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Strategies for targeting T-cells in allergic diseases and asthma

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Abstract

T helper (Th) 2 lymphocytes play a crucial role in the initiation, progression and persistence of allergic diseases, including asthma. Drugs that interfere with the activation of T-cells or more selectively Th2-specific signaling molecules and drugs that prevent the selective migration into lung tissue are promising novel strategies for the treatment of allergic asthma. Although the mainstay asthma therapy of inhaled glucocorticoids is rather effective, targeting Th2 cells may be an important alternative in childhood. Regulatory T-cells (Treg cells) have a physiological role in protection of unwanted immune responses to auto-antigens and allergens. Literature data indicate that an imbalance between Th2 and Treg cells may underlie development and disease expression of allergic asthma. Drugs or immunotherapies that stimulate these counter-Treg cells may limit aberrant Th2 responses leading to suppression of symptoms. Furthermore, these types of treatments may offer the perspective of disease modification and long-term relief of symptoms.
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Abbreviations: AHR, airway hyperreactivity; AP, activator protein; APC, antigen-presenting cell; BAL, bronchoalveolar lavage; cAMP, cyclic adenosine monophosphate; CBP, CREB-binding protein; CpG DNA, CpG-containing immunostimulatory deoxyribonucleic acid; CsA, cyclosporin A; CTLA4, cytotoxic T lymphocyte antigen 4; DC, dendritic cell; ERK, extracellular-regulated kinase; GR, glucocorticoid receptor; GRE, glucocorticoid response elements; ICAM, intercellular adhesion molecule; Ig, immunoglobulin; IL, interleukin; ITAM, immunoreceptor tyrosine-based activation motif; JNK, Jun N-terminal kinase; LFA, leukocyte function-associated antigen; MAPK, mitogen-activated kinase; NF-AT, nuclear factor for activated T-cells; PAMP, pathogen-associated molecular patterns; PBMC, peripheral blood mononuclear cells; PDE, phosphodiesterase; PGD2, prostaglandin D2; PI3-K, phosphoinositide-3 phosphate; SAPK, stress activated protein kinase; STAT, signal transducer and activator of transcription; TARC, thymus and activation-regulated chemokine; TCR, T-cell receptor; TGF-β, transforming growth factor β; Th, T helper; TLR, toll-like receptor; Treg cells, regulatory T-cell; VCAM, vascular cell adhesion molecule; VLA, very late antigen.

Contents

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1. Introduction

High serum levels of immunoglobulin E (IgE) antibodies to common environmental allergens like house-dust or pollen is a key determinant of allergic diseases like allergic asthma, allergic rhinitis and atopic dermatitis. IgE mediates the type-I immediate hypersensitivity reaction that is characterized by crosslinking of receptor-bound IgE on mast cells with allergen, inducing the release of preformed and newly generated mediators that elicit the symptoms of allergic disease. The prevalence of allergic diseases is very high and has shown a considerable increase during the last decades, especially in children, although this increase appears to level off (van Schayck & Smit, 2005). Allergic rhinitis alone affects more than 155 million people worldwide and over 80 million people in Europe have some form of allergic disease. Asthma is one of the most common chronic diseases with over 300 million people worldwide (GINA report 2004). While approximately 90% of children with asthma are allergic, only 50–60% of adult asthmatics display elevated serum levels of allergen-specific IgE. Asthma is further characterized by reversible airway obstruction, chronic eosinophilic airway inflammation, airway remodelling, mucus hypersecretion, and airway hyperrespon-siveness (AHR) to bronchospasmogenic stimuli. T-helper (Th) 2 lymphocytes play a critical role in the initiation, progression and persistence of allergic diseases, asthma included. Initially, a disturbed balance between Th1- and Th2-mediated immune responses has been postulated to underlie aberrant Th2 reactions to harmless inhaled allergens. Indeed, allergen-specific T-cell clones isolated from the blood of allergic individuals express a typical Th2 cytokine profile secreting interleukin (IL)-4, IL-5 and minimal IFN-γ and IL-2, whereas those clones from non-atopic individuals displayed a Th1 profile (Kapsenberg et al., 1992). Furthermore, allergic asthma is associated with expres-sion of IL-3, IL-4, IL-5 and GM-CSF in bronchoalveolar cells, strongly supporting Th2 activation (Robinson et al., 1992). Nowadays, Th2-type cytokines Il-4, IL-5, IL-13 are known to be critical for IgE production, airway eosinophilia, mucus hypersecretion and non-specific airway hyperreactivity (AHR). However, it appears that susceptibility to allergic diseases cannot solely be explained by an imbalance between Th1 and Th2 responses (Wills-Karp et al., 2001; Herrick & Bottomly, 2003). Recently, an important immunoregulatory role for regulatory T-cells (Treg cells) has been put forward, capable of suppressing both Th1- and Th2-mediated adaptive immune responses (van Oosterhout & Bloksma, 2005). Targeting these different T-cell subsets for the treatment of allergic asthma is an interesting strategy that has not yet been widely explored. Interestingly, some T-cell-directed therapies harbour the potential to induce long-lasting suppression or even complete remission of disease.

2. Modulation of T-cell receptor-induced signal transduction during T-cell activation

Activation of T-cells is initiated by processed antigen-derived peptides presented by antigen-presenting cells (APC) to the T-cell receptor (TCR)/CD3 complex. An accessory signal provided by co-stimulatory molecules on APC leads to full T-cell activation and this prevents the induction of T-cell tolerance (T-cell unresponsiveness, also called anergy), which normally occurs when T-cells are stimulated by antigen-derived peptide in absence of an appropriate accessory signal. The most potent accessory signal is provided by CD80/CD86, which interacts with CD28 on the T-cell. TCR/CD3 protein complex activation induces a cascade of phosphorylation reactions. First, non-receptor tyrosine kinases, for example, the Src family kinases Lck and Fyn, phosphorylate immunoreceptor tyrosine-based activation motifs (ITAM) located in the CD3 complex (Pitcher and van Oers, 2003), which serve as a docking site for downstream adaptor molecules containing SH2 and phospho-tyrosine domains. This induces recruitment of Syk family tyrosine kinases and activation of adaptor molecules, leading to activation of more downstream signaling molecules, for example, phospholipase C and phosphoinositide-3 phosphate kinase (PI3-K) (Nel, 2002). These molecules can activate multiple signaling cascades, including the Ca^{2+} mobilization/calmodulin pathway, the mitogen-activated kinases (MAPK) extracellular-regulated kinase (ERK) pathway, Jun N-terminal kinase (JNK)/stress activated protein kinase (SAPK) pathway and the p38/Mpk2 MAPK pathway, finally resulting in activation of the transcription factors, nuclear factor for activated T-cells (NF-AT) and activator protein (AP)-1. NF-AT and AP-1 can bind to the promoter of many T-cell cytokine genes and enhance their transcription. Full activation of AP-1 and NF-AT requires co-stimulation of the TCR-induced signal by CD28. Similar to TCR/CD3 signaling, CD28 signaling is mediated by tyrosine kinases (e.g. Lck and Ltk) and subsequent

activation of the PI3K, JNK and Rac-1/p38 MAPK pathways (Zhang et al., 2001). In addition to the co-stimulatory role of CD28, CD28-mediated activation of PI3-kinase is thought to induce downregulation of transcription factors involved in the suppression of T-cell activity. This has for instance been described for the expression of cyclin-dependent kinase inhibitor p27[kip], a factor that is involved in tolerance induction (Boussiotis et al., 2000; Appleman et al., 2002; Heijink et al., 2003; Kubsch et al., 2003).

2.1. Inhibition of signal transduction in T-cells

Many novel strategies of asthma therapy are directed towards the inhibition of TCR/CD3-induced pathways. Targeting of the signal transduction pathways involved in the transcription of cytokines genes, in particular Th2-type cytokines, is of interest because of the central role of these cytokines in role in IgE synthesis, mucus production, activation and attraction of eosinophils and airway hyperresponsiveness. It is important to note that the kinase signaling pathways described below not only mediate TCR activation, but also activation of B cell receptor signaling, FcεRI activation, cytokine receptors, G protein-coupled receptors and receptor tyrosine kinases.

Activation of non-receptor tysrosine kinase Syk is involved TCR signaling and inhibition of this molecule has potential interest in the treatment of asthma. Syk inhibitor BAY61-3606 as well as Syk-selective oligonucleotides have been demonstrated to reduce airway eosinophilia in a rat model of acute asthma (Stenton et al., 2002; Yamamoto et al., 2003; Ulanova et al., 2005). In addition, inhibition of Lyn, a tyrosine kinase that acts upstream of Syk, resulted in a reduction of eosinophilic inflammation in a murine model of asthma (Adachi et al., 1999). Inhibitors of more downstream molecules of TCR signaling, that is, the PI3-K inhibitor LY294002, p38 MAPK inhibitor SB203580 and ERK-1/2 inhibitor PD98059 may also have relevant effects for the treatment of asthma. All these inhibitors reduce α-CD3/α-CD28-induced IL-5 secretion in freshly isolated human T-cells (Heijink et al., 2002). In addition, the use of LY924002 and ERK inhibitor U0126 has been demonstrated to reduce airway infiltration of inflammatory cells, IL-4, IL-5 and IL-13 production and airway hyperresponsiveness in mouse models of asthma (Kwak et al., 2003; Duan et al., 2004; Chialda et al., 2005; Duan et al., 2005). Targeting of JNK by selective inhibitor SP600125 has revealed anti-inflammatory effects in a rat model of asthma (Eynott et al., 2004; Chialda et al., 2005). The drugs SB203850, SB239063 and RWJ67657 (Barnes, 2004) block activity of p38 and are of particular interest in the treatment of asthma, given their potential preference to inhibit Th2-type cytokines (Schafer et al., 1999). Although p38 inhibitor SB203580 antagonized the beneficial effect of ERK-1/2 inhibitor U0126 on lung inflammation and cytokine mRNA levels in bronchoalveolar lavage (BAL) in a murine model of asthma (Chialda et al., 2005), p38 inhibition has also been described to reduce airway inflammation in another mouse study (Underwood et al., 2002). In addition, p38-selective antisense oligonucleotides showed significant beneficial effects in mice, through reduction

of airway inflammatory cell infiltration, IL-4, IL-5 and IL-13 production, mucus secretion and airway hyperresponsiveness (Duan et al., 2005). Several p38 MAP kinase inhibitors are now in Phase II development (Barnes, 2004). Because of the wide distribution of the above described signaling cascades in the immune system, the safety of these selective inhibitors is still of concern.

T-cell selective immunosuppressive drugs have also proven beneficial in asthma. For instance, cyclosporin A (CsA), which blocks activation of NF-AT by inhibiting its dephosphorylation, has strong anti-inflammatory effects and inhibits IL-5 production in peripheral blood mononuclear cells (PBMC) of asthma patients (Mori et al., 1995). CsA has established clinical efficacy in improving lung function in chronic, severe, glucocorticoid-dependent asthma (Powell et al., 2001). FK506, which also inhibits dephosphorylation of NF-AT, has been described to reduce asthma symptoms in a guinea pig model of asthma (Fukuda et al., 1991). However, both CsA and FK506 have serious side effects, including nephrotoxicity, which limits their use for chronic asthma therapy.

2.2. Effects of glucocorticoids and cyclic AMP elevating drugs

At the moment, glucocorticoids are the mainstay of asthma therapy. They have broad anti-inflammatory effects, including suppression of cytokine genes. One of the mechanisms by which this is mediated is the binding to elements in cytokine gene promoters that are recognized by the NF-AT/AP-1 complex. Repression of the IL-5 gene has been shown to involve recruitment of the glucocorticoid receptor (GR) to the NF-AT and AP-1 binding site (Jee et al., 2005). In addition, activated GR can bind to glucocorticoid response elements (GRE) in the promoter of several genes and regulate their expression. Another way of GR to modulate gene transcription is by competitive binding to cofactor CREB-binding protein (CBP)/p300, which is also involved in AP-1-dependent transcription. GR binding to CBP results in histone deacetylation, leading to restricted access of transcription factors to promoter regions of proinflammatory genes (Popescu, 2003). Histone deacetylation is at least in part involved in the repression of transcription of the IL-5 gene by glucocorticoids (Jee et al., 2005). In contrast, however, it has recently been demonstrated that histone deacetylase trichostatin A has T-cell suppressive effects and reduces airway inflammation in a mouse model of asthma by reducing T-cell infiltration, IL-4, IL-5 and IgE levels in BAL fluid (Choi et al., 2005). In addition to histone acetylase inhibition, the upregulatory effect that is exerted by GR on β_2-adrenoreceptor (β_2-AR) expression might be an interesting feature of corticosteroids with respect to the inhibition of T-cell activity. β_2-AR activation results in cyclic adenosine monophosphate (cAMP) production, a 2nd messenger with many anti-inflammatory effects. Although inhaled β_2-agonists have no proven efficacy in reducing airway inflammation in asthma (Caramori & Adcock, 2003), inhibitory effects of β_2-agonists have been observed on TCR/CD28-dependent signaling pathways, Th2-type cytokine production and Th2 cell migration (Staples et al., 2001; Heijink et al., 2004;

Loza et al., 2005). Possibly, the inability of β_2-agonists to efficiently inhibit airway inflammation in asthma is a consequence of the allergen-induced desensitization of the β_2-adrenergic receptor on Th2 cells that has been observed in asthma (Meurs et al., 1982; Heijink et al., 2004). Improvement of β_2-adrenergic function by glucocorticoid treatment may enhance the anti-inflammatory effects of β_2-agonists. Similar to β_2-agonists, phosphodiesterase (PDE) 4 inhibitors increase cAMP accumulation and exert anti-inflammatory effects, including inhibition of Th2 cytokine production (Staples et al., 2001; Heijink et al., 2003). There are now emerging preliminary data on the beneficial effect of PDE4 inhibitors on lung function in asthma patients (Lipworth, 2005). Also the non-selective PDE inhibitor Theophylline is used for the treatment and has been described to reduce the number of IL4$^+$ and IL-5$^+$ cells in bronchial biopsies of asthma patients (Djukanovic et al., 1995; Finnerty et al., 1996). Similar to glucocorticoids, Theophylline has been described to exert anti-asthma effects by increasing the activation of histone deacety-lase, which is subsequently recruited by glucocorticoids to suppress inflammatory genes (Ito et al., 2002).

Together, therapies directed towards antigen activation of T-cells and downstream signal transduction pathways may be promising, although the broad immunologic activity might limit their use in some cases.

3. Modulation of T-cell differentiation into functional subsets and expression of Th2 specific transcription factors

While NF-AT and AP-1 are involved in the expression of both Th1 and Th2 cytokines, the production of a restricted Th1 or Th2 cytokine pattern requires expression of specific transcription factors. Differentiation of uncommitted Th cells into Th1 or Th2 cells is induced when T-cells undergo cell cycle progression a specific cytokine environment. The best characterized cytokine to induce Th2 differentiation is IL-4, while IL-12 is well known to induce differentiation towards a Th1 phenotype. Specific transcription factors induced by the Th1- and Th2-directing cytokines IL-12 and IL-4 are signal transducer and activator of transcription (STAT) 4 and STAT6, respectively. STAT6 is essential for the development of Th2 cells in response to IL-4 and has been described to negatively regulate a Th1-specific IL-4 silencer (Kubo et al., 1997). Other transcription factors that are selective for differentiated Th2 cells and Th2 clones are NF-IL-6 (C/EBP-β), c-Maf and GATA-3. GATA-3 may be a key regulator of Th2 cytokine production. GATA-3 expression is enhanced in polarized Th2 cells and downregulated in Th1 cells. Moreover, forced expression of GATA-3 in polarized Th1 cells is sufficient to initiate Th2 cytokine expression (Zhang et al., 1997). GATA-3 is thought to play an important role in the expression of Th2 cytokines in asthma. Enhanced expression of GATA-3 has been observed in the airways of asthma patients, with a further increase upon segmental allergen challenge (Nakamura et al., 1999; Erpenbeck et al., 2003). A Th1-specific transcription factor is T-bet, which may be key in the development of Th1

cells, since T-cells from T-bet$^{-/-}$ mice show defective IFN-γ production. Vice versa, retroviral expression of T-bet has been described to suppress expression of the Th2-type cytokines IL-4 and IL-5 (Szabo et al., 2000).

3.1. Inhibition of Th2 specific effector molecules

Modulating the expression of Th1-, Th2- or Treg-specific transcription factors may become an important tool in the treatment of Th1- or Th2-deviated immune responses, including asthma. New asthma drugs that act on Th2 effector molecules include peroxisome proliferator-activated receptor (PPAR) agonists (e.g. cyclopentenone prostaglandins and thiazolidine-diones) and antisense oligonucleotides (Popescu, 2003). Most studies are directed towards inhibition of Th2-specific transcription factor GATA-3. In a murine model of asthma, PPAR-treatment downregulated expression of GATA-3, leading to decreased antigen-induced airway hyperresponsiveness, lung inflammation, eosinophilia, cytokine production as well as serum levels of antigen-specific IgE (Woerly et al., 2003). In a similar model, treatment with PPAR agonists inhibited GATA-3 and decreased Th2-driven IgE production (Woerly et al., 2003). In addition, PPARγ ligands have been described to reduce IL-5 production in in vitro activated T-cells (Mueller et al., 2003). Both PPAR agonists and glucocorticoids may exert their anti-inflammatory effects through inhibition of histone acetylation (Nie et al., 2005) and similar to PPAR, GR activation has been described to repress IL-5 transcription by histone deacetylase activity and inhibition of GATA-3 transcriptional activation (Jee et al., 2005). Also, treatment with antisense oligonucleotides to GATA-3 has been reported to reduce Th2 cytokine production, lung inflammation and airway hyperresponsiveness in OVA-sensitized mice (Finotto et al., 2001). These data indicate that drugs acting on GATA-3 are promising in the treatment of asthma. In addition, a thiadiazole inhibitor of the Th2-specific transcription factor STAT6 may be a potential drug for asthma treatment, as it has been described in vitro to repress IL-4-induced genes (Popescu, 2003). No data on selective inhibitors for Th2-specific transcription factor c-Maf have been published. Although the inhibition of Th2 activity, in particular inhibition of GATA-3, may be an important tool to relieve asthma symptoms and even prevent the disease, risks to develop Th1-mediated immune diseases, such as Crohn's disease and autoimmune diseases should be considered.

4. Targeting regulatory T-cells

With the initial discovery of Th1 and Th2 cells in the mouse that exert mutual inhibitory effects (Mosmann & Coffman, 1989), an imbalance between these 2 arms of the immune response has been postulated to underlie both Th1-mediated autoimmune diseases as well as Th2-mediated allergic diseases and asthma (Kapsenberg et al., 1992; Romagnani, 1992). The balance between Th1 and Th2 cells as an immunoregulatory system to control immune responses to self or foreign antigens, however, appears insufficient to explain many experimental observations (Wills-Karp et al., 2001; Gor et al., 2003; Herrick

& Bottomly, 2003; van Oosterhout & Motta, 2005). CD4+ Treg cells are crucial immunoregulatory cells that suppress Th1- and Th2-mediated adaptive immune responses. Treg cells are subdivided in naturally occurring Treg cells and adaptive Treg cells, that are postulated to prevent immune responses against self-antigens and adaptive immune responses, respectively (Bluestone & Abbas, 2003; Sakaguchi, 2005). Adaptive Treg cells are further subdivided into type-1 regulatory T-cells (Tr1 cells) and T-helper type-3 cells (Th3 cells) that mediate suppression via the cytokines IL-10 and transforming growth factor β (TGF-β), respectively. Natural Treg cells, unlike adaptive Treg cells, exert their immunosuppressive effects through T-cell:T-cell/APC contact, but the exact molecular mechanism of suppression has remained incompletely understood (Bluestone & Tang, 2005). As previously reviewed in detail (Hawrylowicz, 2005; van Oosterhout & Bloksma, 2005), literature data indicate that different Treg cell subsets interfere with the development of asthma and other allergic diseases at different stages, like allergic sensitisation, progression to established allergic disease and asthma and severity and persistence of disease.

4.1. Drugs that stimulate regulatory T-cells

The transcription factor FOXP3 appears to be selectively expressed by natural Treg cells both in mice and humans and is a master regulatory gene for the development and function of natural Treg cells (Fontenot et al., 2003; Hori et al., 2003; Walker et al., 2003; Yagi et al., 2004). This makes FOXP3 an interesting therapeutic target protein and may open novel therapeutic strategies aimed at the induction of FOXP3 to convert conventional CD4+ T-cells into natural Treg cells with the ultimate goal to reverse aberrant Th2-mediated allergic asthmatic responses (Fig. 1).

As mentioned above, glucocorticoids are widely used in the treatment of allergic asthma for their potent anti-inflammatory effect. Interestingly, glucocorticoids potentiate the suppressive effects of natural Treg cells on allergen-stimulated conventional (CD4$^+$ CD25$^-$) T-cells from atopic as well as non-atopic donors (Dao Nguyen & Robinson, 2004). The glucocorticoid-induced potentiation of suppressive activity was mediated by enhanced IL-10 production. This mechanism may also be operative in vivo, since Karagiannidis et al. (2004) demonstrated that inhalation or systemic glucocorticoid treatment of asthma patients increased the levels of FOXP3 as well as IL-10 mRNA in peripheral blood CD4+ T-cells. In addition, synergistic effects of glucocorticoids and β$_2$-adrenoceptor agonists on IL-10 secretion by T-cells have been demonstrated (Peek et al., 2005). Furthermore, glucocorticoids not only enhance IL-10 production in T lymphocytes but also generate new IL-10 producing Treg cells when given in combination with the active form of vitamin D3, 1,25(OH$_2$) vitamin D3 (Barrat et al., 2002). Recently, an interesting interaction between glucocorticoids and 1,25(OH$_2$) vitamin D3 has been shown (Xystrakis et al., 2005). The failure of T lymphocytes from steroid-resistant asthma patients to upregulate IL-10 production by glucocorticoids in vitro could be restored by 1,25(OH$_2$) vitamin D3 leading to levels of IL-10 production comparable to those observed in steroid-sensitive asthma patients.

Altogether, the data demonstrate that glucocorticoid treatment either alone or in combination with β$_2$-agonists or 1,25 (OH$_2$) vitamin D3 potentiates the immunosuppressive effects of Treg cells by increasing IL-10 production and/or FOXP3 expression. Besides glucocorticoids, other hormones, including estrogen and dehydroepiandrosterone and the immunosuppressant rapamycin, can induce FOXP3 in T lymphocytes (Polanczyk et al., 2004; Battaglia et al., 2005; Coenen et al., 2005; Coles et al., 2005). It remains to be established whether
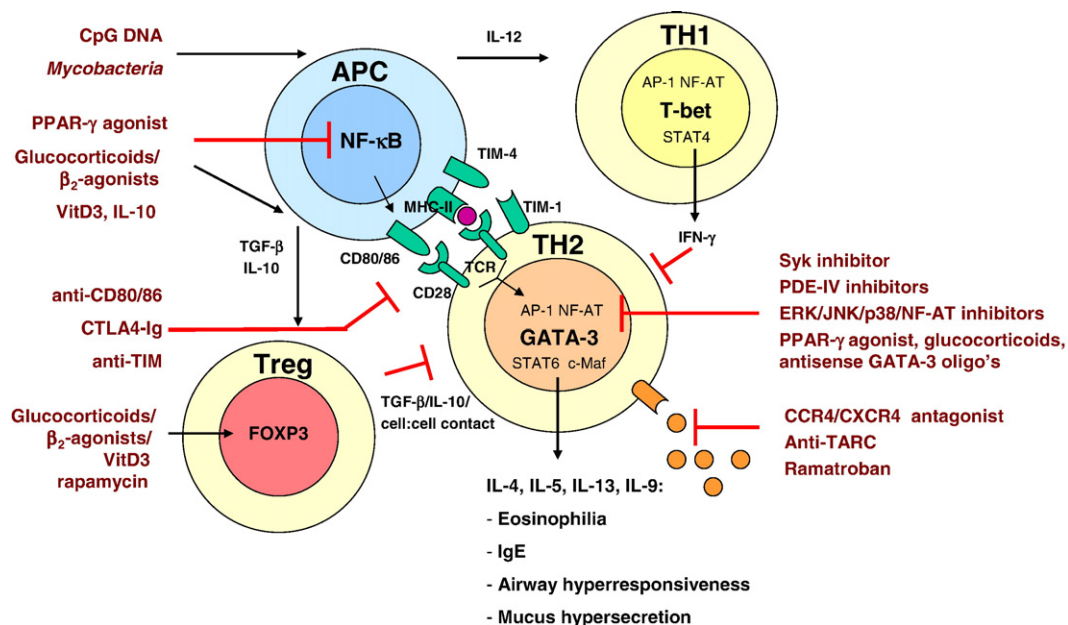

Fig. 1. Overview of therapeutic strategies to limit Th2 activity in allergic diseases, including asthma. Drugs can directly inhibit Th2 effector function or modulate APC function. Otherwise, drugs may act to promote Th1 or Treg cell activities. See text for further details.

failure of different Treg cell subsets in allergic asthma can be restored by these agents. Nonetheless, novel therapeutic strategies aiming at transient or stable induction of Treg cell subsets or potentiation of their immunosuppressive capacities may offer new perspectives for the treatment of allergic asthma.

5. Targeting T-cell costimulation

As described above, CD4+ T-cells require 2 independent signals for optimal activation, one through the TCR provided by engagement of the peptide-MHC class II complex on APC and a second costimulatory signal. Although various receptor-ligand pairs for T-cell costimulation have been identified, the CD28 pathway is crucial for primary activation of naive T-cells (Riley & June, 2005). CD28 is constitutively present on the cell-surface of T-cells. The 2 ligands of CD28, CD80 and CD86, are expressed by APCs. On most APC populations, CD86 is expressed constitutively at low levels and is rapidly upregulated upon APC activation, whereas CD80 is inducibly expressed later after activation. CD28 signals promote T-cell activation by augmenting and sustaining T-cell responses initiated by TCR signaling. Blockade of CD28 during activation of naive T-cells renders these cells anergic to secondary activation, even when restimulated in the presence of CD28 ligation. Furthermore, costimulation with CD28 has been described to prevent the induction of Foxp3 expression and suppressive function of Tregs in mice (Fu et al., 2004). Thus, prevention of CD28 costimulation may contribute to tolerance induction and lead to suppressed cytokine expression in T-cells. Another receptor for the CD80 and CD86 is called cytotoxic T lymphocyte antigen 4 (CTLA4; CD152) and delivers inhibitory signals. In contrast to CD28, CTLA4 is only expressed on activated T-cells and is a powerful negative regulator of T-cell activation. Thus, CD28 and CTLA4 compete for the same ligands, CD80 and CD86, but have counterregulatory effects on T-cell activation.

In addition to the CD28 pathway, many different receptor-ligand pairs of T-cell co-stimulation have been identified and are implicated in mouse models of allergic asthma (Deurloo & van Oosterhout, 2004). These costimulatory interactions can be divided into stimulatory signals for T lymphocytes including inducible costimulator (ICOS):ICOS ligand, OX40:OX40 ligand and CD40:CD40 ligand and inhibitory signals including programmed death (PD)-1:PD-1 ligand-1 and -2 and B and T lymphocyte attenuator (BTLA):herpes virus entry mediator (HVEM) (Deurloo & van Oosterhout, 2004; Sedy et al., 2005). However, the CD28 pathway is the only pathway that is currently therapeutically targeted in the clinic and is further discussed in detail (Riley & June, 2005).

5.1. CTLA4-Ig

CTLA4-Ig is a chimeric fusion protein that consists of the extracellular domain of CTLA4 and the heavy-chain constant region of IgG. CTLA4 has a higher affinity for CD80/CD86 when compared to CD28, which makes CTLA4-Ig a potent inhibitor of CD28-mediated T-cell costimulation by blocking of CD80/CD86 on APC. CTLA4-Ig (Abatacept, Orencia®) is currently in clinical development for the treatment of rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus, which are also immunological disorders maintained by T-cell activation (Kremer et al., 2005). Moreover, a variant of CTLA4-Ig (Belatacept®) has been developed with 10-fold higher binding avidity for CD80/CD86 as the standard CTLA4-Ig (Larsen et al., 2005). So far, both compounds have shown little apparent toxicity and exhibit substantial clinical activity in these autoimmune diseases as well as renal transplant rejection (Riley & June, 2005).

5.2. CTLA4-Ig in allergic asthma

The therapeutic potential of CTLA4-Ig in the development of Th2-dominated allergic inflammatory responses and AHR in mice has been unequivocally demonstrated (Tsuyuki et al., 1997; van Oosterhout et al., 1997; Keane-Myers et al., 1998). In all of these studies decreased Th2 cytokine production was observed in vivo as shown in BAL fluid or in vitro in supernatants of antigen re-stimulated lung T-cells. The importance of the CD28 costimulatory pathway in allergic asthma has been studied in vitro using PBMC, BAL T-cells and bronchial tissue explants. Larche and colleagues (Larche et al., 1998) demonstrated that, in contrast to peripheral blood CD4+ T-cell lines, allergen-induced proliferation and IL-5 production of PBMC were inhibited by CTLA4-Ig. Similarly, van Neerven et al. (1998) demonstrated that allergen- and antigen-specific T-cells of allergic patients and non-allergic control persons are equally dependent on costimulation via the CD28 pathway for their proliferation and cytokine mRNA production. The group of Holgate compared the role of CD28 costimulation in allergen-induced IL-5 production between PBMC cultures and ex vivo bronchial explants. CTLA4-Ig inhibited allergen-induced IL5 production by bronchial tissue explants from mild atopic asthmatic subjects (Jaffar et al., 1999), but not by those of subjects with moderately severe asthma (Lordan et al., 2001). In contrast, blocking of CD28 costimulation by CTLA4-Ig effectively inhibited allergen-induced IL-5 production in PBMC cultures from the same subjects. In agreement herewith, the suppressive effects of CTLA4-Ig on serum IgE levels and airway eosinophilia are more pronounced in a "mild" compared to a more "severe" mouse model of established antigen-induced asthma manifestations (Deurloo et al., 2001).

Together, these data suggest that CTLA4-Ig may have therapeutic potential at least for the treatment of mild forms of asthma. However, to the best of our knowledge no clinical trials on the use of CTLA4-Ig in asthma are currently reported.

5.3. T-cell immunoglobulin and mucin-containing molecules

T-cell immunoglobulin (Ig) and mucin-containing molecules (TIM) comprise a recently described family of molecules expressed predominantly on T-cells (Kuchroo et al., 2003). Th1 cells selectively express TIM-3, while Th2 cells selectively express TIM-1 (Khademi et al., 2004; Chakravarti et al., 2005). Ligation of TIM-3 with its ligand, galectin 9, provides a

negative costimulatory signal to the T-cell leading to down-regulation of Th1 responses (Zhu et al., 2005). TIM-1 was previously identified as the hepatitis A virus receptor and is genetically linked to asthma in humans as well as to experimental asthma in a mouse model (McIntire et al., 2001; McIntire et al., 2004). Recently, TIM-4, which is expressed by APC, was discovered as the endogenous ligand for TIM-1 (Meyers et al., 2005). TIM-1:TIM-4 interaction behaves as a costimulatory signal to T-cells leading to enhanced proliferation and cytokine production (Meyers et al., 2005; Umetsu et al., 2005). Interestingly, blockade of TIM-1 by a monoclonal antibody at the time of allergen challenge inhibits Th2 mediated allergic airway inflammation, mucus hypersecretion and Th2 cytokine production in a mouse model of asthma (Encinas et al., 2005). In contrast, blockade of TIM-1 in a model of respiratory tolerance reversed tolerance induction and increased allergic inflammation (Umetsu et al., 2005). More research is definitely needed to unravel these apparent discrepancies before TIM-1 and its ligand, TIM-4, can be regarded as a novel therapeutic target protein for allergic asthma.

6. Modulation of T-cell trafficking

Chemokines orchestrate immune responses by the attraction of inflammatory cells to target organ. Chemokines are small chemotactic cytokines that induce adhesion and transmigration of leukocytes through the endothelium Arrest on the endothelium is a prerequisite for the transmigration of leukocytes. A process of rolling precedes this and specialized integrins are involved in the adhesion of T-cells to the endothelium (Luster et al., 2005). These include very late antigen (VLA)-4 and leukocyte function-associated antigen (LFA)-1 (integrin $\alpha4\beta1$ and integrin $\alpha L\beta2$, respectively), which are ligands for the adhesion molecules, vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM)-1, respectively. VLA-4 and LFA-1 are expressed on memory T-cells and their expression is induced during antigenic activation of T-cells in the lymph nodes. High affinity adhesive interactions have been described between VCAM-1 and VLA-4 expressed on differentiated Th2 cells, whereas VLA-4 expressing Th1 were less capable of sustained interactions with VCAM-1 (Lim et al., 2000). Another adhesion molecule expressed on the endothelium and involved in the transendothelial migration of T-cells is P-selectin. Unlike VLA-4, P-selectin glycoprotein ligand-1 (PSGL-1) appears to be selectively involved in the migration of Th1 cells, since PSGL-1 on Th1 cells, but not Th2 cells, could bind to P-selectin and support migration (Borges et al., 1997). Another study demonstrated that PSGL-1 is functional on both Th1 and Th2 cells, but is expressed more abundantly on Th1 cells (Mangan et al., 2005). Thus, differences in adhesion molecule signaling may contribute to the selective recruitment of T-cell subsets to the lung. In addition to integrins, chemokine receptors are expressed in different patterns on T-cell subsets. While the lymph node-homing chemokine receptor CCR7 is expressed on both naive and memory T-cells, most chemokine receptors (e.g. CCR2, CCR3, CCR4, CCR5, CXCR3 and CXCR5) are induced by antigenic stimulation and only expressed

on memory T-cells. Th0 cells and nonpolarized Th cells preferentially express the chemokine receptors CCR2, CCR4, CCR7 and CXCR3. Instead, CCR5 may be a marker of Treg cells and it has been suggested that ligands for this receptor play a central role in the recruitment of Treg cells (Bystry et al., 2001). Th1 cells abundantly express CCR2, CCR5, CCR7 and CXCR3, with the latter being present on almost all Th1 cells. A substantial percentage of Th2 cells also expresses CCR7 and CCR2. Receptors that are preferentially expressed on Th2 cells are CCR4, CCR8, CXCR4 and (on a low percentage) CCR3 (Annunziato et al., 1999; Kim et al., 2001). Infiltration of CCR4^{+} T-cells has been observed in bronchial tissue after allergen challenge in asthma patients (Panina-Bordignon et al., 2001). There is growing evidence that one of the ligands for CCR4, that is, thymus and activation-regulated chemokine (TARC)/CCL17, plays a key role in recruitment of Th2 cells to the asthmatic airways (Sekiya et al., 2000; Berin et al., 2001; Panina-Bordignon et al., 2001; Sekiya et al., 2002; Bochner et al., 2003; Morgan et al., 2005).

6.1. Inhibition of T-cell lung infiltration

Strategies to inhibit T-cell infiltration in the asthmatic lung are directed to T-cell adhesion or chemoattraction. T-cell adhesion can be targeted by inhibiting the interaction between adhesion molecules and their ligands. In this respect, the use of VLA-4 inhibitors may be relevant in asthma, as demonstrated by their effects in allergen-induced inflammatory responses in sheep (Lin et al., 1999; Singh et al., 2004). However, VLA-4 antagonist IVL745 did not affect the early and late response to inhaled allergen or markers of airway inflammation in patients with mild-to-moderate atopic asthma, except for a modest reduction in sputum eosinophils (Diamant et al., 2005; Norris et al., 2005). The clinical development of inhibitors of VLA-4 or the treatment of asthma was discontinued. While OVA-sensitized/challenged ICAM-1-deficient mice have a dramatically reduced inflammatory influx into the airway/lung and a corresponding attenuation of AHR as compared to wild-type controls (Tang & Fiscus, 2001), no clinical data on blocking integrins or antibodies to ICAM-1 or VCAM-1 are available for asthma.

Treatment with specific antagonists to chemokine receptors is in development for asthma. Trafficking of Th2 cells into the lung is mediated by $G_{alpha\ i}$-coupled chemoattractant receptors. $G_{alpha\ i}$-signaling is inhibited by pertussis toxin. Pertussis toxin-treated Th2 cells have been described to be unable to traffic into the airways and to induce eosinophilic airway inflammation following OVA challenge in a mouse model of asthma (Mathew et al., 2002). Thus, chemokine receptor antagonism may become an important approach in the treatment of asthma. CCR3 antagonists are likely to be safe and efficient in allergic diseases and their pharmacology has already been defined in phase I/II studies in patients (Erin et al., 2002). However, anti-inflammatory activity of CCR3 antagonists may be predominantly due to reduction in eosinophilic infiltration, since the majority of eosinophils but only a minority of T-cells expresses CCR3. Moreover, in CCR3 deficient mice Th2 trafficking

seems to be intact and these mice can even mount a tissue-specific allergic response (Humbles et al., 2002; Ma et al., 2002). Increased expression of 2 other Th2 specific chemokine receptors, that is, CCR8 and CCR4, has been observed in the asthmatic airway upon allergen challenge. However, CCR8 ligand I-309 was hardly detectable in BAL and serum of asthma patients and knockout of CCR8 did not affect allergic inflammation in mice (Bochner et al., 2003; Chung et al., 2003). In contrast, although blocking of CCR4 failed to reduce Th2 inflammation in the airways of guinea pigs, CCR4 deficiency and monoclonal antibodies against TARC have been described to do so in mouse models of asthma (Kawasaki et al., 2001; Schuh et al., 2002; Conroy et al., 2003). Thus, blocking of CCR4-mediated chemotaxis may be promising in the treatment of asthma. In addition, a CXCR4 agonist has been demonstrated to inhibit allergen-induced inflammation in a murine model of asthma, although increased CXCR4 expression in the asthmatic lung has only been described for eosinophils. Another chemokine receptor that characterizes Th2 cells is CRTH2 (Caramori et al., 2004). Prostaglandin D2 (PGD2), which is produced by mast cells, is believed to be one of the ligands of CRTH2 (Hirai et al., 2001). Ramatroban, an orally active antagonist of receptors activated by PGD2, is marketed in Japan for the treatment of rhinitis (Sugimoto et al., 2003). This drug has also been shown to inhibit airway hyperresponsiveness in humans, but no studies in asthma patients are available yet. Thus, although drugs interfering with chemokines and chemokine receptors have been proven beneficial in animal models, no clinical data on these compounds in asthma have been reported so far (Caramori et al., 2004; Garcia et al., 2005).

7. Indirect modulation of T-cells by targeting dendritic cells

Dendritic cells (DC) are considered to be essential for the priming of naïve CD4+ T lymphocytes by delivering signal 1 (T-cell receptor) and 2 (costimulatory). Moreover, they also provide T-cell polarizing signal 3, of which IL-12 family members (IL-12, IL-23 and IL-27) for Th1 generation and IL-10 and TGF-β for respectively Tr1 and Th3 generation, are well-known examples (Kapsenberg, 2003). Herewith, DCs play a crucial role in generation of different effector T-cell subsets, Th1 and Th2, and adaptive Treg cells, Tr1 and Th3 cells (Kapsenberg, 2003; De Jong et al., 2005). DCs that promote Treg cells are generally immature or semi-mature and display lower antigen uptake capacity, lower expression of costimulatory molecules and lower T-cell stimulatory capacity (Lutz & Schuler, 2002). Inhibition of DC maturation by inhibiting NF-κB activation with 1,25(OH$_2$) vitamin D3, glucocorticoids, or PPAR-γ agonist induces Tr1-like cells in vitro and tolerance in mouse models of transplantation, auto-immune diseases and asthma in vivo (Barrat et al., 2002; Gregori et al., 2002; Hammad et al., 2004; Pedersen et al., 2004). The induction of Tr1 or Th3 cells is related to the production of IL-10 or TGF-β, respectively, by these DC (Lutz & Schuler, 2002). Many pathogens, including viruses, parasites, fungi and bacteria, and isolated, so-called regulato-

ry-type pathogen-associated molecular patterns (PAMP), have been shown to induce the production of either one or both of these cytokines by DC and to facilitate induction of Treg cells in this way (Mills, 2004). It will be of interest to know whether some of these PAMP can be therapeutically exploited for the treatment of allergic asthma.

7.1. Mycobateria

The hygiene hypothesis implies that there is an inverse relation between the extent of exposure to microbial agents during early childhood and the prevalence of allergic diseases, asthma included. One of the early observations that spurred research in this area was the strong inverse association between Th1-mediated delayed hypersensitivity to *Mycobacterium tuberculosis* and atopy in Japanese schoolchildren (Shirakawa et al., 1997). Consequently, it was anticipated that Th2-dominated allergic responses could be downregulated by exposure to specific bacteria such as heat-killed or live *Mycobacteria* (Rook & Stanford, 1998; Matricardi et al., 2003). It has been shown that prophylactic treatment with *Mycobacterium bacillus calmette-guerin* (BCG) or *Mycobacterium vaccae* can suppress AHR and inflammation in mouse models. The mechanism of action may not be related to the induction of Th1 responses, but rather the production of IL-10 and TGF-β by antigen-specific Treg cells (Zuany-Amorim et al., 2002). Despite these encouraging preclinical data, clinical trials using BCG vaccination or injection of the non-pathogenic mycobacterium *M. vaccae* (SRP299) for the treatment of allergic asthma have been disappointing so far (Renz, 2004). It remains to be seen whether treatment in early childhood, in particular before the onset of allergic disease, offers more perspective for this type of treatment.

7.2. CpG-containing immunostimulatory deoxyribonucleic acid

CpG-containing immunostimulatory deoxyribonucleic acid (CpG DNA) are derived from bacterial genomic DNA and exert their biological effects through interaction with toll-like receptor (TLR) 9. In mouse models, CpG DNA has been shown to inhibit antigen-induced AHR, acute and persistent airway inflammation, airway remodelling, goblet cell hyperplasia and Th2-type cytokine production (Jain et al., 2002; Ikeda et al., 2003; Hessel et al., 2005). The precise mechanisms of action are still incompletely understood, but include the promotion of Th1 or Treg cell responses, inhibition of APC-mediated Th2 cell activation and inhibition of IgE-dependent release of Th2 cytokines from mast-cells. In addition to this direct therapeutic intervention, another successful approach is the combination of CpG DNA with specific allergen immunotherapy as demonstrated in a mouse model (Kline et al., 2002) and patients with allergic rhinitis (Tulic et al., 2004). Currently at least 2 companies have TLR-9 ligands in clinical development for the treatment of allergy and asthma, either alone or in combination with specific allergen immunotherapy (Aventis: AVE7279 and AVE0675 and Dynavax: 1018 ISS).

8. Concluding remarks

Considering the central role of T lymphocytes in the regulation of disease manifestation in allergic asthma, drugs targeting disease-inducing Th2 cells or Treg cells are promising therapeutic strategies (see Fig. 1). Th2 cells can be targeted by inhibition of their activation through inhibition of TCR-induced signalling or inhibition of specific transcription factors as well as by prevention of their migration into the lung tissue. Although these interventions may not lead to long-term beneficial effects, they may be of particular interest when given at the time of disease progression in childhood. A more promising approach to limit aberrant Th2 responses and provide long-term relief of symptoms may be the induction of disease-regulatory T lymphocytes, in particular Treg cells. Although mouse models of allergic asthma suggest that this is feasible, the translation of these types of studies for the treatment of human asthma remains poor. Furthermore, whether these strategies can compete with the mainstay asthma therapy of inhaled glucocorticoids remains to be seen in the future.

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