

The pharmacological modulation of allergen-induced asthma

L. L. Ma · Paul M. O'Byrne

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Abstract Aeroallergens are the most common triggers for the development of asthma. Recent birth cohort studies have identified viral infections occurring against a background of aeroallergen sensitization as a potent risk factor for initiation of asthma. Viral infection enhances immunopathogenic potential of pre-existing inhalant allergy via modulating airway mucosal dendritic cells. By using an allergen inhalation challenge clinical model, studies have shown that the late asthma response (LAR) is associated with more pronounced allergen-induced airway inflammation and airway hyperresponsiveness. The degree of airway eosinophilia, regulated by bone marrow progenitor cells and interleukin-5 level, correlates with the magnitude of the LAR and the increase in hyperresponsiveness. Both myeloid and plasmacytoid dendritic cell subsets have been involved in the pathogenesis of allergen-induced LAR. Myeloid dendritic cells are responsible for the allergen presentation and induction of inflammation and plasmacytoid dendritic cells play a role in the resolution of allergen-induced inflammation. A variety of potential new classes of asthma medication has also been evaluated with the allergen inhalation challenge in mild asthmatic subjects. Examples are TPI ASM8, an inhaled anti-sense oligonucleotide drug product, which attenuated both early and LARs via inhibition of the target gene mRNA of chemokine receptor 3, and the common β chain of

interleukin-3, interleukin-5 and granulocyte-macrophage colony-stimulating factor receptor. Anti-human antibody interleukin-13 (IM-638) significantly attenuated both early and late allergen-induced asthma response. Pitakinra, which targets both interleukin-4 and interleukin-13, substantially diminishes allergen-induced airway responses. Allergen-induced airway responses are a valuable way to evaluate the activity of possible new therapies in asthmatic airways.

Keywords Inflammation · Eosinophils · Airway hyperresponsiveness · Therapy

Introduction

Asthma is a common, complex disease affecting millions of children and adults worldwide. In most developed countries the prevalence of asthma is at least 10 % (Janson et al. 2001). The most commonly identified stimuli for the development of asthma are environmental allergens.

Allergenic sensitization which triggers asthma can present at any age, but its highest prevalence is during childhood and young adulthood. An interaction between early exposure to allergens, viral infections and family history are probably important in the initiation of allergic asthma, but the mechanisms by which some children develop asthma, whereas others have non-respiratory allergic manifestations, are not understood (Braun-Fahrlander et al. 2002). Recently, results of a series long-term birth cohort studies have shed new lights on the initiation of allergen-induced asthma. The primary risk factor for allergen-induced asthma is sensitization to aeroallergens, as a result from a failure to generate protective immunologic tolerance. This tolerance is orchestrated by airway

L. L. Ma · P. M. O'Byrne
Firestone Institute of Respiratory Health,
St. Joseph's Healthcare, Department of Medicine,
McMaster University, Hamilton, ON, Canada

P. M. O'Byrne (✉)
Rm 3W10, McMaster University Medical Center,
1280 Main Street West, Hamilton, ON L8S 4K1, Canada
e-mail: obyryp@mcmaster.ca

mucosal dendritic cells and normally results in programming of regulatory T cells, which inhibit activation of T helper (Th)2 memory cells that drive IgE production and initiate inflammatory cascade results to IgE-mediated tissue damage (Holt and Strickland 2010).

The origin of allergen-induced asthma

Respiratory viral infections have been recognized as a potential initiating factor of asthma for many years. This hypothesis has been supported by results from long-term birth cohort studies in the US (Copenhaver et al. 2004; Stern et al. 2007), Australia (Holt et al. 2010; Oddy et al. 2002) and Europe (Illi et al. 2001), which provide a more comprehensive understanding of the impact of infections in the origins of asthma. These studies have collectively shown that wheeze is very common in infancy, especially when associated with respiratory infection. Usually the wheeze is transient and resolves spontaneously, usually by age 3. These children generally have normal lung function at school age, and are non-atopic (Illi et al. 2006). However, in a subgroup of children, wheeze becomes persistent which indicates the early onset of asthma, and this phenotype is strongly associated with early sensitization to aeroallergens. This subset of children tend to have lower lung function at school age (Illi et al. 2006; Kusel et al. 2007; Martinez et al. 1995; Rhodes et al. 2002).

Increased risk of development of allergic asthma associated with early sensitization may be related to the more intensely Th2 cell-polarized long-term memory responses during the early sensitization (Turner et al. 2007). The risk for persistence of symptoms is more pronounced if sensitization is also persistent (Illi et al. 2006; Kulig et al. 1998; Rhodes et al. 2002). Longitudinal follow-up of birth cohorts have shown that early sensitization (Illi et al. 2006; Kusel et al. 2007; Peat et al. 1990; Rhodes et al. 2002; Sherrill et al. 1999) and severe sensitization (Lowe et al. 2007) are risk factors for persistence of asthma. The most severe childhood asthma, and the highest risk for progression to persistent asthma, is encountered when lower respiratory viral infections occur against a background of pre-existing aeroallergen sensitization (Holt et al. 2010; Jackson et al. 2012; Oddy et al. 2002; Sly et al. 2008), especially during the most rapid proceeding period of postnatal lung growth and differentiation (Holt et al. 2010; Oddy et al. 2002; Sly et al. 2008).

Interactions between atopy and infections in this context are not completely clear. Atopic individuals with ongoing aeroallergen exposure sustain a state of relatively low level T cell-mediated and granulocyte-mediated inflammation in the airway epithelium; however, the local expression of high-intensity Th2 cell-mediated inflammation is

controlled by the resident airway mucosal dendritic cells (DCs). These DCs maintain the immature antigen-surveillance until they migrate to the regional lymph nodes (RLNs), where they deliver a signal that is Th2 polarized that precedes the induction of tolerance (Holt et al. 2008).

Respiratory viral infection markedly up-regulates local innate immune functions, especially the activity of airway mucosal DCs (McWilliam et al. 1996, 1997). Parainfluenza, or RSV infection markedly accelerated normal turnover of resident myeloid dendritic cells (mDCs), which are replacements for emigrating antigen-bearing mDCs trafficking to the RLNs (Beyer et al. 2004; Gill et al. 2005; McWilliam et al. 1997; Smit et al. 2006), and also prolong the expansion of the airway mucosal DCs well beyond parainfluenza clearance (McWilliam et al. 1997). Airway mucosal DCs also provide the initial trigger for the CD4+ Th2-cell component of the late-phase response of asthma in atopic patients (Holt and Thomas 2005). Therefore, via these routes, viral infections may enhance the immunopathogenic potential of pre-existing inhalant allergy (Fig. 1), creating a heterogeneous inflammatory milieu associated with greatest risk of asthma development (Holt et al. 2008; Oddy et al. 2002).

When an individual is consistently exposed to allergens or other airborne irritant stimuli, which trigger repeated cycles of inflammation, the resolution of inflammation between each cycle becomes less complete. Persistent inflammation leads to repeated cycles of tissue repair and regeneration which leads to persistent pathological changes, resulting in the progressive deterioration in respiratory function. Once these changes become irreversible, persistent asthma develops (Holt and Sly 2012).

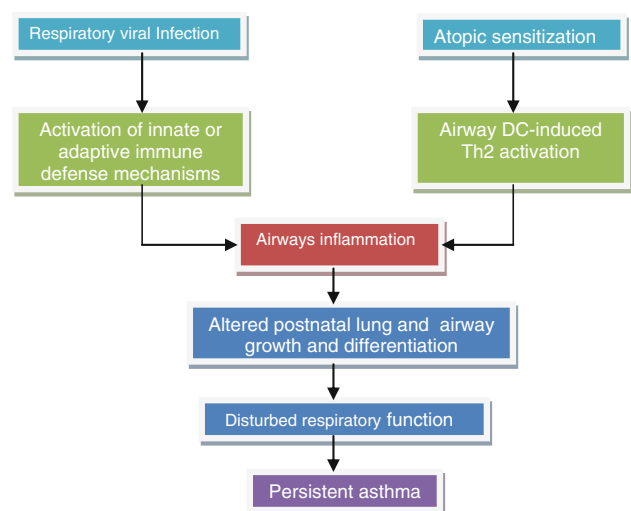


Fig. 1 Interactions between early respiratory viral infections and atopic sensitization on the pathway to persistent asthma. DC dendritic cell, Th2 T helper-2 cell. (Sly et al. 2008)

Pathogenesis of allergen-induced asthma

The pathogenesis of allergic asthma involves complex interactions between inflammatory and resident airway cells which lead to the development of characteristic pathologic features, airway inflammation, airway hyperresponsiveness, and airway remodeling.

Airway inflammation

Asthmatic airway inflammation is a multi-cellular process involving mainly mast cells, eosinophils, lymphocytes, neutrophils, monocytes, and basophils, with eosinophilic infiltration being the most striking feature (Kay 2001). From a mechanistic standpoint, early aeroallergen sensitization presumably indicates a series of early life cellular events in the airway mucosa to initiate the recruitment of Th2 cells with their capacity to secrete a panel of characteristic cytokines, including IL-3, IL-4, IL-5, IL-13 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Robinson 2010), among which IL-4 and IL-13 drive the isotype switching of B cells to IgE synthesis. It is well known that these events require allergen uptake and processing by DCs with subsequent peptide epitope presentation to naive T cells in association with major histocompatibility complex class II (Lambrecht and Hamad 2009).

Allergens, e.g., house dust, can activate Toll-like receptors (TLRs) on airway epithelial cells, leading to the release of the IL-7-like cytokine, thymic stromal lymphopoietin (TSLP) (He and Geha 2010) that, on interacting with its receptor on DCs, up-regulates the expression of the costimulatory molecules cluster of differentiation (CD)40, OX40, and CD80 to enhance Th2 polarization (Ziegler and Artis 2010) as well as activating mast cells to initiate the inflammatory cascade (Holgate 2007).

Activated mast cells start the inflammatory process with cytokine synthesis, degranulation and mediator release. The cytokines are synthesized and secreted by mast cells, including IL-4, IL-5 and IL-13, which regulate IgE synthesis, Th2 lymphocyte differentiation, and development of eosinophilic inflammation (Bradding and Holgate 1996).

The mediator release by mast cells, including preformed mediators, e.g., histamine, and rapidly synthesized mediators, e.g., prostaglandin (PGD)₂ and leukotriene (LT)C₄, can induce bronchoconstriction, mucus secretion, and mucosal edema (Boyce 2005).

The eosinophil is the most characteristic cell that accumulates in allergic asthma inflammation; its presence is often related to disease severity (Bousquet et al. 1990). Eosinophils are recruited and activated by IL-5, the eotaxin family of chemokines via the eosinophil-selective chemokine receptor CCR3, and TLRs (Wong et al. 2007).

Activated eosinophils produce lipid mediators, such as LTs, and platelet activating factor (PAF), that mediate smooth muscle contraction; toxic eosinophil granule products (e.g., major basic protein, eosinophil-derived neurotoxin, eosinophil peroxidase, or eosinophil cationic protein) can damage airway epithelium and nerves; cytokines, such as GM-CSF, transforming growth factors (TGF)- α and β , and ILs that maybe involved in airway remodeling and fibrosis (Bousquet et al. 1990).

An important, and consistent, feature of allergic asthma is the production of excess and altered mucus that blocks peripheral airways and is difficult to expectorate. Goblet cell hyperplasia usually involve the conducting airways (Ordonez et al. 2001), but in chronic persistent disease, goblet cells also spread down to the more peripheral airways where they normally do not exist (Jeffery and Zhu 2002; Shimura et al. 1996). Submucosal glands in asthma are also larger and contain a greater proportion of mucin as compared to serous cells. The net result is the secretion of abnormally large amounts of the highly viscous mucins 5AC and 5B (Kirkham et al. 2002), which, together with DNA and eosinophil basic proteins, contribute to the tenacious and viscous mucus (Voynow and Rubin 2009). Genetic studies reveal evidence for the role of four highly polymorphic genes (MUC-6,-2, 5AC, and 5B) encoding gel forming mucins on chromosome 11p15.5 (Rousseau et al. 2007). Goblet cell differentiation and increased mucus secretion is tightly associated with the enhanced expression of calcium-activated chloride channel 1 (hCLC A1; Gob 5) (Choy et al. 2011; Lai and Rogers 2010; Thai et al. 2005).

Airway hyperresponsiveness

Airway hyperresponsiveness (AHR) is another defining feature of allergic asthma and is a manifestation of reversible airflow obstruction due to smooth muscle contraction (Black et al. 2012). AHR represents an exaggerated constrictor response to a variety of physical, chemical, or environmental stimuli (Black et al. 2012). While airway smooth muscle is clearly involved in AHR, the precise mechanism is unknown. Alterations in smooth muscle function or mass, enhanced sensitivity of neural pathways leading to bronchoconstriction, loss of airway parenchymal interdependence, loss of bronchodilating effect of deep breaths, and exaggerated airway narrowing from smooth muscle contraction as a consequence of remodeling and structural abnormalities of the airway may all contribute to the AHR (Black et al. 2012; Ozier et al. 2011).

Airway inflammatory and structural changes are believed to be important in the development of AHR. A number of persisting airway inflammation markers have been correlated to the AHR. These include increasing the

number of eosinophils, mast cells and T lymphocytes in bronchial fluids and bronchial biopsies and the extent of deposition of collagen and other extracellular matrix proteins below the basement membrane (Black et al. 2012; Ozier et al. 2011). Eosinophils may also contribute to AHR through interactions with parasympathetic nerves and acetylcholine release (Jacoby et al. 2001). However, the precise role of each inflammatory cell in causing AHR has not yet been clarified.

A number of structural changes have also been described with persistence of AHR. These include patchy desquamated epithelium, thickening of the reticular collagen layer of the basement membrane, and airway smooth muscle hypertrophy and hyperplasia (Boulet et al. 2000). The mediators released from the inflammatory and structural cells, such as cytokines, eicosanoids, and eosinophil basic proteins, maybe responsible for these changes (Black et al. 2012).

Airway remodeling

Airway remodeling refers to structural changes in the airway that may cause irreversible airflow limitation, superimposed on the effects of inflammation and smooth muscle contraction (Limb et al. 2005). Histopathology changes include damage or loss of the normal pseudostratified structure of airway epithelium, an increase in the proportion of mucus-producing goblet cells (Ordonez et al. 2001), fibrotic thickening of the subepithelial reticular basement membrane or “lamina reticularis” (Brewster et al. 1990; Molina et al. 1977), increased numbers of myofibroblasts (Brewster et al. 1990), increased vascularity (Li and Wilson 1997; Salvato 2001; Vrugt et al. 2000), increased airway smooth muscle mass and increased extracellular matrix (Pepe et al. 2005; Pini et al. 2007).

These structural changes contribute to bronchial wall thickening, alterations in the physiologic consequences of smooth muscle contraction, or loss of airway–parenchymal interdependence (e.g., the tethering forces exerted by the lung parenchyma that maintain airway patency) (Ozier et al. 2011). Structural narrowing of airway associated with remodeling appears to increase AHR, and may lead to the irreversible airflow limitation (Brown et al. 2006).

Allergen inhalation challenge

Allergen inhalation challenge is a useful clinical model to study the mechanisms of allergen-induced asthma. The airway response to inhaled allergen is characterized by airflow obstruction [usually measured by a decrease in the forced expired volume in 1 s (FEV₁)] that starts shortly after the allergen inhalation, peak at 20–30 min, and

resolves spontaneously by 2–3 h (O'Byrne et al. 1987). This is referred to as the early asthma response (EAR). The clinical symptoms correlate with the allergen-induced EAR, including wheezing, chest tightness, and coughing. The EAR is mediated by immunoglobulin (Ig)E-mediated mast cell degranulation, release of mediators, (e.g., histamine, LTs), which subsequently induce bronchoconstriction, plasma exudation and mucus hypersecretion (Boulet et al. 1997; Kirby et al. 1986; Pepys et al. 1968).

The allergen-induced LAR is a subsequent episode of airflow obstruction which develops after spontaneous resolution of the EAR (O'Byrne et al. 1987). The LAR usually appears between 3 and 4 h after allergen inhalation, peaking at 8–12 h; and can persist for 24 h. The airflow obstruction can be reversed by inhaled β_2 agonists (Dorsch et al. 1980; Twentyman et al. 1990). The mechanisms of LAR are not that clear as that of EAR, but immune responses and inflammation are likely major contributors. Dual allergic response occurs when an individual develops both EAR and LAR after allergen inhalation challenge.

The advances in the pathogenic mechanisms of allergen-induced asthma

The role of inflammatory cells and their regulation in allergen-induced LAR

Previous studies have shown that EAR can be synergistically suppressed by the combination of an antihistamine (desloratadine) and a leukotriene receptor antagonist (montelukast) (Davis et al. 2005). Recently, desloratadine and montelukast combination, which were given in a single dose prior to allergen inhalation, completely abolished the LAR (Davis et al. 2005). This observation indicates antihistamine and leukotriene combination provides protection against LAR, presumably by blocking the bronchoconstrictor responses to these mediators (Davis et al. 2009).

The possible underlying mechanisms of this effective suppression regimen of LAR has been investigated in our center (Cartier et al. 1982; Gauvreau et al. 1999, 2000). An early study has shown that the magnitude and duration of increase in histamine responsiveness was related to the magnitude of the LAR (Cartier et al. 1982). The more severe the LAR, the greater the increase in histamine responsiveness, and the greater the duration of increase in histamine responsiveness (Cartier et al. 1982). Therefore, airway inflammation, mucosal edema and mucus secretions all potentially contribute to the development of LAR in addition to smooth muscle constriction (Cartier et al. 1982). Further studies have explored the role of inflammatory cells in the LAR and their relations with AHR. Sputum eosinophils and mast cells significantly increased

at 7 h after allergen inhalation in dual responders (Gauvreau et al. 1999), correlated with AHR (Gauvreau et al. 1999). IL-5, eotaxin, and regulated and normal T cell expressed and secreted (RANTES), which are specific for the activation and chemotaxis of eosinophils regulate the recruitment of activated eosinophils and airway mucosal inflammation (Gauvreau et al. 1999). Basophils and mast cells also significantly increased in sputum at 7 and 24 h after allergen inhalation in dual responders and sputum basophils correlated significantly with AHR (Gauvreau et al. 2000). Thus, the LAR is likely associated with more severe allergen-induced airway inflammation and AHR (Cartier et al. 1982; Gauvreau et al. 1999, 2000).

The role of eosinophilia and its regulation in allergen-induced LAR

In contrast to isolated early responders, dual responders develop significant sputum and blood eosinophilia and prolonged AHR (Dorman et al. 2004). The degree of airway eosinophilia correlates with the size of the LAR and the increase in AHR (O'Byrne et al. 1987). Airway eosinophilic responses are regulated by bone marrow progenitor cells. IL-3 responsive eosinophil/basophil progenitors were detected in bone marrow as early as 5 h after allergen inhalation, and IL-5 responsive eosinophil/basophil progenitors detected in bone marrow at 12 and 24 h after allergen inhalation (Dorman et al. 2004). Bone marrow IL-5 level has also increased simultaneously with their progenitor cells, indicating the cytokine levels (e.g., IL-5) regulate allergen-induced activation of eosinophilopoietic process in bone marrow (Dorman et al. 2004). Bone marrow CD34+ cells expressing IL-5 α significantly increased at 24 h after allergen inhalation in dual responders (Sehmi et al. 1997). The increased expression of IL-5 α on CD34+ cells favors eosinophilopoiesis and contribute to the subsequent blood and tissue eosinophilia (Sehmi et al. 1997).

The role of DCs in allergen-induced LAR

Recently, it has been shown that DCs are essential in the initiation and maintenance of the T cell-mediated immune response to inhaled allergen in animal models (Dorman et al. 2004; Hammad et al. 2004; Lambrecht et al. 2000a, b). The two subpopulations of DCs in humans are myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs), which represent ~0.5–1 and <0.3 % of circulating mononuclear cells, respectively (de Heer et al. 2004; Hammad and Lambrecht 2006). In animal models, mDCs are critical in allergic Th2 sensitization and are responsible for the development of secondary immune responses to

inhaled allergens (Hammad et al. 2004; Lambrecht et al. 2000a, b). In contrast, pDCs may play a role in providing tolerance to inhaled allergens (de Heer et al. 2004).

The homing properties of DCs are mainly regulated by the chemokine receptors they express. Chemokine ligand (CCL)20 is an important chemokine for the recruitment of chemokine receptor (CCR)6-expressing immature DCs to tissues for subsequent antigen presentation (Greaves et al. 1997; Power et al. 1997). Once DCs have entered airway and captured antigen, maturation commences. Maturing DCs lose responsiveness to inflammatory chemokines and concomitantly upregulate CCR7, which is absolutely expressed in mature DC (Hammad and Lambrecht 2006). CCL19, expressed in T cell-rich areas of the lymph nodes, and CCL21 are known ligands for the CCR7 receptor (Leigh et al. 2002; Yoshida et al. 1998). Mature DCs traffic to RLNs to present the antigen and initiate a T cell response.

Circulating mDCs and immature CCR6+ mDCs have been shown to be significantly reduced up to 24 h after allergen inhalation (Farrell et al. 2007). No differences have been observed in the number of circulating mature CCR7+ mDCs or pDCs after allergen inhalation. Thus, allergen inhalation causes trafficking of immature CCR6+ mDCs from blood into the airway (Farrell et al. 2007). Both mDCs and pDCs in sputum have significantly increased at 24 h after allergen inhalation in dual responders (Dua et al. 2010). Sputum levels of CCL20, which is the CCR6+ mDC recruiter, also significantly increased at 24 h post-allergen inhalation, possibly leading to the significant increase of CCR6+ mDC at 72 h after allergen inhalation (Dua et al. 2010). In contrast, CCR7+ mDC, as well as the number of CCR6+ pDCs and CCR7+ pDCs, were not different between challenges (Dua et al. 2010). These data suggest that both mDC and pDC subsets have been involved in the pathogenesis of allergen responses in LAR. mDCs are responsible for the allergen presentation and induction of inflammation, pDCs more possibly play a role in the resolution of allergen-induced inflammation (Dua et al. 2010).

Pharmacological manipulation of allergen-induced asthma

There are a variety of effective medications for the treatment of asthma. These include short-term symptom relief medications, short-acting inhaled β_2 -agonists (SABAs), which provide rapid relief of bronchoconstriction, but have no anti-inflammatory effects in asthma. The most widely used and effective anti-inflammatory medications are the inhaled corticosteroids (ICS), used for maintenance treatment of asthma. Other maintenance treatment options include ICS, in combination with long-acting inhaled β_2

Table 1 New drugs/approaches for asthma evaluated in the AllerGen Clinical Investigator Collaborative

Modifications of existing drugs	New approaches
Ultra long-acting inhaled β 2-agonists	Anti-sense against IL-3, IL-5 GM-CSF, and CCR3
Non-steroidal GR agonist	Anti-sense against IL-4R Anti-IL-9 Anti-IL-13 Anti-C5a Anti-OX40L CXCR2 antagonist α 7 Nicotinic agonist Anti-M1 prime Anti-TSLP

GR glucocorticoid receptor, IL interleukin, GM-CSF granulocyte-macrophage colony-stimulating factor, CCR chemokine receptor, TSLP thymic stromal lymphopoietin

agonists (LABAs) in a single inhaler, leukotriene receptor antagonists (LTRAs) and anti IgE monoclonal antibodies.

Each of these classes of treatment have been evaluated in their ability to protect against allergen-induced airway responses, and all have been demonstrated to be effective in attenuating the bronchoconstrictor responses after inhaled allergen (Fahy et al. 1997; Gauvreau et al. 1996, 1997; Kelly et al. 2010; Leigh et al. 2002) and for the ICS and LTRAs, also the allergen-induced airway eosinophilia and AHR (Gauvreau et al. 1996; Leigh et al. 2002). Overall, ICS have been the most effective of all drugs tested and are also the most effective class of drugs to treat asthma (Bateman et al. 2008). For these reasons, allergen inhalation challenge has become an established way of evaluation the potential efficacy of new classes of drugs for asthma treatment, usually early (in Phase II) of their development. This is because of the fact that no drug which did not modify the allergen-induced airway responses has ever proven to be a useful addition to asthma therapy.

A group of Canadian clinical scientists, with expertise in using the allergen inhalation challenge clinical model, have developed a Clinical Investigators Consortium, under the auspices of the AllerGen Network of Centers of Excellence (AllerGen CIC), to evaluate possible new therapies for asthma (Boulet et al. 2007). These centers are based in Hamilton, Quebec City, Saskatoon, Edmonton, Calgary, and Vancouver. This group has evaluated a variety of potential new classes of asthma medication (Table 1), the most novel of which are described below.

Oligonucleotide therapeutic approaches

Oligonucleotides (ODNs) comprise a diverse family of emerging drug candidates for the treatment of respiratory

diseases, including asthma. ODN therapies include the anti-sense, the RNA interference (RNAi) [small interfering RNA (siRNA) and microRNA (miRNA)], the immuno-modulatory [cytosine-phosphate-guanosine (CpG) ODN], the aptamer and the decoy approaches (Fig. 2). There is a commonality in the chemistry of ODN drug molecules, but each individual candidate is distinct in its molecular targets and also possesses different mechanisms of actions (Ferrari et al. 2011).

The concept of anti-sense ODNs technology is using a complementary sequence by virtue of base pair hybridization to specific mRNA and inhibit target mRNA expression and subsequently induce a blockade in translation of genetic information from DNA to protein (Ferrari et al. 2011). siRNA, double-stranded RNA like ODN, unwound into two single-stranded (ss) ssRNAs, namely the passenger strand and the guide strand. The passenger strand is degraded, and the guide strand is incorporated into a protein complex, RNA-induced silencing complex (RISC), which subsequently mediates the target mRNA degradation (Elbashir et al. 2001). CpG containing oligonucleotide (CpG DNA), potent activator of natural killer (NK) cell, B cell and DC, bind to TLR9 resulting in activation of intracellular signaling which leads to an immune response (Krieg et al. 1995, 1996). Decoy ODNs bearing the consensus binding sequence of a specific transcription factor can bind the target transcription factor and titrate its activity resulting in gene expression changes (Mann and Dzau 2000). Finally, aptamers, also termed as 'chemical antibodies', are short DNA or RNA ODNs or peptides that assume a specific and stable 3D shape in vivo, providing specific tight binding to secreted protein targets to inhibit their function (Jayasena 1999).

TPI ASM8 is an inhaled anti-sense drug product, which contains two modified anti-sense ODNs directly against human eotaxin receptor CCR3 and the common β (β c) chain of IL-3, IL-5 and GM-CSF receptor. Previous studies have demonstrated that LAR is associated with the elevated levels of eosinophils, as well as with other inflammatory cells, including basophils and mast cells (Gauvreau et al. 1999, 2000). Migration and proliferation of these effector cells in the airways may depend on signaling through the chemokine receptor CCR3 (Lamkhieou et al. 1997, 2003), and IL-3, IL-5 and GM-CSF, which stimulate their respective receptors composed of a common β chain (β c) and an individual α chain (Lamkhieou et al. 1997, 2003). TPI ASM8 is designed to decrease allergic inflammation by inhibiting the migration and survival of eosinophils, basophils and mast cells via down-regulating CCR3 and IL-3, IL-5 and GM-CSF receptor (Allakhverdi et al. 2002, 2006; Allam and Renzi 2001; Fortin et al. 2006).

TPI ASM8 has been shown to inhibit sputum eosinophil influx by 46 %, blunted the increase in total sputum

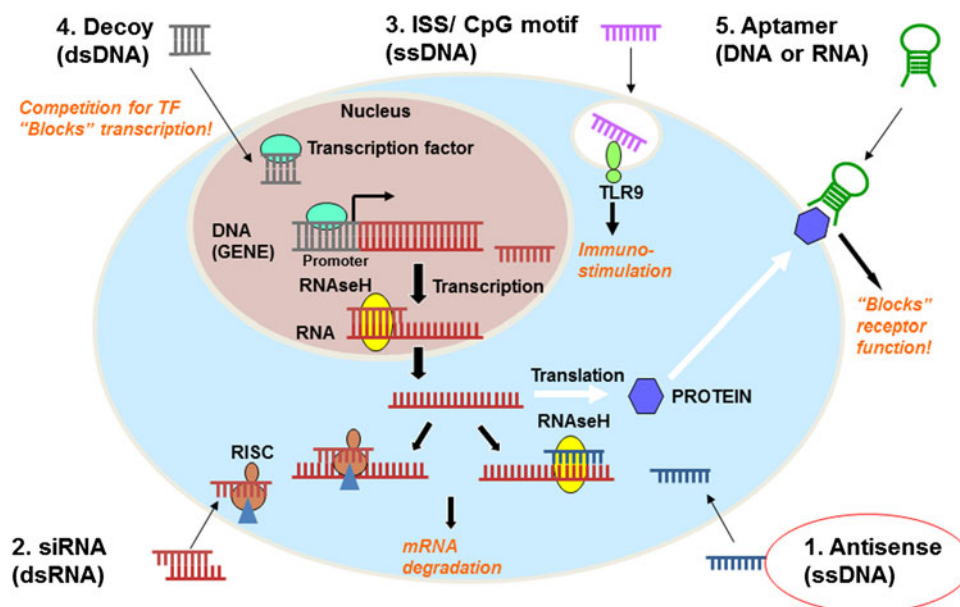


Fig. 2 The mechanisms of different oligonucleotide therapeutic approaches (Ferrari et al. 2011). The concept of anti-sense ODNs technology is using a sequence complementary by virtue of Watson–Crick base pair hybridization to specific mRNA can inhibit its expression and subsequently induce a blockade in translation of genetic information from DNA to protein. siRNA, double-stranded RNA like ODN, unwound into two single-stranded (ss) ssRNAs, namely the passenger strand and the guide strand. The passenger strand is degraded, and the guide strand is incorporated into a protein complex, RNA-induced silencing complex (RISC), which mediate the target mRNA degradation (Elbashir et al. 2001). CpG containing

oligonucleotide (CpG DNA), potent activator of NK cell, B cell and DC, bind to toll-like receptor 9 (TLR9) resulting in activation of intracellular signaling which leads to an immune response. Decoy ODNs bearing the consensus binding sequence of a specific transcription factor can bind the target transcription factor and titrate its activity resulting in gene expression changes. Finally, aptamers, also termed as ‘chemical antibodies,’ are short DNA or RNA ODNs or peptides that assume a specific and stable 3D shape in vivo, providing specific tight binding to secreted protein targets to inhibit their function

leukocytes after allergen inhalation, and significantly reduced the EAR (Gauvreau et al. 2008). And allergen-induced β c mRNA and CCR3 mRNA levels in sputum-derived cells have also been inhibited (Gauvreau et al. 2008). These data suggest TPI ASM8 suppresses asthma airway response via inhibition of allergen-induced increase in target gene mRNA of CCR3, and IL-3, IL-5, GM-CSF (Gauvreau et al. 2008).

A dose–response relationship has been observed to attenuate sputum eosinophils, eosinophil cationic protein (ECP), and basophils post-allergen inhalation at almost all the dosages of TPI ASM8 (1, 2, 4 mg BID and 8 mg OD), and efficacy can be maintained with once per day administration (Gauvreau et al. 2011b). TPI ASM8 has attenuated both the EAR and LAR (Gauvreau et al. 2011b). It is safe and well tolerated at all dosages (Gauvreau et al. 2011b).

Targeting IL-13 and IL-4 with monoclonal antibodies

It is widely accepted that Th2 cytokines play an important role in the maintenance of asthma and allergy. IL-13 is one of the Th2 cytokines and the emerging evidence has highlighted its critical role in the pathogenesis of asthma.

IL-13 is produced by activated Th2 cells, NKT cells, mast cells, and basophils (Wills-Karp 2004). IL-13 shares approximately 20 % amino acid sequence identity, and a range of cellular activities, with IL-4 (Wills-Karp 2004; Wynn 2003; Zurawski and de Vries 1994), which is also a Th2 cytokine. Both IL-4 and IL-13 were originally described based on their ability to promote IgE switch recombination in B cells and remain the only cytokines known to possess this function. IL-13 and IL-4 signal through the IL-4R α /IL-13R α 1 receptor complex shared by both cytokines (LaPorte et al. 2008), activate Jak-STAT signaling pathway (Palmer-Crocker et al. 1996) to initiate nuclear transcription of the target genes (Hebenstreit et al. 2006), leading to IgE production, fibrosis, eosinophilia and AHR. IL-4 also can signal through the IL-4R α / γ common receptor complex, which is not shared by IL-13 (Zurawski and de Vries 1994).

Extensive animal studies have indicated that IL-13 is involved in the regulation of IgE synthesis (Fish et al. 2005), eosinophil infiltration (Grunig et al. 1998; Wills-Karp et al. 1998), inflammation (Grunig et al. 1998; Wills-Karp et al. 1998), AHR (Webb et al. 2000), mucus hypersecretion (Webb et al. 2000; Whittaker et al. 2002), and subepithelial fibrosis (Wynn 2003).

IL-4 and IL-13 appear to have redundant roles in promoting specific IgE synthesis, airway eosinophilia, AHR and pulmonary inflammation in animal models (Fish et al. 2005; Foster et al. 2003; McKenzie et al. 1999; Perkins et al. 2006; Webb et al. 2000, 2004). Mucus hypersecretion is more likely regulated by IL-13, but not IL-4 in mice (Webb et al. 2000; Whittaker et al. 2002). IL-13 is also more essential than IL-4 in driving fibrotic changes in vivo (Wynn 2003).

Thus, targeting IL-13, IL-4 or both will be the potential therapeutic approach to the treatment of allergic asthma. Various strategies targeting IL-13 and its associated receptors have been investigated, including soluble (s)IL-13R α 2-Fc (Grunig et al. 1998; Wills-Karp et al. 1998; Zheng et al. 2008), siRNA (Lively et al. 2008), antibody (Bree et al. 2007; Kasaian et al. 2007, 2008; Yang et al. 2004), or peptide-based vaccines (Ma et al. 2007a, b). Additional indirect strategies would antagonize IL-13 generation by targeting IL-4 or other Th2-modulating cytokines, including TSLP, IL-25, and IL-33 (Nakajima and Takatsu 2007). Animal data are promising. In murine systems, blockade of IL-13 by sIL-13R α 2-Fc (Grunig et al. 1998; Wills-Karp et al. 1998) or by antibody (Blanchard et al. 2005; Yang et al. 2004) effectively limits asthmatic responses, including IgE production, eosinophilia, AHR, mucus production and fibrosis (Blanchard et al. 2005; Grunig et al. 1998; Wills-Karp et al. 1998; Yang et al. 2004).

IM-638 is a fully humanized IgG1 monoclonal antibody, which is specific for the IL-13 epitope that binds IL-4Ra and neutralizes IL-13 bioactivity in the animal model (Kasaian et al. 2008). It has no neutralization activity against IL-4 (Kasaian et al. 2008). To evaluate the effect of blockade of IL-13 on LAR, AHR, and airway inflammation in mild atopic asthma patients, IMA-638 was given subcutaneously in 2 doses, 7 days apart. IMA-638 significantly attenuated the early maximum percent fall in FEV₁, the early-phase area under the curve (AUC_{0–2h}) and late-phase AUC (AUC_{3–7h}) on Day 14, but this effect was lost by Day 35 (Gauvreau et al. 2011a). IMA-638 treatment did not significantly affect allergen-induced AHR or sputum eosinophilia (Gauvreau et al. 2011a).

IL-13 can induce bronchial epithelial cells to secrete periostin, a matricellular protein (Sidhu et al. 2010; Woodruff et al. 2007), into the underlying matrix, where it has autocrine effects on epithelial cell function and paracrine effects on fibroblasts (Sidhu et al. 2010). Therefore, periostin may contribute to the airway remodeling (Sidhu et al. 2010; Takayama et al. 2006), and has been used as a marker for high IL-13 activity in asthmatic airways. Lebrikizumab is an IgG4 humanized anti-IL-13 monoclonal antibody (Corren et al. 2011). After 12-week treatment with lebrikizumab, high pre-treatment periostin group of patients with uncontrolled asthma has greater lung function

improvement (as per FEV₁) compared to the low periostin group (Corren et al. 2011). Pitrakinra, an IL-4 variant, targets both IL-4 and IL-13 via binding to IL-4R α receptor complexes (Wenzel et al. 2007). Pitrakinra significantly reduced LAR after 4 weeks treatment by nebulization twice daily, in contrast, when it was given by subcutaneous injection at same dose and duration, it did not inhibit LAR significantly compared to the placebo group (Wenzel et al. 2007). Pitrakinra also reduced asthma-related adverse events and the use of rescue medications following allergen inhalation (Wenzel et al. 2007). Thus, targeted local treatment by inhibition of both IL-4 and IL-13 in the lung, substantially diminish allergen-induced airway responses.

Conclusions

Allergen inhalation challenge in mild asthmatic subjects is an established and effective way to examine the pathogenesis and therapeutic approaches of asthma. Allergen-induced LAR is associated with more severe airway inflammation and AHR. The degree of airway eosinophilia, correlates to the size of airway inflammation, is regulated by bone marrow progenitor cells. Both myeloid and plasmacytoid dendritic cells are involved in the pathogenesis of allergen-induced LAR, but play different roles. Early study (Phase II) results of the potential new classes of asthma medications, such as anti-sense ODN drug product (TPI ASM8), anti-human IL-13 (IM-638, lebrikizumab), and anti-IL-4 and IL-13 (pitrakinra), are promising in terms of their efficacy and safety for asthma treatment.

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