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DNA-Based Immunization for Asthma

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Key Words

DNA Immunization · Asthma · Eosinophils · IL-5 · T lymphocytes

Abstract

Background: Immunostimulatory DNA sequences (ISS) containing a CpG motif are able to inhibit Th2-mediated airway eosinophilia and bronchial hyperresponsiveness in a mouse model of asthma. ***Methods:*** To determine the optimal frequency and timing of intervention with ISS in inhibiting Th2 cytokine production and airway eosinophilia, we used ISS administration protocols which differed in the frequency (one vs. two doses), route (systemic vs. mucosal) and timing of ISS administration (before or together with antigen) in a mouse model of ovalbumin-induced eosinophilic airway inflammation. ***Results:*** ISS induced Th1 cytokine production (IFN-γ) and effectively inhibited Th2 cytokine production (IL-5) as well as eosinophilic inflammation when ISS was administered before or coadministered with inhaled allergen challenge. Although ISS was effective when coadministered with inhaled allergen, it was most effective when administered once 6 days prior to allergen challenge. Mucosal (intranasal and intratracheal) delivery of ISS was as effective as systemic (intraperitoneal) ISS delivery in inhibiting airway eosinophilia and switching cytokine responses from a Th2 to a Th1 response. ***Conclusions:*** ISS is most effective in inhibiting airway eosinophilia when administered as a single dose 6 days prior to antigen inhalation. However, ISS can also significantly inhibit eosinophilic inflammation, when coadministered with antigen inhalation. Thus, ISS administered prior or together with allergen should be considered as a novel method of allergen-based immunotherapy.

Introduction

The ability of noncoding DNA sequences to stimulate the innate immune system was suggested more than a decade ago by Yamamoto et al. [1] who discovered that DNA purified from mycobacteria induced IFN (α, β and γ) production and NK cell activation [1]. Fractionation of the mycobacterial DNA led to the isolation of several short DNA sequences (containing CpG dinucleotide cores) which mediated the immunostimulatory activity [2]. Subsequent experiments have shown that oligodeoxynucleotides (ODN) containing immunostimulatory DNA sequences (ISS-ODN), induce the release of IFN-α,/β [1, 2], IL-6 [3], IL-12, [4–6] and IL-18 [10] primarily from monocytes [2, 6, 7] and IFN-γ from NK cells [2]. The immune response to ISS is similar to the innate response against intracellular pathogens [6], triggering the release of cytokines which bias the immune response toward development of an antigen-specific Th1 effector and memory response [6].

We [8] and others [9] have recently shown that ISS containing CpG motifs inhibit airway eosinophilia and bronchial hyperresponsiveness in a mouse model of allergen-induced eosinophilic airway inflammation. ISS inhibits airway eosinophilia by inhibiting Th2 cytokine production (i.e. IL-5), resulting in inhibition of bone marrow production of eosinophils [8]. In this study, we have investigated the optimal frequency and timing of intervention with ISS

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in inhibiting Th2 cytokine production and airway eosinophilia using ISS immunization protocols which differed in the frequency (one vs. two doses), route (systemic vs. mucosal) and timing of ISS administration (before or together with antigen) in a mouse model of ovalbumin (OVA)-induced eosinophilic airway inflammation.

Material and Methods

Oligonucleotides

Endotoxin-free (<1 ng/mg DNA) ISS-ODN (5′-TGACTGTG-AACGTTCCGAGATGA-3′) or mutated (M)-ODN (5′-TGACTGTG-AAGGTTAGAGATGA-3′, (Trilink, San Diego, Calif., USA) have been previously described [6] and were used in the in vivo and in vitro experiments described below.

Animals

Female Balb/c mice (Jackson Laboratory, Bar Harbor, Me, USA) were used when they reached 8–10 weeks of age. All animal experimental protocols were approved by the University of California, San Diego Animal Subjects Committee.

Induction of Pulmonary Allergic Inflammation

Basic Protocol. Mice were immunized subcutaneously on days 0, 7, 14 and 21 with 25 µg of OVA (grade V, Sigma) adsorbed to 1 mg of alum (Aldrich) in 200 µ1 PBS. The OVA inhalation challenge (days 26 and 31) consisted of three 30-min inhalations of OVA at a concentration of 10 µg/ml in an inhalation chamber, separated by 30-min rest intervals. This basic protocol was applied to all the experimental groups (tables 1, 2) but not to the naive mice. Mice were sacrificed and the bronchoalveolar lavage fluid, lungs, peripheral blood and bone marrow analyzed 24 h after the second OVA inhalation.

Therapeutic intervention with ISS-ODN or M-ODN. ISS-ODN or M-ODN were injected intraperitoneally (100 µg in 100 µl of sterile, endotoxin-free PBS) or instilled intranasally (50 µg of ODN/naris, in 20 µ1 PBS, under metofane anesthesia). The ODNs were administered either twice (on days 25 and 30, 1 day before each OVA inhalation challenge), or once (once on day 25, 1 day before the first OVA inhalation challenge, once on day 30, 1 day before the second OVA inhalation challenge, or once on day 31, 30 min prior to the second OVA inhalation challenge).

Eosinophil Counts

At 24 h after the final OVA inhalation challenge, mice were sacrificed. Eosinophil counts were enumerated in the lung, bronchoalveolar lavage fluid, blood and bone marrow by detection of eosinophil peroxidase using the peroxidase substrate DAB (Vector Lab, Burlingame, Calif., USA) staining and microscopic examination as previously described [l0].

Cytokine ELISAs

Cytokine ELISA assays (Pharmingen) were used to measure individual cytokines (IL-5 and IFN-γ) as previously described [6, 8].

Statistical Analysis

Statistical analysis was performed with ANOVA as previously described [6, 8]. A p value of <0.05 was considered statistically significant.

Results

As in our previous studies [8], ISS induced Th1 cytokine production (IFN-γ) and effectively inhibited Th2 cytokine production (IL-5, table l) as well as eosinophilic inflammation (table 2) when ISS was administered before or coadministered with inhaled allergen challenge. Although ISS was effective when coadministered with inhaled allergen, it was most effective when administered once 6 days prior to allergen challenge. This single administration of ISS (6 days prior to termination of experiment) was as effective as ISS administered on 2 occasions (6 days and 3 days prior to termination of the experiment). Mucosal (intranasal) delivery of ISS was as effective as systemic ISS in inhibiting airway eosinophilia and switching cytokine responses from a Th2 to a Th1 response (tables1, 2).

Discussion

The ability of protein immunization to protect against allergic inflammation is well recognized [11]. However, as protein desensitization may induce anaphylaxis and is not effective in many patients with allergic rhinitis and asthma, the ability of DNA immunization to inhibit allergic inflammation has recently been investigated. Two general approaches to DNA immunization have been taken. In the first approach, we [12] and others [13], using plasmid DNA coding for a specific antigen or allergen protein, (i.e. β-galactosidase or dermatophagoides), have demonstrated efficacy of this DNA vaccine in mouse [12] and rat [13] models of allergic inflammation. A second alternative DNA-based immunization approach, which we have used in this study, is to utilize noncoding immunostimulatory DNA sequences containing a CpG motif (ISS) to induce a protective Th1 response to allergen [8, 9]. In previous studies, immunization of mice with ISS has demonstrated that it protects them from developing Th2 cytokine production, eosinophilic inflammation and bronchial hyperreactivity [8, 9]. This study demonstrates that ISS is most effective in inhibiting airway eosinophilia when administered as a single dose 6 days prior to antigen inhalation. However, ISS can also significantly inhibit eosinophilic inflammation when coadministered with antigen inhalation.

The ability to temporally dissociate ISS-ODN delivery and allergen challenge (the prepriming effect) may provide important therapeutic advantages and even simplify the treatment of allergic diseases, including asthma. The activation of innate immunity by ISS-ODN would initially prevent the development of asthmatic symptoms by preventing

Table 1. Effect of ISS on allergen-induced eosinophilia

Group	Therapy	Timing of ISS	BALF, %	PB, %	BM, %	Lung, n
No ISS						
A	naive	no ISS	0	2±1	3±2	2±1
B	allergen	no ISS	42±4	14±3	12±1	67±5
Systemic ISS (intraperitoneal)						
C	M-ODN	before each challenge	43±3	10±1	13±1	52±14
D	ISS-ODN	before each challenge	3±1[a]	2±1[a]	5±1[a]	6±1[a]
E	ISS-ODN	before 1st challenge	4±1[a]	3±1[a]	6±1[a]	7±2[a]
F	ISS-ODN	before 2nd challenge	10±1[a]	4±1[a]	10±1	16±4[a]
G	ISS-ODN	with 2nd challenge	24±4[a]	12±1	7±1[a]	21±6[a]
Mucosal ISS (intranasal)						
H	M-ODN	before each challenge	51±3	10±1	13±1	88±12
I	ISS-ODN	before each challenge	10±2[a]	3±1[a]	5±1[a]	8±1[a]
J	ISS-ODN	before 1st challenge	10±5[a]	9±1[a]	9±1	13±4[a]
L	ISS-ODN	before 2nd challenge	17±4[a]	6±2[a]	12±1	19±5[a]
L	ISS-ODN	with 2nd challenge	22±6[a]	7±1[a]	10±1	22±10[a]

The effect of the route (intraperitoneal or intranasal) and the timing of ISS-ODN administration on eosinophil counts in bronchoalveolar lavage fluid (BALF), peripheral blood (PB), bone marrow (BM) and lung in the mouse model of allergen-induced eosinophilic airway inflammation. Naive mice (group A) were not immunized nor challenged. All other groups of mice (group B–L) were immunized and challenged by OVA inhalation. Figures are means ± SD.

[a] $p < 0.05$ in comparison to group B (OVA sensitized and challenged).

Table 2. Effect of ISS on allergen-induced Th1 (IFN-γ) and Th2 (IL-5) cytokine levels

Group	Therapy	Timing of ISS	IL-5, pg/ml	IFN-γ, pg/ml
No ISS				
A	naive	no ISS	<20	<20
B	allergen	no ISS	750±124	24±21
Systemic ISS (intraperitoneal)				
C	M-ODN	before each challenge	820±122	33±33
D	ISS-ODN	before each challenge	121±18[a]	730±99[a]
E	ISS-ODN	before 1st challenge	190±52[a]	664±61[a]
F	ISS-ODN	before 2nd challenge	421±102[a]	252±24[a]
G	ISS-ODN	with 2nd challenge	629±110	104±15[a]
Mucosal ISS (intranasal)				
H	M-ODN	before each challenge	795±138	31±22
I	ISS-ODN	before each challenge	200±66[a]	443±128[a]
J	ISS-ODN	before 1st challenge	466±40[a]	246±86[a]
K	ISS-ODN	before 2nd challenge	531±109	168±22[a]
L	ISS-ODN	with 2nd challenge	575±90	98±44[a]

The effect of the route and the timing of ISS-ODN administration on levels of IL-5 and IFN-γ produced in vitro by OVA-stimulated CD4+ splenocytes. Figures are means ± SD.

[a] $p < 0.05$ in comparison to group B.

eosinophil accumulation, and subsequently could induce an allergen-specific Th1 response to allergens encountered by the host in his own natural environment [8].

ISS were initially identified and isolated from mycobacterial DNA [1, 2]. The ISS DNA sequence appears at the expected frequency in many pathogenic bacteria and viruses, while it is underrepresented in the vertebrate genome [3]. A recent study demonstrated an inverse epidemiological association between exposure to mycobacteria and the prevalence of atopic disorders, arguing that the relatively recent decline in infections (e.g. tuberculosis) in developed countries is a factor underlying the rising severity and prevalence of allergic diseases [14]. Thus, the natural exposure to ISS-enriched DNA from normal intracellular pathogens, through infection, may play a role in shaping the immune response away from an allergic phenotype and allergic responses following allergen challenge.

Why does ISS inhibit allergic inflammation? Recognition of ISS motifs appears to have evolved in order to defend against invading intracellular pathogens [6]. In this respect, it appears that ISS provides a 'danger/alarm-like signal' [15] required for this vital Th1 immune response. The importance of the Th1 immune response in infections has been demonstrated in studies of *Leishmania* and other intracellular pathogens [16], where biasing of the immune response toward Th2 (allergic) responses may compromise the body's defenses against invading organisms.

In summary, the results of ISS therapy presented here provide an alternative to the current practice of allergen protein desensitization for allergic asthma which has relatively low efficacy and high potential for significant side effects, including anaphylaxis [11]. Prepriming with ISS via the systemic (intraperitoneal), local (intranasal) or even via the pulmonary route (intratracheal; data not shown) in allergic patients may inhibit the allergic inflammatory response in asthmatics after natural allergen exposure. The future application of ISS therapy to human allergic diseases will depend on how well the inhibitory effects of ISS in murine models of experimental allergy translate to related human diseases.

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