

CpG Motifs as Possible Adjuvants for the Treatment of Allergic Diseases

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Key Words

CpG motif · Type I allergy · Specific immunotherapy · Th1/Th2 · Immunomodulation

Abstract

DNA containing unmethylated CpG motifs and synthetic oligodeoxynucleotides derived thereof (CpG ODN) have intensively been investigated for their immunostimulatory properties in the recent past. CpG ODN were shown to induce strong Th1 immune responses in mammals. The downregulation of the antigen-driven Th2 response of type I allergies represents one important therapeutic goal of specific immunotherapy (SIT). Hence, CpG ODN represent promising substances which support the modification of the pathogenic Th2 immune profile toward a Th1 profile when used as adjuvants for SIT. This article discusses how the use of CpG ODN in immunotherapeutics could improve the treatment of type I allergy.

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Introduction

Rodent and primate immune systems react strongly to certain sequences (immunostimulatory DNA sequences, ISS) that are rare in vertebrates but present in many lower, potentially pathogenic organisms. Therefore, ISS were associated with dangerous invaders and thus represented

powerful adjuvants for activation of the innate arm of the immune response. In response to stimulation with ISS, professional antigen-presenting cells (APC), such as macrophages and dendritic cells (DCs) produce IL-12, IL-18, and IFN- α/β , while altering their surface molecule profile [1–6]. B cells also alter their surface markers for enhanced antigen presentation while proliferating and producing IL-6 and IL-12 [7, 8]. Natural killer (NK) cells become cytotoxic and produce early IFN- γ [9, 10]. This concerted response then biases the adaptive arm of the immune system toward a Th1 response against any locally encountered foreign protein.

Type I allergy is caused by a characteristic immune response to particular antigens that is mainly mediated by Th2 cells. This subset of CD4⁺ T lymphocytes synthesizes high levels of IL-4, IL-5 and IL-13, subsequently leading to the production of allergen-specific IgE. Specific immunotherapy (SIT) has been successfully used in the treatment of type I allergies for more than 80 years [11]. SIT consists in the administration of increasing doses of allergen extract to sensitized subjects. Besides an observed increase of allergen-specific antibody levels, different immunological mechanisms at the T cell level have been discussed to contribute to the success of SIT: the treatment leads to diminished allergen-induced lymphoproliferative responses indicating induction of peripheral tolerance to the injected allergen [12]. In addition, the Th2-like response to allergenic molecules was shifted to a Th0/Th1-like response, characterized by the production of higher

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amounts of IFN- γ or reduced levels of IL-4 [13, 14]. This phenomenon has been termed immune deviation.

Consequently, the modulation of T cells towards a Th0/Th1-like phenotype represents one crucial therapeutic strategy of type I allergy. An approach to accomplish this immunomodulation at the T cell level is the use of adjuvants supporting the desired type of immune profile. In this respect, ISS represent potential candidate molecules to be used in SIT.

Immunostimulatory Nucleotides

The observation that certain unmethylated prokaryotic DNA sequences induced strong Th1-associated immune responses in vertebrates started off a new field of immunological research. DNA was not seen as an immunologically inert molecule but thought to serve as 'danger signal' for the vertebrate immune system [15]. In general, ISS consist of a central unmethylated pair of nucleotides, cytosine and guanosine (CpG), and either methylation or reversion into GpC abrogates their immunostimulatory activities [7]. In vertebrates, methylation of DNA at cytosine residues in the CpG sequence plays an important role for the long-term gene regulation of different cell types [16]. DNA methylation patterns are normally transmitted to daughter cells via cell division leading to clonally inherited patterns of gene expression [17]. Furthermore, transcription of some genes was repressed by the presence of 5-methylcytosine at certain CpG dinucleotides in the promoter region [17, 18]. Approximately 70% of mammalian CpG motifs are methylated. These methylated dinucleotides represent hotspots for mutation to TpG during replication, therefore probably leading to a drastic reduction of CpG motifs in the vertebrate genome. As a consequence, unmethylated CpG sequences are 20 times more common in bacterial than in mammalian DNA. The concept emerged that immune recognition of unmethylated CpG motifs served as an early detection of evidence for microbial invasion and subsequently activates defense mechanisms. The fact that certain intracellular pathogens such as small DNA viruses have extraordinarily low CpG contents indicates an evolutionary adaptation to avoid the induction of CpG-based immune defenses [19].

The concept of how CpG DNA and short synthetic oligodeoxynucleotides (CpG ODN) derived thereof were recognized by mammalian cells has been intensively investigated in the past years. It was established that cell types which directly responded to stimulation with CpG motifs expressed Toll-like receptor (TLR) 9 [20–

22]. TLRs are a family of closely related proteins recognizing conserved patterns on microorganisms. So far, 10 members of the TLR family have been reported representing a combinatorial repertoire to discriminate between a wide spectrum of pathogen-associated microbial molecules [23, 24]. Certain TLRs cooperate with each other by forming functional pairs between cytoplasmatic domains [25]. In this context, a possible cooperation in CpG DNA recognition between TLR 9 and TLR 7 was suggested only recently [26, 27].

TLR and the formation of combinatorial sets between different TLRs could be involved in the discrimination between different CpG sequences [22]. Indeed, immune recognition of CpG motifs varies between different species and human PBMC respond poorly to ODN that are very active in mice [28]. Thus, intensive attempts to precisely define the DNA sequence structure required for optimal immune stimulation were done [5, 29–31]. At present, CpG sequences can be divided into at least 2 distinct classes. One class induces weak activation of B cells but high levels of NK cell activation and synthesis of IFNs. By contrast, the second class induces high levels of B cell activation and IgM production but only low levels of IFNs [6, 28, 30]. These findings suggest that a combination of several CpG sequences will probably be necessary to achieve optimal immunostimulatory effects.

CpG ODN and Type I Allergy

T lymphocytes are activated by CpG ODN but not directly. Their bias towards Th1 cells rather depends on CpG ODN-induced cytokines and upregulation of cellular factors by APC. IL-12, which induces the production of IFN- γ and drives the development of Th1 cells is of key importance [32]. Therefore, in order to support the emergence of a Th1 immune response in atopic individuals, the induction of IL-12 remains a necessary first step. However, the atopic state was associated with a reduced synthesis of Th1 cytokines [33]. Therefore, it was tempting to investigate whether CpG ODN displayed Th1-fostering activities in atopic individuals. We could demonstrate that CpG ODN were capable of inducing IL-12, IL-18 and subsequently IFN- γ in peripheral blood mononuclear cells (PBMC) derived from atopic individuals to the same extent as in cells from healthy individuals [4]. As a consequence, IgE synthesis in vitro was downregulated [4, 34]. The addition of CpG ODN to *Dermatophagoides pteronyssinus* group-1-specific T cell lines differentiated CD4⁺ T cells into Th1 effector cells [35]. Although the

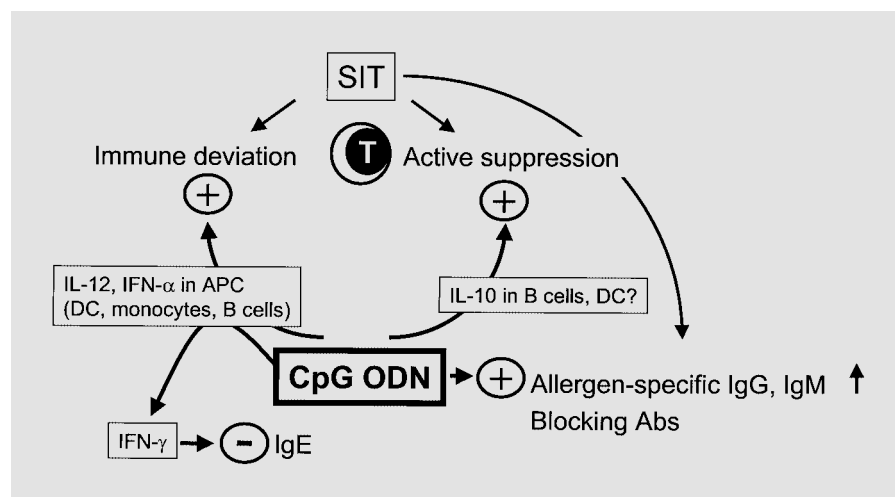


Fig. 1. Potential supporting effects of CpG ODN on the treatment of type I allergy.

induction of the Th1 shift in these allergen-specific T cells depended on IL-12 synthesis, an additional role for IFN- α was suggested. Further studies supported that IFN- α mediates Th1-fostering properties of CpG ODN [34, 36, 37]. Type I IFNs are essential in anti-viral innate immunity, and plasmacytoid dendritic cells (PDC) were found to be the major source for IFN- α production in PBMC [38]. This cell type is also responsible for the production of high amounts of IFN- α in response to certain CpG ODN [6, 39]. Nevertheless, allergic asthma was associated with reduced IFN- α levels in plasma [40]. Hence, the selection for IFN- α -inducing CpG sequences as adjuvants for SIT might have an additional effect on the Th1 bias in allergic individuals [41, 42].

Besides the expansion of naïve T lymphocytes towards Th1 cells, the modulation of allergen-specific Th2 memory cells towards the Th0/Th1 cell type represents an additional target for SIT. The persistence of long-lived allergen-specific T cells in vivo was demonstrated for atopic allergy [43, 44]. Human Th2 populations were shown to lack the expression of the signaling IL-12 receptor $\beta 2$ subunit and consequently IL-12 responsiveness [45]. IFN- α , on the other hand, acted directly on Th2 cells and drove Th1 development by activating STAT-4, a transcription factor necessary for IFN- γ induction [46]. Although several studies suggested that established Th2 populations remained susceptible to IL-12-dependent reversal to IFN- γ producing phenotypes [47, 48] additional support to accomplish this immunomodulation could result from the induction of CpG-ODN-induced IFN- α in APC.

Very encouraging data on Th1-inducing capabilities of CpG ODN as adjuvants were obtained from murine mod-

els of pollen allergy. In a mouse model for birch pollen allergy we could demonstrate that co-administration of CpG ODN with Bet v 1 in Al(OH)₃ resulted in markedly increased Th1 activities (high IgG2a levels) and subsequently in reduced airway inflammation [49]. The Th1-like immune response was also reflected by the cytokine patterns synthesized in cell cultures in response to the allergen, i.e. by decreased IL-5 and increased IFN- γ levels [49]. In a murine model of ragweed allergy, systemic or mucosal administration of ODN containing ISS after sensitization inhibited both the immediate hypersensitivity response and the late-phase cellular infiltration [50]. The administration of CpG ODN skewed the ragweed-specific response to Th1 and suppressed the rise of specific IgE titers after repeated allergen challenge. When mice were treated with anti-IL-12 neutralizing antibodies, the CpG-ODN-induced immunomodulatory effects were abolished, again indicating the pivotal role of IL-12 in this scenario [50]. Direct conjugation of an ODN containing CpG motifs to Amb a 1, the major ragweed allergen, represented an attempt which enhanced the immunotherapeutic potential of CpG ODN [51]. This allergen-ODN conjugate displayed stronger therapeutic activity than the mixture of Amb a 1 and CpG ODN and reversed established Th2-driven allergic airway responses in a murine model of asthma [52]. Taken together, these data show that CpG ODN are capable of modulating established allergen-specific Th2 immune responses in vivo. Results obtained from ragweed-allergic patients in vitro revealed that this modulation also applied for humans [53]. Initial phase I clinical trials of ragweed-allergic patients with the Amb a 1-CpG ODN conjugate confirmed that CpG ODN

are potent agents for inducing Th1-type anti-allergen immune responses [54]. The immunological mechanisms of successful SIT comprise the induction of peripheral tolerance and a Th1 immune response. Nevertheless, it remains to be clarified whether upregulating the production of Th1-type cytokines is the right way to redirect the predominant Th2 response in atopic individuals. Stimulation of strong Th1 cytokine production includes the risk to promote Th1-cell-mediated inflammation, which can cause serious tissue damage. Deduced from the compelling evidence that CD4⁺ T lymphocytes specialised in the suppression of immune responses play a critical role in immune regulation, strategies to downregulate ongoing Th2 responses should also include the potential promotion of regulatory T lymphocytes (T_{reg}). In general, T_{reg} are characterized by CD25 expression, low proliferative capacity in response to T cell receptor activation, production of high levels of IL-10, but low levels of IL-2 and no IL-4 [55–57]. Furthermore, these cells actively suppress naïve as well as memory Th1 immune responses to foreign antigens and self-antigens in vitro and in vivo [58–60]. Successful SIT against insect venom allergy was associated with the induction of IL-10 producing CD4⁺CD25⁺ T cells [61, 62]. However, the involvement of IL-10-producing CD4⁺ T cells for SIT of atopic allergy in humans is not so clear. In asthmatic patients, a diminished synthesis of IL-10 was observed [63]. Despite being the product of T_{reg} cells, IL-10 represents a crucial cytokine for the generation of T_{reg} cells since repetitive activation of CD4⁺ T cells in the presence of IL-10 promoted the differentiation of T_{reg} cells [58, 64]. This maturation was additionally supported by the presence of IFN- α [64]. Certain CpG ODN are shown to induce IL-10 as well as

IFN- α in cells from atopic patients [65]. These results raise the hope that a repeated stimulation of the immune system with such CpG sequences might – in addition to immunostimulatory activities – support the induction of immune suppressive effects.

Concluding Remarks

CpG ODN represent promising adjuvants for type I allergy treatment because they induce ‘first line’ Th1 cytokines such as IL-12 and IFN- α . Via these cytokines the differentiation of naïve T lymphocytes towards Th1 or the redirection of Th2 memory cells towards Th0/Th1 was accomplished, both in murine models and in allergic patients. However, the detailed characterization of different CpG ODN sequences in the recent past revealed that immunostimulatory effects depend on the DNA sequence. Therefore, optimal sequences for certain types of immune stimulation – modulation or suppression – can be defined. It remains an open question whether one CpG ODN of defined sequence will fulfill all the requirements for a potential adjuvant of allergy vaccines (fig. 1). Thus, there might still be a long way until the optimal CpG ODN and/or mixtures of CpG ODN sequences for use as adjuvants for type I allergy treatment are found.

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References

- 1 Sparwasser T, Miethke T, Lipford G, Erdmann A, Hacker H, Heeg K, Wagner H: Macrophages sense pathogens via DNA motifs: Induction of tumor necrosis factor- α -mediated shock. *Eur J Immunol* 1997;27:1671–1679.
- 2 Sparwasser T, Koch ES, Vabulas RM, Heeg K, Lipford GB, Ellwart JW, Wagner H: Bacterial DNA and immunostimulatory CpG oligonucleotides trigger maturation and activation of murine dendritic cells. *Eur J Immunol* 1998;28:2045–2054.
- 3 Hartmann G, Weiner GJ, Krieg AM: CpG DNA: A potent signal for growth, activation, and maturation of human dendritic cells. *Proc Natl Acad Sci USA* 1999;96:9305–9310.
- 4 Bohle B, Jahn-Schmid B, Maurer D, Kraft D, Ebner C: Oligodeoxynucleotides containing CpG motifs induce IL-12, IL-18 and IFN- γ production in cells from allergic individuals and inhibit IgE synthesis in vitro. *Eur J Immunol* 1999;29:2344–2353.
- 5 Bauer M, Heeg K, Wagner H, Lipford GB: DNA activates human immune cells through a CpG sequence-dependent manner. *Immunology* 1999;97:699–705.
- 6 Krug A, Rothenfusser S, Hornung V, Jahrsdorfer B, Blackwell S, Ballas ZK, Endres S, Krieg AM, Hartmann G: Identification of CpG oligonucleotide sequences with high induction of IFN- α / β in plasmacytoid dendritic cells. *Eur J Immunol* 2001;31:2154–2163.
- 7 Krieg AM, Yi AK, Matson S, Waldschmidt TJ, Bishop GA, Teasdale R, Koretzky GA, Klinman DM: CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature* 1995;374:546–549.
- 8 Liang H, Nishioka Y, Reich CF, Pisetsky DS, Lipsky PE: Activation of human B cells by phosphorothioate oligodeoxynucleotides. *J Clin Invest* 1996;98:1119–1129.
- 9 Ballas ZK, Rasmussen WL, Krieg AM: Induction of NK activity in murine and human cells by CpG motifs in oligodeoxynucleotides and bacterial DNA. *J Immunol* 1996;157:1840–1845.

- 10 Chace JH, Hooker NA, Mildestein KL, Krieg AM, Cowdery JS: Bacterial DNA-induced NK cell IFN-gamma production is dependent on macrophage secretion of IL-12. *Clin Immunol Immunopathol* 1997;84:185-193.
- 11 Noon L: Prophylactic inoculation against hay fever. *Lancet* 1911;i:1572-1573.
- 12 Akdis CA, Blaser K: IL-10-induced anergy in peripheral T cell and reactivation by microenvironmental cytokines: Two key steps in specific immunotherapy. *FASEB J* 1999;13:603-609.
- 13 Durham SR, Varney VA, Gaga M, Jacobson MR, Varga EM, Frew AJ, Kay AB: Grass pollen immunotherapy decreases the number of mast cells in the skin. *Clin Exp Allergy* 1999;29:1490-1496.
- 14 Secrist H, DeKruyff RH, Umetsu DT: Interleukin 4 production by CD4+ T cells from allergic individuals is modulated by antigen concentration and antigen-presenting cell type. *J Exp Med* 1995;181:1081-1089.
- 15 Wagner H: Bacterial CpG DNA activates immune cells to signal infectious danger. *Adv Immunol* 1999;73:329-368.
- 16 Bird AP: Functions for DNA methylation in vertebrates. *Cold Spring Harb Symp Quant Biol* 1993;58:281-285.
- 17 Cross SH, Bird AP: CpG islands and genes. *Curr Opin Genet Dev* 1995;5:309-314.
- 18 Siegfried Z, Eden S, Mendelsohn M, Feng X, Tsuberi BZ, Cedar H: DNA methylation represses transcription in vivo. *Nat Genet* 1999;22:203-206.
- 19 Karlin S, Doerfler W, Cardon LR: Why is CpG suppressed in the genomes of virtually all small eukaryotic viruses but not in those of large eukaryotic viruses? *J Virol* 1994;68:2889-2897.
- 20 Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, Akira S: A Toll-like receptor recognizes bacterial DNA. *Nature* 2000;408:740-745.
- 21 Takeshita F, Leifer CA, Gursel I, Ishii KJ, Takeshita S, Gursel M, Klinman DM: Cutting edge: Role of Toll-like receptor 9 in CpG DNA-induced activation of human cells. *J Immunol* 2001;167:3555-3558.
- 22 Bauer S, Kirschning CJ, Hacker H, Redecke V, Hausmann S, Akira S, Wagner H, Lipford GB: Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *Proc Natl Acad Sci USA* 2001;98:9237-9242.
- 23 Kirschning CJ, Bauer S: Toll-like receptors: Cellular signal transducers for exogenous molecular patterns causing immune responses. *Int J Med Microbiol* 2001;291:251-260.
- 24 Akira S, Takeda K, Kaisho T: Toll-like receptors: Critical proteins linking innate and acquired immunity. *Nat Immunol* 2001;2:675-680.
- 25 Ozinsky A, Underhill DM, Fontenot JD, Hajar AM, Smith KD, Wilson CB, Schroeder L, Aderem A: The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. *Proc Natl Acad Sci USA* 2000;97:13766-13771.
- 26 Ito T, Amakawa R, Kaisho T, Hemmi H, Tajima K, Uehira K, Ozaki Y, Tomizawa H, Akira S, Fukuhara S: Interferon-alpha and interleukin-12 are induced differentially by toll-like receptor 7 ligands in human blood dendritic cell subsets. *J Exp Med* 2002;195:1507-1512.
- 27 Hornung V, Rothenfusser S, Britsch S, Krug A, Jahrsdorfer B, Giese T, Endres S, Hartmann G: Quantitative expression of toll-like receptor 1-10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *J Immunol* 2002;168:4531-4537.
- 28 Hartmann G, Krieg AM: Mechanism and function of a newly identified CpG DNA motif in human primary B cells. *J Immunol* 2000;164:944-953.
- 29 Hartmann G, Weeratna RD, Ballas ZK, Payette P, Blackwell S, Suparto I, Rasmussen WL, Waldschmidt M, Sajuthi D, Purcell RH, Davis HL, Krieg AM: Delineation of a CpG phosphorothioate oligodeoxynucleotide for activating primate immune responses in vitro and in vivo. *J Immunol* 2000;164:1617-1624.
- 30 Verthelyi D, Ishii K, Gursel M, Takeshita F, Klinman D: Human peripheral blood cells differentially recognize and respond to two distinct CpG motifs. *J Immunol* 2001;166:2372-2377.
- 31 Gursel M, Verthelyi D, Gursel I, Ishii KJ, Klinman DM: Differential and competitive activation of human immune cells by distinct classes of CpG oligodeoxynucleotide. *J Leukoc Biol* 2002;71:813-820.
- 32 Manetti R, Parronchi P, Giudizi MG, Piccinini MP, Maggi E, Trinchieri G, Romagnani S: Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J Exp Med* 1993;177:1199-1204.
- 33 Teramoto T, Fukao T, Tashita H, Inoue R, Kaneko H, Takemura M, Kondo N: Serum IgE level is negatively correlated with the ability of peripheral mononuclear cells to produce interferon gamma (IFN-gamma): Evidence of reduced expression of IFN-gamma mRNA in atopic patients. *Clin Exp Allergy* 1998;28:74-82.
- 34 Horner AA, Widhopf GF, Burger JA, Takabayashi K, Cinman N, Ronaghy A, Spiegelberg HL, Raz E: Immunostimulatory DNA inhibits IL-4-dependent IgE synthesis by human B cells. *J Allergy Clin Immunol* 2001;108:417-423.
- 35 Parronchi P, Brugnolo F, Annunziato F, Manuelli C, Sampognaro S, Mavilia C, Romagnani S, Maggi E: Phosphorothioate oligodeoxynucleotides promote the in vitro development of human allergen-specific CD4+ T cells into Th1 effectors. *J Immunol* 1999;163:5946-5953.
- 36 Van Uden JH, Tran CH, Carson DA, Raz E: Type I interferon is required to mount an adaptive response to immunostimulatory DNA. *Eur J Immunol* 2001;31:3281-3290.
- 37 Rothenfusser S, Hornung V, Krug A, Towarowski A, Krieg AM, Endres S, Hartmann G: Distinct CpG oligonucleotide sequences activate human gamma delta T cells via interferon-alpha/beta. *Eur J Immunol* 2001;31:3525-3534.
- 38 Siegal FP, Kadowaki N, Shodell M, Fitzgerald-Bocarsly PA, Shah K, Ho S, Antonenko S, Liu YJ: The nature of the principal type 1 interferon-producing cells in human blood. *Science* 1999;284:1835-1837.
- 39 Bauer M, Redecke V, Ellwart JW, Scherer B, Kremer JP, Wagner H, Lipford GB: Bacterial CpG-DNA triggers activation and maturation of human CD11c-, CD123+ dendritic cells. *J Immunol* 2001;166:5000-5007.
- 40 Bufe A, Gehlhar K, Grage-Griebenow E, Ernst M: Atopic phenotype in children is associated with decreased virus-induced interferon-alpha release. *Int Arch Allergy Immunol* 2002;127:82-88.
- 41 Parronchi P, De Carli M, Manetti R, Simonelli C, Sampognaro S, Piccinini MP, Macchia D, Maggi E, Del Prete G, Romagnani S: IL-4 and IFN (alpha and gamma) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones. *J Immunol* 1992;149:2977-2983.
- 42 Demeure CE, Wu CY, Shu U, Schneider PV, Heusser C, Yssel H, Delespessie G: In vitro maturation of human neonatal CD4 T lymphocytes. II. Cytokines present at priming modulate the development of lymphokine production. *J Immunol* 1994;152:4775-4782.
- 43 Wedderburn LR, O'Hehir RE, Hewitt CR, Lamb JR, Owen MJ: In vivo clonal dominance and limited T-cell receptor usage in human CD4+ T-cell recognition of house dust mite allergens. *Proc Natl Acad Sci USA* 1993;90:8214-8218.
- 44 Bohle B, Schwihla H, Hu HZ, Friedl-Hajek R, Sowka S, Ferreira F, Breiteneder H, Bruijnzeel-Koomen CA, de Weger RA, Mudde GC, Ebner C, Van Reijsen FC: Long-lived Th2 clones specific for seasonal and perennial allergens can be detected in blood and skin by their TCR-hypervariable regions. *J Immunol* 1998;160:2022-2027.
- 45 Rogge L, Barberis-Maino L, Biffi M, Passini N, Presky DH, Gubler U, Sinigaglia F: Selective expression of an interleukin-12 receptor component by human T helper 1 cells. *J Exp Med* 1997;185:825-831.
- 46 Rogge L, D'Ambrosio D, Biffi M, Penna G, Minetti LJ, Presky DH, Adorini L, Sinigaglia F: The role of Stat4 in species-specific regulation of Th cell development by type I IFNs. *J Immunol* 1998;161:6567-6574.
- 47 Yssel H, Fasler S, de Vries JE, de Waal Malefyt R: IL-12 transiently induces IFN-gamma transcription and protein synthesis in human CD4+ allergen-specific Th2 T cell clones. *Int Immunol* 1994;6:1091-1096.

- 48 Smits HH, van Rietschoten JG, Hilken CM, Sayilir R, Stiekema F, Kapsenberg ML, Wierenga EA: IL-12-induced reversal of human Th2 cells is accompanied by full restoration of IL-12 responsiveness and loss of GATA-3 expression. *Eur J Immunol* 2001;31:1055–1065.
- 49 Jahn-Schmid B, Wiedermann U, Bohle B, Repa A, Kraft D, Ebner C: Oligodeoxynucleotides containing CpG motifs modulate the allergic Th2 response of BALB/c mice to Bet v 1, the major birch pollen allergen. *J Allergy Clin Immunol* 1999;104:1015–1023.
- 50 Magone MT, Chan CC, Beck L, Whitcup SM, Raz E: Systemic or mucosal administration of immunostimulatory DNA inhibits early and late phases of murine allergic conjunctivitis. *Eur J Immunol* 2000;30:1841–1850.
- 51 Tighe H, Takabayashi K, Schwartz D, Van Nest G, Tuck S, Eiden JJ, Kagey-Sobotka A, Creticos PS, Lichtenstein LM, Spiegelberg HL, Raz E: Conjugation of immunostimulatory DNA to the short ragweed allergen Amb a 1 enhances its immunogenicity and reduces its allergenicity. *J Allergy Clin Immunol* 2000;106:124–134.
- 52 Santeliz JV, Nest GV, Traquina P, Larsen E, Wills-Karp M: Amb a 1-linked CpG oligodeoxynucleotides reverse established airway hyperresponsiveness in a murine model of asthma. *J Allergy Clin Immunol* 2002;109:455–462.
- 53 Marshall JD, Abtahi S, Eiden JJ, Tuck S, Milley R, Haycock F, Reid MJ, Kagey-Sobotka A, Creticos PS, Lichtenstein LM, Van Nest G: Immunostimulatory sequence DNA linked to the Amb a 1 allergen promotes T(H)1 cytokine expression while downregulating T(H)2 cytokine expression in PBMCs from human patients with ragweed allergy. *J Allergy Clin Immunol* 2001;108:191–197.
- 54 Spiegelberg HL, Raz E: DNA-based approaches to the treatment of allergies. *Curr Opin Mol Ther* 2002;4:64–71.
- 55 Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL: Regulatory T cell clones induced by oral tolerance: Suppression of autoimmune encephalomyelitis. *Science* 1994;265:1237–1240.
- 56 Lombardi G, Sidhu S, Batchelor R, Lechler R: Anergic T cells as suppressor cells in vitro. *Science* 1994;264:1587–1589.
- 57 Levings MK, Roncarolo MG: T-regulatory 1 cells: A novel subset of CD4 T cells with immunoregulatory properties. *J Allergy Clin Immunol* 2000;106:S109–S112.
- 58 Asseman C, Powrie F: Interleukin 10 is a growth factor for a population of regulatory T cells. *Gut* 1998;42:157–158.
- 59 Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG: A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997;389:737–742.
- 60 Roncarolo MG, Bacchetta R, Bordignon C, Narula S, Levings MK: Type 1 T regulatory cells. *Immunol Rev* 2001;182:68–79.
- 61 Bellinghausen I, Metz G, Enk AH, Christmann S, Knop J, Saloga J: Insect venom immunotherapy induces interleukin-10 production and a Th2- to Th1-shift, and changes surface marker expression in venom-allergic subjects. *Eur J Immunol* 1997;27:1131–1139.
- 62 Akdis CA, Joss A, Akdis M, Faith A, Blaser K: A molecular basis for T cell suppression by IL-10: CD28-associated IL-10 receptor inhibits CD28 tyrosine phosphorylation and phosphatidylinositol 3-kinase binding. *FASEB J* 2000;14:1666–1668.
- 63 Borish L, Aarons A, Rumblyrt J, Cvietusa P, Negri J, Wenzel S: Interleukin-10 regulation in normal subjects and patients with asthma. *J Allergy Clin Immunol* 1996;97:1288–1296.
- 64 Levings MK, Sangregorio R, Galbiati F, Squadrone S, de Waal Malefyt R, Roncarolo MG: IFN- α and IL-10 induce the differentiation of human type 1 T regulatory cells. *J Immunol* 2001;166:5530–5539.
- 65 Horner AA, Widhopf GF, Burger JA, Takabayashi K, Cinman N, Ronaghy A, Spiegelberg HL, Raz E: Immunostimulatory DNA inhibits IL-4-dependent IgE synthesis by human B cells. *J Allergy Clin Immunol* 2001;108:417–423.