res.* 296 (2004) 134–138.
- [491] O. Akbari, J.L. Faul, E.G. Hoyte, G.J. Berry, J. Wahlstrom, M. Kronenberg, et al., CD4+ invariant T-cell-receptor+ natural killer T cells in bronchial asthma, *N. Engl. J. Med.* 354 (2006) 1117–1129.
- [492] J.U. Shim, Y.I. Koh, Increased Th2-like invariant natural killer T cells in peripheral blood from patients with asthma, *Allergy, Asthma Immunol. Res.* 6 (2014) 444–448.
- [493] H. Wei, J. Zhang, W. Xiao, J. Feng, R. Sun, Z. Tian, Involvement of human natural killer cells in asthma pathogenesis: natural killer 2 cells in type 2 cytokine predominance, *J. Allergy Clin. Immunol.* 115 (2005) 841–847.
- [494] T. Hoshino, R.T. Winkler-Pickett, A.T. Mason, J.R. Ortaldo, H.A. Young, IL-13 production by NK cells: IL-13-producing NK and T cells are present in vivo in the absence of IFN- γ , *J. Immunol.* 162 (1999) 51–59.
- [495] A. Nagler, L.L. Lanier, J.H. Phillips, The effects of IL-4 on human natural killer cells. A potent regulator of IL-2 activation and proliferation, *J. Immunol.* 141 (1988) 2349–2351.
- [496] T.A. Doherty, N. Khorram, S. Lund, A.K. Mehta, M. Croft, D.H. Broide, Lung type 2 innate lymphoid cells express cysteinyl leukotriene receptor 1, which regulates TH2 cytokine production, *J. Allergy Clin. Immunol.* 132 (2013) 205–213.
- [497] K.R. Bartemes, G.M. Kephart, S.J. Fox, H. Kita, Enhanced innate type 2 immune response in peripheral blood from patients with asthma, *J. Allergy Clin. Immunol.* 134 (2014). 671–678 e4.

- [498] D.P. Harris, S. Goodrich, K. Mohrs, M. Mohrs, F.E. Lund, Cutting edge: the development of IL-4-producing B cells (B effector 2 cells) is controlled by IL-4, IL-4 receptor alpha, and Th2 cells, *J. Immunol.* 175 (2005) 7103–7107.
- [499] O. Hajoui, R. Janani, M. Tulic, P. Joubert, T. Ronis, Q. Hamid, et al., Synthesis of IL-13 by human B lymphocytes: regulation and role in IgE production, *J. Allergy Clin. Immunol.* 114 (2004) 657–663.
- [500] T. Defrance, J.P. Aubry, F. Rousset, B. Vanbervliet, J.Y. Bonnefoy, N. Arai, et al., Human recombinant interleukin 4 induces Fc epsilon receptors (CD23) on normal human B lymphocytes, *J. Exp. Med.* 165 (1987) 1459–1467.
- [501] T. Defrance, B. Vanbervliet, J.P. Aubry, Y. Takebe, N. Arai, A. Miyajima, et al., B cell growth-promoting activity of recombinant human interleukin 4, *J. Immunol.* 139 (1987) 1135–1141.
- [502] J. Pene, F. Rousset, F. Briere, I. Chretien, J. Wideman, J.Y. Bonnefoy, et al., Interleukin 5 enhances interleukin 4-induced IgE production by normal human B cells. The role of soluble CD23 antigen, *Eur. J. Immunol.* 18 (1988) 929–935.
- [503] S. Fujieda, K. Zhang, A. Saxon, IL-4 plus CD40 monoclonal antibody induces human B cells gamma subclass-specific isotype switch: switching to gamma 1, gamma 3, and gamma 4, but not gamma 2, *J. Immunol.* 155 (1995) 2318–2328.
- [504] L.C. Mitchell, L.S. Davis, P.E. Lipsky, Promotion of human T lymphocyte proliferation by IL-4, *J. Immunol.* 142 (1989) 1548–1557.
- [505] R. Enelow, D.F. Baramki, L.C. Borish, Inhibition of effector T lymphocytes mediated through antagonism of IL-4, *J. Allergy Clin. Immunol.* 113 (2004) 560–562.
- [506] T. Jinquan, S. Quan, G. Feili, C.G. Larsen, K. Thestrup-Pedersen, Eotaxin activates T cells to chemotaxis and adhesion only if induced to express CCR3 by IL-2 together with IL-4, *J. Immunol.* 162 (1999) 4285–4292.
- [507] Y.T. Lin, C.T. Wang, J.H. Lee, C.Y. Chu, W.C. Tsao, Y.H. Yang, et al., Higher Bcl-2 levels decrease staphylococcal superantigen-induced apoptosis of CD4+ T cells in atopic dermatitis, *Allergy* 62 (2007) 520–526.
- [508] D.C. Newcomb, M.G. Boswell, W. Zhou, M.M. Huckabee, K. Goleniewska, C.M. Sevin, et al., Human TH17 cells express a functional IL-13 receptor and IL-13 attenuates IL-17A production, *J. Allergy Clin. Immunol.* 127 (2011). 1006–1013 e1–e4.
- [509] M. Vukmanovic-Stejic, B. Vyas, P. Gorak-Stolinska, A. Noble, D.M. Kemeny, Human Tc1 and Tc2/TC0 CD8 T-cell clones display distinct cell surface and functional phenotypes, *Blood* 95 (2000) 231–240.
- [510] N. Lee, S. You, M.S. Shin, W.W. Lee, K.S. Kang, S.H. Kim, et al., IL-6 receptor alpha defines effector memory CD8+ T cells producing Th2 cytokines and expanding in asthma, *Am. J. Respir. Crit. Care Med.* 190 (2014) 1383–1394.
- [511] A. Dakhama, M.L. Collins, H. Ohnishi, E. Goleva, D.Y. Leung, R. Alam, et al., IL-13-producing BLT1-positive CD8 cells are increased in asthma and are associated with airway obstruction, *Allergy* 68 (2013) 666–673.
- [512] S.L. Seneviratne, L. Jones, A.S. Bailey, R.V. Samuel, A.P. Black, G.S. Ogg, Interleukin-4 induced down-regulation of skin homing receptor expression by human viral-specific CD8 T cells may contribute to atopic risk of cutaneous infection, *Clin. Exp. Immunol.* 141 (2005) 107–115.
- [513] I. Bellinghausen, P. Brand, I. Bottcher, B. Klostermann, J. Knop, J. Saloga, Production of interleukin-13 by human dendritic cells after stimulation with protein allergens is a key factor for induction of T helper 2 cytokines and is associated with activation of signal transducer and activator of transcription-6, *Immunology* 108 (2003) 167–176.
- [514] E. Guenova, Y. Skabytska, W. Hoetzenecker, G. Weindl, K. Sauer, M. Tham, et al., IL-4 abrogates T(H)17 cell-mediated inflammation by selective silencing of IL-23 in antigen-presenting cells, *Proc. Natl. Acad. Sci. USA* 112 (2015) 2163–2168.
- [515] L. Piemonti, S. Bernasconi, W. Luini, Z. Trobonjaca, A. Minty, P. Allavena, et al., IL-13 supports differentiation of dendritic cells from circulating precursors in concert with GM-CSF, *Eur. Cytokine Netw.* 6 (1995) 245–252.
- [516] K. Sato, H. Nagayama, K. Tadokoro, T. Juji, T.A. Takahashi, Interleukin-13 is involved in functional maturation of human peripheral blood monocyte-derived dendritic cells, *Exp. Hematol.* 27 (1999) 326–336.
- [517] L. Aoudjehane, P. Podevin, O. Scatton, P. Jaffray, I. Dusanter-Fourt, G. Feldmann, et al., Interleukin-4 induces human hepatocyte apoptosis through a Fas-independent pathway, *FASEB J.: Off. Publ. Fed. Am. Soc. Exp. Biol.* 21 (2007) 1433–1444.
- [518] C. Gabay, B. Porter, D. Guenette, B. Billir, W.P. Arend, Interleukin-4 (IL-4) and IL-13 enhance the effect of IL-1beta on production of IL-1 receptor antagonist by human primary hepatocytes and hepatoma HepG2 cells: differential effect on C-reactive protein production, *Blood* 93 (1999) 1299–1307.
- [519] R. Sugimoto, M. Enjoji, M. Nakamuta, S. Ohta, M. Kohjima, M. Fukushima, et al., Effect of IL-4 and IL-13 on collagen production in cultured LI90 human hepatic stellate cells, *Liver Int.: Off. J. Int. Assoc. Study Liver* 25 (2005) 420–428.
- [520] M.I. de Moraes-Pinto, G.S. Vince, B.F. Flanagan, C.A. Hart, P.M. Johnson, Localization of IL-4 and IL-4 receptors in the human term placenta, decidua and amniochorionic membranes, *Immunology* 90 (1997) 87–94.
- [521] C.U. Henriques, G.E. Rice, M.H. Wong, K. Bendtzen, Immunolocalisation of interleukin-4 and interleukin-4 receptor in placenta and fetal membranes in association with pre-term labour and pre-eclampsia, *Gynecol. Obstet. Invest.* 46 (1998) 172–177.
- [522] G. Papacleovoulou, K. Hogg, K.S. Fegan, H.O. Critchley, S.G. Hillier, J.I. Mason, Regulation of 3beta-hydroxysteroid dehydrogenase type 1 and type 2 gene expression and function in the human ovarian surface epithelium by cytokines, *Mol. Hum. Reprod.* 15 (2009) 379–392.
- [523] H. Itoh, K. Nasu, A. Yuge, Y. Kawano, J. Yoshimatsu, H. Narahara, Interleukin-13 stimulates the secretion of vascular endothelial growth factor and soluble fms-like tyrosine kinase-1 by human oviductal epithelial cells, *Eur. J. Obstet. Gynecol. Reprod. Biol.* 133 (2007) 208–212.
- [524] N. Chagini, C. Ma, M. Roberts, R.S. Williams, B.A. Ripps, Differential expression of interleukins (IL) IL-13 and IL-15 throughout the menstrual cycle in endometrium of normal fertile women and women with recurrent spontaneous abortion, *J. Reprod. Immunol.* 56 (2002) 93–110.
- [525] K. Nasu, B. Sun, M. Nishida, J. Fukuda, H. Narahara, I. Miyakawa, Cultured human endometrial epithelial cells produce thymus and activation-regulated chemokine with stimulation of interleukin-4 and interleukin-13, *Fertil. Steril.* 82 (Suppl. 3) (2004) 1014–1018.
- [526] C.E. Juge-Aubry, E. Somme, R. Chicheportiche, D. Burger, A. Pernin, B. Cuenod-Pittet, et al., Regulatory effects of interleukin (IL)-1, interferon-beta, and IL-4 on the production of IL-1 receptor antagonist by human adipose tissue, *J. Clin. Endocrinol. Metab.* 89 (2004) 2652–2658.
- [527] Y. Nolan, F.O. Maher, D.S. Martin, R.M. Clarke, M.T. Brady, A.E. Bolton, et al., Role of interleukin-4 in regulation of age-related inflammatory changes in the hippocampus, *J. Biol. Chem.* 280 (2005) 9354–9362.
- [528] B.E. Morrison, M.C. Marcondes, D.K. Nomura, M. Sanchez-Alavez, A. Sanchez-Gonzalez, I. Saar, et al., Cutting edge: IL-13Ralpha1 expression in dopaminergic neurons contributes to their oxidative stress-mediated loss following chronic peripheral treatment with lipopolysaccharide, *J. Immunol.* 189 (2012) 5498–5502.
- [529] W.H. Shin, D.Y. Lee, K.W. Park, S.U. Kim, M.S. Yang, E.H. Joe, et al., Microglia expressing interleukin-13 undergo cell death and contribute to neuronal survival in vivo, *Glia* 46 (2004) 142–152.
- [530] G. Sebire, J.F. Delfraissy, J. Demotes-Mainard, A. Oteifeh, D. Emilie, M. Tardieu, Interleukin-13 and interleukin-4 act as interleukin-6 inducers in human microglial cells, *Cytokine* 8 (1996) 636–641.
- [531] B.P. Barna, M.L. Estes, J. Pettay, K. Iwasaki, P. Zhou, G.H. Barnett, Human astrocyte growth regulation: interleukin-4 sensitivity and receptor expression, *J. Neuroimmunol.* 60 (1995) 75–81.
- [532] S.J. Millward-Sadler, M.O. Wright, H. Lee, K. Nishida, H. Caldwell, G. Nuki, et al., Integrin-regulated secretion of interleukin 4: a novel pathway of mechanotransduction in human articular chondrocytes, *J. Cell Biol.* 145 (1999) 183–189.
- [533] B. Relic, J. Guicheux, F. Mezin, E. Lubberts, D. Togninalli, I. Garcia, et al., IL-4 and IL-13, but not IL-10, protect human synoviocytes from apoptosis, *J. Immunol.* 166 (2001) 2775–2782.
- [534] J.P. Courneya, I.G. Luzina, C.B. Zeller, J.F. Rasmussen, A. Bocharov, L.C. Schon, et al., Interleukins 4 and 13 modulate gene expression and promote proliferation of primary human tenocytes, *Fibrogenesis Tissue Repair* 3 (2010) 9.
- [535] O. Nemoto, H. Yamada, T. Kikuchi, M. Shinmei, K. Obata, H. Sato, et al., Suppression of matrix metalloproteinase-3 synthesis by interleukin-4 in human articular chondrocytes, *J. Rheumatol.* 24 (1997) 1774–1779.
- [536] L.S. Mangashetti, S.M. Khapli, M.R. Wani, IL-4 inhibits bone-resorbing activity of mature osteoclasts by affecting NF-kappa B and Ca²⁺ signaling, *J. Immunol.* 175 (2005) 917–925.
- [537] J.L. Moreno, M. Kaczmarek, A.D. Keegan, M. Tondravi, IL-4 suppresses osteoclast development and mature osteoclast function by a STAT6-dependent mechanism: irreversible inhibition of the differentiation program activated by RANKL, *Blood* 102 (2003) 1078–1086.
- [538] A. Frost, K.B. Jonsson, H. Brandstrom, S. Ljunghall, O. Nilsson, O. Ljunggren, Interleukin (IL)-13 and IL-4 inhibit proliferation and stimulate IL-6 formation in human osteoblasts: evidence for involvement of receptor subunits IL-13R, IL-13Ralpha, and IL-4Ralpha, *Bone* 28 (2001) 268–274.
- [539] L. Rifas, S.L. Cheng, IL-13 regulates vascular cell adhesion molecule-1 expression in human osteoblasts, *J. Cell. Biochem.* 89 (2003) 213–219.
- [540] A. Frost, K.B. Jonsson, H. Brandstrom, C. Ohlsson, S. Ljunghall, O. Ljunggren, Interleukin-13 inhibits cell proliferation and stimulates interleukin-6 formation in isolated human osteoblasts, *J. Clin. Endocrinol. Metab.* 83 (1998) 3285–3289.