

Expert Opinion

1. Overview
2. CpG DNA therapy
3. Antisense oligonucleotide
4. RNA interference
5. Application of siRNA to clinical therapeutics
6. Therapeutic potential of siRNA for allergy
7. Reduction of allergic responses using siRNA
8. Clinical potential of siRNA-based therapy
9. Expert opinion

informa
healthcare

Oligonucleotide based-strategies for allergy with special reference to siRNA

Motohiko Suzuki, Xiufen Zheng, Xusheng Zhang, Thomas E Ichim, Marianne E Beduhn & Weiping Min[†]

University of Western Ontario, London, Ontario, Canada

Background: Allergic diseases are a significant global health care problem. Current pharmacological approaches address symptoms but do not alter the underlying immune dysregulation. Current allergen-specific immunotherapy has several drawbacks. Therefore, approaches that attenuate allergic responses safely and effectively at the level of upstream causative events are desirable. Oligonucleotide-based therapies [CpG DNA, antisense oligonucleotides, and small interfering RNA (siRNA)] are promising approaches. **Objective/methods:** We review developments in oligonucleotide-based therapies and the potential of siRNA for treating allergy. **Results/conclusions:** Strategies with oligonucleotides basically aim to reduce T helper type 2 (Th2) responses. It is controversial whether the reduction of Th2 responses does, in fact, attenuate allergic diseases. Increased understanding of allergic mechanisms will enhance the efficacy of oligonucleotide-based therapy

Keywords: allergy, CpG DNA, dendritic cells, gene silencing, RNA interference, siRNA

Expert Opin. Biol. Ther. (2009) 9(4):441-450

1. Overview

Allergic diseases, which include allergic rhinitis, asthma and atopic dermatitis, affect people on a widespread and global scale. Allergy is considered to be a hypersensitivity reaction produced by antibodies or cells in response to environmental allergens. Allergic patients have a tendency to produce IgE antibody upon stimulation with allergens, in addition to developing symptoms of asthma, rhinoconjunctivitis, and/or eczema.

Allergic diseases are generally associated with CD4⁺ T helper type 2 (Th2) cells, rather than Th1 cells which appear in immune responses to virus and bacteria (Figure 1). The Th2 cell appears soon after sensitization with environmental allergens and is characterized by production of IL-4, IL-5 and IL-13. B cells play a critical role in IgE synthesis, and require two signals for this synthesis [1-3]. The first signal is provided by cytokines, such as IL-4. The second signal is provided by ligation of CD40 (Figure 2). IL-5 plays a central role not only in eosinophil differentiation and proliferation, but also in eosinophil accumulation [4]. The functional importance of this cytokine was demonstrated in studies where exogenous administration of IL-5 resulted in eosinophilic infiltration and activation [4-6]. Conversely, IL-5 deficiency results in abrogation of eosinophilic accumulation in murine models of allergy [4,7]. The eosinophil has been known to be a primary effector in terms of inducing airway damage by releasing detrimental mediators such as major basic protein, eosinophilic peroxidase and eosinophil cationic protein [4,8,9]. The ability to manipulate or suppress expression of Th2 cells could inhibit allergic responses and symptoms.

Despite the increased understanding of the pathophysiology of allergic responses, current therapy targets late events within the allergic cascade. Clinically, the main

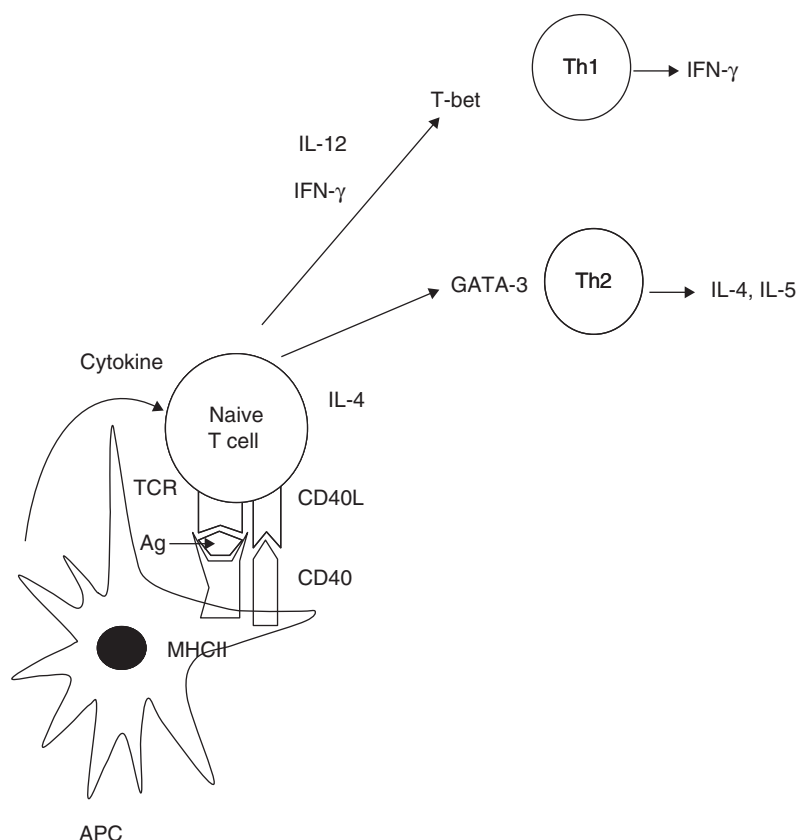


Figure 1. Dendritic cells (DCs) can potentially initiate naive T cells to differentiate into effector T cells in immune responses.

DCs provide three signals to T cells during activation: (1) the antigenic signal that is communicated via the high expression of MHC molecules found on DCs; (2) the costimulatory signal, comprising membrane-bound molecules such as CD40, CD80/86 and CD-40L, which are essential for T cell expansion and escape from anergy; and (3) soluble cytokines. T cell differentiation into a T helper type 1 (Th1) phenotype is induced by IFN- γ via upregulation of T-box expressed in T cells (T-bet). In contrast, the Th2 phenotype is caused by IL-4 through the regulation of GATA binding factor 3 (GATA-3). Th2 cells can then produce IL-4 and IL-5, which stimulate allergic sensitization and symptoms. Ag: antigen; APC: antigen presenting cell,

therapy for allergic disorders includes pharmacological antihistamines, antileukotrienes and corticosteroids. These are effective for controlling allergic disease manifestations, but only reduce the symptoms of asthma, rhinoconjunctivitis, and eczema. Anti-IgE antibody therapy also reduces allergic symptoms and inflammatory reactions [10]. However, these approaches do not alter the underlying causative factors of immune dysregulation. Current allergen-specific immunotherapy is performed using extracts from natural sources that are not standardized and contain allergenic and non-allergenic components, requiring treatment that lasts for a period of about 3 years. Current allergen-specific immunotherapy is the only treatment that might be able to cure the allergic disease itself. However, it is controversial whether current allergen-specific immunotherapy is useful method or not, although this phenomenon has been widely applied in the treatment of allergic diseases. The long duration of treatment and frequent injections result in low rates of patient compliance. Therefore, new strategies have been

developed and published regarding the clinical treatment of allergy. For example, intralymphatic allergen administration increased safety and efficacy, and reduced treatment time from 3 years to 8 weeks [11].

The human genome project, combined with the exponential rise in genomic and proteomic technologies, enabled scientists to associate numerous genes and their products with pathological processes. Conventional means of drug development after identifying pathology-associated genes involves creation of recombinant protein products and attempting to target the protein with small-molecule libraries. A more specific approach to targeting expression of undesired genes appeared with the development of antisense oligonucleotides. Unfortunately, the relatively low *in vivo* efficacy of this approach has held back significant clinical progress. A more potent, self-amplifying means of gene-silencing involves introduction of a double stranded RNA into a cell to activate the process of RNA interference (RNAi). Double stranded RNA strands of approximately 21 nucleotide base pairs are sufficient to

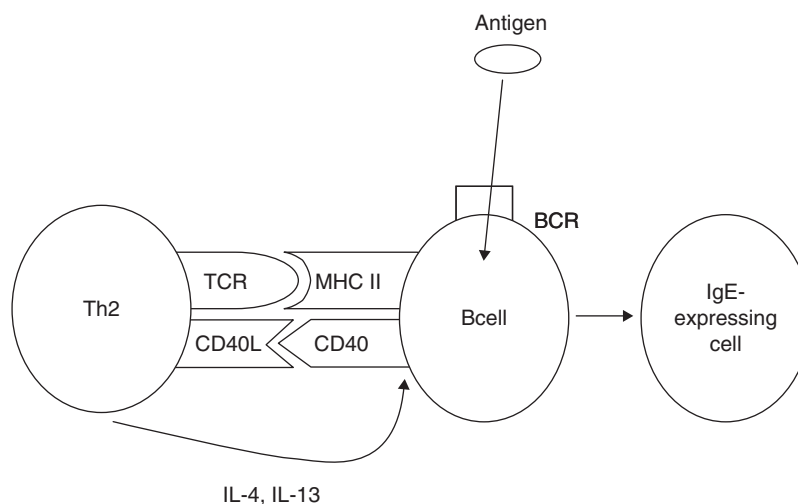


Figure 2. B cell differentiation into IgE-expressing cells is induced by several important signals. The first is an antigen-specific signal which is provided via B cell antigen receptor (BCR). Signals delivered through the interaction of co-stimulatory molecule CD-40 and CD40 ligand (CD40L) are also critical. Cytokines such as IL-4 and IL13, which are produced by Th2 cells, are important signals.

TCR: T-cell receptor.

activate RNAi, without activating innate IFN- α responses. These small interfering RNA (short interference RNA, siRNA) have proven to be a potent, selective, and easily-inducible method for specifically blocking expression of desired genes. siRNA has been shown to have superior gene silencing capability when compared with antisense oligonucleotides and ribozymes [12-14]. In this paper, we review the recent developments oligonucleotide-based therapies, and the potential of siRNA for allergy attenuation.

2. CpG DNA therapy

Toll-like receptors (TLRs) are transmembrane signaling molecules that play an important role in the initiation of innate and adaptive immune responses. TLRs recognize conserved molecular patterns associated with either pathogens or tissue injury. For example, TLR-2 and -4 recognize lipoproteins found on bacteria, as well as endogenous extracellular matrix fragments, TLR-3 is activated by double stranded RNA, as found on viruses, and TLR-5 recognizes bacterial flagellin. Many TLRs induce Th1 cytokines, such as IL-12 and IFN- γ . Among the TLRs, TLR9 is prominent for its involvement in stimulating innate responses after detecting DNA oligodeoxynucleotides that contain CpG motifs (CpG DNA), which are commonly associated with DNA of bacterial origin. CpG DNA induces antigen presenting cells to promote Th1-like responses characterized by the production of IL-12, IFN- γ and IgG2a. IL-12 and IFN- γ induce naive T cells to differentiate into Th1 cells [15,16]. This strategy also inhibits IgE, IgG1 and passive cutaneous anaphylaxis (PCA) reactions, and reduces IL-4 levels [15,16]. The mechanism by which these effects occur has been recently investigated. CpG DNA undergoes

endocytosis and interacts with toll-like receptor 9 (TLR-9), which further interacts with the adaptor protein, myeloid differentiation primary response protein 88 (MyD88) [15]. Through MAPK, p38, JUN transcription factor homologs 1 and 2 (JUN1/2), I κ B kinase (IKK), activator protein 1 (AP-1), and NF- κ B translocate to the nucleus, where they promote the transcriptional activation of target proinflammatory genes like TNF- α , IL-12 and CD 40 [15]. CpG DNA alone can induce non-specific inhibition of allergic responses. Conjugates of CpG DNA and allergenic proteins are more effective in allergen-specific immunotherapy, compared with non-conjugated molecules [17]. Clinical trials with CpG DNA have been performed. Tulic *et al.* [18] showed that immunotherapy with conjugates of Amb a 1 oligonucleotide and CpG DNA modifies the response of nasal mucosa to allergen challenge by increasing Th1 cytokine production while decreasing both Th2 cytokine production and eosinophilia in patients with ragweed allergy. Creticos *et al.* [19] also demonstrated clinical benefit of CpG DNA in patients with allergic rhinitis. Gauvreau *et al.* also showed regulation of IFN by CpG DNA, although CpG DNA did not regulate allergic airway responses [20]. Furthermore, clinical trials to evaluate the safety and efficiency of Tolamba, a conjugate of CpG DNA and ragweed, have been performed by Dynavax Technologies Corporation. However, Dynavax discontinued its Tolamba program because of failure in Phase II clinical trial [21].

2.1 Side effects

Fever, a mild increase in circulating neutrophils and elevated serum haptoglobin were recorded in mice, cattle and sheep treated with CpG DNA [22-24]. Since CpG DNA can

activate B cells and antigen-presenting cells, it might trigger or aggravate autoimmune processes [25].

2.2 Chemical modification of CpG DNA

Modification of CpG DNA has been investigated to improve its immunostimulatory activity, stability and toxicity [26,27]. Chemical modification may enhance the clinical potential of CpG DNA.

2.3 Dendritic cells pulsed with CpG DNA

Dendritic cells (DCs) derived from bone marrow (BM-DCs) pulsed with CpG DNA together with the allergen downregulated Th2 immune responses and reduced airway inflammation [28]. Therefore, allergen-specific therapy using allergen plus CpG-pulsed DCs appears promising. However, Trujillo-Vargas *et al* [29] showed that allergen-pulsed DCs matured with CpG DNA did not result in reduction of airway eosinophilia nor allergen-specific IgE in the serum. Further experiments and trials are required to fully elucidate the effects of this therapy.

2.4 Conjugation of CpG DNA and T-cell epitope peptide

Conventional allergen-specific immunotherapy has limited safety because of side effects such as anaphylactic reactions. Therefore, current allergen-specific immunotherapy generally starts with a low dose of allergen which is subsequently increased to a higher dose. T-cell peptide epitopes are short linear structures, and are not readily able to crosslink IgE, which is bound to mast cells or basophils. T-cell peptide epitopes can, however, induce immunologic tolerance. The tolerogenic effects of peptides on T cells and antibody responses against whole protein allergens have been demonstrated using allergenic epitopes [30,31], suggesting that the underlying mechanism of peptide immunotherapy is the induction of tolerance within a specific T-cell population. The major advantage of this therapy is the reduction in IgE-mediated events compared with whole-allergen therapy. Clinical trials using peptide immunotherapy have been performed for bee venom [32] and cat allergy [33]. Tarzi *et al.* [32] showed that peptide immunotherapy reduced late-phase skin reactions following an allergen challenge with bee venom, and reduced the production of both IL-13 and IFN- γ by peripheral blood mononuclear cells.

Since the mechanisms by which CpG DNA and peptide immunotherapy mediate activity are different [15,30,31], we investigated the use of conjugate of CpG DNA and T-cell epitope peptides. Consequently, we showed that conjugates of CpG DNA and T-cell epitope peptides reduced allergic responses compared with T-cell epitope peptide alone, and that conjugates of CpG DNA and T-cell epitope peptide had therapeutic effects in mice [34].

3. Antisense oligonucleotide

Immune modulation has conventionally been restricted to relatively non-specific approaches, for example chemical agents

that induce multiple cascades, or cytokines that are administered systemically at non-physiological levels. The advent of gene-specific silencing has provided immunologists a novel method of intervening in biological processes that is more selective and 'refined' as compared with previous approaches.

Antisense oligonucleotides are single-stranded synthetic nucleic acids which are 18 – 30 bp in length and can specifically hybridize to the target gene through Watson–Crick base-pair hybridization. This results in specific inhibition of gene expression through activation of RNase H which cleaves the target mRNA [35]. Antisense is useful method for attenuating allergy and immune diseases. For example, Gauvreau *et al.* [36] examined the drug TPI ASM8, containing two phosphorothioate antisense oligonucleotides against the chemokine receptor CCR3 and β (c) subunit of IL-3, IL5 and IL-13; they found it inhibited β (c) and CCR3 mRNA in sputum, allergen-induced eosinophilic response, and early asthmatic response in patients with mild asthma.

4. RNA interference

In 1998, Fire *et al.* demonstrated that injecting *Caenorhabditis elegans* with a combination of both sense and antisense RNA specific to endogenous genes led to a more potent suppression of gene expression than the administration of sense or antisense alone. Inhibition of gene expression was so potent that ~1 – 3 molecules of duplexed RNA per cell were effective at knocking down gene expression. One of the striking findings was that suppression of gene expression was capable of migrating from cell to cell and that the inhibitory effect was transferred to progeny cells. This was, to our knowledge, the first description of RNAi [37]. This phenomenon is an endogenous cellular defense mechanism against both viruses that invade the cell and transposable elements in the genome [38,39]. Upon recognition of these 'dangerous' double-stranded RNA (dsRNA), enzymatic complexes degrade any mRNA transcripts containing homology to the dsRNA. Although RNAi was first detected in *C. elegans*, it has been discovered that RNAi is conserved in many species, including insects, plants, and fungi [40] and also mammalian cells [41]. This implies that artificial induction of RNAi can be useful for silencing pathological genes in a therapeutic manner. One problem arising from the initial description of RNAi was that in order to induce RNAi, long pieces (200 – 800 base pairs) of dsRNA had to be used. This was impractical for therapeutic uses owing to the sensitivity of long RNA to cleavage by plasma and intracellular RNases. In addition, long pieces of dsRNA induce a cellular 'panic response', part of which includes nonspecific inhibition of gene transcription, which was associated with production of IFN- α [42]. In 2001, it was demonstrated that following the entry of long dsRNA duplexes into the cytoplasm, a ribonuclease III-like enzymatic activity cleaved the duplex into smaller 21 – 23 base pair fragments, which are active in blocking endogenous gene expression. These small pieces of RNA, siRNA,

can be administered exogenously to block gene expression in mammalian cells without triggering the panic response [41].

There are two pathways for inducing RNAi. The first is the naturally occurring pathway that is triggered when viral or long dsRNA enters a cell. Upon crossing the membrane, the dsRNA activates several enzymatic components: i) (2',5')oligoadenylate synthetase, an enzyme that initiates a biochemical cascade leading to inhibition of protein synthesis [43]; ii) protein kinase R (PKR), which also results in nonspecific shut-down of cellular activity [44]; and iii) Dicer, a nuclease that cuts the dsRNA into 21 – 23 base pair lengths which then become active in blocking endogenous gene expression [45]. Long dsRNA also activates TLR-3, resulting in the production of IFN- α by a variety of cells, such as plasmacytoid dendritic cells [46]. Gene silencing by long dsRNA is not advantageous for therapeutic or experimental purposes owing to its non-specific effects.

Another pathway of RNAi induction is initiated by synthetic siRNA of 21 – 23 base-pairs in length [47]. The siRNA forms and guides the RNA-induced silencing complex (RISC) to dock with endogenous mRNA transcripts. RISC subsequently cleaves the mRNA transcript in a sequence- and length-specific manner. This approach is highly selective in that only endogenous mRNA transcripts with strict homology to the siRNA are destroyed. Furthermore, because siRNA does not activate (2',5')oligoadenylate synthetase or PKR, the cell maintains normal protein synthesis and function.

5. Application of siRNA to clinical therapeutics

Application of siRNA as a treatment was demonstrated for various diseases. The therapeutic promise of siRNA is dependent upon the following conditions: i) bioavailability, ii) lack of toxicity, iii) specificity of silencing effects, and iv) efficacy *in vivo*. It appears that siRNA meets all of these criteria. Gene silencing strategies using siRNA have been successful in inducing therapeutic benefits in animal models of various conditions, such as transplantation [48-50], cancer [51-53], and viral models.

Mice inoculated intravaginally with herpes simplex virus type 2 (HSV-2) develop infection and apparent disease. Only 25 % of mice treated with siRNA intravaginally 2 h before challenge and 4 h after challenge with HSV-2 died, compared with 75% of mice in the control groups [54]. In the case of HIV, effective silencing of both the primary HIV receptor, CD4, and the HIV coreceptor, CXCR-4 [55], has been successfully accomplished with siRNA, resulting in the prevention of viral entry into target cells. Clinical translation of RNAi therapies for viral infections have already begun. For example, a triple combination vector of anti-HIV shRNA, anti-CC5 ribozyme, and nucleolar-localizing transactivating response (TAR) decoy was approved by the US FDA to evaluate RNAi for HIV infections [56]. A Phase II trial testing RNAi for respiratory syncytial virus (RSV) infection was also reported [57]. The field of antiviral siRNA therapeutics is intensely studied and is a newly-developing area of investigation. Investigations of siRNA for controlling

allergy are quite recent, although therapeutic applications of siRNA have been studied for various other diseases.

6. Therapeutic potential of siRNA for allergy

Allergic diseases are caused by an excessive Th2 response. It has been reported that siRNA suppression of various immunological genes can be used to shift naïve T cell commitment to either Th1 or Th2. Of relevance to allergy, gene silencing of signal transducer and activator of transcription 6 (STAT6) or GATA binding protein 3 (GATA3) with siRNA reduced Th2 cytokines and responses *in vitro*, as discussed below.

6.1 STAT6 siRNA

STAT proteins are considered to be transcription factors mediating cytokine signaling [58]. The IL-4 response fails in STAT6 deficient mice, since STAT6 is important for mediating the responses causing IL-4 and Th2 production [59]. Gene expression of STAT6 was silenced in lymphocytes transfected with STAT6 siRNA. *In vitro*, STAT6 siRNA increased the production of IFN- γ , a Th1 cytokine, from lymphocytes [60].

6.2 GATA3 siRNA

The transcription factor GATA3 is required for the differentiation of Th2 cells from naive precursors and for the expression of Th2 cytokines in differentiated Th2 cells [61-63]. GATA3 siRNA knocked down GATA3 gene expression in human T cells. Expression of the genes encoding the Th2 cytokines IL-4 and IL-13 was downregulated in GATA3-silenced human T cells [64].

7. Reduction of allergic responses using siRNA

siRNA has therapeutic potential to attenuate allergy not only *in vitro* but also *in vivo*. Several authors have shown the usefulness of siRNA *in vivo* as described below.

7.1 GATA3 short hairpin siRNA (GATA3 shRNA)

Administration of GATA3 shRNA after ovalbumin (OVA) sensitization inhibited GATA3 expression in lung tissue, resulting in the suppression of eosinophil, lymphocyte, and neutrophil infiltration in bronchoalveolar lavage (BAL) fluid. GATA3 shRNA also reduced levels of IL-4, IL-5, and IL-13 in BAL fluid as well as decreasing airway hyper-responsiveness [65].

7.2 IL-5 short hairpin siRNA (IL-5 shRNA)

IL-5 shRNA inhibited IL-5 mRNA from being produced in lung tissue *in vivo*. IL-5 shRNA also reduced airway hyper-responsiveness, eosinophil and inflammatory cells into the lung, and eotaxin levels in the BAL fluid. However, IL-5 shRNA did not decrease the OVA-specific IgE level [66].

7.3 CD40 short hairpin siRNA (CD40 shRNA)

We have recently reported the use of CD40 shRNA [67] to systemically inhibit Th2 development *in vivo*. CD40 in

dendritic cells (DCs) activates T cells through CD40 ligand (CD40L, CD154), which is expressed on T cells [68]. CD40 crosslinking on isolated T cells has been demonstrated to increase Th1 and Th2 cytokines and stimulate proliferation [69]. To date, blockade of the CD40-CD40L interaction is being aggressively pursued as a tolerance-inducing strategy [70,71]. Inhibition of this bidirectional interaction not only suppresses T cell responses [72] and Th2 cytokines [73], but also actively generates regulatory T cells (Treg) [74].

Allergic symptoms improved significantly in mice that received CD40 shRNA compared with mice that received control shRNA or PBS. The therapeutic significance of CD40 shRNA was also demonstrated by the remarkable reduction of eosinophils infiltrating the nasal mucosa. OVA-specific T cell responses and anti-OVA-specific IgE levels were significantly decreased in sera of mice that received CD40 shRNA. Spleen cell production of IL-4 and IL-5 was suppressed in mice given CD40 shRNA. Finally, CD40 shRNA therapy facilitated the generation of regulatory T cells.

We also showed that the mechanisms of CD40 shRNA include; i) impaired antigen presenting cell (APC) function of dendritic cells; ii) impaired function of B cells; and iii) facilitation of regulatory T cells.

7.4 CD86 siRNA

CD86 is involved in the development of allergic inflammation and hyper-responsiveness [75]. CD86 plays an important role in APC-T cell interaction through CD28/cytotoxic T lymphocyte antigen 4 (CTLA-4)-CD80/CD86 costimulatory pathway. Administering cream-emulsified CD86 siRNA directly to ears significantly reduced ear swelling and attenuated contact hypersensitivity [76]. Atopic dermatitis was also induced by repeated exposure to *Dermatophagoides farine* (Df) antigen. In mice with atopic dermatitis, topical CD86 siRNA application inhibited total IgE and Df-specific IgG1.

7.5 Sphingosine kinase (SPHK) siRNA

Activation of FcεRI increases the amount of intracellular second messengers through sphingosine kinase (SPHK) stimulation. siRNA against SPHK suppressed anti-OVA-specific IgE in serum, in addition to decreasing the number of total cells and eosinophil infiltration in BAL. SPHK1 siRNA also significantly inhibited levels of IL-4, IL-5 and eotaxin in BAL [77].

7.6 Natriuretic peptide receptor (NPR) siRNA

Atrial natriuretic peptide (ANP) is associated with inflammatory responses [78], suggesting the importance of natriuretic peptide receptor (NPR) in inflammation. Imiquimod cream (TLR-7/8 agonist) mixed with chitosan nanoparticles containing NPRA siRNA inhibited airway hyper-reactivity, eosinophilia in lung tissue, and goblet hyperplasia of lung tissue. NPRA siRNA also reduced IL-4 in BAL fluid, and IL-5 in lung homogenates [79].

7.7 Summary

siRNA that is specific for GATA3, IL-5, CD40, CD86, SPHK and NPR inhibited allergic responses *in vivo*. These results will contribute to developing therapies for allergy.

8. Clinical potential of siRNA-based therapy

siRNA based-therapy has entered clinical trials for some diseases such as viral infection [56,57], but siRNA-based therapies for allergy have not yet entered clinical trials. Direct administration of siRNA has the potential for deleterious side effects, but holds much promise for the future.

Administration of modified DCs with antisense oligonucleotide targeting CD80 or CD86 prolongs allograft survival [80]. We also showed that administration of donor-derived v-rel reticuloendotheliosis viral oncogene homolog B (RelB)-silenced DCs significantly prevented allograft rejection in murine heart transplantation [81]. Also, we demonstrated that CD40 siRNA attenuated allergic diseases, and that this effect is associated with the immunomodulation of DCs transfected with CD40 siRNA [67]. This finding indicates the potential of DC therapy for allergy whilst excluding side effects caused by direct administration of siRNA. DCs are the major APC capable of activating naive T cells [82]. This is due to the high concentration of MHC II, as well as secondary signals and cytokine production by DCs, in comparison with other APCs. DCs are highly phagocytotic in the immature state. The role that DCs seem to have in allergic diseases can be largely attributed to their ability to sample the environment. Receptors, such as DEC-205 or CD36, are used by the DCs for internalizing bacterial products or apoptotic cells [83]. The DCs are also able to acquire components shed by live cells using scavenger receptors such as scavenger receptor A (SRA) [83]. In addition to receptor-mediated endocytosis, direct acquisition of environmental components surrounding the immature DC occurs through pinocytosis [84]. This high endocytotic capacity is illustrated by experiments assessing immature DCs in which fluorescein isothiocyanate (FITC)-dextran is constitutively acquired by immature DCs but not by mature DCs [85]. It is known that intradermal administration of naked DNA plasmids using either injection or gene-gun approaches leads to preferential gene expression in DCs [86], indicating that DCs take up genetic material efficiently. Furthermore, the ability of mRNA-pulsed DCs to transcribe and express the encoded allergens demonstrates that DCs can actively sequester and utilize exogenous RNA instead of leading it to cytoplasmic destruction. This approach is considered to be a valuable method for *in vivo* 'DC vaccination' for use in the treatment of cancer patients [87]. DCs are a novel target in the development of therapies for allergy [88,89].

9. Expert opinion

Allergy, which includes asthma, allergic rhinitis and atopic dermatitis, is a common condition. Quality of life is negatively

correlated with allergic diseases. Asthma can cause severe symptoms, dyspnea and even death. The effects of allergy treatments, such as antihistamines, leukotriene receptor antagonists, and corticosteroids, are insufficient, highlighting the need for new strategies. Recently, immunomodulatory therapies are the center of attention for treating allergic diseases. These therapies include oligonucleotide-based CpG DNA, antisense oligonucleotide, and siRNA.

The 2006 Nobel Prize in Medicine was awarded to Andrew Fire and Craig Mello for their discovery of RNAi. RNAi using siRNA is a potent method of specifically knocking down molecular targets. Gene silencing via siRNA allows for the development of a novel therapies for various immunological problems such as cancer, transplantation and viral diseases. siRNA can inhibit specific gene expression in the early events of an allergic cascade; clinically-used agents only target the late events. The interaction between APCs and T cells plays an important role in allergic sensitization, and this interaction is considered to be the source of allergic responses. Blocking the APC–T cell interaction can suppress allergic responses and attenuate allergic diseases by targeting upstream events in the allergic cascade. In fact, it has been reported that CD40 and CD86 siRNA attenuated allergic responses. siRNA against the genes that are associated with APC–T cell interaction has great potential for attenuating immune diseases such as allergy.

Although we focused on siRNA specific for CD40 in our previous study, there are numerous other attractive targets. Indeed, siRNA specific for IL-10, STAT6 and GATA3 have been shown to inhibit Th2 responses *in vitro*. Several authors also reported that administration of siRNA specific for IL-5, GATA3, sphingosine kinase, natriuretic peptide receptor, CD40 and CD86 suppressed allergic responses *in vivo*. There are other promising molecules including, but not limited to, cell surface receptors, adhesion molecules,

ligands, ion channels, cytokines and intracellular signal transduction molecules.

siRNA-based therapy is an attractive approach for attenuating allergy. However, direct administration may cause some side effects *in vivo*. There are ongoing experiments in our laboratory examining therapies using DCs transfected with siRNA for the purpose of excluding the side effects of direct administration. It should be noted that it is controversial whether this type of DC therapy is effective, since the mechanisms of allergy are quite complex. DCs are the most potent APCs, largely bearing the responsibility of initiating protective immune responses [90]. Interestingly, DCs also play a critical role in the induction of immune tolerance [91], drawing attention to the use of DCs for inducing tolerance. It may be possible to generate dendritic cells possessing tolerogenic properties (tol-DCs) that lack a specific cytokine, a costimulatory molecule, etc. by transfecting DC with gene-specific siRNA. Allergic responses may be blocked by tol-DCs at the source of allergic diseases, since DCs play an important upstream role.

Strategies with oligonucleotides basically aim to reduce Th2 responses. It is controversial whether the reduction of Th2 responses does, in fact, attenuate allergic diseases. Considering this, there are many issues to be resolved for oligonucleotide-based therapies: i) route of administration; ii) timing/frequency; iii) inclusion/exclusion criteria; and iv) selection for specific immunological profiles. Solutions for these issues will allow for further development of this therapy for clinical treatment. Moreover, increased understanding of allergic mechanisms will enhance the efficacy of oligonucleotide-based therapy.

Declaration of interest

Dr Wei-Ping Min's research is partially supported by a grant from the Canadian Institutes of Health Research (CIHR).

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers

- Poulsen LK, Hummelshoj L. Triggers of IgE class switching and allergy development. *Ann Med* 2007;39:440-56
- Bacharier LB, Geha RS. Molecular mechanisms of IgE regulation. *J Allergy Clin Immunol* 2000;105:S547-58
- Jelinek DF. Regulation of B lymphocyte differentiation. *Ann Allergy Asthma Immunol* 2000;84:375-85; quiz 385-7
- Hamelmann E, Gelfand EW. IL-5-induced airway eosinophilia—the key to asthma? *Immunol Rev* 2001;179:182-91
- Shi H, Qin S, Huang G, et al. Infiltration of eosinophils into the asthmatic airways caused by interleukin 5. *Am J Respir Cell Mol Biol* 1997;16:220-4
- Shi HZ, Xiao CQ, Zhong D, et al. Effect of inhaled interleukin-5 on airway hyperactivity and eosinophilia in asthmatics. *Am J Respir Crit Care Med* 1998;157:204-9
- Foster PS, Hogan SP, Ramsay AJ, et al. Interleukin 5 deficiency abolishes eosinophilia, airways hyperactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996;183:195-201
- Corrigan CJ, Kay AB. T cells and eosinophils in the pathogenesis of asthma. *Immunol Today* 1992;13:501-7
- Nishioka K, Saito C, Nagano T, et al. Eosinophil cationic protein in the nasal secretions of patients with mite allergic rhinitis. *Laryngoscope* 1993;103:189-92
- Belvisi MG, Hele DJ, Birrell MA. New anti-inflammatory therapies and targets for asthma and chronic obstructive pulmonary disease. *Expert Opin Ther Targets* 2004;8:265-85
- Senti G, Prinz Vavricka BM, Erdmann I, et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled trial. *Proc Natl Acad Sci USA* 2008;105:17908-12
- Tong AW, Zhang YA, Nemunaitis J. Small interfering RNA for experimental

- cancer therapy. *Curr Opin Mol Ther* 2005;7:114-24
13. Bertrand JR, Pottier M, Vekris A, et al. Comparison of antisense oligonucleotides and siRNAs in cell culture and in vivo. *Biochem Biophys Res Commun* 2002;296:1000-4
14. Thompson JD. Applications of antisense and siRNAs during preclinical drug development. *Drug Discov Today* 2002;7:912-7
15. Tsalik EL. DNA-based immunotherapy to treat atopic disease. *Ann Allergy Asthma Immunol* 2005;95:403-10; quiz 410-411, 451
16. Fonseca DE, Kline JN. Use of CpG oligonucleotides in treatment of asthma and allergic disease. *Adv Drug Deliv Rev* 2009, published online 9 January 2009 doi:10.1016/j.addr.2008.12.007
17. Shirota H, Sano K, Hirasawa N, et al. Novel roles of CpG oligodeoxynucleotides as a leader for the sampling and presentation of CpG-tagged antigen by dendritic cells. *J Immunol* 2001;167:66-74
18. Tulic MK, Fiset PO, Christodoulouopoulos P, et al. Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. *J Allergy Clin Immunol* 2004;113:235-41
- **A clinical study showing that Amb a 1-immunostimulatory oligonucleotide conjugate decreased Th2 cytokine production and eosinophilia in patients with ragweed sensitivity.**
19. Creticos PS, Schroeder JT, Hamilton RG, et al. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. *N Engl J Med* 2006;355:1445-55
- **A clinical trial of ragweed-toll-like receptor 9 agonist.**
20. Gauvreau GM, Hessel EM, Boulet LP, et al. Immunostimulatory sequences regulate interferon-inducible genes but not allergic airway responses. *Am J Respir Crit Care Med* 2006;174:15-20
- **A clinical study showing that CpG DNA regulated IFN.**
21. An Evaluation of the efficacy and safety of Tolamba™ for ragweed-allergic rhinitis in an environmental exposure chamber. National Institute of Health. Bethesda, MD: 2008; Available from: <http://clinicaltrials.gov/ct2/show/NCT00537355?term=NCT00537355&rank=1>
22. Kozak W, Wrotek S, Kozak A. Pyrogenicity of CpG-DNA in mice: role of interleukin-6, cyclooxygenases and nuclear factor-κB. *Am J Physiol Regul Integr Comp Physiol* 2006;290:R871-80
23. Nichani AK, Dar MA, Krieg AM, et al. Systemic innate immune responses following intrapulmonary delivery of CpG oligodeoxynucleotides in sheep. *Vet Immunol Immunopathol* 2007;115:357-68
24. Nichani AK, Mena A, Popowich Y, et al. In vivo immunostimulatory effects of CpG oligodeoxynucleotide in cattle and sheep. *Vet Immunol Immunopathol* 2004;98:17-29
25. Segal BM, Klinman DM, Shevach EM. Microbial products induce autoimmune disease by an IL-12-dependent pathway. *J Immunol* 1997;158:5087-90
26. Zimmermann S, Heeg K, Dalpke A. Immunostimulatory DNA as adjuvant: efficacy of phosphodiester CpG oligonucleotides is enhanced by 3' sequence modifications. *Vaccine* 2003;21:990-5
27. Bartz H, Mendoza Y, Gebker M, et al. Poly-guanosine strings improve cellular uptake and stimulatory activity of phosphodiester CpG oligonucleotides in human leukocytes. *Vaccine* 2004;23:148-55
28. Chiang DJ, Ye YL, Chen WL, et al. Ribavirin or CpG DNA sequence-modulated dendritic cells decrease the IgE level and airway inflammation. *Am J Respir Crit Care Med* 2003;168:575-80
- **A study showing the potential of modulated dendritic cells with CpG DNA as an antigen-specific therapy for allergy.**
29. Trujillo-Vargas CM, Ramirez-Pineda JR, Palmethofer A, et al. Mice vaccinated with allergen-pulsed myeloid dendritic cells are not protected from developing allergen-induced Th2 responses. *Int Arch Allergy Immunol* 2005;137:219-28
- **A report demonstrating no reduction of airway eosinophilia and allergen-specific IgE by modulated dendritic cells with CpG DNA.**
30. Larche M, Wraith DC. Peptide-based therapeutic vaccines for allergic and autoimmune diseases. *Nat Med* 2005;11:S69-76
31. Norman PS. Immunotherapy: 1999-2004. *J Allergy Clin Immunol* 2004;113:1013-23; quiz 1024
32. Tarzi M, Klunker S, Texier C, et al. Induction of interleukin-10 and suppressor of cytokine signalling-3 gene expression following peptide immunotherapy. *Clin Exp Allergy* 2006;36:465-74
33. Alexander C, Tarzi M, Larche M, Kay AB. The effect of Fel d 1-derived T-cell peptides on upper and lower airway outcome measurements in cat-allergic subjects. *Allergy* 2005;60:1269-74
34. Suzuki M, Ohta N, Min WP, et al. Immunotherapy with CpG DNA conjugated with T-cell epitope peptide of an allergenic Cry j 2 protein is useful for control of allergic conditions in mice. *Int Immunopharmacol* 2007;7:46-54
35. Lebedeva I, Stein CA. Antisense oligonucleotides: promise and reality. *Annu Rev Pharmacol Toxicol* 2001;41:403-19
36. Gauvreau GM, Boulet LP, Cockcroft DW, et al. Antisense therapy against CCR3 and the common beta chain attenuates allergen-induced eosinophilic responses. *Am J Respir Crit Care Med* 2008;177:952-8
- **A clinical study of antisense oligonucleotide.**
37. Fire A, Xu S, Montgomery MK, et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 1998;391:806-11
- **This is the first study, to the authors' knowledge, demonstrating RNA interference.**
38. Voinnet O. Induction and suppression of RNA silencing: insights from viral infections. *Nat Rev Genet* 2005;6:206-20
39. Ullu E, Djikeng A, Shi H, Tschudi C. RNA interference: advances and questions. *Philos Trans R Soc Lond B Biol Sci* 2002;357:65-70
40. Waterhouse PM, Wang MB, Lough T. Gene silencing as an adaptive defence against viruses. *Nature* 2001;411:834-42
41. Elbashir SM, Harborth J, Lendeckel W, et al. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 2001;411:494-8
- **The first study, to the authors' knowledge, demonstrating that 21 – 23 base pairs, termed small interfering RNA (siRNA), are capable of blocking gene expression**

- without triggering the nonspecific panic response.
42. Proud CG. PKR: a new name and new roles. *Trends Biochem Sci* 1995;20:241-6
 43. Adah SA, Bayly SF, Cramer H, et al. Chemistry and biochemistry of 2',5'-oligoadenylate-based antisense strategy. *Curr Med Chem* 2001;8:1189-212
 44. Williams BR. Signal integration via PKR. *Sci STKE* 2001;2001:RE2, published online 3 July 2001, doi: 10.1126/stke.2001.89.re2
 45. Saunders LR, Barber GN. The dsRNA binding protein family: critical roles, diverse cellular functions. *FASEB J* 2003;17:961-83
 46. Schlee M, Hornung V, Hartmann G. siRNA and isRNA: two edges of one sword. *Mol Ther* 2006;14:463-70
 47. Timmons L. The long and short of siRNAs. *Mol Cell* 2002;10:435-7
 48. Zheng X, Zhang X, Sun H, et al. Protection of renal ischemia injury using combination gene silencing of complement 3 and caspase 3 genes. *Transplantation* 2006;82:1781-6
 49. Zheng X, Zhang X, Feng B, et al. Gene silencing of complement C5a receptor using siRNA for preventing ischemia/reperfusion injury. *Am J Pathol* 2008;173:973-80
 50. Zhang X, Zheng X, Sun H, et al. Prevention of renal ischemic injury by silencing the expression of renal caspase 3 and caspase 8. *Transplantation* 2006;82:1728-32
 51. Scherr M, Battmer K, Winkler T, et al. Specific inhibition of bcr-abl gene expression by small interfering RNA. *Blood* 2003;101:1566-9
 52. Choudhury A, Charo J, Parapuram SK, et al. Small interfering RNA (siRNA) inhibits the expression of the Her2/neu gene, upregulates HLA class I and induces apoptosis of Her2/neu positive tumor cell lines. *Int J Cancer* 2004;108:71-7
 53. Martinez LA, Naguibneva I, Lehrmann H, et al. Synthetic small inhibiting RNAs: efficient tools to inactivate oncogenic mutations and restore p53 pathways. *Proc Natl Acad Sci USA* 2002;99:14849-54
 54. Palliser D, Chowdhury D, Wang QY, et al. An siRNA-based microbicide protects mice from lethal herpes simplex virus 2 infection. *Nature* 2006;439:89-94
 55. Ji J, Wernli M, Klimkait T, Erb P. Enhanced gene silencing by the application of multiple specific small interfering RNAs. *FEBS Lett* 2003;552:247-52
 56. Rossi JJ, June CH, Kohn DB. Genetic therapies against HIV. *Nat Biotechnol* 2007;25:1444-54
 57. de Fougerolles A, Vornlocher HP, Maraganore J, Lieberman J. Interfering with disease: a progress report on siRNA-based therapeutics. *Nat Rev Drug Discov* 2007;6:443-53
 58. Hoey T, Grusby MJ. STATs as mediators of cytokine-induced responses. *Adv Immunol* 1999;71:145-62
 59. Kaplan MH, Schindler U, Smiley ST, Grusby MJ. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. *Immunity* 1996;4:313-9
 60. Kozma N, Halasz M, Polgar B, et al. Progesterone-induced blocking factor activates STAT6 via binding to a novel IL-4 receptor. *J Immunol* 2006;176:819-26
 - A study demonstrating suppression of Th2 cytokines by STAT6 siRNA *in vitro*.
 61. Zhu J, Min B, Hu-li J, et al. Conditional deletion of Gata3 shows its essential function in TH1-TH2 responses. *Nat Immunol* 2004;5:1157-65
 - A report showing suppression of Th2 cytokines by GATA3 siRNA *in vitro*.
 62. Ferber IA, Lee HJ, Zonin F, et al. GATA-3 significantly downregulates IFN- γ production from developing Th1 cells in addition to inducing IL-4 and IL-5 levels. *Clin Immunol* 1999;91:134-44
 63. Lee HJ, O'Garra A, Arai K, Arai N. Characterization of cis-regulatory elements and nuclear factors conferring Th2-specific expression of the IL-5 gene: a role for a GATA-binding protein. *J Immunol* 1998;160:2343-52
 64. Maneechotesuwan K, Xin Y, Ito K, et al. Regulation of Th2 cytokine genes by p38 MAPK-mediated phosphorylation of GATA-3. *J Immunol* 2007;178:2491-8
 65. Lee CC, Huang HY, Chiang BL. Lentiviral-mediated GATA-3 RNAi decreases allergic airway inflammation and hyperresponsiveness. *Mol Ther* 2008;16:60-5
 - The first report, to the authors' knowledge, showing *in vivo* reduction of allergic inflammation with GATA3 RNAi.
 66. Huang HY, Lee CC, Chiang BL. Small interfering RNA against interleukin-5 decreases airway eosinophilia and hyper-responsiveness. *Gene Ther* 2008;15:660-7
 - The first study, to the author's knowledge, describing an *in vivo* efficacious strategy with IL-5 siRNA for asthma.
 67. Suzuki M, Zheng X, Zhang X, et al. Novel vaccination for allergy through gene silencing of CD40 using small interfering RNA. *J Immunol* 2008;180:8461-9
 - This is the first study, to the author's knowledge, demonstrating therapy with siRNA specific for CD40 can reduce allergic symptoms and responses *in vivo*.
 68. Caux C, Massacrier C, Vanbervliet B, et al. Activation of human dendritic cells through CD40 cross-linking. *J Exp Med* 1994;180:1263-72
 69. Peng X, Kasran A, Warmerdam PA, et al. Accessory signaling by CD40 for T cell activation: induction of Th1 and Th2 cytokines and synergy with interleukin-12 for interferon- γ production. *Eur J Immunol* 1996;26:1621-7
 70. Kirk AD, Blair PJ, Tadaki DK, et al. The role of CD154 in organ transplant rejection and acceptance. *Philos Trans R Soc Lond B Biol Sci* 2001;356:691-702
 71. Lanschuetzer CM, Olsz EB, Lazarova Z, Yancey KB. Transient anti-CD40L co-stimulation blockade prevents immune responses against human bullous pemphigoid antigen 2: implications for gene therapy. *J Invest Dermatol* 2008, published online 27 November 2008, doi: 10.1038/jid.2008.364
 72. Blazar BR, Taylor PA, Panoskaltsis-Mortari A, et al. Blockade of CD40 ligand-CD40 interaction impairs CD4⁺ T cell-mediated alloreactivity by inhibiting mature donor T cell expansion and function after bone marrow transplantation. *J Immunol* 1997;158:29-39
 73. Pochanke V, Hatak S, Hengartner H, et al. Induction of IgE and allergic-type responses in fur mite-infested mice. *Eur J Immunol* 2006;36:2434-45
 74. Taylor PA, Friedman TM, Korngold R, et al. Tolerance induction of alloreactive T cells via ex vivo blockade of the CD40:CD40L costimulatory pathway results in the generation of a potent immune regulatory cell. *Blood* 2002;99:4601-9
 75. Chen YQ, Shi HZ. CD28/CTLA-4-CD80/CD86 and ICOS-B7RP-1

- costimulatory pathway in bronchial asthma. *Allergy* 2006;61:15-26
76. Ritprajak P, Hashiguchi M, Azuma M. Topical application of cream-emulsified CD86 siRNA ameliorates allergic skin disease by targeting cutaneous dendritic cells. *Mol Ther* 2008;16:1323-30
 - **The first report, to the authors' knowledge, demonstrating the *in vivo* therapeutical potential of CD86 siRNA for contact hypersensitivity and atopic dermatitis.**
 77. Lai WQ, Goh HH, Bao Z, et al. The role of sphingosine kinase in a murine model of allergic asthma. *J Immunol* 2008;180:4323-9
 - **The first study, to the authors' knowledge, demonstrating the *in vivo* therapeutical potential of sphingosine kinase siRNA in allergic airway disease.**
 78. Ohbayashi H, Suito H, Takagi K. Compared effects of natriuretic peptides on ovalbumin-induced asthmatic model. *Eur J Pharmacol* 1998;346:55-64
 79. Wang X, Xu W, Mohapatra S, et al. Prevention of airway inflammation with topical cream containing imiquimod and small interfering RNA for natriuretic peptide receptor. *Genet Vaccines Ther* 2008;6:7, published online 15 February 2008, doi:10.1186/1479-0556-6-7
 - **The first report, to the author's knowledge, to show the *in vivo* anti-inflammatory effect of natriuretic peptide receptor in asthma.**
 80. Liang X, Lu L, Chen Z, et al. Administration of dendritic cells transduced with antisense oligodeoxyribonucleotides targeting CD80 or CD86 prolongs allograft survival. *Transplantation* 2003;76:721-9
 - **A report demonstrating the therapeutic potential of dendritic cells transduced with antisense oligonucleotide.**
 81. Li M, Zhang X, Zheng X, et al. Immune modulation and tolerance induction by RelB-silenced dendritic cells through RNA interference. *J Immunol* 2007;178:5480-7
 - **This is the first study, to the authors' knowledge, demonstrating that immunomodulated DCs by siRNA can prevent allograft rejection.**
 82. Inaba K, Metlay JP, Crowley MT, et al. Dendritic cells as antigen presenting cells in vivo. *Int Rev Immunol* 1990;6:197-206
 83. Urban BC, Willcox N, Roberts DJ. A role for CD36 in the regulation of dendritic cell function. *Proc Natl Acad Sci USA* 2001;98:8750-5
 84. Norbury CC, Chambers BJ, Prescott AR, et al. Constitutive macropinocytosis allows TAP-dependent major histocompatibility complex class I presentation of exogenous soluble antigen by bone marrow-derived dendritic cells. *Eur J Immunol* 1997;27:280-8
 85. Kato M, Neil TK, Fearnley DB, et al. Expression of multilectin receptors and comparative FITC-dextran uptake by human dendritic cells. *Int Immunol* 2000;12:1511-9
 86. Condon C, Watkins SC, Celluzzi CM, et al. DNA-based immunization by in vivo transfection of dendritic cells. *Nat Med* 1996;2:1122-8
 87. Sullenger BA, Gilboa E. Emerging clinical applications of RNA. *Nature* 2002;418:252-8
 88. Lambrecht BN. Lung dendritic cells: targets for therapy in allergic disease. *Curr Mol Med* 2008;8:393-400
 89. Hammad H, Kool M, Soullie T, et al. Activation of the D prostanoid 1 receptor suppresses asthma by modulation of lung dendritic cell function and induction of regulatory T cells. *J Exp Med* 2007;204:357-67
 90. Lechler R, Ng WF, Steinman RM. Dendritic cells in transplantation—friend or foe? *Immunity* 2001;14:357-68
 91. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol* 2003;21:685-711.

Affiliation

Motohiko Suzuki^{1,2}, Xiufen Zheng¹, Xusheng Zhang¹, Thomas E Ichim^{1,3}, Marianne E Beduhn¹ & Weiping Min^{†1}

[†]Author for correspondence

¹University of Western Ontario, University Hospital C9 – 136, Departments of Surgery, Pathology and Oncology, 339 Windermere Road, London, Ontario, N6A 5A5, Canada
Tel: +1 519 663 2985; Fax: +1 519 663 3295; E-mail: weiping.min@uwo.ca

²Nagoya City University, Department of Otorhinolaryngology, Nagoya, Japan

³Medistem Laboratories, San Diego, CA, USA