Immunostimulatory sequence DNA linked to the Amb a 1 allergen promotes T_H1 cytokine expression while downregulating T_H2 cytokine expression in PBMCs from human patients with ragweed allergy

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Background: Recent studies have demonstrated that bacterially derived immunostimulatory sequences (ISSs) of DNA can activate the mammalian innate immune system and promote the development of T_H1 cells. Promotion of T_H1 immunity by means of immunotherapy in allergic patients has led to the alleviation of symptoms that result from allergen-specific T_H2 responses.
Objective: Our purpose was to investigate whether the T_H1-enhancing properties of ISSs could be used to alter the T_H2-dominated immune response of allergic PBMCs in vitro.
Methods: Ragweed protein-linked ISS (PLI) was generated from a specific, highly active 22-base ISS and Amb a 1, the immunodominant allergen in ragweed pollen, to combine the T_H1-enhancing properties of ISSs with allergen selectivity, and its activity was investigated in PBMC cultures from subjects with ragweed allergy.
Results: PLI was markedly successful at reversing the dominant allergen-induced T_H2 profile while greatly enhancing IFN-γ production. Delivering ISSs in a linked form proved to be much more effective at modulating the resulting cytokine profile than delivering free ISSs in a mixture with unlinked Amb a 1. PLI also demonstrated cytokine-modulating properties, even when used to stimulate cells that had already been primed for 6 days with Amb a 1. The antigen specificity of the action of PLI was confirmed by the observations that PLI enhances Amb a 1–specific T-cell proliferation.
Conclusion: These data indicate that delivery of ISSs within an antigen-specific context exhibits potent cytokine-modulating activity and, combined with its reduced allergenicity, makes this molecule a strong candidate for use in improved immunotherapy applications. (J Allergy Clin Immunol 2001;108:191-7.)

Key words: *Allergy, T_H1/T_H2, cytokines, immunotherapy*

The immunostimulatory properties of bacterial DNA were first demonstrated by Yamamoto et al,[1] who found that the nucleic acid fraction of 6 species of bacteria, but not of vertebrates, could stimulate increased natural killer (NK) lytic and antiviral activity in murine splenocytes caused by enhanced production of IFNs. In later studies, they discovered that human PBLs were also responsive to bacterial DNA and released large quantities of IFN-α/β after incubation with oligodeoxynucleotides (ODNs) containing palindromic hexamers with CpG at the third and fourth positions of the hexamer.[2,3] Several investigators have expanded on these studies in murine models and have found that plasmid or ODNs containing CpG motifs exhibit an impressive array of immunostimulatory activities, including induction of IL-6, IL-12, and IFN-γ production from splenocytes[4]; adjuvant activity for increased IgG2a production[5,6]; activation of macrophage and dendritic cell maturation[7-9]; antitumor activity[10]; promotion of cytotoxic T-lymphocyte generation[11]; and reduction of eosinophilia and airway hyperreactivity in mice with induced asthma-like pathology.[12-14]

Numerous recent studies have been devoted to analyzing the effects of immunostimulatory sequences (ISSs) on human cellular immune function. These reports have described the ability of CpG ODNs to enhance proliferation and Ig production by B cells[15,16]; to promote expression of activation markers on B cells,[17] macrophages,[18] and dendritic cells[19]; and to increase NK lytic activity.[16,20] Another property of ISSs with profound therapeutic implications is its ability to induce the production of a wide array of cytokines from multiple cell types. Bauer et al[21] demonstrated that human monocytes would secrete IL-6, IL-12, and TNF-α in response to CpG ODNs. IFN-γ production from PBMCs of both subjects allergic to birch and grass pollen and control subjects was detected by Bohle et al,[22] who also reported an increase in mRNA expression of IL-12 p40, IL-12 p35, and IL-18, although no IL-12 protein production was reported. Purified NK cells or T cells that are costimulat-

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Abbreviations used
ISS: Immunostimulatory sequence
NK: Natural killer
ODN: Oligodeoxynucleotide
OVA: Ovalbumin
PLI: Protein-linked immunostimulatory sequence
PLN: Protein-linked nonimmunostimulatory sequence
PMA: Phorbol myristate acetate
TT: Tetanus toxoid

ed with anti-CD3 also can produce IFN-γ in response to CpG ODNs.[20]

Because several pro-T_H1 activities have been attributed to ISSs, we proposed to determine whether it could be effective at modulating the cytokine profile of allergen-stimulated PBMCs from atopic subjects. Allergic reactions are characterized by IgE-mediated activation of mast cells and basophils, a process that has been shown to be largely controlled by overproduction of T_H2 cytokines like IL-4 and IL-5.[23] Because IL-12 and IL-12 inducers, such as varicella zoster, have been shown to partially or completely reverse production of T_H2 cytokines by cells from allergic individuals,[24,25] we investigated the effect of ISS, another IL-12 inducer, on PBMC populations from donors with ragweed allergy. Such PBMCs typically respond to in vitro ragweed allergen stimulation with secretion of T_H2 cytokines. ISS ODNs were covalently linked to a purified form of the immunodominant short ragweed allergen Amb a 1 to form ragweed to ensure that ISS stimulation would occur within the context of antigen recognition by ragweed allergen–specific T cells (Amb a 1) protein-linked ISS (PLI). Previous work has shown that ragweed PLI immunization of mice induced a strong T_H1 response characterized by elevated synthesis of IFN-γ and Amb a 1–specific IgG2a concomitant with reductions in IL-5 and antigen-specific IgE and IgG1.[26] Here, we present data that demonstrate that PLI can similarly alter the T_H1/T_H2 ratio of cytokine expression in cultures of PBMCs from antigen-primed subjects with ragweed allergy. ISSs delivered in linked form was found to be more effective than a free mixture of ISS ODNs and Amb a 1. Additionally, PLI enhanced the expansion of Amb a 1–specific T cells and modulated cytokine profiles, even during ongoing T_H2 stimulation. Finally, the enhancement of IFN-γ by PLI was shown to be dependent on IL-12.

METHODS

Reagents

Amb a 1, the primary allergenic component of ragweed pollen, was extracted and purified from whole ragweed pollen extract.[26] Tetanus toxoid (TT) was purchased from Calbiochem and Signal Transduction Products. ISS 1018 (TGA CTG TGA ACG TTC GAG ATG A),[6] ISS 2006 (TCG TCG TTT TGT CGT TTT GTC GTT),[21] and ISS C069 (TCC ATA ACG TTC GCC TGA TGC T) are ISS sequences with CpG motifs, which have been shown to have cytokine-inducing potential in vitro with murine splenocytes and human PBMCs, whereas non-ISS 1019 (TGA CTG TGA AGG TTA GAG ATG A)[6] and non-ISS 1040 (TGA CTG TGA ACC TTA GAG ATG A) do not have CpG motifs and demonstrate little T_H1-inducing activity (Marshall JD and Abbate C, unpublished observations). The phosphorothioate ODNs 1018, 1019, and 1040 were synthesized by Hybridon Specialty Products, whereas 2006 and C069 were synthesized by Trilink Biotechnologies. Ragweed PLI and protein-linked non-ISS (PLN) were created by linking Amb a 1 and ISS or non-ISS ODNs through standard heterobifunctional chemistry, as previously described.[26] For clarity, in this article a covalent linkage of 1018 ODN and Amb a 1 is termed PLI-1018, a covalent linkage of 1040 ODN and Amb a 1 is termed PLN-1040, and so on. The molar ratio of ODNs to molecules of Amb a 1 in these conjugates is approximately 3 to 5:1. Anti-IL-12 mAb (clone C8.6) and mouse IgG1 isotype control antibody (α-TNP, clone 107.3) were purchased from PharMingen. The cell culture media used was RPMI-1640, with 50 U/mL penicillin, 50 μg/mL streptomycin, 300 μg/mL L-glutamine, 1 mmol/L sodium pyruvate, 1× nonessential amino acids (all from Bio-Whittaker), and 10% human AB serum (Gemini).

Allergic subjects

Individuals atopic for short ragweed *(Ambrosia artemisiifolia)*, as determined by skin test positivity, were recruited under conditions of informed consent at the Johns Hopkins University Asthma & Allergy Center and at the practice of Michael Reid, MD. Subjects were bled through venipuncture into heparanized syringes, and PBMCs were isolated by means of Ficoll centrifugation.

Cytokine induction

The culture periods and conditions have been optimized and described in a previous study.[27] Briefly, PBMCs from individuals with ragweed allergy were cultured at 2×10^6/mL for 6 days with 5 μg/mL Amb a 1, TT, PLI, or PLN. Cultures were harvested on day 6, and supernatants were removed and frozen, later to be analyzed for IFN-γ content by means of ELISA (Biosource Cytoscreen). Some cells were washed, counted, and restimulated at 2×10^6/mL per well in 24-well plates for 24 hours with 5 μg/mL PHA (Sigma) and 50 ng/mL phorbol myristate acetate (PMA; Sigma). Supernatants were then removed and frozen, later to be analyzed for IL-4 and IL-5 content by means of ELISA (Biosource Cytoscreen).

In some experiments other cells from these cultures were restimulated on day 6 with irradiated autologous feeder cells at a 1.25:1 ratio (cultured cell/feeder cell), maintaining the 2×10^6/mL concentration. These cells were also restimulated with fresh antigen and 10 ng/mL rIL-2 (PharMingen) and cultured for an additional 6 days. At day 12, supernatants were removed and frozen, later to be analyzed for IFN-γ content by means of ELISA (Biosource Cytoscreen). Some cells were washed, counted, and restimulated at 2×10^6/mL per well in 24-well plates for 24 hours with 5 μg/mL PHA and 50 ng/mL PMA. Supernatants were then removed and frozen, later to be analyzed for IL-4 and IL-5 content by means of ELISA (Biosource Cytoscreen).

Proliferation

PBMCs from individuals with ragweed allergy were cultured at 2×10^6/mL for 6 days with 20 μg/mL Amb a 1, the optimal dose to induce proliferation, or 5 μg/mL PLI-1018, PLI-C069, or PLN-1019. Cultures were harvested on day 6, washed, and restimulated at 2×10^5/200 μL per well in 96-well round-bottomed polystyrene plates for 5 days with 20 μg/mL Amb a 1. On day 5, all wells were pulsed with 1 μCi tritiated thymidine (Amersham Pharmacia) and harvested 24 hours later.

RT-PCR

Total RNA was extracted by means of the Ultraspec isolation system (Biotecx), purified with isopropanol precipitation and ethanol

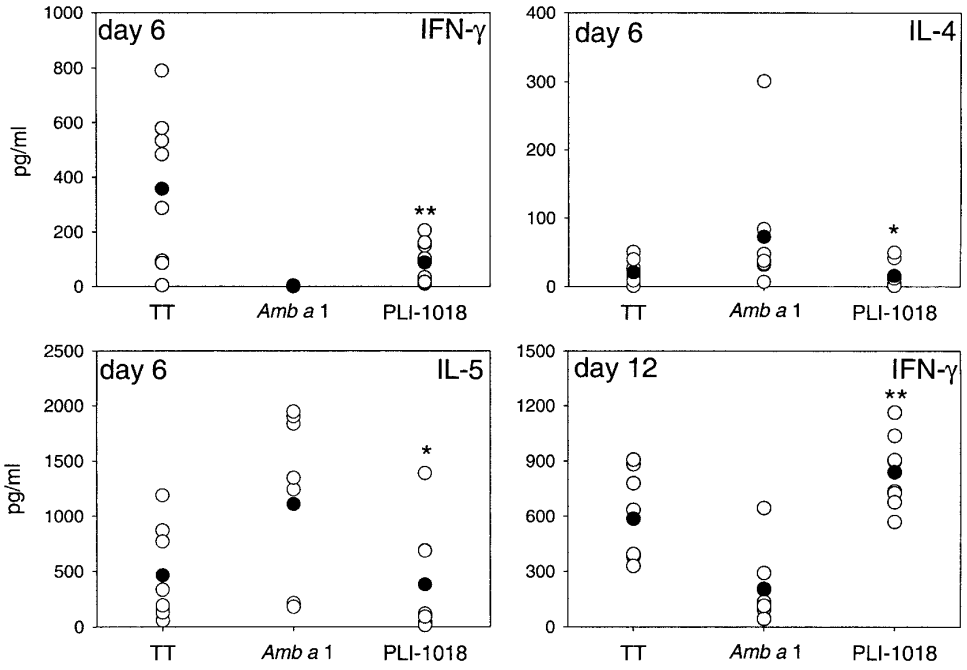

FIG 1. PLI induces a T_H1-like response in PBMC cultures from 8 subjects with ragweed allergy after 6 to 12 days of culture in contrast to the T_H2 response induced by Amb a 1. Each *open circle* represents an individual donor, with the mean of each group designated by a *filled circle*. Statistical significance comparing Amb a 1 with PLI-1018 stimulation was calculated by using 1-way ANOVA. $*P < .05$; $**P < .005$.

washes, and quantitated by means of spectrophotometry. RT reactions were performed with 4 μg of RNA from each sample with TaqMan Reverse Transcription reagents from PE Biosystems (catalog No. N808-0234, kit manufactured by Roche). PCR reactions were conducted with TaqMan Universal PCR Master Mix and the TaqMan Cytokine Gene Expression Plate I (PE Biosystems) and run in duplicate on the 7700 Sequence Detector (PE Biosystems) after instrument protocol. Expression of each cytokine gene was first normalized to expression of 18S ribosomal RNA, which was amplified in the same well to verify equal RNA loading. Relative quantification of cytokine gene expression was calculated with the formula $2^{\text{average}\Delta\Delta C_T}$, where average$\Delta\Delta C_T$ is the average of duplicate values of threshold cycles for the PCR of one cytokine gene after subtracting the threshold cycles for the PCR of 18S ribosomal RNA. Because one sample is chosen as the calibrator and thus will be arbitrarily assigned a value of 1, the values of the samples to be compared with the calibrator are expressed as fold changes in expression compared with the calibrator. These calculations are performed automatically with the Sequence Detection Systems software supplied with the 7700 Sequence Detector.

RESULTS

PLI reverses the T_H2-dominant cytokine secretion profile induced by Amb a 1

PBMCs from individuals with ragweed allergy were stimulated with Amb a 1 or PLI-1018 for 6 days and then restimulated with fresh antigen and irradiated syngeneic PBMCs as feeders for another 6 days. Cell-free supernatants were removed at both days 6 and 12 and assayed for IFN-γ content. Because IL-4 and IL-5 levels are extremely low in such supernatants, a portion of cells harvested on days 6 and 12 was also stimulated with PHA and PMA for 24 hours, and the resultant supernatants were tested for levels of IL-4 and IL-5. PBMCs were also cultured with TT, which has been shown to promote the expression of a T_H1-like pattern of cytokines (high IFN-γ/low IL-4).[27] As expected, exposure of PBMCs from these subjects to TT resulted in substantial IFN-γ production with lower levels of IL-4 by day 6, whereas Amb a 1 promoted an opposing cytokine profile dominated by IL-4 and IL-5 with little IFN-γ induction (Fig 1). Stimulation with PLI-1018, on the other hand, resulted in a marked reduction of allergen-induced IL-4 ($P < .05$) and IL-5 ($P < .05$) production by day 6 with an accompanying increase in titers of IFN-γ ($P < .005$). By day 12, secretion of IFN-γ after PLI-1018 stimulation was higher even than that from TT-stimulated cultures, whereas IL-4 and IL-5 continued to show significant reductions ($P < .05$, data not shown). Thus linkage of ISS 1018 to Amb a 1 appears to alter the immune response to this allergen, downregulating the resultant T_H2 cytokine response and instead promoting the secretion of IFN-γ.

Further experiments were performed with other PLIs (Amb a 1 linked to the ISS ODNs 2006 and C069) and PLNs (Amb a 1 linked to the non-ISS ODNs 1040 and 1019). PLI-2006 and PLI-C069 exhibited equivalent activity compared with PLI-1018, whereas PLN-1040 and PLN-1019 demonstrated little or no cytokine-modulating activity (data not shown). These results demonstrate that a conjugate with cytokine-modulating properties requires an ODN with ISS activity.

IL-12–inducing properties have been ascribed to free ISS,[28] and enhancement of IFN-γ by ISS has been linked to a dependence on the presence of IL-12.[22] To determine

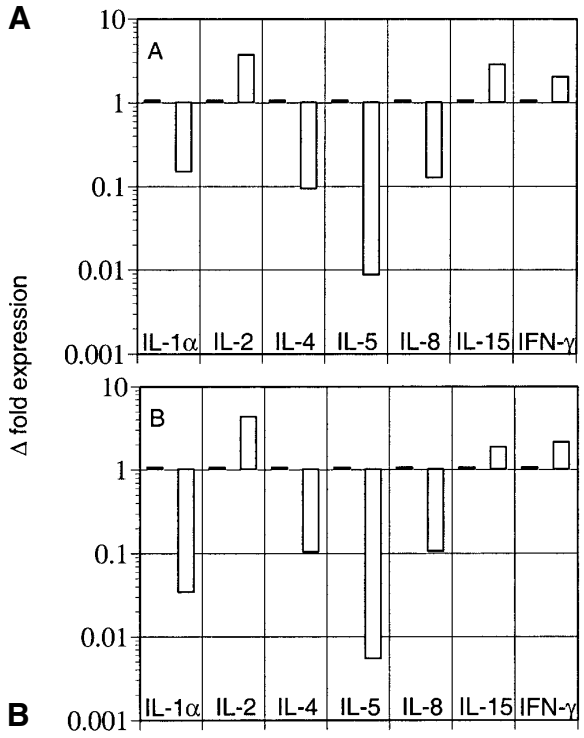

FIG 2. PLI modulates the expression of several cytokine mRNAs in comparison with Amb a 1. PBMCs from 2 subjects with ragweed allergy (**A** and **B**) were cultured for 6 days with Amb a 1 or PLI-1018 and then underwent PCR analysis. Data are expressed as change in fold expression of cytokine RNA of PLI-1018–stimulated cells *(right-hand bars)* compared with RNA expression of Amb a 1–stimulated cells (*left-hand bars,* designated as the calibrator and arbitrarily assigned a value of 1).

whether IL-12 is critical for the altering of the T_H2/T_H1 ratio by PLI in our system, we performed stimulations of PBMCs from subjects with ragweed allergy with PLI-1018 in the presence and absence of neutralizing anti-IL-12 mAb. Upregulation of IFN-γ production by PLI-1018 was indeed substantially curtailed during the IL-12 blockade; however, the reduction of IL-4 and IL-5 by PLI-1018 was virtually unchanged in the presence of anti-IL-12 (data not shown), indicating PLI may reduce T_H2 cytokine secretions in an IL-12–independent manner.

PLI substantially alters the cytokine mRNA profile of Amb a 1–stimulated cells

The cytokine-modulating properties of PLI on human PBMCs were also analyzed by measurement of cytokine mRNA expression. PBMCs from 2 individuals with ragweed allergy were stimulated for 6 days with either Amb a 1 or PLI-1018, and then total RNA was isolated from these cells, and RT-PCR was performed by using the TaqMan 7700 Sequence Detector. In cells from both subjects, PLI-1018 stimulation resulted in substantially lower expression of IL-1α, IL-4, IL-5, and IL-8 mRNA compared with Amb a 1 stimulation, whereas levels of IL-2, IL-15, and IFN-γ were enhanced (Fig 2). These data verified the T_H1-enhancing and T_H2-suppressing functions of PLI that were observed by measurement of in vitro cytokine secretion from PBMCs.

Linkage of ISSs to Amb a 1 is optimal for cytokine modulation

Previously, we have found that covalent linkage of Amb a 1 and ISS enhances the in vivo antigen-specific T_H1 response in mice in comparison with a simple mixture of ISSs and Amb a 1.[26] To investigate whether linkage also enhanced the in vitro response of PBMCs to ISSs, we compared stimulation with PLI-1018 to treatment with an unlinked mixture of Amb a 1 and 1018 ODN. The molar ratio of ODN delivered in the mix was approximately twice as much as that delivered by PLI-1018. Stimulation with a mix of Amb a 1 and 1018 ODN (Fig 3) did not substantially enhance IFN-γ or decrease IL-4 at day 6 or 12, which is in contrast to those effects demonstrated by PLI-1018 (day 6: IFNγ, $P < .005$; IL-4, $P < .05$). Therefore covalent linkage of the ISS ODN to Amb a 1 is required for optimal suppression of IL-4 and enhancement of IFN-γ in our culture system.

PLI enhances Amb a 1–specific T-cell proliferation

In an attempt to determine whether PLI induces the expansion of Amb a 1–specific T cells in vitro, PBMCs from individuals with ragweed allergy were stimulated with Amb a 1 or with various species of PLI/PLN for 6 days and then restimulated with Amb a 1. As shown in Fig 4, cells that had been expanded by PLIs had increased proliferative ability when restimulated with Amb a 1, indicating either the presence of higher numbers of Amb a 1–specific T cells or a higher activated state of T cells in those cultures. In contrast, cells stimulated by PLN-1019 responded to Amb a 1 in the proliferation assay no better than control cells. Thus PLI not only stimulates the expansion of Amb a 1–specific T cells but also does so more efficiently than stimulation with allergen alone.

PLI retains cytokine-modulating activity in the midst of an ongoing T_H2 response

In previous experiments PBMCs from atopic individuals were exposed to PLI alone without accompanying free Amb a 1. We next investigated whether PLI would retain its IFN-γ–enhancing and T_H2-reducing properties when administered during an ongoing stimulation with allergen. First, PBMCs from individuals with ragweed allergy were stimulated with Amb a 1 alone or with a combination of Amb a 1 and PLI-1018 (Fig 5, *A*). Even in the presence of Amb a 1, PLI-1018 clearly induced a cytokine profile much different from that induced by allergen alone: IFN-γ was significantly enhanced ($P < .05$), whereas levels of IL-4 ($P < .005$) and IL-5 ($P < .05$) were both suppressed. PBMCs were stimulated for 6 days with Amb a 1 and then restimulated at day 6 with either allergen alone again or with Amb a 1 plus PLI-1018 to further explore these properties of PLI (Fig 5, *B*). Although it was introduced to these cultures at a point

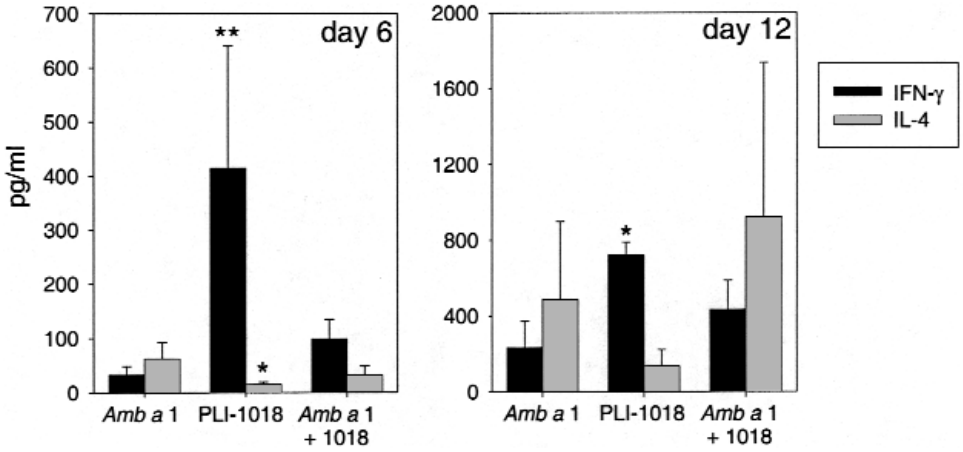

FIG 3. PLI is more effective at enhancing IFN-γ and reducing IL-4 than stimulation with Amb a 1 and free ISSs. PBMCs from subjects with ragweed allergy were cultured for 6 (n = 9) or 12 (n = 4) days. Data are reported as means ± SEM. Statistical significance comparing Amb a 1 with PLI-1018 or with mix stimulation was calculated by using 1-way ANOVA. **P* < .05; ***P* < .005.

where Amb a 1–specific T_H2 cells were dominant, PLI-1018 still significantly enhanced the secretion by T cells of IFN-γ ($P < .05$) and partially inhibited IL-4 and IL-5 production. These results indicate that although primed and stimulated T_H2 cells are actively secreting IL-4 and IL-5 in these allergen-stimulated cultures, the cytokine profile of the antigen-specific population taken as a whole is not completely immutable, and enhanced IFN-γ production can still be obtained from some cells after exposure to PLI.

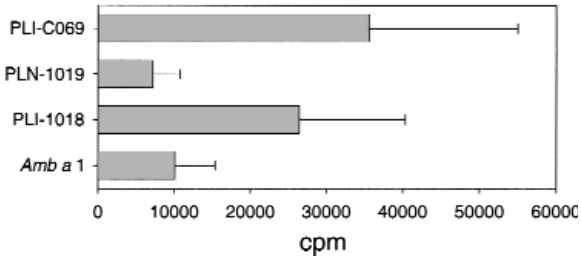

FIG 4. ISS-specific PLIs promote Amb a 1–specific lymphocyte expansion. Data are reported as means ± SEM (n = 3).

DISCUSSION

This study characterized the in vitro modulation by ragweed (Amb a 1) PLI of cytokine production by Amb a 1–specific T cells from individuals with ragweed allergy. Parronchi et al[29] reported on the effects of CpG ODNs in cultures of cells from allergic individuals in a similar culture system, although a mix of free ODNs and allergen was used to stimulate in that case instead of an ISS linked to allergen. That study indicated that a mix of ISS-type ODNs and Der p 1–enhanced IFN-γ protein expression while decreasing production of IL-4 in 14-day cultures of PBMCs from individuals with dust mite allergy, as measured by cytofluorometric analysis of intracellular cytokine staining. These results are in agreement with our observations on the ability of ISSs to alter the profile of cytokines induced by allergen from T_H2 to T_H1. However, these investigators demonstrated this effect with a nonlinked allergen plus ISS mix,[29] a formulation that had poor activity in our culture system in comparison with PLI. This may be due to several factors, including differences in immunogenicity between Amb a 1 and Der p 1; the constant exposure of individuals with dust mite allergy to dust mite antigen compared with the strictly seasonal appearance of ragweed pollen (September-October); and differences in experimental assays (ELISA vs intracellular cytokine staining). PBMCs from subjects bled outside of ragweed allergy season may have far fewer Amb a 1–primed T cells to respond to the in vitro stimulation of recombinant allergen in comparison with Der p 1–atopic individuals. Our results illustrate a major potential benefit of ISS linkage to allergen as opposed to a free mixture: simultaneous delivery to the same target antigen-presenting cell of both antigen and ISS. This feature may overcome problems inherent in situations of poorly immunogenic antigens or low frequencies of antigen-primed T cells.

Other recent reports have examined the role of ISS-antigen conjugates in antigen-specific murine models. Similarly to our study, Cho et al[11] found that although immunization of a mix of free ISS and ovalbumin (OVA) could significantly increase IFN-γ and IgG2a in subsequent in vitro splenocyte assays, a conjugate of ISS DNA and OVA was clearly more effective at enhancing cytotoxic lymphocyte generation and promoting antitumor immunity. Shirota et al[30] proved the effectiveness of an ISS-OVA conjugate in a murine asthma model in which BALB/c mice primed and challenged intratracheally with OVA have asthma-like lung eosinophilia and airway hyperresponsiveness. At equivalent concentrations, conjugate was 50- to 100-fold more effective than mix at

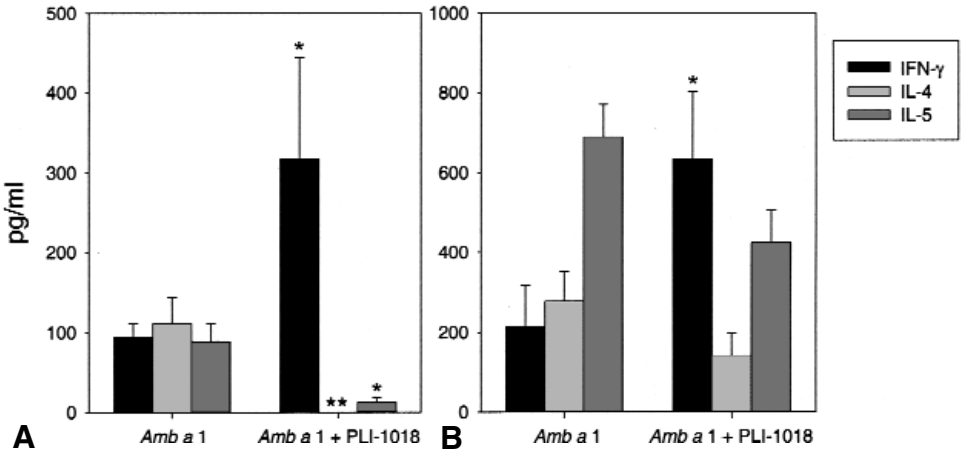

FIG 5. The cytokine-modulating activity of PLI is dominant over that of Amb a 1 when simultaneously administered, and it retains activity, even when introduced during an ongoing Amb a 1–stimulated T_H2 response. **A**, PBMCs from 4 subjects with ragweed allergy were cultured for 6 days with Amb a 1 alone or Amb a 1 plus PLI-1018. **B**, PBMCs from 4 subjects with ragweed allergy were cultured for 6 days with Amb a 1 and then restimulated for 6 days with Amb a 1 or Amb a 1 plus PLI-1018. Data are reported as means ± SEM. Statistical significance comparing Amb a 1 with Amb a 1 plus PLI-1018 stimulation was calculated by using 1-way ANOVA. $*P < .05$; $**P < .005$.

reducing eosinophilia and priming for elevated IFN-γ secretion by lymph node cells in vitro. Similarly, coinjection of ISS ODN and Amb a 1 was much less effective than conjugate at promoting antigen-specific T_H1 immunity in mice.[26] These data agree with our observation that proximity of ISS and antigen is critical to achieve optimal allergen-specific immunity.

In addition to the T_H1-enhancing immunogenic properties of PLI, which are attributable to its ISS component, PLI also has the additional benefit of decreased allergenicity. In vitro studies conducted with human basophils from individuals with ragweed allergy indicate that approximately 50-fold more PLI is needed to induce histamine release levels comparable with that observed with native allergen.[26] Furthermore, an initial safety study performed with 6 subjects with ragweed allergy undergoing puncture skin tests demonstrated that approximately 95- to 300-fold greater quantities of PLI ($P < .05$) were required to achieve skin test allergic reactions similar to those induced by ragweed extract.[31] These data point to a much higher threshold for activation of IgE-armed mast cells and basophils by PLI in contrast to Amb a 1 or native ragweed allergen, most likely because Amb a 1–specific IgE molecules are sterically hindered from binding to the allergen by the approximately 3 to 5 ISS molecules that are covalently linked per Amb a 1 molecule. This characteristic of PLI suggests that it might be tolerated much better than Amb a 1 at similar concentrations if administered as an immunotherapeutic agent to patients.

Conventional immunotherapy comprises a series of subcutaneous injections of small quantities of allergen extracts, a process that may require as many as 36 injections until maintenance is achieved, followed by monthly shots for 3 to 5 years. Because of its potent T_H1-enhancing properties, much lower, fewer, or both doses of ragweed PLI might be required for an immunotherapeutic protocol compared with standard allergenic extracts. The other major drawback of conventional immunotherapy is the risk of anaphylaxis, which necessitates beginning each regimen with suboptimal doses of allergen that further prolong the treatment without significantly contributing to antiallergy immunity.[32] The substantially reduced allergenicity of PLI coupled with its immunoenhancing, T_H1-directing properties might allow for high initial doses closer to immunogenic levels, thus reducing the total number of injections required.

These results indicate that in the context of allergen-stimulated in vitro cultures, ragweed PLI can reverse the allergen-induced T_H2 profile of cytokines and replace it with enhanced T_H1 immunity characterized by elevated levels of IFN-γ and IL-2, as well as improved allergen-specific proliferation by T cells. PLI is able to direct this response, even in the presence of Amb a 1 and does so in an antigen-specific manner. Studies are currently underway to investigate the nature of the target antigen-presenting cells of PLI and the mechanism by which ISSs amplify IFN-γ production at the expense of T_H2 cytokine secretion. In addition, phase I safety studies of ragweed PLI have begun with patients with ragweed allergy as the initial step in assessment of PLI for immunity. If immunization with PLI is well tolerated, further studies will continue to examine a potential role for ragweed PLI in standard immunotherapeutic regimens.

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Correction

In the article in the June issue entitled "Immunochemical characterization of edible bird's nest allergens" (2001;107:1082-8), the listing of the author names was incomplete. The article was written by Denise Li Meng Goh, MMed (Paeds), MRCP, Kaw Yan Chua, PhD, Fook Tim Chew, PhD, Rosa Cynthia Mui Yee Liang, BSc, Teck Keong Seow, PhD, Ke Li Ou, PhD, Fong Cheng Yi, BSc (Hons), and Bee Wah Lee, MD.