Selective immune redirection in humans with ragweed allergy by injecting Amb a 1 linked to immunostimulatory DNA

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Background: In animal models administration of immunostimulatory DNA sequences preferentially elicits T_H1-dominated (type 1-dominated) immunity and can inhibit developing or ongoing T_H2 (type 2) responses.
Objective: Our objective was to investigate this phenomenon in humans.
Methods: In a randomized, third party-blinded, placebo-controlled, proof-of-concept study conducted entirely in the winter in 19 adults with ragweed allergy, we administered 6 subcutaneous injections of purified Amb a 1 linked to the 22-base-long immunostimulatory phosphorothioate oligodeoxyribonucleotide 1018 (Amb a 1–immunostimulatory DNA sequence conjugate [AIC]). Before the course of AIC or placebo injections and 2 and 16 weeks afterward, we measured recall responses to ragweed, streptokinase, and PHA in short-term primary culture of fresh PBMCs after restimulation with antigen. We quantified regulatory cytokine and chemokine responses characteristic of T_H2 immunity (IL-5, IL-13, CCL17 [TARC], and CCL22 [MDC]), and T_H1 immunity (IFN-γ, CXCL9 [Mig], and CXCL10 [IP-10]), as well as IL-10, a cytokine sometimes linked to regulatory T-cell populations.
Results: We demonstrated for the first time that human systemic in vivo ragweed-specific T_H2 responses were selectively redirected toward T_H1 responses, with significant increases in IFN-γ, CXCL9, and CXCL10 and significant decreases in IL-5, CCL17, and CCL22 found at 2 and 16 weeks after the sixth injection. Cytokine and chemokine responses to the unrelated bacterial antigen streptokinase and the global capacity to mount immune responses on polyclonal activation with PHA did not change. No clinically significant systemic or local allergic reactions were associated with AIC or placebo injections.
Conclusions: AIC, injected in concentrations that were approximately 40-fold lower than those used in most murine studies published to date, led to a prolonged shift from T_H2 immunity toward T_H1 immunity and appeared to be safe. This novel approach has the potential for immune redirection in human immediate hypersensitivity diseases. (J Allergy Clin Immunol 2004;113:1144-51.)

Key words: *Allergen-specific immunotherapy, allergen vaccines, Amb a 1, chemokines, cytokines, DNA vaccines, humans, immunostimulatory DNA sequences, ragweed allergy*

Redirecting the underlying immune dysfunction that elicits and maintains immediate hypersensitivity diseases in humans remains an unmet challenge.[1] The immunologic mechanisms associated with these diseases involve a spectrum of $CD4^+$ T cells, including regulatory T cells, $CD25^+$ cells, natural killer T cells, and T_H1, T_H2, and T_H3 cells.[2] Allergen-specific immunotherapy aims to correct this underlying immune imbalance, particularly by attempting to redirect the established T_H2-dominated response to a more balanced T_H1/T_H2 response.[3-6] Although conventional allergen-specific immunotherapy has proved dose-related efficacy, it is neither optimally convenient nor perfectly safe. Considerable attention has therefore been focused on development of novel immunotherapy strategies.[7,8]

In animal studies immunostimulatory DNA sequences (ISSs), which occur naturally in prokaryotes but are largely absent from vertebrates, induce T_H1-biased (type 1-biased) immune responses and inhibit development of T_H2 (type 2) immunity when administered before antigen exposure,[9-13] thus effectively inducing a strong and long-lasting commitment to T_H1-biased immunoregulatory responses. ISS administration to animals with established immediate hypersensitivity, in which the allergen-specific response is already committed to a T_H2 phenotype, has been less consistently effective. Although some investigators have had little success in redirecting pre-established T_H2-dominated immunity,[14,15] others[16,17] have elicited 2- to 4-fold reductions in T_H2 cytokines, such as IL-5, concomitant with similar increases in IFN-γ, suggesting that DNA-based therapeutics are potentially useful for the treatment of human immediate hypersensitivity diseases.[18,19]

Amb a 1 predominates among approximately 20 allergens in *Ambrosia artemisiifolia*. It has a molecular mass of 37,800 d, has been sequenced and otherwise thoroughly studied, and is currently used to standardize short ragweed allergen extracts and to define the target dose of short ragweed allergen in conventional immunotherapy.[7]

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Abbreviations used

Ag: Antigen
AIC: Amb a 1–immunostimulatory DNA sequence conjugate
Amb a 1: Immunodominant allergen from *Ambrosia artemisiifolia* (short ragweed)
ANA: Anti-nuclear antibody
ANOVA: Analysis of variance
C: Complement
CD: Cluster of differentiation
DNA: Deoxyribonucleic acid
ELISA: Enzyme-linked immunosorbent assay
IFN-γ: Interferon-gamma
IgE: Immunoglobulin E
IL: Interleukin
ISS: Immunostimulatory DNA sequence
MHC: Major histocompatibility complex
PBS: Phosphate-buffered saline
PHA: Phytohemagglutinin
SE: Standard error
T_H: T helper

Preclinical rodent studies have demonstrated that conjugation of Amb a 1 and ISS leads to more potent induction of Amb a 1–specific T_H1 immune responses and greater suppression of T_H2 immune responses than a simple mixture of Amb a 1 and ISS.[20] The effect of ISSs added to cultures of human cells suggests their potential for revolutionizing allergen-specific immunologic treatment.[11,19-24] We hypothesized that Amb a 1 linked to ISS would have the capacity to redirect pre-established allergen-specific immunoregulatory cytokine and chemokine recall responses in humans with ragweed allergy.

METHODS

This randomized, third party–blinded, placebo-controlled Phase I investigation was approved by the University of Manitoba Research Ethics Board, and written informed consent was obtained from all participants. The study was conducted entirely in midwinter, when there was no exposure to ragweed or other pollens. Individuals received 6 injections of investigational vaccine or placebo administered at weekly intervals (Fig 1).

Investigational vaccine

The Amb a 1 immunostimulatory DNA sequences conjugate (AIC) vaccine consisted of purified Amb a 1 linked to the immunostimulatory phosphorothioate oligodeoxyribonucleotide 1018 (22 bases in length with sequence of 5′-TGACTGTG-AACGTTCGAGATGA-3′; prepared at Avecia, Boston, Mass, for Dynavax Technologies Corp, Berkeley, Calif). Each vial contained 0.7 mL of AIC at a concentration of 30 μg/mL by protein in PBS. Thirty micrograms per milliliter of AIC contained approximately 28 μg/mL ISS 1018. The AIC was stored at −60°C, thawed at room temperature, and diluted serially with PBS. PBS was also used for control injections. Throughout the study, all participants and clinical and laboratory personnel involved were blinded as to whether AIC or placebo was injected, with the exception of one nurse who prepared and injected the AIC or placebo but had no other involvement.

Participants

We included individuals age 18 to 60 years who had a history of fall allergic rhinitis symptoms and a positive epicutaneous test result of at least Σ_{10} (sum of longest diameter of erythema plus width of erythema measured perpendicularly to longest diameter) to licensed standardized ragweed extract (Greer Laboratories, Inc, Lenoir, NC). Individuals were excluded if they had any clinically significant illness, were pregnant, were breast-feeding, or required daily treatment for asthma at the time of the study; had ever had a hospital admission for asthma or received anti-IgE antibody; had received ragweed immunotherapy within the previous 5 years; or had taken immunosuppressive medication, including systemic corticosteroids, within the previous month. Throughout the study, AIC or placebo injections were administered only if participants continued to meet these criteria. Women of child-bearing potential had negative pregnancy test results at study entry and before each injection.

Study plan

At baseline before the first subcutaneous injection of AIC or placebo and 2 and 16 weeks after the sixth and last injection, medical history was taken, physical examination was performed, and 40 mL of blood was obtained for immunologic tests (Fig 1). Weekly AIC or placebo injections in incremental doses of 0.06, 0.3, 1.2, 3.0, 6.0, and 12.0 μg were scheduled. Adjustments in the dose regimen were made for missed injections and for local or systemic reactions to injections, if any. The AIC or placebo dose was increased if the preceding injection resulted in a local reaction of less than 2 cm in mean diameter, repeated if the injection resulted in a local reaction of 2 to 4 cm in mean diameter, and decreased by 50% if a local reaction of greater than 4 cm in mean diameter or a systemic reaction occurred. Participants who experienced a second reaction on dose escalation (following the 50% dose reduction after their first reaction) had their dose decreased by 50% and subsequently received the reduced dose until the end of the study.

Immunologic tests

Fresh PBMCs were isolated and used for short-term primary culture at 5×10^6/mL for antigen and 2.5×10^6/mL for polyclonal stimulation, with at least 2 wells per condition. Cells were cultured (1) in the absence of stimuli; (2) with standardized short ragweed extract (309 antigen units/mL, 25 mg/mL; stock from Greer Laboratories Inc, Lenoir, NC), titrating each subject over a concentration range of 1, 10, and 100 μg/mL; and (3) with recall antigen streptokinase (Aventis Pharma, Montreal, Quebec, Canada) at 5000 U/mL or PHA (Sigma-Aldrich Canada Ltd, Oakville, Ontario, Canada) at suboptimal (2 μg/mL) and optimal (10 μg/mL) concentrations. Culture supernatants were harvested at the times we identified in previous experiments to yield optimal cytokine and chemokine responses.[25-27] Standardized short ragweed extract, rather than Amb a 1, was used as the stimulating antigen because it is readily available, involves a broader panel of antigens than Amb a 1, and, as the extract widely used in skin testing and conventional immunotherapy, is highly clinically relevant.[7]

Allergen-driven and polyclonally stimulated T_H2 immunity-associated IL-4, IL-5, IL-13, and the chemokines CCL1 (I-309), CCL2 (MCP-1), CCL11 (eotaxin-1), CCL17 (TARC), and CCL22 (MDC) and T_H1 immunity-associated IFN-γ and the chemokines CXCL9 (Mig) and CXCL10 (IP-10), as well as T regulatory-associated IL-10, were quantified. Ultrasensitive absorbance or chemiluminescense ELISAs developed in our laboratory and described in detail in previous publications[25-27] were used. World Health Organization standards were used where available. In all

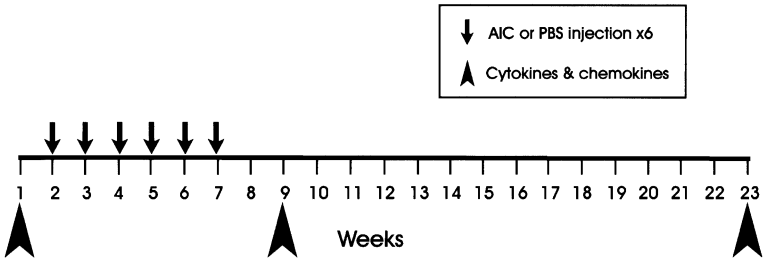

FIG 1. Time course of the study: six injections of AIC in incremental doses from 0.06 to 12 μg per injection or of placebo (PBS) were given at weekly intervals during the winter. Before the first injection and at 2 and 16 weeks after the sixth injection, ragweed-specific immunoregulatory recall cytokine and chemokine responses were evaluated in antigen-driven, short-term primary culture.

participants at all sample times, immunologic tests were performed in at least 2 independent assays, with the concentration calculated from a minimum of 3 points falling on the linear portion of sample titration curves calibrated against standards run on each plate. SEs ranged from 3% to 10%.

Statistical analysis

Data analysis included all participants, regardless of the maximum AIC dose injected during the study. Geometric means for the absolute levels of given cytokines and logs of ratios (ie, pairwise comparisons of IFN-γ/IL-5 production) are presented. *P* values are derived from a priori contrasts from repeated-measures ANOVA on logarithmically transformed data comparing AIC and placebo groups by using SAS V8.2. Significance levels are 1-tailed for cytokine and chemokine responses elicited by the sensitizing allergen (on the basis of the literature and our hypothesis that AIC administration would lead to increased T_H1 and decreased T_H2 responses) and 2-tailed for all other comparisons.

Safety

At each visit, diaries of symptoms and events were reviewed. Laboratory tests for safety, performed before the first, third, and sixth AIC injections and 2 weeks after the last injection, included complete blood count, platelet count, erythrocyte sedimentation rate, serum electrolytes, creatinine, blood urea nitrogen, glucose, aspartate transaminase, alanine transaminase, bilirubin, alkaline phosphatase, serum and total iron-binding capacity, C3, C4, and urinalysis. In addition, immunoassays for anti-nuclear antibody, anti-single-stranded DNA, and anti-double-stranded DNA were performed before the first and third study injections and repeated 4 and 16 weeks after the last injection.

RESULTS

The AIC and placebo groups were demographically and clinically similar (AIC [n = 9]: 4 men, 41 ± 13 y, 76 ± 20 kg; placebo [n = 10]: 6 men, 41 ± 13 y, 79 ± 18 kg). They did not differ significantly in the intensity of cytokine and chemokine production or in the balance between their T_H1 and T_H2 immunity at preinjection baseline ($P > .05$); however, when ragweed-specific responses were evaluated 2 and 16 weeks after the sixth and last injection, the individuals in the AIC group exhibited striking reorientation of their ragweed-specific recall responses (Table I and Fig 2).

Ragweed-specific T_H2-associated responses are reduced after injection of ISS linked to Amb a 1

Under conditions of both optimal and suboptimal allergen concentrations (100 and 10 μg/mL, respectively, with the latter used for enhanced sensitivity to possible changes in allergen-specific responses), ragweed-dependent production of the T_H2 chemokine CCL17 was markedly lower (medians, 4230 vs 1808 pg/mL, $P = .005$) in the AIC group than in the placebo group (Table I). The median intensity of these reductions was similar at 2 and 16 weeks after treatment, ranging from 2- to 4-fold under the various conditions assessed; indeed, CCL17 levels were suppressed to background levels 16 weeks after AIC injections ($P = .004$). Similarly, ragweed-dependent CCL22 responses, also initially indistinguishable in the AIC and placebo groups ($P = .14$), were reduced after AIC injections but not after placebo injections (medians of 22,925 vs 7746 pg/mL [$P < .0002$] at 2 weeks and medians of 16,317 vs 8103 [$P < .007$] at 16 weeks).

In addition, 200% to 400% dampening of some T_H2 immunity-associated cytokine responses was found. Under conditions of maximal recall responses (ragweed, 100 μg), median IL-5 recall responses were reduced by one third (medians, 293 pg/mL in the placebo group vs 198 pg/mL in the AIC group, $P < .05$) 2 weeks after the last injection, with somewhat stronger reductions evident 16 weeks after the last injection (351 vs 148 pg/mL, $P = .001$). Baseline IL-5 recall responses were indistinguishable between the groups ($P > .05$). The same picture emerged by using threshold concentrations (10 μg of ragweed: 247 vs 100 pg/mL, $P = .005$ at 16 weeks). Ragweed-driven IL-13 responses were not detectably altered before treatment ($P = .24$ and $P = .48$ for 10 and 100 μg of ragweed, respectively) at 2 weeks ($P = .46$ and $P = .48$, respectively) or 16 weeks after the last injections ($P = .12$ and $P = .27$, respectively). IL-4, CCL1, CCL2,

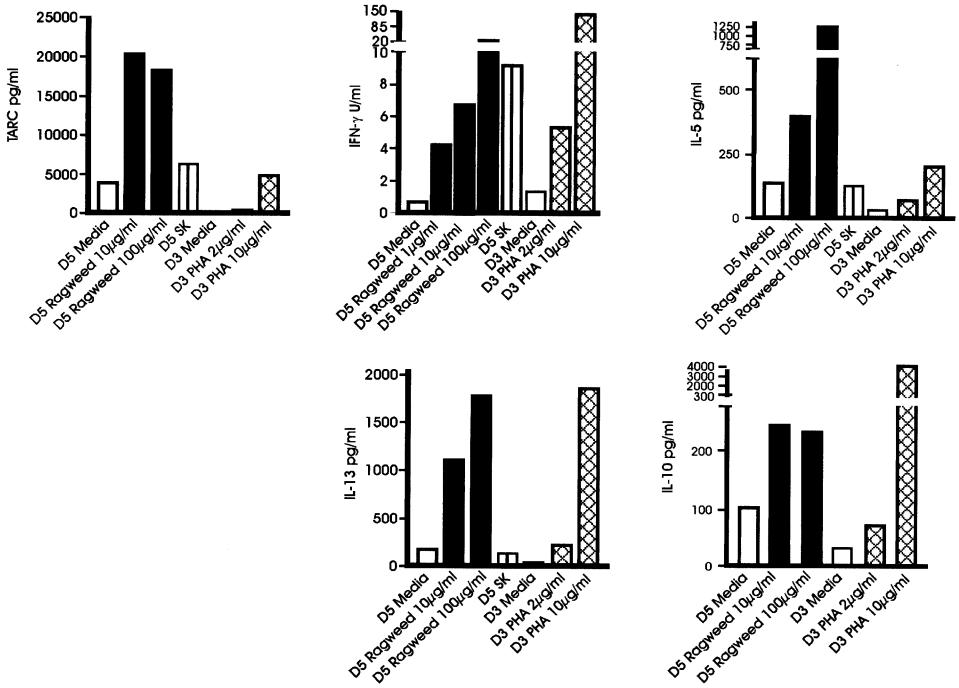

FIG 2. Expression of cytokine responses by fresh PBMCs from an individual with ragweed allergy representative of baseline results. Geometric mean responses from 5×10^5 PBMCs after 3 to 5 days of stimulation with sensitizing allergen at suboptimal (1 and 10 μg/mL) and optimal (100 μg/mL) conditions, streptokinase (5000 U/mL), and polyclonal activator PHA (suboptimal, 2 μg/mL; optimal, 10 μg/mL) and responses in unstimulated control wells were determined in tissue culture supernatants.

TABLE I. Ragweed-specific recall responses

	2 Wk after injection (pg/mL), PBS vs AIC	AIC effect	16 Wk after injection (pg/mL), PBS vs AIC	AIC effect
T_H1				
CXCL10	10,715 vs 11,561, $P > .05$	~	11,142 vs 27,925, $P < .004$	↑
CXCL9	2344 vs 4915, $P < .02$	↑	1998 vs 3751, $P > .05$	~
IFN-γ	161 vs 483, $P = .004$ to $P = .015$	↑	219 vs 207, $P > .05$	~
T_H2				
CCL17	4230 vs 1808, $P = .005$	↓	6836 vs 2697, $P = .004$	↓
CCL22	22,925 vs 7746, $P < .0002$	↓	16,317 vs 8103, $P < .007$	↓
IL-5	293 vs 198, $P < .05$	↓	351 vs 148, $P = .001$	↓
IL-13	226 vs 265, $P = .48$	~	120 vs 122, $P = .27$	~
T regulatory				
IL-10	179 vs 473, $P = .03$	↑	403 vs 590, $P > .05$	~

AIC versus placebo: ↑, significant increase; ↓, significant decrease; ~, no significant effect.

and CCL11 responses were less than the levels of detection in virtually all allergen-driven responses examined (1, 10, and 100 μg/mL ragweed).

Ragweed-specific T_H1-associated responses are increased after injection of ISS linked to Amb a 1

CXCL10 levels were similar in the treatment groups at study entry, but by 16 weeks after the last injection, as compared with the placebo group, there were 3-fold increases in the AIC group (medians, 11,142 vs 27,925 pg/mL; $P < .004$; Table I). CXCL9 responses, usually coregulated with CXCL10 and also highly IFN-γ dependent, were substantial in both treatment groups and also trended higher after AIC. Classical markers of T_H1 immunity, such as IFN-γ, were enhanced on ragweed restimulation, with individuals in the AIC group exhibiting median responses 2- or 3-fold higher than those in the placebo group (161 vs 483 pg/mL, $P = .004$ to $P = .015$) at 2 weeks after treatment. Interestingly, unlike the changes in T_H2 immunoregulatory cytokines and chemokines, these increases in IFN-γ did not persist at 16 weeks (219 vs 207 pg/mL, $P = .24$).

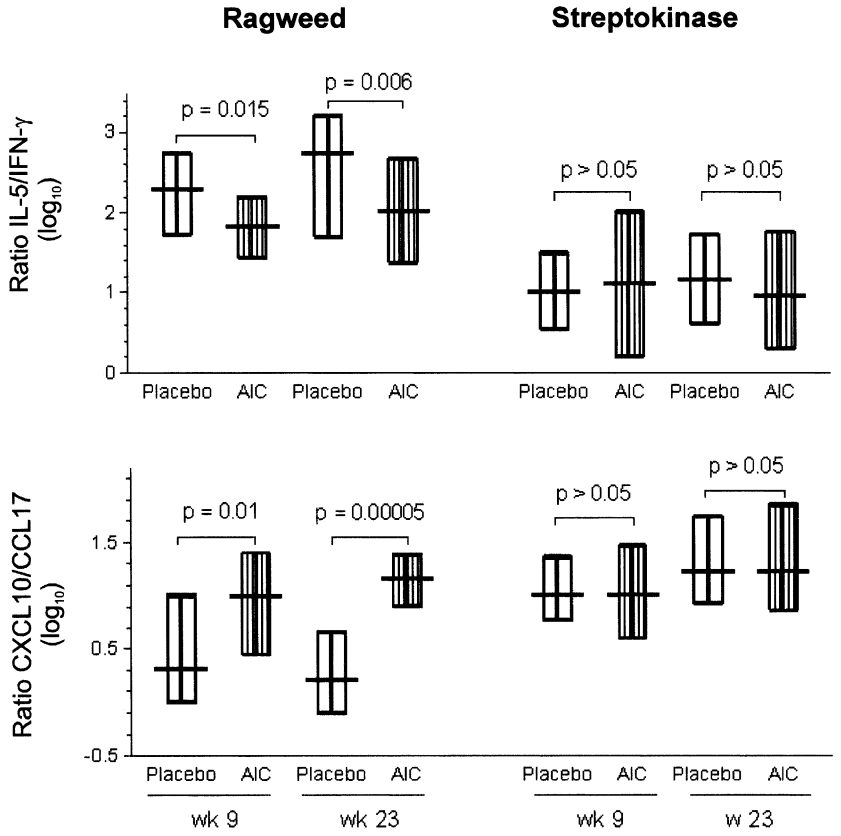

FIG 3. Modification of ragweed-driven, but not streptokinase-driven, cytokine and chemokine responses in individuals with ragweed allergy after AIC administration. Results are displayed as the median log ratio of IL-5/IFN-γ production and CXCL10/CCL17 production. *Boxes* indicate the limits of the 25th and 75th percentiles (AIC group, n = 9; placebo [PBS] group, n = 10) for each comparison. *P* values represent statistical significance from repeated-measures ANOVA on logarithmically transformed data.

IL-10 responses, of interest because of their potential role in regulatory T-cell biology, exhibited a transient increase in the AIC group compared with the placebo group at 2 weeks (473 vs 179 pg/mL, $P = .03$) that was not evident at 16 weeks.

Shifting the immunologic balance away from T_H2-dominated immunity

The ratios of cytokine and chemokine responses were examined to obtain a broader measure of the effect of AIC administration on the balance of immunity than is possible through examination of individual cytokines. Median ratios of ragweed allergen-driven T_H2/T_H1 (IL-5/IFN-γ) recall responses (Fig 3) demonstrate striking reductions. Whereas responses to sensitizing allergen were indistinguishable in the 2 groups before AIC or placebo injection ($P = .22$), AIC led to 3- to 10-fold changes in the balance of the ragweed-specific recall response, shown as ragweed-stimulated IL-5/IFN-γ. This redirection continued to the end of the study (median ratios of 200:1 vs 69:1 and 562:1 vs 64:1 at 2 and 16 weeks, respectively, after the last injection). Use of threshold antigen stimulation conditions (ragweed, 10 μg/mL) also demonstrated placebo and AIC groups to be indistinguishable initially ($P = .24$), followed by redirection of the T_H2/T_H1 response at 2 and 16 weeks (257 vs 100, $P = .025$) after treatment.

The ratios of T_H1/T_H2 chemokine responses were indistinguishable between the groups before the injections (all $P > .05$). The AIC group exhibited a 5-fold shift in the ragweed-specific balance 2 weeks after the last injection ($P = .01$) and a 9-fold shift 16 weeks after the last injection (median CXCL10/CCL17 ratios of 1.6 vs 14.5 in the placebo group vs the AIC group, respectively; $P < .00005$; Fig 3). Comparison of a range of other T_H1/T_H2 indicators revealed similar 2- to 9-fold shifts in allergen-driven recall responses (IFN-γ/IL-5, $P < .05$, and CXCL10/CCL17, $P < .01$ to $P < .0001$).

Global effect of AIC administration

In contrast to the effect of AIC on ragweed-specific recall responses, neither T_H1 nor T_H2 recall responses to streptokinase (Fig 3 and Table II) nor the global capacity to respond after polyclonal activation with PHA were generally altered. In streptokinase-driven recall responses, IFN-γ ($P = .75$ to $P = .97$) and CXCL10 ($P = .39$ to $P = .99$), as well as CCL17 ($P = .51$ to $P = .93$), and IL-5

($P = .21$ to $P = .44$), were unaffected when comparing the AIC group and the placebo group before the injections and at 2 and 16 weeks after the last injection.

Mirroring cytokine responses, the ratios of CXCL10/CCL17 production in unstimulated medium controls ($P = .38$ to $P = .78$), in streptokinase-stimulated cultures ($P = .99$ to $P = .94$; Fig 3), or in cultures stimulated with PHA under threshold ($P = .59$ to $P = .64$) or maximal ($P = .94$ to $P = .65$) activation conditions did not differ detectably between the placebo and AIC groups.

Adverse effects

All 19 participants completed the series of injections and all related tests. Five of the 9 participants receiving AIC did not require any dose adjustments and tolerated the sixth and final dose of 12 μg (total dose, 28 μg), whereas 4 of the 9 participants required a reduced AIC dose as per protocol because of local reactions involving some combination of itch, pain, warmth, and redness. Nine of the 10 participants receiving placebo did not require a dose reduction. No clinically significant systemic or local allergic reactions were associated with AIC or placebo injections. No individual withdrew from the study because of adverse effects. Reported adverse events were generally mild and considered to be unrelated to AIC or placebo injections. No clinically relevant changes in hematology or blood chemistry test results were found.

DISCUSSION

In this proof-of-concept study of a novel approach to immune modulation in humans, we determined the effect of AIC on ongoing immunoregulatory responses and investigated its safety. We demonstrated for the first time that human systemic in vivo T_H2 cytokine and chemokine ragweed-specific recall responses and IL-5, CCL17, and CCL22 levels readily detectable 6 months after the end of the ragweed season were markedly reduced after AIC, but not placebo, injections. In some individuals, reductions to background levels occurred. Interestingly, IL-13 recall responses to ragweed were largely unaffected under the conditions tested, and IL-4 responses to ragweed were generally less than detection limits. In contrast, ragweed-specific T_H1 immune responses included transiently increased IFN-γ and CXCL9 production and long-term increases in CXCL10 production in the AIC group compared with the placebo group. These allergen-dependent responses are strictly dependent on $CD4^+$ T-cell activation, MHC class II antigen presentation, and costimulation through CD80/CD86 pathways (Stinson MJ, et al, manuscript in preparation). Given the small number of individuals in the study, no post hoc analysis of the relationship between maximum total dose of AIC injected and immunologic responses was attempted. Although only 5 individuals received the full dose of AIC, data from all individuals in the AIC group, regardless of the total AIC dose injected, were compared with data from all individuals in the placebo group.

Different ISSs exhibit different levels of activity in different species.[17,28-31] In some murine studies, ISS-driven changes have been global rather than allergen specific, a potential concern in human immunotherapy. In marked contrast, in this study immunologic changes were mainly restricted to ragweed-specific responses, possibly because the amount of ISS administered was substantially less than that in most animal studies to date. Although mice are typically given 2 to 5 injections of 50 to 100 μg (approximately 10-15 mg/kg total) of ISSs mixed with antigen, participants in this study who received the full course of AIC injections received approximately 0.3 μg/kg ISS linked to Amb a 1. Selection of this lower dose was based on animal studies demonstrating that linkage of antigen to ISS markedly enhances its immunogenicity and reduces its allergenicity in comparison with simply mixing it with an ISS.[20,28,32,33] Therefore the 2- to 5-fold increases in T_H1 cytokine and chemokine profiles and similar reductions in T_H2 cytokines (predominately IL-5) seen in successful murine studies modifying established T_H2-biased immunity[17,18,29] were achieved in this human study by using 3000-fold less ISS linked to antigen (comparable with 30,000- to 50,000-fold less ISS mixed with antigen). Clearly, only very small amounts of linked ISSs are required to alter the immunoregulatory cytokine and chemokine recall response to sensitizing allergen in humans, and under these conditions, the immune redirection achieved does not result in a potentially dangerous global redirection of immune capacity but rather is restricted to the specific immune responses targeted.

Another important distinction between murine studies and the human study reported here concerns the nature of the experimental protocol and duration of biologic effects induced. Most studies of ISS administration aiming to downregulate ongoing T_H2 responses in animals have involved injections immediately (usually 1-3 days) before evaluation of recall cytokine responses,[17,18,29] irrespective of whether ISSs were given without allergen,[16,34] with allergen added, or with allergen chemically linked to the ISS. However, in contrast to ISSs used to influence initial responses in naive animals in which induction of T_H1-biased immunity persists for more than 1 year, activity of ISSs in ongoing murine allergy models has generally been found to be short lived, persisting between 1 and 2 months, after which time the specific recall responses in animals receiving ISSs are often indistinguishable from the responses in those receiving placebo.[17] We therefore designed this study to evaluate potential short-term (2 weeks after completion of treatment) and sustained (16 weeks later) alterations in responses to ragweed and unrelated antigen. Several observations are apparent. First, changes in allergen-dependent recall responses, the expression of which is associated with T_H1 (CXCL9, CXCL10) and T_H2 (CCL17, CCL22) responses, were readily observed indicators of T_H1/T_H2 immunity. Second, unlike in murine studies, ISS administration in humans resulted in sustained alterations in the T_H1/T_H2 balance of recall responses, which lasted for at least 16

TABLE II. Responses to streptokinase and PHA

	2 Wk after injection (pg/mL), PBS vs AIC	AIC effect	16 Wk after injection (pg/mL), PBS vs AIC	AIC effect
Streptokinase				
T_H1				
CXCL10	27,227 vs 21,380, $P = .39$	~	33,113 vs 32,359, $P = .99$	~
IFN-γ	736 vs 724, $P = .97$	~	518 vs 449, $P = .75$	~
T_H2				
CCL17	2617 vs 2059, $P = .51$	~	1920 vs 1984, $P = .93$	~
IL-5	63 vs 81, $P = .44$	~	64 vs 40, $P = .21$	~
IL-13	130 vs 210, $P = .02$	↓	50 vs 77, $P = .05$	~
T regulatory				
IL-10	246 vs 328, $P = .69$	~	307 vs 357, $P = .53$	~
PHA				
T_H1				
CXCL10	7244 vs 7079, $P = .98$	~	15,488 vs 12,882, $P = .57$	~
IFN-γ	3738 vs 2553, $P = .53$	~	6325 vs 3347, $P = .28$	~
T_H2				
CCL17	2208 vs 1959, $P = .71$	~	4064 vs 3415, $P = .57$	~
IL-5	147 vs 145, $P = .97$	~	103 vs 128, $P = .34$	~
IL-13	556 vs 706, $P = .38$	~	399 vs 424, $P = .79$	~
T regulatory				
IL-10	5653 vs 3678, $P < .05$	↓	5767 vs 3328, $P < .02$	↓

AIC versus placebo: ~, no significant effect; ↓, decrease.

weeks. Interestingly, although enhanced T_H1 cytokine and chemokine synthesis and reduced T_H2 cytokine and chemokine synthesis were both clearly evident in the ragweed-specific response 2 weeks after AIC treatment, these changes were relatively transient. The most durable effect of AIC injections, seen 16 weeks later, was the reduced T_H2 regulatory responses.

This may have important implications for immunomodulation in humans. Although immediate hypersensitivity diseases are classically defined as T_H2 dependent, there is considerable evidence of enhanced IFN-γ synthesis during chronic allergen stimulation.[1] Excessive T_H1 cytokine and chemokine production could potentially have negative consequences by exacerbating, rather than reducing, this inflammatory process. Our observations in this translational research suggest that ISS administration by means of an allergen-linked molecule may lead to pronounced inhibition of allergen-specific T_H2 responses without chronically high levels of IFN-γ expression. ISSs are markedly lower in toxicity than other danger signals such as LPSs and other pathogen-associated molecular patterns to which the innate immune system is programmed to respond promptly and vigorously.

AIC appeared to have a good safety profile, as indicated by 100- to 300-fold decreased allergenicity[23] and few adverse effects, despite the high doses of ragweed allergen injected in this study relative to those injected in conventional allergen-specific immunotherapy regimens. History, physical examination, and clinical laboratory evaluations did not suggest any evidence of global immune stimulation. The local reactions observed with AIC injections were similar to those observed with licensed ragweed allergen extracts and were consistent with pre-existing ragweed allergy.

In summary, we have demonstrated for the first time that injections of AIC in humans with ragweed allergy resulted in a marked shift in systemic in vivo allergen-specific immunoregulatory responses characterized by transiently enhanced IFN-γ and longer-term inhibition of T_H2 cytokine and chemokine responses, including IL-5, CCL17, and CCL22. The net effect of this intervention was a shift in the T_H1/T_H2 immunoregulatory cytokine and chemokine balance of the ragweed-specific response without global effects on the immune system because the effects of AIC administration were restricted to the sensitizing allergen, with minimal detectable differences in responses to an unrelated antigen or to broader immune activation.

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