A Randomized Controlled Trial to Evaluate Inhibition of T-Cell Costimulation in Allergen-induced Airway Inflammation

Amit D. Parulekar[1]*†, Jonathan S. Boomer[1]*, Brenda M. Patterson[1], Huiqing Yin-Declue[1], Christine M. Deppong[1], Brad S. Wilson[2], Nizar N. Jarjour[3], Mario Castro[1], and Jonathan M. Green[1]

[1]Department of Internal Medicine and [2]Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, Missouri; and [3]Department of Internal Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin

***Rationale*: T lymphocytes are important in the pathogenesis of allergic asthma. Costimulation through CD28 is critical for optimal activation of T cells, and inhibition of this pathway with CTLA4Ig has been shown to be effective in preventing airway inflammation and hyperresponsiveness in animal models of asthma. Abatacept, a humanized version of CTLA4Ig, has been approved for treatment of rheumatoid arthritis, providing the opportunity to test whether inhibition of costimulation is an effective strategy to treat people with asthma.**

***Objectives*: To determine if 3 months of treatment with abatacept reduced allergen-induced airway inflammation in people with mild atopic asthma.**

***Methods*: Randomized, placebo-controlled, double-blinded study. Bronchoscopically directed segmental allergen challenge was performed on 24 subjects followed by bronchoalveolar lavage 48 hours later. Subjects were randomized 1:1 to receive abatacept or placebo, followed by a second allergen challenge protocol after 3 months of study drug.**

***Measurements and Main Results*: There was no significant reduction in allergen-induced eosinophilic inflammation in the abatacept-treated group compared with placebo (17.71% ± 17.25% vs. 46.39% ± 29.21%; $P = 0.26$). In addition, we did not detect an effect of abatacept on FEV_1, provocative concentration of methacholine sufficient to induce a 20% decline in FEV_1, or asthma symptoms. Subjects treated with abatacept had an increased percentage of naive and a corresponding decrease in memory $CD4^+$ T cells in the blood compared with placebo.**

***Conclusions*: Inhibition of CD28-mediated costimulation with abatacept does not seem to alter the inflammatory response to segmental allergen challenge or clinical measures of asthma symptoms in people with mild atopic asthma.**

Clinical trial registered with ClinicalTrials.gov (NCT 00784459).

Keywords: asthma; allergic inflammation; T lymphocyte

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Inhibition of CD28-mediated costimulation has been shown to be effective at preventing airway inflammation and hyperresponsiveness in animal models. However, the effectiveness of such a strategy in humans is unknown.

What This Study Adds to the Field

This study in humans with asthma tested the hypothesis that blockade of costimulation would diminish allergen-induced airway inflammation. This study demonstrated that treatment of subjects with mild atopic asthma with abatacept, an inhibitor of CD28-mediated costimulation, had no effect on inflammation induced by segmental allergen challenge, or on clinical measures of asthma symptoms or severity.

Asthma and other allergic diseases are mediated by a complex inflammatory response in which the innate and adaptive immune systems have important roles (1, 2). Exposure to allergen in subjects who are atopic results in IgE-mediated activation of mast cells and basophils with the release of soluble mediators that lead to many of the acute manifestations of allergic disease (3). However, although this may initiate the allergic cascade, these events alone are not sufficient to account for the chronic inflammation characteristic of asthma.

Depending on the combination of signals received, T cells may differentiate down one of several effector lineages. In the presence of IL-4, T cells develop along the Th2 lineage, which are central to allergic inflammation and are capable of secreting IL-4, IL-5, IL-9, and IL-13 (4). These cytokines orchestrate the Th2 inflammatory response, which is characterized by the recruitment of eosinophils. Although the presence and contribution of Th2 cells to airway disease in asthma is well established (5, 6), clinical trials testing agents that block IL-4, IL-5, or IL-13 have had mixed results (reviewed in [7]).

T cells require an ordered series of signals to be fully activated and develop effector function. Binding of the T-cell receptor (TCR) to the antigen–major histocompatibility complex expressed on the surface of an antigen-presenting cell delivers a required but insufficient signal to fully activate the T cell (8). Additional receptors, termed "costimulatory receptors," deliver essential signals that result

(*Received in original form July 12, 2012; accepted in final form December 20, 2012*)

* A.D.P. and J.S.B. contributed equally to this work and should be considered co–first authors.

† Present address: Department of Internal Medicine, Baylor College of Medicine, Houston, TX 77030.

Supported by an investigator-initiated grant awarded by Bristol-Myers Squibb Corporation.

Author Contributions: A.D.P. enrolled subjects, performed segmental allergen challenge protocols, analyzed specimens and data. J.S.B. developed and performed the research assays. H.Y.-D. and C.M.D. performed research assays and analyzed data. B.M.P. was the lead research coordinator and was involved in all aspects involving participants. B.S.W. performed the statistical analysis. N.N.J. was the lead investigator at University of Wisconsin. M.C. and J.M.G. collaboratively designed the study and provided oversight over the conduct of the entire study. All authors reviewed and contributed to the writing of the manuscript.

Correspondence and requests for reprints should be addressed to Jonathan M. Green, M.D., Pulmonary and Critical Care Medicine, Washington University School of Medicine, 660 S. Euclid Avenue, Box 8052, St. Louis, MO 63110. E-mail: jgreen@wustl.edu

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 187, Iss. 5, pp 494–501, Mar 1, 2013
Copyright © 2013 by the American Thoracic Society
Originally Published in Press as DOI: 10.1164/rccm.201207-1205OC on January 4, 2013
Internet address: www.atsjournals.org

in clonal expansion, augmented cytokine secretion, and enhanced cell survival (9). The most critical of these is CD28, which when engaged by its ligands (CD80 or CD86) concomitant with TCR signaling is sufficient to fully activate a resting naive T cell (10). Conversely, inhibition of CD28 prevents T-cell activation *in vitro* and *in vivo* (11, 12).

CD28 has been shown to be essential to the development of allergic airway inflammation in a number of preclinical models (13–15). Mice deficient in CD28 fail to develop airway inflammation hyperresponsiveness (16, 17). In addition, treatment of mice with CTLA4Ig, a soluble inhibitor that interferes with the binding of CD28 to CD80/CD86, also prevented *in vivo* responses to inhaled allergen (18). Interestingly, CTLA4Ig was effective even if administered only at the time of inhaled challenge and not at sensitization, suggesting a mechanism beyond just prevention of T-cell priming (19). Furthermore, e*x vivo* treatment of bronchial biopsies obtained from atopic people with asthma with CTLA4Ig reduced allergen stimulated secretion of chemokines important in the recruitment of T cells to the lung (20).

Given the preclinical data demonstrating the importance of CD28-mediated costimulation in allergic airway inflammation, we designed a pilot study to test the efficacy of manipulating this pathway in patients with atopic asthma. This is the first trial reported of which we are aware that directly tests whether blockade of costimulation in humans might be an effective therapy for asthma. Mild atopic people with asthma underwent a segmental allergen challenge (SAC) to determine the baseline inflammatory response. Participants were then treated with the humanized version of CTLA4Ig (abatacept) to block CD28 costimulation or placebo in a randomized, double-blinded fashion for 3 months. This drug has been approved for the treatment of rheumatoid arthritis in adults and juvenile idiopathic arthritis in children (21). After 3 months of treatment participants underwent a second SAC to determine if their baseline inflammatory response was altered. The primary endpoint was recruitment of eosinophils to the lung after allergen challenge. In contrast to data from animal models, our data demonstrate that treatment with abatacept had no effect on allergen-induced airway inflammation.

METHODS

Patients

Nonsmoking males and females between 18 and 50 years of age with previously diagnosed mild asthma were enrolled (22, 23). Key inclusion criteria included an FEV_1% of greater than or equal to 70; a provocative concentration of methacholine sufficient to induce a 20% decline in FEV_1 (PC_{20}) of less than or equal to 8 mg/ml ($\leq$16 mg/ml if taking inhaled corticosteroids); a history of atopic symptoms; and a positive skin prick test to cat, ragweed, or dust mite allergen. Individuals were excluded if they had any diagnosed lung disease other than allergic asthma, evidence of an upper or lower respiratory tract infection, or chronic use of oral or inhaled corticosteroids at a dose greater than 440 μg/day of fluticasone. Full inclusion and exclusion criteria can be found in the METHODS section in the online supplement.

Study Design

The study was a randomized, double-blind, placebo-controlled parallel group trial (Figure 1A). After screening, eligible patients underwent bronchoscopy with SAC to determine the baseline inflammatory response to instilled allergen. Those that increased the percentage of eosinophils recovered in bronchoalveolar lavage (BAL) fluid by at least 50% 48 hours after allergen challenge were eligible for randomization. Randomization was performed in a 1:1 ratio by the research pharmacist who prepared the study medications but otherwise had no involvement in the trial. Participants received abatacept (Orencia, Bristol-Myers Squibb, New York, NY), 10 mg/kg (intravenously), or placebo for 3 months administered at Days 0, 14, 28, 56, and 84. The dose administered was chosen because it is the approved dose for treatment of rheumatoid arthritis. A second SAC was performed on Day 98 using the same lot and dose of allergen as had been used during the initial SAC. Participants were followed until Day 154. All participants were enrolled at University of Wisconsin, Madison (five participants) or Washington University in St. Louis (19 participants). Institutional review board approval was obtained at each site and each participant provided written informed consent.

Outcome Measures and Study Endpoints

The primary endpoint was the change in recovery of eosinophils in BAL fluid in response to allergen challenge. Secondary endpoints included changes in total cells recovered into BAL fluid, FEV_1, PC_{20}, changes in asthma control scores as determined by the Asthma Control Questionnaire, changes in exhaled nitric oxide, and changes in serum IgE levels. Exploratory endpoints included changes in specific subsets of inflammatory cells recruited to the lung after allergen challenge as determined by flow cytometry; changes in the cytokine profile of cells isolated from BAL fluid after allergen challenge; and changes in peripheral blood T cells as determined by flow cytometry, proliferation assays, and stimulated cytokine secretion.

Titrated Skin Prick Testing and SAC Protocol

Skin prick testing of cat allergen extract, short ragweed allergen extract (*Ambrosia artemisiifolia*), and standardized dust mite allergen extracts (*Dermatophagoides farinae* or *Dermatophagoides pteronyssinus*) were performed at screening (all glycerinated stocks from Greer Laboratories, Lenoir, NC) and reactivity determined using standard methods (24–26). Titrated skin prick testing was performed using the allergen that induced the strongest positive result to skin prick testing. The most concentrated dilution administered during the titration was a 1:100,000 dilution of the stock allergen. A full description of the titrated skin prick protocol is provided in the online supplement.

Bronchoscopically directed SAC was performed using a modification to the protocol described by Jarjour and coworkers (27) as described in detail in the online supplement. The most dilute solution of allergen that yielded a positive skin test reaction was used to calculate the dose for SAC. A 5-ml solution of the final allergen dilution was instilled bronchoscopically as fully described in the online supplement. At the start of the study, participants were administered a SAC dose 100 times greater than the minimum concentration that provoked a positive result in the titrated skin test. After the first three subjects were enrolled, we modified the protocol to increase the dose to 1,000 times the minimum reactive skin test dose, with a maximum dose being a 1:100 dilution of the stock allergen. This modification was made because the first three subjects had limited eosinophil responses (<10%) in response to the SAC. In all cases, the second SAC was performed at the same allergen dose as the first SAC. All subjects were included in the final data analysis.

Measurement of Lung Function

Spirometry was performed at each visit using standard methods (28). Methacholine bronchoprovocation challenge testing was performed as previously described and the dose required to induce a 20% decrease in FEV_1 (PC_{20}) determined by inhalation of increasing doses of methacholine (0.03125–16 mg/ml) (29).

Analysis of Blood and BAL Fluid

BAL fluid obtained at each bronchoscopy (four bronchoscopies per participant) was analyzed for total cell counts and cell subsets as described briefly below and in detail in the online supplement. Manual differentials were performed on cytospin preparations and immune cell subsets determined by flow cytometry. Peripheral blood was collected before each SAC protocol. Whole blood and cells recovered from BAL fluid were incubated with antibodies directed against the immune cell subsets as indicated in the result tables and described in detail in the online supplement. The markers used to define each cell type are detailed in Table E7 in the online supplement. The cells were then analyzed on a four-color FACSCalibur flow cytometer using CellQuest software (Becton-Dickinson Corporation, Mountainview, CA). Data were further analyzed using Winlist v7 software (Verity Software Corporation, Topsham, ME). Cytokine levels

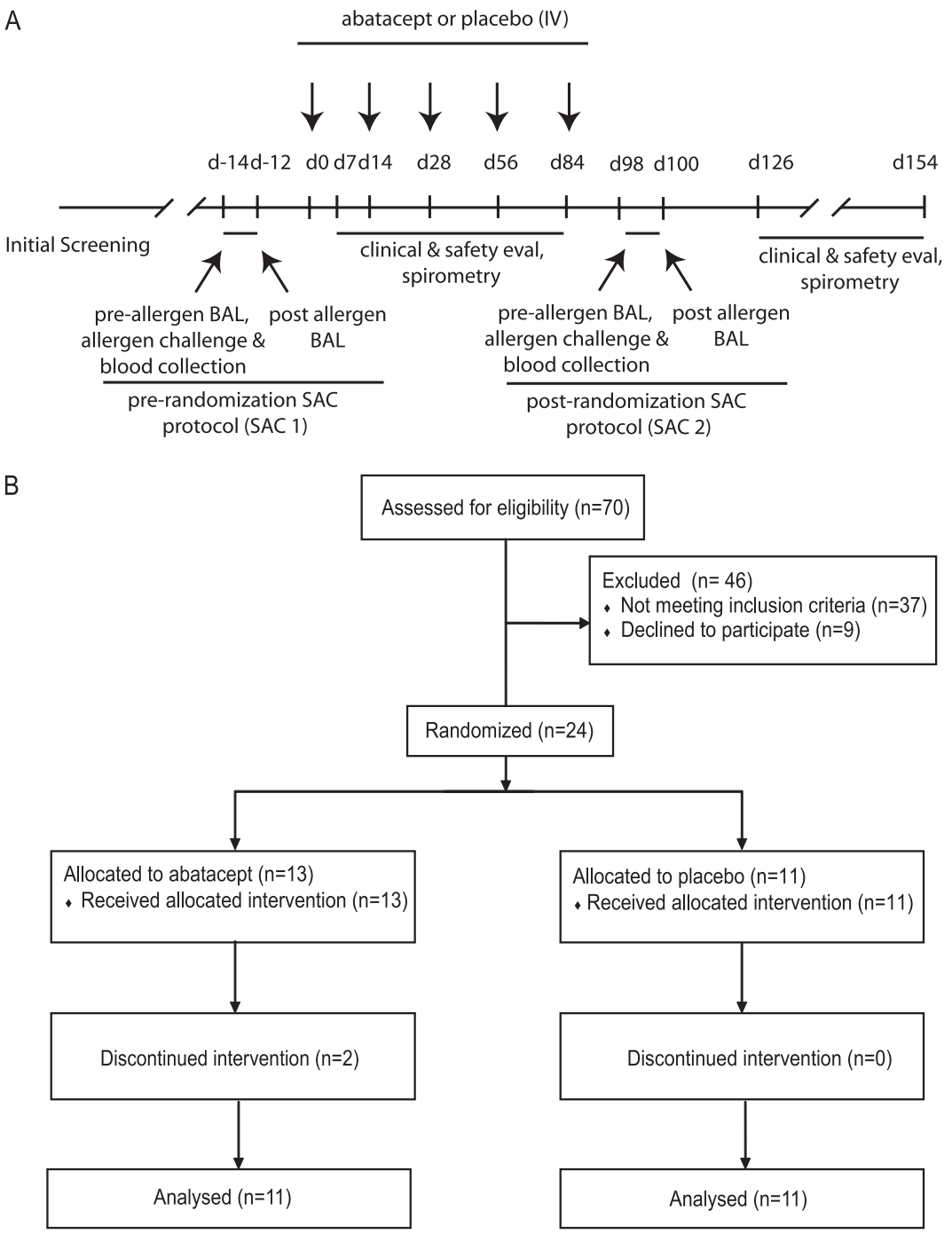

Figure 1. (*A*) Overview of study design. (*B*) Disposition of participants evaluated for the study. BAL = bronchoalveolar lavage; IV = intravenous; SAC = segmental allergen challenge.

were determined on serum, concentrated BAL fluid, and stimulated culture supernatants by ELISA and T-cell proliferation was determined by tritiated thymidine incorporation as described in the online supplement.

Statistical Analysis

All data were analyzed using SAS version 9.3 (SAS Institute, Cary, NC). Continuous outcomes were presented as the mean ± standard deviation. The comparison of baseline values between placebo and abatacept was done using a two-group *t* test for continuous outcomes and a chi-square test for categorical outcomes. Comparison of prerandomization, end of treatment intervention, and change from prerandomization to end of treatment values between placebo and abatacept was done using two-group *t* tests.

The primary analysis was the change between prerandomization and postrandomization BAL fluid eosinophilia. This was done using an analysis of covariance, where the postrandomization outcome was compared between placebo and abatacept groups after adjusting for the prerandomization outcome. A Wilcoxon nonparametric two-group analysis was performed on the differences between prerandomization and postrandomization BAL fluid cytokine level outcomes of IL-4, IL-10, IL-17A, and tumor necrosis factor-α.

Secondary analyses were done for BAL % eosinophils and total cells. For % eosinophils, analyses were performed to compare the level of change in pre- to post-SAC values between placebo and abatacept. This was done using an analysis of covariance, where the post-SAC outcome was compared between placebo and abatacept groups after adjusting for the preallergen BAL % eosinophil. This analysis was done for both SAC1 and SAC2 separately. For total BAL cells, matched pair *t* tests were done to test for change between SAC1 and SAC2 for placebo and abatacept groups separately. The same approach was used to test for change in total BAL cells between preallergen and postallergen outcomes within each SAC and treatment group separately.

A two-tailed *P* value less than 0.05 was considered significant.

RESULTS

A total of 70 participants were screened with 24 randomized; two withdrew after the first infusion of study medication because of an anaphylactoid reaction, both of whom were randomized to abatacept (Figure 1B). The remaining 22 participants, 11 in each arm, completed the entire study protocol. Overall, there were no significant differences in the baseline characteristics of the participants (Table 1). The results of the titrated skin testing for participants randomized to abatacept or placebo are shown in Table E8. These are the concentrations used to determine the allergen challenge dose used for the SAC protocol.

Primary Outcome

The prespecified primary endpoint was the change in percentage of eosinophils recovered from BAL fluid after allergen challenge between the abatacept and placebo groups. We hypothesized that treatment with abatacept would blunt the response to allergen resulting in a decrease in eosinophils recovered in BAL fluid. However, there was no significant difference in the recovery of eosinophils in BAL fluid in participants treated with abatacept compared with placebo (change in eosinophils post-SAC: 17.53% ± 17.27% vs. 44.84% ± 28.16%; $P = 0.26$) (Figure 2). When assessed using the change in the absolute number of eosinophils recovered instead of percent eosinophils, no difference in response was observed ($P = 0.32$). The analysis remained not significant when adjusted for race, age, sex, or allergen. Overall, participants randomized to abatacept had a less robust inflammatory response, as determined by total cell counts and cell differential analysis of the postallergen BAL fluid (Figure 3; *see* Figure E1). However, this diminished response was apparent before and after randomization, and when adjusted for the prerandomization response to allergen challenge, the post randomization inflammatory response to allergen did not differ between treatment arms. In addition, there was no difference in the cellular composition of the preallergen BAL fluid sample between abatacept- or placebo-treated participants at either SAC1 or SAC2 time points (Figure 3; *see* Figure E3 and data not shown). Thus, our data suggest that abatacept does not alter the inflammatory response in the lung to SAC.

Secondary Outcomes

We assessed a number of clinical endpoints to determine if treatment with abatacept affected lung function or asthma symptoms (Table 2). There was no difference in FEV_1 at baseline, nor did treatment with abatacept change the FEV_1 when compared with placebo ($P = 0.12$). To assess airway hyperresponsiveness, methacholine challenge tests were performed before initiation of study drug and again before the second SAC protocol. Although participants randomized to abatacept were less reactive at baseline, as determined by PC_{20}, compared with those randomized to placebo, this difference was not statistically significant ($P = 0.19$). Treatment with abatacept did not alter the PC_{20}, suggesting no effect on airway hyperresponsiveness. We also assessed asthma control using the Asthma Control Questionnaire. Both study arms were similar at baseline and did not change over the course of the trial. No difference in serum IgE or exhaled nitric oxide between groups or in response to treatment with abatacept was observed (data not shown). Thus, abatacept did not significantly affect asthma symptoms or indicators of control airway inflammation.

Safety

The study protocol was well tolerated with six adverse events reported in those receiving abatacept and 21 in those receiving placebo (*see* Table E1). Among those receiving abatacept two participants experienced an anaphylactoid reaction during the first infusion of study drug, prompting a change in the protocol to premedicate all participants with cetirizine and acetaminophen before study drug administration. No further reactions were observed. According to the investigators brochure for abatacept, acute infusion-related reactions occur between 0.1% and 1% of patients, with anaphylactoid reactions occurring less frequently. Premedication is not routinely used in clinical practice, raising the possibility that in individuals who are atopic, there may be a higher incidence of infusion reactions. However, our study was not designed to detect this. The remaining adverse events were mild in severity and occurred primarily in the placebo group. Many of the adverse events that were reported are common in an asthmatic population, and although they occurred mostly in the placebo group, are of such low number that no conclusions can be drawn based on these data.

TABLE 1. BASELINE CHARACTERISTICS OF PARTICIPANTS ENROLLED INTO THE TRIAL

	Abatacept ($n = 13$)	Placebo ($n = 11$)	*P* Value
Age (range)	35 ± 9.8 (20–47)	28 ± 7.8 (19–42)	0.08
Sex			0.34
Female	7 (54%)	8 (73%)	
Male	6 (46%)	3 (27%)	
Race			0.13
White	8 (62%)	5 (45%)	
African American	5 (38%)	3 (28%)	
Mixed	0	3 (27%)	
Inhaled corticosteroids (number receiving)	3	2	0.77
FEV_1, L	3.19 ± 0.92	3.05 ± 0.81	0.71
FEV_1, % predicted	89.1 ± 12.3	87.4 ± 14.9	0.81
PC_{20}, mg/ml	5.00 ± 10.52	1.46 ± 2.12	0.29
IgE, mg/ml	313.2 ± 339	312 ± 302	0.79
Allergen			0.20
Cat	7 (54%)	3 (27%)	
Ragweed	6 (46%)	5 (45%)	
Dermatophagoides farina	0	2 (18%)	
Dermatophagoides pternyssinus	0	1 (9%)	

Definition of abbreviations: PC_{20} = provocative concentration of methacholine sufficient to induce a 20% decline in FEV_1. For FEV_1, PC_{20}, and IgE, the data are presented as mean ± standard deviation.

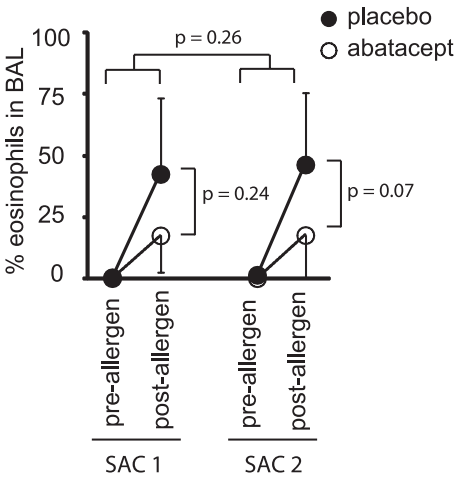

Figure 2. The percentage of eosinophils recovered in the bronchoalveolar lavage (BAL) fluid from bronchoscopy performed before and after challenge at the start of the study (before randomization) and again at the end of the intervention period (after randomization). Values shown are the mean ± standard deviation, although for clarity of presentation, the overlapping segments of the error bars have been omitted from the figure. SAC = segmental allergen challenge.

Exploratory Outcomes

To determine if abatacept altered the nature of the inflammatory response to SAC, we performed a comprehensive analysis of the BAL fluid, analyzing cellular composition by flow cytometry (*see* Table E2) and cytokine content by ELISA (*see* Table E3). Overall, the change in most cell subsets was similar between abatacept- and placebo-treated participants (*see* Table E3). Although some of the differences may be statistically significant, the magnitude of the difference between abatacept and placebo are relatively small and confounded by baseline differences observed for the placebo arm. In addition, although we detected higher levels of IL-2, IL-10, and IL-6 in the BAL fluid of the placebo-treated group, these are driven by a few outliers. Thus, these data are unlikely to indicate a meaningful difference between groups. Therefore, we conclude that abatacept had little biologic effect on the local inflammatory response to allergen challenge.

We assessed whether treatment with abatacept affected the percentages of specific immune cell subsets in the peripheral blood (Table 3). Blood was collected at two time points, before allergen challenge at the start of the study treatment period (SAC1) and again at the end (SAC2). The objective of this sample collection and analysis was to determine the effect of abatacept on the circulating immune cell profile. After treatment with abatacept, there was an increase in naive $CD4^+$ T cells in the blood accompanied by a decrease in memory $CD4^+$ T cells. No change was observed in placebo-treated participants,

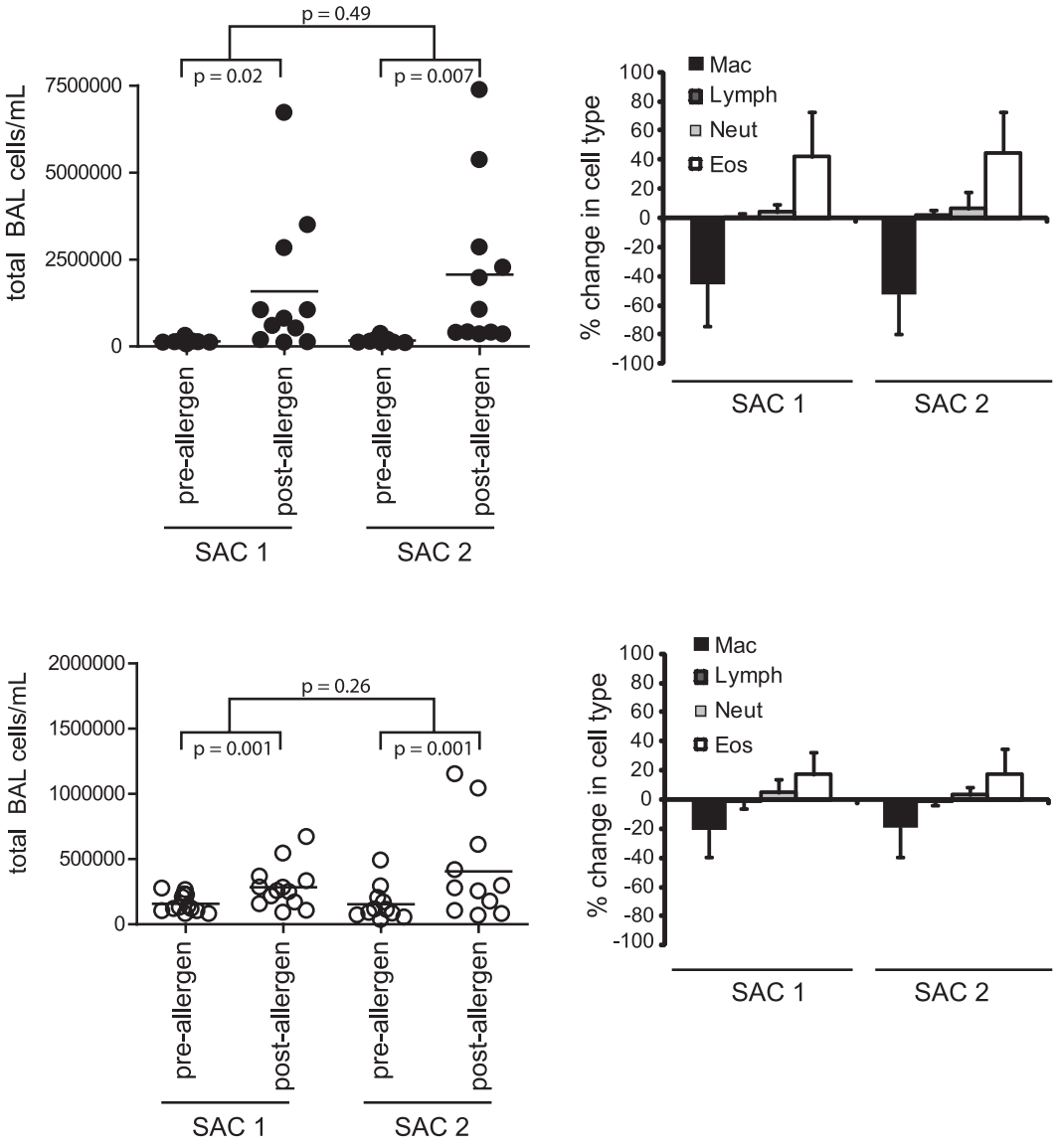

Figure 3. Bronchoalveolar lavage (BAL) cell counts and cellular differential analysis from participants randomized to either placebo (*top*) or abatacept (*bottom*) for the preintervention and postintervention segmental allergen challenge (SAC). (*Left*) Total number of cells recovered in the BAL. (*Right*) Change in the percentage of each cell type recovered in the BAL after allergen challenge, calculated by subtracting the preallergen value from the postallergen value (postallergen % cell type − preallergen % cell type). *P* values were calculated by matched paired *t* test.

TABLE 2. CLINICAL ENDPOINT DATA

	Abatacept ($n = 11$)	Placebo ($n = 11$)	*P* Value
FEV_1, L			
Before randomization (V4)	3.01 ± 0.84	3.03 ± 0.86	0.97
End of treatment intervention (V10)	2.89 ± 0.83	2.99 ± 0.88	0.79
Change (V10 − V4)	−0.12 ± 0.17	−0.04 ± 0.27	0.40
FEV_1, % predicted			
Before randomization (V4)	85.7 ± 14.9	85.5 ± 10.5	0.97
End of treatment intervention (V10)	82.0 ± 14.6	85.5 ± 9.60	0.51
Change (V10 − V4)	−3.70 ± 5.0	0 ± 5.60	0.12
PC_{20}, mg/ml			
Before randomization (V4)	5.87 ± 11.41	1.46 ± 2.12	0.19
End of treatment (V9)	7.06 ± 12.33	1.45 ± 1.80	0.17
Change (V9 − V4)	0.65 ± 4.9	0.09 ± 1.89	0.92
Asthma Control Questionnaire			
Before randomization (V4)	0.87 ± 0.50	1.03 ± 0.58	0.51
End of treatment (V9)	0.77 ± 0.61	0.95 ± 0.84	0.57
Change (V9 − V4)	−0.10 ± 0.62	−0.08 ± 0.78	0.94

Definition of abbreviation: PC_{20} = provocative concentration of methacholine sufficient to induce a 20% decline in FEV_1; V = visit.

FEV_1, PC_{20}, and Asthma Control Questionnaire scores are shown for the 11 participants in each arm of the study who completed the trial. The two subjects who withdrew after the first infusion of study drug are not included in the analysis. Data are presented as the mean ± standard deviation.

suggesting this was an effect specific to abatacept treatment. We did not observe any other significant changes in the distribution of cell types. Expression of the T-cell activation marker CD25 was not affected by abatacept treatment, suggesting no effect on the baseline activation status of the circulating T cells. T-cell function was assessed by stimulating peripheral blood mononuclear cells *in vitro* with either α-CD3 alone, in combination with α-CD28 antibody, or with CTLA4Ig to block any endogenous CD80- or CD86-mediated costimulation (*see* Table E4). There were no differences in proliferation in response to α-CD3 or α-CD3/α-CD28 stimulation or when stimulated with α-CD3 in the presence of CTLA4Ig. However, the CD80/CD86-dependent proliferation approached statistical significance ($P = 0.08$) in those treated with abatacept compared with placebo. Cytokines were measured from α-CD3 and α-CD3/CD28 antibody-stimulated cultures at 48 hours. No differences were detected with the exception of IL-17A, which increased in the placebo-treated group but remained unchanged in abatacept-treated over the course of the study intervention.

DISCUSSION

Data from clinical and animal studies support the central role of T lymphocytes in the pathogenesis of allergic asthma. Therefore, interventions that target T-cell function have been attractive candidates for development as new therapeutics. Many of these agents have been directed at individual T cell–derived cytokines, with clinical trials yielding mixed results (7, 30, 31). Given that multiple effector molecules are involved in the pathogenesis of asthmatic inflammation, a more broad inhibition of T-cell responses to antigen might potentially be an effective therapeutic strategy.

TABLE 3. ANALYSIS OF PERIPHERAL BLOOD CELL SUBSETS

	Before Randomization (SAC 1)		After Randomization (SAC 2)		
Cell Type %	Placebo	Abatacept	Placebo	Abatacept	*P* Value
$CD4^+$ T cell	47.3 ± 6.6	44.2 ± 7.3	45.5 ± 15.1	45.3 ± 10.7	
Naive	48.8 ± 14.2	37.0 ± 10.3	49.7 ± 14.5	44.2 ± 10.4	0.041
Memory	46.2 ± 13.5	54.7 ± 10.3	45.2 ± 14.1	47.3 ± 11.0	0.013
$CD28^+CD25^+$	14.7 ± 8.5	13.9 ± 4.2	15.3 ± 6.1	15.4 ± 5.0	
$CD28^+CD25^-$	83.9 ± 8.6	83.5 ± 5.3	83.3 ± 6.9	81.6 ± 7.9	
$CD8^+$ T cell	26.0 ± 3.7	32.0 ± 6.1	23.7 ± 8.5	28.1 ± 6.1	
Naive	71.9 ± 11.4	69.1 ± 12.5	71.8 ± 8.9	69.6 ± 11.9	
Memory	21.6 ± 9.5	22.5 ± 9.0	22.0 ± 8.0	22.9 ± 9.8	
$CD28^+CD25^+$	0.7 ± 0.8	0.7 ± 0.6	0.7 ± 0.7	1.1 ± 0.6	0.08
$CD28^+CD25^-$	57.5 ± 17.0	53.0 ± 13.5	61.3 ± 14.2	55.3 ± 9.4	
B cell	17.6 ± 5.1	15.8 ± 6.0	15.7 ± 5.5	17.4 ± 6.8	
Nk cell	15.7 ± 12.2	19.9 ± 13.3	18.7 ± 16.4	16.1 ± 9.5	
Treg	3.2 ± 3.1	3.7 ± 1.5	3.9 ± 2.2	3.1 ± 1.9	
MDSC ($Lin1^-$)	17.8 ± 16.7	28.3 ± 20.3	13.5 ± 14.2	13.3 ± 16.3	
Myeloid DC ($Lin1^-$)	2.4 ± 2.1	3.0 ± 3.4	3.1 ± 2.8	1.9 ± 1.2	
Plasmacytoid DC ($Lin1^-$)	1.4 ± 1.2	1.3 ± 1.0	1.3 ± 1.3	1.1 ± 0.9	

Definition of abbreviations: DC = dendritic cell; MDSC = myeloid-derived suppressor cell; SAC = segmental allergen challenge.

Peripheral blood was collected before bronchoscopy at the prerandomization allergen challenge time point and again before the second SAC at the end of the study intervention period (after randomization) and analyzed by flow cytometry for specific cell subsets. Shown are the mean ± standard deviation of each subset as a percentage of the lymphocyte gate. DC and MDSC are shown as a percentage of the lineage cocktail negative gate. *P* values were calculated comparing whether the postrandomization values were different between placebo and abatacept after correcting for differences present at the prerandomization time point.

Optimal T-cell expansion and function requires the delivery of costimulatory signals provided by CD28. CTLA4Ig is a soluble fusion protein that binds the ligands for CD28 and is thought to work primarily by preventing receptor engagement and downstream signaling by CD28 (12, 32). The humanized version of this protein, abatacept, was approved for use in the treatment of rheumatoid arthritis and juvenile idiopathic arthritis in 2005 (21). Trials of abatacept have demonstrated efficacy in autoimmune diseases including psoriasis, psoriatic arthritis, systemic lupus erythematosus, and being effective in reducing β-cell loss in early diabetes mellitus (21, 33–36). A modified version of the drug, belatacept, was approved in 2011 to prevent kidney transplant rejection (37).

Given the numerous animal studies that demonstrated CTLA4Ig could potently inhibit allergic airway inflammation (13–15, 17, 19, 38), we tested the efficacy of abatacept in preventing inflammation in response to allergen challenge in humans with mild atopic asthma. In contrast to the results from animal models, we found that administration of abatacept had no effect on the inflammatory response in the lung, nor did we observe a clinical benefit as measured by bronchial hyperreactivity, lung function, or asthma control. One possible explanation for the lack of efficacy is that the drug did not exert its intended biologic effect, perhaps because of an inadequate dosing regimen. However, the dose used was the same as that used for several treatment trials of inflammatory diseases (33–36, 39, 40) and is the approved dose for rheumatoid arthritis (http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=0836c6ac-ee37-5640-2fed-a3185a0b16eb). An additional possibility is that the small sample size studied may have precluded our ability to detect a treatment effect. A *post hoc* analysis of the data demonstrated that the study was 80% powered to detect a between-group difference of 29.4% in the % BAL eosinophils at a 0.05 level of significance. Thus, although we may have missed a lesser effect, it seems unlikely that a smaller reduction in eosinophil recruitment would be clinically meaningful.

CD28 costimulation markedly enhances T-cell proliferation and cytokine secretion *in vitro*, and blockade with CTLA4Ig diminishes both responses (11, 12, 41, 42). Therefore, we predicted that participants who had received abatacept would have reduced cytokine levels in BAL fluid, and that T-cell function would be impaired when peripheral blood mononuclear cells were *ex vivo* stimulated. However, neither effect was observed suggesting that *in vivo* treatment with abatacept did not substantially modulate these aspects of T-cell function. Although most of our exploratory endpoints were negative, we did observe a change in the percentage of memory and naive $CD4^+$ T cells detected in the blood. In addition, we observed a dampening of CD80/CD86-dependent proliferative responses. Although no assay has been developed for either clinical or research use to determine whether abatacept has exerted its intended biologic effect *in vivo*, these data would suggest that abatacept did in fact exert a clinically detectable effect.

An additional consideration is recent data that suggest alternative mechanisms of action for abatacept. CTLA4Ig binding to CD80/CD86 on dendritic cells has been shown to induce indoleamine 2,3-dioxygenase, an immunoregulatory enzyme that can inhibit T-cell function through a number of pathways, including alteration of dendritic cell function and promotion of Treg development (43–46). However, we did not detect changes in Treg number in the BAL or blood, although these data do not exclude the possibility of changes in Treg function. Recently we demonstrated in a murine model that CTLA4Ig can inhibit airway inflammation independent of CD28 by activation of nitric oxide synthase in macrophages (38). This pathway is independent of indoleamine 2,3-dioxygenase and distinct from the conventional mechanism by which abatacept blocks engagement of CD28 on the T cell by CD80/CD86 expressed on the antigen-presenting cell. The parameters we measured would not detect alterations in this pathway.

There are limitations to this study. The SAC model provides a very strong stimulus that activates numerous effector pathways of the innate and adaptive immune system. All of the participants were atopic and the T-cell responses would be expected to be predominantly memory responses, which are less dependent on CD28-mediated costimulation than activation of naive T cells (47–49). Thus, the intensity of the provoked inflammatory stimulus may have overridden any requirement for T-cell costimulation. Patients with asthma may in reality experience a more chronic low level of exposure and airway inflammation, punctuated by intermittent severe exacerbations. It is certainly possible that long-term treatment with abatacept may modulate the underlying chronic inflammatory response favorably in a more severely affected asthma population, perhaps resulting in decreased exacerbations and improving asthma control. Such an effect would not have been detected in the present study but the negligible differences after 3 months of therapy suggest this is unlikely.

In conclusion, the current study demonstrated that treatment with abatacept did not significantly alter the inflammatory response to SAC in people with mild atopic asthma. Thus, modulation of CD28-mediated costimulation is unlikely to be an effective strategy in manipulating the T-cell response for therapeutic benefit in allergic inflammation.

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank Julie Nobbe for preparing study drug and randomizing subjects, Chandrika Christie for help with research assays, and Jamie Tarsi for assistance in conducting this study.

References

1. Barnes PJ. Pathophysiology of allergic inflammation. *Immunol Rev* 2011; 242:31–50.
2. Minnicozzi M, Sawyer RT, Fenton MJ. Innate immunity in allergic disease. *Immunol Rev* 2011;242:106–127.
3. Amin K. The role of mast cells in allergic inflammation. *Respir Med* 2012;106:9–14.
4. Seder RA, Paul WE. Acquisition of lymphokine-producing phenotype by CD4+ T cells. *Annu Rev Immunol* 1994;12:635–673.
5. Robinson D, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley A, Corrigan C, Durham S, Kay A. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298–304.
6. Wills-Karp M. Interleukin-13 in asthma pathogenesis. *Immunol Rev* 2004;202:175–190.
7. Nguyen T-HT, Casale TB. Immune modulation for treatment of allergic disease. *Immunol Rev* 2011;242:258–271.
8. Schwartz RH. A cell culture model for T lymphocyte clonal anergy. *Science* 1990;248:1349–1356.
9. Nurieva RI, Liu X, Dong C. Yin–yang of costimulation: crucial controls of immune tolerance and function. *Immunol Rev* 2009;229:88–100.
10. Boomer JS, Green JM. An enigmatic tail of CD28 signaling. *Cold Spring Harb Perspect Biol* 2010;2:a002436.
11. Lenschow DJ, Zeng Y, Thistlethwaite JR, Montag A, Brady W, Gibson MG, Linsley PS, Bluestone JA. Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4Ig. *Science* 1992;257:789–792.
12. Turka LA, Linsley PS, Lin H, Brady W, Leiden JM, Wei R-Q, Gibson ML, Zheng X-G, Myrdal S, Gordon D, *et al.* T-cell activation by the CD28 ligand B7 is required for cardiac allograft rejection in vivo. *Proc Natl Acad Sci USA* 1992;89:11102–11105.
13. Harris N, Campbell C, Le Gros G, Ronchese F. Blockade of CD28/B7 co-stimulation by mCTLA4-Hγ1 inhibits antigen- induced lung eosinophilia but not Th2 cell development or recruitment in the lung. *Eur J Immunol* 1997;27:155–161.
14. Harris N, Peach R, Naemura J, Linsley PS, Le Gros G, Ronchese F. CD80 costimulation is essential for the induction of airway eosinophilia. *J Exp Med* 1997;185:177–182.

15. Keane-Myers A, Gause WC, Linsley PS, Chen SJ, Wills-Karp M. B7–CD28/CTLA-4 costimulatory pathways are required for the development of T helper cell 2-mediated allergic airway responses to inhaled antigens. *J Immunol* 1997;158:2042–2049.
16. Green JM. The B7/CD28/CTLA4 T-cell activation pathway. Implications for inflammatory lung disease. *Am J Respir Cell Mol Biol* 2000; 22:261–264.
17. Burr JS, Kimzey SL, Randolph DR, Green JM. CD28 and CTLA4 coordinately regulate airway inflammatory cell recruitment and T-helper cell differentiation after inhaled allergen. *Am J Respir Cell Mol Biol* 2001;24: 563–568.
18. Van Oosterhout AJ, Hofstra CL, Shields R, Chan B, Van Ark I, Jardieu PM, Nijkamp FP. Murine CTLA4-IgG treatment inhibits airway eosinophilia and hyperresponsiveness and attenuates IgE upregulation in a murine model of allergic asthma. *Am J Respir Cell Mol Biol* 1997;17:386–392.
19. Kimzey SL, Liu P, Green JM. Requirement for CD28 in the effector phase of allergic airway inflammation. *J Immunol* 2004;173:632–640.
20. Hidi R, Riches V, Al-Ali M, Cruikshank WW, Center DM, Holgate ST, Djukanovic R. Role of B7–CD28/CTLA-4 costimulation and NF-kappa B in allergen-induced T cell chemotaxis by IL-16 and RANTES. *J Immunol* 2000;164:412–418.
21. Linsley PS, Nadler SG. The clinical utility of inhibiting CD28-mediated costimulation. *Immunol Rev* 2009;229:307–321.
22. National Asthma Education and Prevention Program. Expert panel report: guidelines for the diagnosis and management of asthma update on selected topics–2002. *J Allergy Clin Immunol* 2002;110(Suppl. 5):S141–S219.
23. Janson S. National Asthma Education and Prevention Program. Expert panel report. II: Overview and application to primary care. *Lippincotts Prim Care Pract* 1998;2:578–588.
24. Weiland SK, Bjorksten B, Brunekreef B, Cookson WO, von Mutius E, Strachan DP. Phase II of the International Study of Asthma and Allergies in Childhood (ISAAC II): rationale and methods. *Eur Respir J* 2004;24: 406–412.
25. Romanet-Manent S, Charpin D, Magnan A, Lanteaume A, Vervloet D. Allergic vs nonallergic asthma: what makes the difference? *Allergy* 2002;57:607–613.
26. Pepys J, Roth A, Carroll KB. RAST, skin and nasal tests and the history in grass pollen allergy. *Clin Allergy* 1975;5:431–442.
27. Jarjour NN, Calhoun WJ, Kelly EA, Gleich GJ, Schwartz LB, Busse WW. The immediate and late allergic response to segmental bronchopulmonary provocation in asthma. *Am J Respir Crit Care Med* 1997;155:1515–1521.
28. Hankinson JL, Crapo RO, Jensen RL. Spirometric reference values for the 6-s FVC maneuver. *Chest* 2003;124:1805–1811.
29. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, *et al.* Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000;161:309–329.
30. Flood-Page P, Swenson C, Faiferman I, Matthews J, Williams M, Brannick L, Robinson D, Wenzel S, Busse W, Hansel TT, *et al.* A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. *Am J Respir Crit Care Med* 2007;176:1062–1071.
31. Wenzel S, Wilbraham D, Fuller R, Getz EB, Longphre M. Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies. *Lancet* 2007;370:1422–1431.
32. Linsley PS, Wallace PM, Johnson J, Gibson MG, Greene JL, Ledbetter JA, Singh C, Tepper MA. Immunosuppression in vivo by the soluble form of the CTLA-4 T cell activation molecule. *Science* 1992;257:792–795.
33. Álvarez-Quiroga C, Abud-Mendoza C, Doníz-Padilla L, Juárez-Reyes A, Monsiváis-Urenda A, Baranda L, González-Amaro R. CTLA-4-Ig therapy diminishes the frequency but enhances the function of Treg cells in patients with rheumatoid arthritis. *J Clin Immunol* 2011;31:588–595.
34. Genovese MC, Becker JC, Schiff M, Luggen M, Sherrer Y, Kremer J, Birbara C, Box J, Natarajan K, Nuamah I, *et al.* Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. *N Engl J Med* 2005;353:1114–1123.
35. Mease P, Genovese MC, Gladstein G, Kivitz AJ, Ritchlin C, Tak PP, Wollenhaupt J, Bahary O, Becker J-C, Kelly S, *et al.* Abatacept in the treatment of patients with psoriatic arthritis: results of a six-month, multicenter, randomized, double-blind, placebo-controlled, phase II trial. *Arthritis Rheum* 2011;63:939–948.
36. Orban T, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Gottlieb PA, Greenbaum CJ, Marks JB, Monzavi R, *et al.* Costimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *Lancet* 2011;378:412–419.
37. Vincenti F, Larsen C, Durrbach A, Wekerle T, Nashan B, Blancho G, Lang P, Grinyo J, Halloran PF, Solez K, *et al.* Costimulation blockade with belatacept in renal transplantation. *N Engl J Med* 2005;353:770–781.
38. Deppong CM, Parulekar A, Boomer JS, Bricker TL, Green JM. CTLA4-Ig inhibits allergic airway inflammation by a novel CD28-independent, nitric oxide synthase-dependent mechanism. *Eur J Immunol* 2010;40: 1985–1994.
39. Scarsi M, Ziglioli T, Airo P. Decreased circulating CD28-negative T cells in patients with rheumatoid arthritis treated with abatacept are correlated with clinical response. *J Rheumatol* 2010;37:911–916.
40. Scarsi M, Ziglioli T, Airo P. Baseline numbers of circulating CD28-negative T cells may predict clinical response to abatacept in patients with rheumatoid arthritis. *J Rheumatol* 2011;38:2105–2111.
41. Thompson CB, Lindsten T, Ledbetter JA, Kunkel SL, Young HA, Emerson SG, Leiden JM, June CH. CD28 activation pathway regulates the production of multiple T-cell-derived lymphokines/cytokines. *Proc Natl Acad Sci USA* 1989;86:1333–1337.
42. Green JM, Noel PJ, Sperling AI, Walunas TL, Gray GS, Bluestone JA, Thompson CB. Absence of B7-dependent responses in CD28-deficient mice. *Immunity* 1994;1:501–508.
43. Boasso A, Herbeuval JP, Hardy AW, Winkler C, Shearer GM. Regulation of indoleamine 2,3-dioxygenase and tryptophanyl-tRNA-synthetase by CTLA-4-Fc in human CD4+ T cells. *Blood* 2005;105:1574–1581.
44. Munn DH, Sharma MD, Mellor AL. Ligation of B7–1/B7–2 by human CD4+ T cells triggers indoleamine 2,3-dioxygenase activity in dendritic cells. *J Immunol* 2004;172:4100–4110.
45. Mellor AL, Chandler P, Baban B, Hansen AM, Marshall B, Pihkala J, Waldmann H, Cobbold S, Adams E, Munn DH. Specific subsets of murine dendritic cells acquire potent T cell regulatory functions following CTLA4-mediated induction of indoleamine 2,3 dioxygenase. *Int Immunol* 2004;16:1391–1401.
46. Chen W, Liang X, Peterson AJ, Munn DH, Blazar BR. The indoleamine 2,3-dioxygenase pathway is essential for human plasmacytoid dendritic cell-induced adaptive T regulatory cell generation. *J Immunol* 2008;181:5396–5404.
47. Gause WC, Mitro V, Via C, Linsley P, Urban JF Jr, Greenwald RJ. Do effector and memory T helper cells also need B7 ligand costimulatory signals? *J Immunol* 1997;159:1055–1058.
48. Suresh M, Whitmire JK, Harrington LE, Larsen CP, Pearson TC, Altman JD, Ahmed R. Role of CD28–B7 interactions in generation and maintenance of CD8 T cell memory. *J Immunol* 2001;167:5565–5573.
49. Croft M, Bradley LM, Swain SL. Naive versus memory CD4 T cell response to antigen. Memory cells are less dependent on accessory cell costimulation and can respond to many antigen-presenting cell types including resting B cells. *J Immunol* 1994;152:2675–2685.