

Effects of Specific Immunotherapy on the B7 Family of Costimulatory Molecules in Allergic Inflammation¹

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The effect of allergen-specific immunotherapy (IT) on Ag presentation and T lymphocyte stimulation was evaluated by verifying the expression of costimulatory molecules in allergic patients. Thus, CD28 and CTLA-4, B7, and B7-H molecules on immune cells, as well as cytokine production, were analyzed in and out of the pollination period in 30 patients allergic to *Betulaceae* that had or had not undergone specific IT. Results showed that IT attenuated the increase in the percentage of CD28⁺CD4⁺ T cells and the decrease in the percentage of CTLA-4⁺CD4⁺ T cells seen in untreated individuals. CD19⁺/CD80, CD19⁺/CD86⁺, and CD14⁺/CD80⁺ APCs were significantly augmented during pollination in unvaccinated individuals. B7-H1-expressing monocytes (CD14⁺) and B lymphocytes (CD19) as well as CD14 and CD19 B7-H1⁺/IL-10⁺ APC were augmented in *Betulaceae* Ag-stimulated cell cultures of vaccinated patients independently of pollination, and were further increased in these individuals during pollination. As a result, the IL-10-IFN- γ ratio in CD4⁺, CD14⁺, and CD19⁺ cells increased in vaccinated patients, but decreased in unvaccinated individuals during pollination. These data clarify the cellular and molecular basis underlying the recent observation that peripheral expansion of IL-10-producing cells is associated with successful IT. B7-H1 could be an optimal target for IT of allergic diseases using mAbs. *The Journal of Immunology*, 2007, 178: 1931–1937.

T cell activation is induced by the presentation of a peptide/MHC molecule complex on the surface of APCs in the presence of costimulatory molecules (1). The B7/CD28 family of costimulatory molecules plays a pivotal role in the development of optimal T cell immunity as cross-linking of CD28 on the surface of T lymphocytes results in the activation of these cells (2, 3). B7-1 (CD80) and B7-2 (CD86) are expressed on APC and engage CD28 or CTLA-4 with different affinity (4). Ligation of these molecules is associated with different functional outcomes: triggering of CD28 has a stimulatory effect, whereas ligation of CTLA-4 is associated with dampening of T cell activation. These interactions also play an important role in regulating Th cell differentiation. Thus, if CD80 or CD86 molecules interact with CD28, Th2 responses are preferentially elicited (5). In contrast, the interaction between these molecules and CTLA-4 results in the inhibition of Th2 cells (6). In addition, whereas engagement of CD80 stimulates T cells alone to proliferate and to secrete IL-2, the ligation of CD86 delivers a bidirectional signal that results both in T cells stimulation and the elicitation of IgG1 and IgE production by B lymphocytes (7, 8). In humans, it was recently reported that both CD80- and CD86- expressing alveolar macrophages

are augmented in asthmatic patients (9, 10) and that the functional blocking of CD80 reduces T lymphocyte proliferative responses and the release of IL-4 and IL-5. Taken together, these results indicate that B7 molecules play an important role in the pathogenesis of allergic disease.

Beside B7-1 and B7-2 several new members of the B7 family of costimulatory molecules have been described (11–13). Among them B7-H1 (also known as PD-L1 or CD274) and B7-H3 (also known as CD276) play a role of growing importance. B7-H1 is constitutively present on monocytes and can be induced on activated T cells (12). B7-H1 ligation up-regulates IL-10 production and reduces T cell proliferation, while there is no effect on the generation of IL-2 and IL-4 (11, 14). Several studies indicate a negative regulatory role for B7-H1 in T cell responses (14–18). Indeed, the expression of B7-H1 on neoplastic cells can actively inhibit immune responses by promoting the apoptosis of effector CTL (19, 20). B7-H1 may play a major role in promoting Th2 cytokine production and in down-regulating effector responses of peripheral T cells (19). B7-H3 is a costimulatory molecule for T cell responses as well (13) with an immunological effect opposite to that of B7-H1. Thus, in the presence of anti-CD3 mAb, B7-H3/Ig fusion proteins elicit human T cell proliferation and selectively induce IFN- γ production (13). Additionally, expression of B7-H3 on human melanoma cell lines increases their allogenic CTL-mediated lysis.

Allergen-specific immunotherapy (IT),³ the elective treatment for the therapy of allergic diseases, is based on the administration of increasing doses of the disease-eliciting allergen (20). The clinical efficacy of allergen-specific IT has been documented in numerous controlled clinical studies and a variety of immunologic changes have been reported in IT-treated patients. Thus, reduction in mucosal recruitment of inflammatory cells (21), reduced basophile reactivity to allergen (22), decreased IgE serum concentration (23), an increase in the production of allergen-specific Th1

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³ Abbreviation used in this paper: IT, immunotherapy.

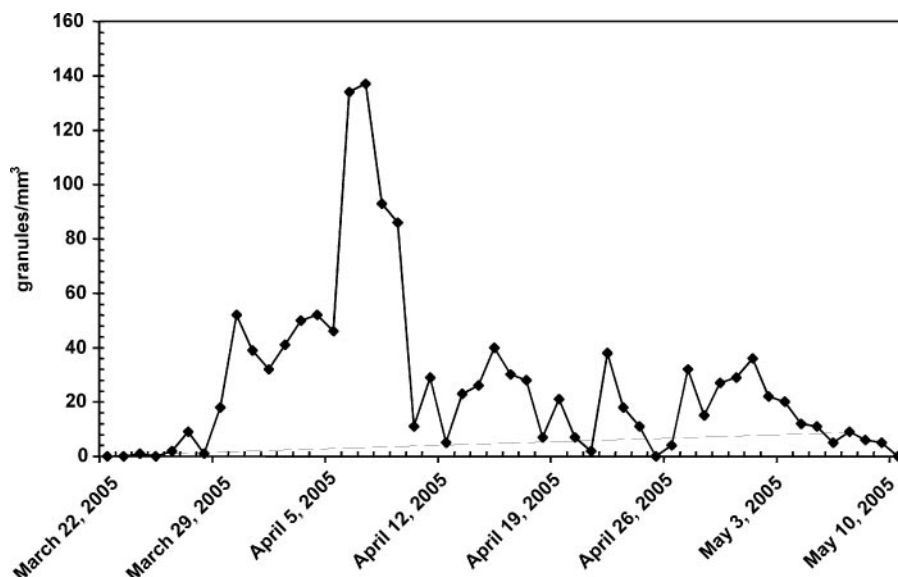


FIGURE 1. Birch pollen count (granules/mm³) for the 2005 season obtained from the Milano 3 Station.

cytokines (24) and IgG4 Abs (25), and the induction of IL-10-producing CD4⁺CD25⁺ regulatory T cell (26, 27) have been reported to take place following IT. Recently, Nouri-Aria et al. (28) showed that conventional IT induced a peripheral expansion of cells able to produce IL-10 in response to specific allergen. Importantly, these cells are recruited and activated in the respiratory mucosa, during pollen season.

To better understand the role of IT in inducing allergen-specific tolerance, we analyzed the expression of a panel of costimulatory molecules and activation markers in allergic patients undergoing or not undergoing IT. In particular, to allow us to analyze the immune correlates of successful IT, we selected, *a priori*, patients who responded positively to IT. These parameters were analyzed in the same individuals before and during pollen season.

Materials and Methods

Study population

Thirty Italian patients were enrolled between September 2004 and January 2005 at the Allergy and Clinical Immunology Clinic of the Luigi Sacco Hospital (Milan, Italy). Inclusion criteria were a clinical history suggesting birch sensitization, age between 18 and 50 years, skin prick test positive to birch pollen, and clinical report of asthma and/or rhinoconjunctivitis. Exclusion criteria were a previous IT treatment, clinical sensitization to other inhalant allergens, severe atopic dermatitis, other severe acute or chronic diseases, and pregnancy. In vivo diagnosis was done by a skin prick test using a standard birch extract (*Betula alba*, 100 index of reactivity/ml, Alyostal; Stallergenes). Prick tests were scored positive if the wheal obtained was equal or larger than the wheal elicited by the positive control (histamine hydrochloride, 10 mg/ml). The patients were subdivided in two groups: group A, patients undergoing symptomatic therapy alone and who had never received IT ($n = 16$); group B, patients who had been undergoing efficacious specific sublingual IT for at least 2 years (Stalolar 300; Stallergenes) ($n = 14$). Patients were instructed to keep a diary during the pollen season for the daily evaluation of the intensity of symptoms according to a 0–3 grading system (0, no symptoms; 1, mild symptoms; 2, moderate symptoms; and 3, severe symptoms). Monitored symptoms were itching, tear flow, and redness of the eyes; sneezing, running and blocked nose; cough, wheezing, asthma, and dyspnea. The Institutional Review Board of Luigi Sacco Hospital approved the study protocol; all patients were informed about the aim of the trial and gave their consent.

Pollen counts

Birch pollen count for the 2005 season was obtained from Milan 3 Station Lofarma; the counts are shown in Fig. 1.

Blood sample collection

Whole blood was collected by venipuncture in Vacutainer tubes containing EDTA (BD Biosciences). PBMCs were separated on lymphocyte separa-

tion medium (Organon Teknika), washed twice in PBS, and the number of viable leukocytes was determined by trypan blue exclusion and a hemocytometer. The samples were kept at room temperature and used within 12 h. Blood samples were obtained from all patients out of pollen season (January 2005) and at the peak of pollination (first decade of April 2005), when the patients were symptomatic.

Lymphocyte proliferation

PBMC (3×10^5) were placed in round-bottom wells of a microtiter culture plate (Costar) in a final volume of 0.2 ml along with: 1) no stimulation (BASAL); 2) mayor birch pollen allergen (Bet-v1, 1 μ g/ml; provided by Dr. F. Frati, Stallergenes, Milan, Italy) (ALLE); and 3) CMV grade II Ag

Table I. Epidemiologic, clinical, and therapeutic characteristics of patients enrolled in the study^a

Group	Patient	Age	Sex	Symptoms	Months in Immunotherapy
A	1	50	M	RC+A	nd
	2	40	F	R+A	nd
	3	45	F	RC+A	nd
	4	34	F	RC	nd
	5	33	F	RC	nd
	6	23	M	RC+A	nd
	7	49	M	RC+A	nd
	8	26	F	RC	nd
	9	28	F	RC+A	nd
	10	34	M	R+A	nd
	11	24	F	RC+A	nd
	12	27	F	RC	nd
	13	50	M	R	nd
	14	43	M	R+A	nd
	15	33	F	RC+A	nd
	16	45	M	RC	nd
B	17	28	F	RC+A	31
	18	41	F	RC+A	30
	19	37	F	RC+A	28
	20	50	M	RC+A	29
	21	50	M	R+A	45
	22	50	M	R+A	50
	23	47	M	RC+A	54
	24	25	F	RC+A	27
	25	22	F	RC+A	32
	26	39	M	RC+A	40
	27	41	M	RC+A	31
	28	35	F	RC+A	46
	29	27	F	RC+A	28
	30	32	F	RC+A	50

^a R, Rhinitis; RC, rhinoconjunctivitis; A, asthma; nd, not done.

Table II. Clinical score of patients enrolled in the study^a

Group	Location	No Symptoms (%)	Mild Symptoms (%)	Moderate Symptoms (%)	Severe Symptoms (%)
A	Eyes	0/16 (0)	3/16 (19)	8/16 (50)	5/16 (31)
	Nose	0/16 (0)	1/16 (6)	7/16 (44)	8/16 (50)
	Chest	6/16 (37)	3/16 (19)	4/16 (25)	3/16 (19)
B	Eyes	10/14 (72)	3/14 (21)	1/14 (7)	0/14 (0)
	Nose	10/14 (72)	3/14 (21)	1/14 (7)	0/14 (0)
	Chest	11/14 (79)	3/14 (21)	0/14 (0)	0/14 (0)

^a 0, No symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms.

(10 μ g/ml; Microbix Biosystem) (control Ag). Three replicate cultures were performed for each condition. Pooled human AB serum (10%) was added to each well. After 6 days of incubation at 37°C and 7% CO₂, cultures were pulsed with 1 μ Ci [³H]thymidine and harvested 18 h later. Incorporated [³H]thymidine was measured with a liquid scintillation beta counter. Results were expressed as stimulation indexes: ratio of mean counts obtained in the presence of Ag to mean counts obtained without Ag.

Immunophenotypic analyses

Lymphocyte subsets were evaluated by flow cytometric analysis, using 50 μ l of EDTA peripheral blood incubated for 30 min at 4°C with fluorochrome-labeled mAbs (CD4 R-PE-Cyanine 5 Tandem-PE-Cy5; CD14 PE-Cy5; CD19 PE-Cy5; CD28 PE; CD80 FITC; CD86 PE; CTLA-4 FITC). After incubation, erythrocyte lysis and fixation of marked cells was performed using the Immuno-Prep EPICS kit (Coulter Electronics) and Q-prep Work Station (Coulter Electronics).

Stimulation of PBMC for FACS analysis

A total of 5×10^6 PBMCs were incubated for 18 h without any Ag or in the presence of ALLE or CMV at the doses specified above. Anti-CD28 Ab (clone 37407.111; R&D Systems) was added during incubation (1 μ g/well) to facilitate costimulation. For cytokine analyses, 10 μ g/ml Brefeldin A (Sigma-Aldrich) was added to the cell cultures during the last 6 h of stimulation to block protein secretion.

Immunofluorescent staining

PBMC were washed in PBS, split in different flow cytometry tubes, and stained with CD4, CD14, CD19, CD80, or CD86 mAbs for 30 min at 4°C in the dark. For indirect immunofluorescence staining, PBMC were first incubated with a mouse anti-human B7-H1 Ab (2.5 μ g/ml) or with a hamster anti-mouse B7-H3 (2.5 μ g/ml) (gifts of Dr. L. Chen). After 30 min at 4°C, the cells were washed and further incubated for 30 min at 4°C with a rat anti-mouse IgG (H+L) Ab conjugated with FITC (1 μ g/ml) or with a mouse anti-hamster IgG conjugated with PE (1 μ g/ml). For the analysis of cytokine-secreting cells, after phenotypic staining, PBMCs were washed and fixed in Reagent A solution (FIX & PERM Cell Permeabilization kits; Caltag Laboratories) for 10 min at room temperature in the

dark. The cells were washed in PBS and resuspended in reagent B (FIX & PERM Cell Permeabilization kits), with mAbs specific for different cytokines (IL-10 PE, IFN- γ FITC, and IL-4 PE). After a 30-min incubation at 4°C in the dark, the cells were washed and fixed in 1% paraformaldehyde in PBS.

Monoclonal Abs

The following mAbs were used in this study: anti-human CD4 (mouse IgG2a isotype), anti-human CD14 (mouse IgG1 isotype), anti-human CD19 (mouse IgG1 isotype) coupled to R-PE-Cyanine 5 Tandem (PE-Cy5; Caltag Laboratories); anti-human CD86 (mouse IgG1 isotype), anti-human CD28 (mouse IgG1 isotype) coupled to R-PE (Serotec); anti-human CD80 (mouse IgG1 isotype; Serotec) and anti-human CTLA-4 (mouse IgG2b; R&D Systems) coupled to FITC; anti-human B7-H1 (mouse IgG1 isotype; provided by Dr. L. Chen) and anti-mouse IgG (H+L) coupled to FITC (eBiosciences); anti-mouse B7-H3 (hamster IgG isotype; provided by Dr. L. Chen) and anti-hamster IgG coupled to PE (BD Pharmingen). The intracellular molecule detection mAbs used were anti-human IL-10 (mouse IgG1 isotype; Caltag Laboratories), coupled to PE and anti-human IFN- γ (mouse IgG1 isotype; Caltag Laboratories) coupled to FITC.

Cytometric analysis

The cytometric analyses of phenotype and cytokine-secreting lymphocytes were performed using an EPICS XL flow cytometer (Beckman Coulter) equipped with a single 15 mW argon ion laser operating at 488 nm interfaced with 486 DX2 IBM computer. For each analysis, 20,000 events were acquired and gated on CD4 (or CD14, CD19) expression and side scatter properties. Green fluorescence from FITC (FL1) was collected through a 525-nm bandpass filter, orange-red fluorescence from R-PE (FL2) was collected through a 575-nm bandpass filter and deep-red fluorescence from PE-Cy5 (FL4) was collected through a 670-nm bandpass filter. Data were collected using linear amplifiers for forward and side scatter and logarithmic amplifiers for FL1, FL2, and FL4. Samples were first run using isotype control or single fluorochrome-stained preparations for color compensation.

Statistical analysis

Procedures were based on nonparametric analyses. Comparisons between the different groups were made using a two-tailed Mann-Whitney *U* test performed for independent samples. Possible relationships were evaluated using a Pearson's correlation test. Statistical analysis was performed using the SPSS statistical package.

Results

Patients

Clinical, epidemiological, and therapeutic characteristics of all the patients enrolled in the study are presented in Table I. Sixteen patients (9 females and 7 males) were included in group A; 14 individuals (8 females and 6 males) who successfully underwent IT were included in group B. Immunological evaluations were

FIGURE 2. Bet-v1- and CMV-specific lymphocyte proliferation in patients who had (●) or had not (○) undergone allergen specific IT. Results obtained in the same individuals in and out of the pollination season are shown; stimulation indexes (SI) are presented. The horizontal bar indicated median values. Statistical significance is shown.

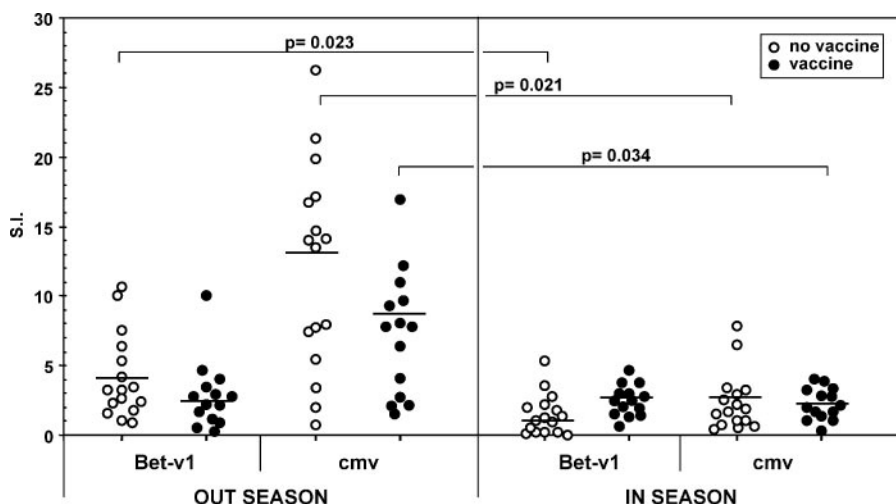
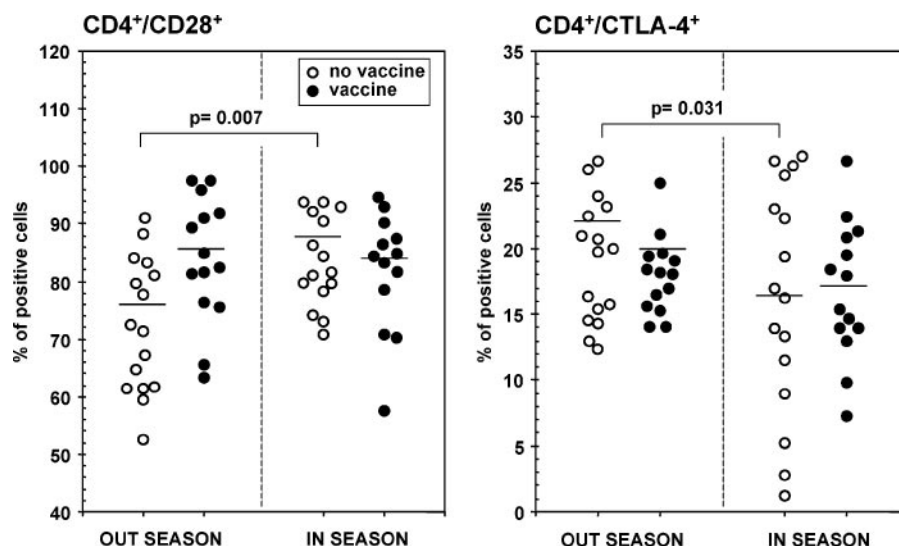


FIGURE 3. Percentages of CD4⁺/CD28⁺ (left panels) and of CD4⁺/CTLA-4⁺ (right panels) lymphocytes in patients who had (●) or had not (○) undergone allergen-specific IT. Results obtained in the same individuals in and out of the pollination season are shown. The horizontal bars indicated median values. Statistical significance is shown.



performed before the initiation of symptomatic therapy. Clinical differences between group A and group B during the 2005 birch pollen season are shown in Table II. The efficacy of IT in group B was evaluated by a significant improvement of a 7-point visual analog scale applied to rhinitis, asthma, and conjunctivitis and need for therapy, according the guidelines of symptom severity assessment of allergic rhinitis (29). The numbers of CD4⁺, CD8⁺, CD14⁺, and CD19⁺ cells was comparable among both groups of patients at both time points.

Lymphocyte proliferation

T cell proliferation in response to Bet-v1 and CMV Ag was analyzed outside of the pollen season or at the peak of pollination in allergic patients who were only receiving symptomatic therapy (group A), or were undergoing allergen specific IT (group B). Bet-v1-specific T cell proliferation was comparable in the two groups of patients but was significantly reduced in unvaccinated individuals during pollination (in season vs out of season: $p = 0.023$)

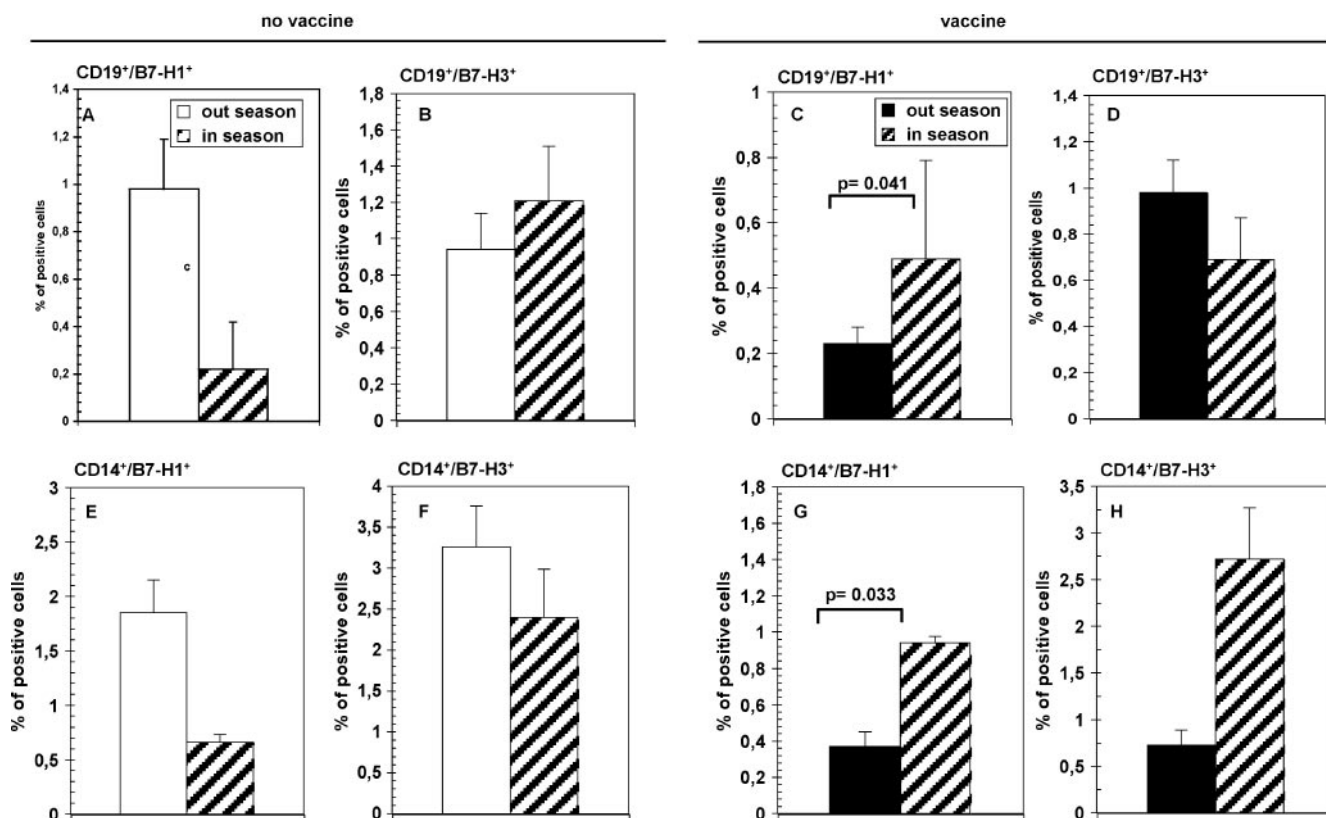


FIGURE 4. In vitro Bet-v1-induced modulation of costimulatory molecules on CD19⁺ and CD14⁺ cells in patients that had or had not undergone allergen specific IT. Upper panels, CD19 lymphocytes expressing B7-H1 (A and C) or B7-H3 (B and D). Lower panels, CD14 cells expressing B7-H1 (E and G) or B7-H3 (F and H). Median results obtained in the same individuals in (▨) and out (□ and ■) of the pollination season are shown. SDs and statistical significance are also shown.

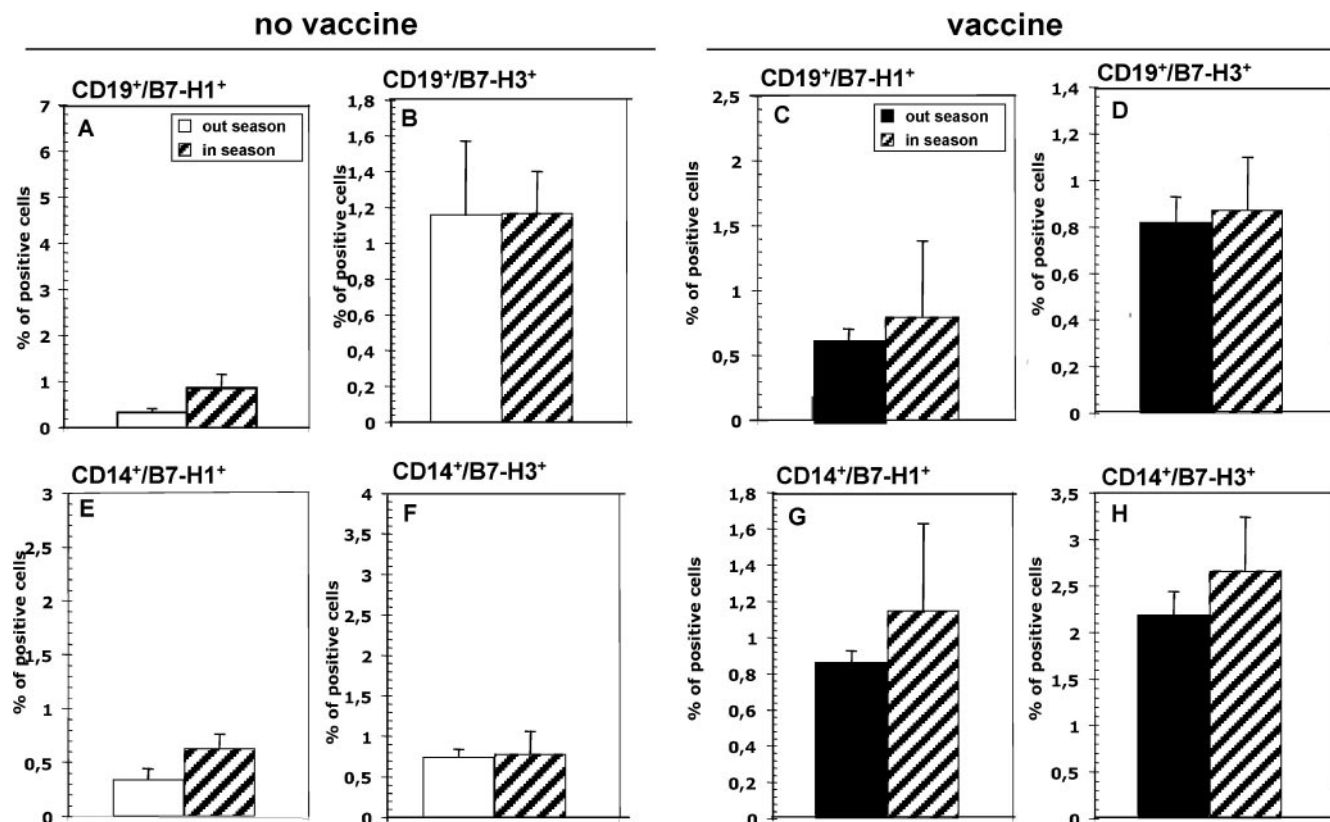


FIGURE 5. In vitro CMV-induced modulation of stimulatory molecules on CD19⁺ and CD14⁺ cells in patients who had or had not undergone allergen-specific IT. Upper panels, CD19 lymphocytes expressing B7-H1 (A and C) or B7-H3 (B and D). Lower panels, CD14 cells expressing B7-H1 (E and G) or B7-H3 (F and H). Median results obtained in the same individuals in (≤) and out (□ and ■) of the pollination season are shown. SDs and statistical significance are also shown.

(Fig. 2). In contrast, CMV-specific responses were reduced in both groups of patients during pollination (in season vs out of season: unvaccinated patients: $p = 0.021$, vaccinated individuals: $p = 0.034$).

Phenotypic analysis: CD28 and CTLA-4 expression on T lymphocytes

CD28 is mainly an activating costimulatory molecule while CTLA-4 is an inhibitory costimulatory molecule. Activation markers (CD28 and CTLA-4) were evaluated on CD4⁺ T lymphocytes of all patients. CD28-expressing CD4⁺ T lymphocytes were in-

creased ($p = 0.007$), whereas CTLA-4-expressing CD4⁺ T cells were reduced ($p = 0.031$) in unvaccinated patients during pollination (Fig. 3). In contrast, both lymphocyte subpopulations were similar over time in vaccinated individuals. These results indicate that a tendency favoring T cell activation is present in unvaccinated individuals during pollination.

Phenotypic analysis: B7 family molecules expression on APC

CD19⁺/CD80⁺ ($p = 0.042$), CD19⁺/CD86⁺ ($p = 0.006$), and CD14⁺/CD80⁺ cells were increased ($p < 0.001$) whereas

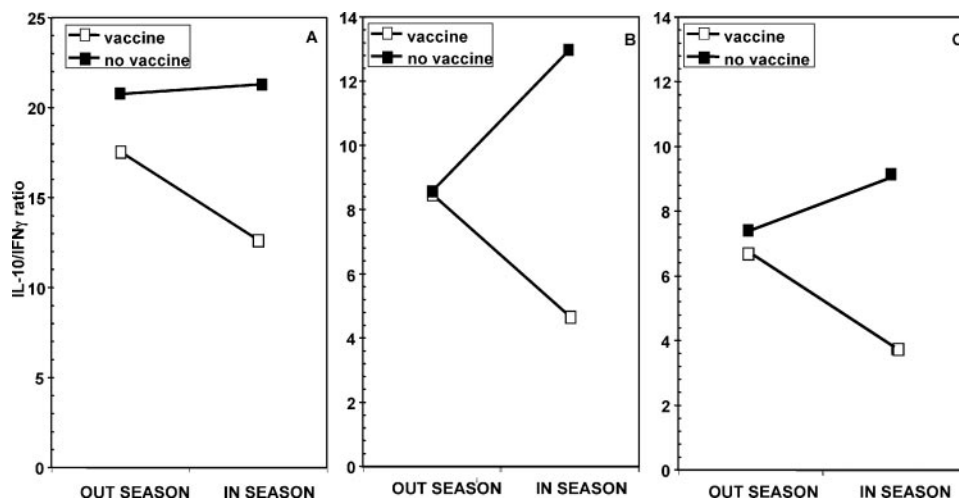


FIGURE 6. Bet-v1-specific IL-10/IFN- γ ratio in CD4⁺ (A), CD14⁺ (B), and CD19⁺ (C) cells of patients that had (□) or had not (■) undergone allergen specific IT. Median results obtained in and out of the pollination season are shown.

CD14⁺/CD86⁺ cells ($p = 0.012$) were diminished during pollination in unvaccinated patients. No significant modification was detected in vaccinated patients, suggesting that vaccination is associated with a stabilization of these populations of APC (data not shown).

Allergen-induced modulation of phenotypic markers

PBMC from all patients were incubated with Bet-v1 to evaluate the specific allergen-induced modulation of costimulatory molecules on B cells and monocytes. Results showed that incubation with Bet-v1 modifies the percentage of CD14⁺/CD86⁺ cells in samples collected during pollination. Thus, similar to results obtained *ex vivo*, CD14⁺/CD86⁺ cells were diminished ($p = 0.022$) in vaccinated and augmented ($p = 0.047$) in unvaccinated patients (data not shown).

Changes observed in B7-H1 and B7-H3 expression were more dramatic. During pollination, in vaccinated individuals CD19⁺/B7-H1⁺ ($p = 0.041$), CD14⁺/B7-H1⁺ ($p = 0.033$), and CD14⁺/B7-H3⁺ cells augmented whereas CD19⁺/B7-H3⁺ cells were reduced. These patterns mirrored those observed in unvaccinated individuals (Fig. 4) and were specific for Bet-v1 stimulation as these populations were not modified by incubation of PBMC with CMV (Fig. 5).

Allergen-stimulated cytokine synthesis

B7-H1-expressing cells preferentially produce IL-10; data presented herein show that B7-H1-expressing APC augment in vaccinated individuals during pollination. To verify whether these changes would be reflected in cytokine production, we analyzed the percentages of B7-H1-expressing and IL-10-producing CD14⁺ and CD19⁺ Bet-v1-stimulated cells. Results showed that CD14⁺/B7-H1⁺/IL-10⁺ as well as CD19⁺/B7-H1⁺/IL-10⁺ cells were increased (>2.5-fold) in vaccinated patients during pollination. As a result, during pollination, the IL-10/IFN- γ ratio in CD4⁺, CD14⁺, and CD19⁺ cells increased in vaccinated patients but decreased secondarily to a reduction in IL-10 production in unvaccinated individuals (Fig. 6). Again, changes in cytokine production were specific for Bet-v1 stimulation as none of these populations were modified by incubation of PBMC with CMV (data not shown). These data suggest that one of the main biological effects of specific IT is to up-regulate an APC-dependent and IL-10-mediated loop.

Discussion

In this study, we evaluated the biological effects of allergen-specific IT on Ag presentation and T lymphocyte stimulation. Thus, we analyzed the expression of CD28, CTLA-4, as well as B7 and B7-H molecules on immune cells in patients with allergy to *Betulaeae* that had or had not undergone specific IT. These parameters were examined twice in the same individuals: in and out of the pollination period. Results shown herein demonstrate that IT has a stabilizing effect on the expression of costimulatory molecules and elicits an APC-dependent and IL-10-mediated loop at least partially responsible for disease control during pollination.

Proliferation to both Bet-v1 and an unrelated Ag (CMV) was reduced during pollination in unvaccinated patients, but was only slightly modified in vaccinated individuals. These data could be explained by the prevalence, in unvaccinated individuals, of Th2 clones of Th lymphocytes, clones that are characterized by a limited proliferative ability (30). The observation that successful IT is associated with a modulation of the Th2/Th1 imbalance, such that Th1 are favored, would justify the observation that proliferation is retained in vaccinated individuals. CD28 and CTLA-4 molecules are expressed on T lymphocytes, bind the B7 receptors (CD80 and CD86) and elicit a stimulatory or an inhibitory effect on T cell

activation, respectively. CD28 is constitutively expressed on cell surfaces whereas CTLA-4 is only present on activated lymphocytes. The reciprocal balance of CD28 and CTLA-4 is pivotal in modulating T cell responses. Expression of these markers on CD4⁺ T lymphocytes was comparable both out and in season in vaccinated individuals, suggesting that one of the effects of IT is to stabilize the reciprocal balance of these costimulatory molecules. In contrast with these results, CD4⁺/CD28⁺ cells were augmented and CD4⁺/CTLA-4⁺ lymphocytes were reduced during pollination in unvaccinated individuals, indicating that the immune response is less prone to suppression in these patients during pollination. These data are apparently in disagreement with the observed loss of Ag-specific proliferation detected in unvaccinated individuals during pollination. Ligation of CTLA-4 inhibits Ag-specific Th2 lymphocytes clones. Thus, the reduced proliferative ability detected in unvaccinated subjects could be explained by an increased activity of the Th2 Bet-v1-specific clones, which are endowed with a minimal proliferative capacity, and would avoid CTLA-4-mediated suppression (8).

To complete this part of the study, we analyzed B7.1 (CD80) and B7.2 (CD86)-expressing monocytes (CD14) and B lymphocytes (CD19) in all patients. The expression of these molecules was comparable out and in season in vaccinated individuals, confirming that IT stabilizes the CD28/CTLA-4 $\approx$ CD80/CD86 T lymphocyte immunomodulatory circuit. In contrast, results obtained in unvaccinated individuals showed that CD19⁺/CD80⁺ and CD14⁺/CD80⁺, as well as CD19⁺/CD86⁺ cells are significantly increased during pollination. These results indicate that an increased susceptibility of the immune system to activation is present in unvaccinated patients during pollination (31, 32); specific IT down-modulates this activation loop.

B7-H1 and B7-H3 are additional co stimulatory molecules that have recently been identified; ligation of B7-H1-expressing APCs results in the preferential production of IL-10, whereas triggering of B7-H3-expressing APC is associated with IFN- γ production. To evaluate changes in Ag-specific responses, we incubated PBMC of all individuals with Bet-v1, the main *Betulaeae* Ag, or a control Ag. Results showed that both CD14⁺/B7-H1⁺ and CD19/B7-H1 cells were increased in vaccinated individuals irrespective of pollination. Interestingly, whereas these cells diminished in unvaccinated subjects during pollination, they showed a further increase in patients undergoing allergen-specific IT. Thus, both types of B7-H1-expressing APC increased only in vaccinated patient whereas these cell types decreased in unvaccinated individuals. Data obtained by analyzing B7-H3-expressing cells were less univocal and a clear pattern could not be identified. This outcome, which needs to be further analyzed, could be a result of the weak pollinosis season that characterized the spring of 2005 in Lombardy.

The results shown herein suggest that the beneficial effect of allergen-specific IT is at least partially secondary to the increase of B7-H1-expressing cells. These data confirm and expand those recently reported by Kim et al. (33) who showed a pivotal role for B7-H1 in the modulation of delayed-type contact hypersensitivity response in primed animals. Thus, in this model: 1) blockade of B7-H1 enhanced the activity of hapten-specific T cells; 2) interaction with a dendritic cell line that expresses high cell surface levels of B7-H1 suppressed the proliferation of activated T cells; and 3) *in vivo* administration of hapten-carrying B7-H1/DC desensitized the response of sensitized animals to hapten challenge.

Ligation of B7-H1 results in IL-10 production. CD14⁺ and CD19, B7-H1⁺ and B7-H1⁺/IL-10⁺ cells were decreased during pollination in unvaccinated patients and, as a likely consequence, the Ag-specific IL-10/IFN- γ ratio was diminished in these patients

during pollination. The observation that modifications of the IL-10-IFN- γ ratio were supported by decreases in IL-10 production, rather than by changes in the generation of IFN- γ suggests that one of the main biological effects of specific IT could be to up-regulate an APC-dependent and IL-10-mediated loop. This hypothesis is further strengthened by the fact that the Ag-specific IL-10-IFN- γ ratio was increased in vaccinated patients, in whom B7-H1-expressing monocytes and B lymphocytes were augmented as well, and clinical symptoms were greatly reduced. Interestingly, it has been shown that IL-10 production is increased in patients undergoing allergen-specific IT; this increase is suggested to be responsible for the beneficial effects of therapy (34–36).

In conclusion, IT in allergic patients results in a stabilization of costimulatory molecules and is associated with a novel immune mechanism potentially resulting in disease control. This mechanism is independent of a Th2/Th1 shift but rather relies on the stimulation of an APC-associated and IL-10-mediated loop that modulates allergic inflammation. The observation that specific IT influences the expression of costimulatory molecules is novel; the fact that B7-H1 seems to be the molecule mostly affected by specific IT suggests that this marker could be used to monitor the biological effects of therapy. B7-H1-directed therapeutic strategies could also be envisioned as a way to design immunosuppressive approaches to allergy and other inflammatory diseases.

Disclosures

The authors have no financial conflict of interest.

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