

Increased expression of the CD80 accessory molecule by alveolar macrophages in asthmatic subjects and its functional involvement in allergen presentation to autologous T_{H2} lymphocytes

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Background: Alveolar macrophages (AMs) are more efficient antigen-presenting cells in allergic individuals than in nonatopic subjects.

Objective: We studied whether this difference may be correlated to increased expression of membrane costimulatory molecules, such as the B7 molecules (CD80 and CD86).

Methods: Eleven subjects with allergic asthma sensitized to *Dermatophagoides pteronyssinus* and 5 healthy nonatopic volunteers underwent bronchoalveolar lavage, and the costimulatory molecule expression on AMs was evaluated. Peripheral blood T cells, either freshly isolated or as established *D pteronyssinus*-specific cell lines, were cultured with autologous monocytes or AMs as antigen-presenting cells. In vitro allergen-induced proliferation and cytokine production were evaluated in the presence of B7-blocking reagents.

Results: Allergic individuals had a significantly higher proportion of AMs expressing the CD80 molecule than control subjects ($28.5\% \pm 14.8\%$ vs $1.4\% \pm 1.2\%$; $P < .001$), whereas no difference was observed in CD86 expression ($2.0\% \pm 2.3\%$ vs $1.1\% \pm 0.6\%$; $P > .1$). In a large proportion of the asthmatic subjects we studied, AMs were presenting soluble antigens (tetanus toxoid and streptolysin-O) to freshly isolated T cells more efficiently than AMs from nonatopic control subjects.

Finally, both T-cell proliferation and cytokine production of *D pteronyssinus*-specific established T-cell lines were inhibited by a CD80-blocking antibody in a dose-dependent manner.

Conclusion: Costimulation by means of CD80 expressed by AMs is probably involved in the amplification of the allergen-specific T-lymphocyte response in the airways of asthmatic subjects. (*J Allergy Clin Immunol* 1999;103:1136-42.)

Key words: Allergy, asthma, monocyte-macrophages, antigen presentation, costimulation, CD80, CD86

Abbreviations used

AM:	Alveolar macrophage
APC:	Antigen-presenting cell
BAL:	Bronchoalveolar lavage
S-O:	Streptolysin-O
³ H-TdR:	Tritiated thymidine
TT:	Tetanus toxoid

Alveolar macrophages (AMs) derived from peripheral blood monocytes are defective cells in terms of their ability to activate memory T lymphocytes and initiate inflammatory responses.^{1,2} T-cell activation requires an interaction between the T-cell receptor and the specific antigen, which occurs in the form of processed peptides associated with MHC molecules. However, for complete T-cell activation, a second signal (known as costimulation) is required.³ One of the best characterized costimulatory molecules is CD28, which is expressed constitutively by T lymphocytes. The natural ligands for this molecule are the B7 molecules (ie, CD80 [B7.1]^{4,5} and CD86 [B7.2]^{6,7}), which are differentially expressed by the various antigen-presenting cells (APCs), such as dendritic cells, monocytes, macrophages, and B cells. Moreover, within the same type of cell, the expression of the B7 molecules may be influenced by activation/differentiation stages.

Defective antigen presentation by AMs has recently been related to the reduced expression of costimulatory molecules.⁸ This characteristic may have the advantage of limiting immune responses in the lung, which is constantly exposed to antigens,⁹ and of providing sufficient levels of protection without impairing respiratory function.¹⁰ AM accessory cell function has been previously studied in normal individuals and in patients with sarcoidosis and asthma.^{11,12} Although the results in sarcoidosis are contradictory, there is evidence that in allergic asthma AMs can be as efficient as peripheral blood monocytes in presenting soluble antigens, at least in a subgroup of patients.¹²

With this background, a study was designed to verify whether the increased antigen-presenting capability of

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AMs in asthmatic subjects was associated with an altered expression of B7 costimulatory molecules.

METHODS

Patients

We studied 11 atopic asthmatic subjects.¹³ All of them were sensitized to both *Dermatophagoides pteronyssinus* and *D farinae*, as shown by measuring allergen-specific serum IgE levels. Specific IgE levels were measured as Phadebas RAST units (PRUs) per milliliter of serum (Pharmacia, Uppsala, Sweden). The characteristics of the patients are shown in Table I. The inclusion criteria were as follows: (1) a RAST class III or higher to *D pteronyssinus* and *D farinae* (RAST class III and IV correspond to the range from 3.5 to 17.5 PRU/mL and above 17.5 PRU/mL, respectively); (2) FEV₁ greater than 70% of the predicted value at the time of the study¹⁴; and (3) no respiratory infections in the 4 weeks preceding the study. At the time of the study, none of the subjects was receiving any anti-asthmatic treatment other than short-acting β_2 -stimulants on an as-needed basis. These were discontinued at least 12 hours before evaluation.

Five healthy, nonsmoking volunteers were included as nonatopic control subjects. These subjects had a negative history for asthma and atopic diseases and negative skin prick test results with a panel of the 12 most common allergens in Italy (*Lolium perenne*, *Parietaria* spp, *Olea* spp, *Betula* spp, *D pteronyssinus*, *D farinae*, *Cupressus* spp, cat dander, dog dander, *Alternaria* spp, *Cladosporium* spp, and *Aspergillus* spp).

All of the subjects were informed about the nature and aims of the study and gave their written consent to participate. The study was approved by the ethical committee of the institution at which the patients and the volunteers were selected.

All of the subjects underwent blood sampling (40 mL, heparinized), spirometry before bronchoscopy, and fiberoptic bronchoscopy with bronchoalveolar lavage (BAL).

Media, mAbs, and antigens

The complete medium used for the cell cultures was prepared as follows. RPMI 1640 (PBI, Milan, Italy) was supplemented with gentamicin 5 μ g/mL, glutamine 2 mmol/L (PBI), and 5% normal human serum (Danish Red Cross Laboratories, Utrecht, Holland). The mAbs used to study the expression of CD80 and CD86 on AMs by means of FACS analysis (FACScan; Becton Dickinson, Mountain View, Calif), as well as for the experiments relating to the inhibition of IL-5 production and allergen-specific proliferation, were clone BB1 (mouse IgM anti-human CD80) and clone BU63 (mouse IgG1 anti-human CD86, both from Ancell Immunology Research Products, Bayport, Minn). The control mouse IgM and IgG1 mAbs with irrelevant specificity were supplied by the same manufacturer. All of the primary antibodies were used at a concentration of 10 μ g/mL. A fluorescein anti-mouse Ig second antibody was used to reveal monoclonal binding (PharminGen, San Diego, Calif).

CTLA4-Ig¹⁵ was kindly provided by Dr Paolo Dellabona (DIBIT; San Raffaele Scientific Institute, Milan, Italy). CD4-Ig or CD4 immunoadhesin, a soluble recombinant molecule containing V1 and V2 domains of human CD4 spliced to the CH3 and CH3 domains of human IgG1, was obtained from Genentech Inc (South San Francisco, Calif).¹⁶

The antigens used for T-cell stimulation assays were as follows: streptolysin-O (S-O; Sigma Italia, Milan, Italy), tetanus toxoid (TT; Connaught Labs, Willowdale, Ontario, Canada), and a *D pteronyssinus* and *D farinae* mixed allergenic extract (Lofarma Allergeni, Milano, Italy). The amounts of Der p 1 and Der f 1 in the *D pteronyssinus* and *D farinae* mixed allergen extract were quantified by ELISA with specific mAbs (32% and 38% of its dry weight; courtesy of Dr P. Falagiani, Lofarma Allergeni).

TABLE I. Patient data

	Asthmatic subjects	Nonatopic control subjects
No. of subjects (M/F)	11 (9/2)	5 (4/1)
Age, y (range)	23.5 $\pm$ 3.3 (17-25)	31.8 $\pm$ 6.6 (25-40)
FEV ₁ , % of predicted value	91 $\pm$ 10.4*	97.0 $\pm$ 5.1
FEV ₁ /FVC	77.3 $\pm$ 7.5†	88 $\pm$ 4.1
BAL cells		
Total	1.17 $\times$ 10 ⁶ (0.94-2.52)	5.3 $\times$ 10 ⁶ (1.2-9.7)
Macrophages	90.5% (87.5%-93.4%)	94% (92%-94%)
Lymphocytes	2.7% (2%-8.3%)	6% (4%-6%)
Neutrophils	1.1% (0%-1.3%)	0% (0%-1%)
Eosinophils	4.4%‡ (1.8%-5.1%)	1% (0%-1%)

FVC, Forced vital capacity.

Data on lung T cells are expressed as median values (lower and upper quartiles are indicated). The other data are mean values.

†P < .01 and ‡P < .05, asthmatic subjects vs control subjects.

The limulus amoebocyte gelification test (PBI) was used to assess the presence of bacterial endotoxins in the allergen extracts. On reconstitution of the allergen extract at 1 mg/mL in culture medium—a concentration 20 times higher than that used for T-lymphocyte stimulation—the assay was performed according to the manufacturer's instructions. It measured 0.01 endotoxin units/mL, a level that was no different from that found in saline solution.

Fiberoptic bronchoscopy and bronchoalveolar lavage

Bronchoscopy and bronchoalveolar lavage (BAL) were performed as follows. After premedication with atropine (0.5 mg administered intramuscularly) and diazepam (10 mg administered intramuscularly), local anaesthesia of the nostrils was obtained by using lidocaine 2% and adrenaline 0.1/1000 (1 mL on each side), and a fiberoptic bronchoscope (Olympus BF, Osaka, Japan) was then passed through the nose. The pharynx and large airways were anesthetized by means of an instillation of 2% lidocaine, and the tip of the bronchoscope was wedged into a subsegmental branch of the right middle lobe and the lingula. Five 20-mL aliquots of sterile warm (37°C) saline were infused through the channel of the bronchoscope and recovered at a negative pressure of 50 to 120 mm Hg.

BAL sample processing

BAL fluid was filtered through 2 layers of sterile gauze and centrifuged at 250g for 5 minutes to separate the cells from the supernatants. The cell pellet was resuspended in ice-cold PBS without Ca²⁺ and Mg²⁺ at a concentration of 10⁶ cells/mL. A small sample of the cell suspension was centrifuged by using a cytocentrifuge (Cytospin; Shandon Southern Instruments, Sewickley, Pa) that spun approximately 100,000 cells at 500 rpm for 5 minutes onto a glass slide. The BAL cells were air-dried and stained with Diff-Quick staining (Merz & Dade A.G., Duding, Switzerland). Cell differentials were determined by counting at least 300 cells per slide by using a light microscope (Carl Zeiss, Oberkochen, Germany). Macrophages, lymphocytes, neutrophils, and eosinophils were expressed as percentages of the total number of recovered cells. Grouped results are shown in Table I.

Preparation of blood CD4 T lymphocytes (responder cells) and 2 distinct populations of APCs (blood monocytes and AMs)

Distinct cell preparations containing CD4 T lymphocytes and monocytes (as APCs) were obtained from PBMCs. Monocyte-enriched cells were isolated by incubating fresh PBMCs (5 to $10 \times 10^6/\text{mL}$) in 60-mm plastic tissue culture dishes in complete medium for 2 hours at 37°C in 5% CO_2 . Adherent cells were detached with cold PBS containing 3.3 mmol/L EDTA and then counted. Nonadherent cells were used to prepare a CD4-enriched T-cell preparation by means of negative immunobead selection with mAbs, according to the manufacturer's instructions (Dynabeads; Unipath, Garbagnate, Italy). Briefly, the nonadherent T cells were incubated for 20 minutes on ice at a cell density of $1 \times 10^7/\text{mL}$ with normal human serum (10%) to saturate the Fc receptors and then with mAbs to CD8, CD56, and CD19 ($1 \mu\text{g}/10^6$ cells) to remove cytotoxic/suppressor T lymphocytes, natural killer cells, and B lymphocytes, respectively. Goat anti-mouse Ig-coated beads were then reacted for 20 minutes on ice (rotating) with mAb-coated nonadherent T cells, and the cells rosetting with beads were removed with a magnet. In the resulting cell population CD4^+ cells together accounted for more than 95% of the cells at FACS analysis (not shown).

AMs from BAL fluid were layered over a 45% Percoll gradient (Pharmacia, Uppsala, Sweden) and spun at $800g$ for 45 minutes at 4°C to remove contaminating lymphocytes and red cells. The cells at the interface were greater than 95% large granular macrophages; they were washed, resuspended in complete medium, and counted.

Determination of the relative capability of AMs to present recall antigens

The relative efficiency of AMs versus blood monocytes as APCs (APC ratio) was determined according to a method previously described by Gant et al,¹² with minor modifications. CD4-enriched T lymphocytes (10^5 per microculture in quadruplicates in 96-well U-bottom plates; Greiner, PBI, Milan, Italy) taken from peripheral blood were used as responder cells. Either blood monocytes or AMs were used ($10^4/\text{well}$) as APCs. A 1:5 ratio of CD4 T cells and AMs or monocytes was used on the basis of the results of preliminary experiments and published data.¹² These cell mixtures were pulsed in a 7-day tritiated thymidine (^3H -TdR) incorporation proliferative assay with the following different soluble antigens: (1) S-O, an ubiquitous antigen ($10 \mu\text{g}/\text{mL}$); (2) TT, an antigen to which the whole population was exposed ($10 \mu\text{g}/\text{mL}$); and (3) the *Dermatophagoides* mixed extract ($20 \mu\text{g}/\text{mL}$).¹⁷ As controls, the proliferation of CD4-enriched T cells, which should not proliferate because of the absence of APCs, and the proliferation of total PBMCs to evaluate the presence of antigen-specific T cells toward a given antigen in unmanipulated peripheral blood were assessed. The APC ratio was expressed by the following formula:

$$\frac{(\text{T cells} + \text{AMs} + \text{antigen}) - (\text{T cells} + \text{antigen})}{(\text{T cells} + \text{monocytes} + \text{antigen}) - (\text{T cells} + \text{antigen})}$$

in which the cpm obtained in ^3H -TdR incorporation proliferative assay were used as the input of each term.

In each proliferation assay the addition of recombinant soluble CTLA4-Ig ($20 \mu\text{g}/\text{mL}$), a soluble chimeric molecule binding with high affinity to CD80 and CD86, made it possible to evaluate whether the proliferation of antigens presented by AMs could be inhibited by blocking the B7 costimulatory molecules. Recombinant soluble CD4, CD4-Ig $20 \mu\text{g}/\text{mL}$ (a chimeric protein containing the constant region of IgG1 fused to 2 extracellular domains of the CD4 molecule), was used as a control for the specificity of the inhibitory effect of CTLA4-Ig. The APC ratio was not calculated

when the proliferation of T cells to the indicated antigens presented by monocytes was less than twice the background proliferation, thus indicating a low intrinsic response of T cells to that antigen.

Establishment of *Dermatophagoides*-specific CD4 T-cell lines and characterization of their cytokine profile

T-cell lines specific to the *Dermatophagoides* mixed extract were established from patients 5 and 9, as previously described.¹⁸ The specificity of proliferation to allergen and lymphokine production by the T-cell lines stimulated twice in vitro were assessed as follows. Three-day in vitro allergen-stimulation assays were set up, in which allergen was added (except in the control wells) to the T cells on day 0 in the presence of autologous irradiated PBMCs as APCs. Phorbol myristate acetate (100 nmol/L, Sigma) and PHA ($10 \mu\text{g}/\text{mL}$, Sigma) were added during the last 18 hours of culture, and the supernatants were collected and kept frozen at -80°C until cytokine determination. In parallel microcultures, ^3H -TdR ($1 \mu\text{Ci}/\text{well}$; Dupont, Cologno M, Italy) was added for the same amount of time to measure cell proliferation. IL-5, IL-4, and IFN- γ were measured by ELISA (in-house Roche ELISA, courtesy of Dr P. Panina, Roche Milano Ricerche, Milan, Italy). Allergen-specific proliferation was determined by using an automated β -plate counter (Pharmacia LKB, Uppsala, Sweden). In the inhibition experiments anti-CD80 and anti-CD86 mAbs in different concentrations (both mAbs were previously described as B7-inhibitory reagents¹⁹) and CTLA4-Ig ($5 \mu\text{g}/\text{mL}$) were added to the T cells ($10^5/\text{well}$) at the beginning of the 3-day in vitro allergen-stimulation assays in the presence of AMs ($10^4/\text{well}$) as APCs. To verify the specificity of inhibition experiments, isotype-matched mAbs with irrelevant binding specificities (see above) were used as controls for anti-CD80 and anti-CD86 mAbs. CD4-Ig was used as a control for CTLA4-Ig.

Statistical analysis

Unpaired t tests and the Mann-Whitney U test were used for between-group comparisons, depending on whether the data were normally distributed. A P value of less than .05 was considered statistically significant. The data are expressed as mean values $\pm$ SD (geometric means) or medians with lower and upper quartiles.

RESULTS

Pulmonary function and BAL analysis

There was no significant difference in the age of the control subjects and allergic asthmatic subjects. Evaluation of the pulmonary function parameters demonstrated that FEV_1 values and $\text{FEV}_1/\text{forced vital capacity}$ ratios were significantly lower in asthmatic subjects compared with control subjects ($P < .01$ and $P < .05$, respectively; Table I). Total cell number recovered by lavage was not statistically different in the 2 study populations (Table I).

Analysis of different BAL cell types in asthmatic patients and control subjects demonstrated similar proportions of macrophages, lymphocytes, and neutrophils ($P > .05$ for each comparison) but an increased eosinophil percentage ($P < .05$; Table I).

Expression of the CD80 and CD86 molecules by AMs

It has been reported that the costimulatory molecules CD80 and CD86 are not physiologically expressed on human AMs. In the subjects here evaluated, very small

TABLE II. CD80 and CD86 expression on AMs

	CD80 (%)	CD86 (%)
Asthmatic subjects		
1	57.1	0.5
2	15.1	ND
3	24.2	1.0
4	15.2	4.1
5	21.0	2.3
6	16.2	0.5
7	45.1	4.6
8	15.2	0.5
9	16.5	0.5
10	24.2	2.0
11	24.1	1.5
Mean $\pm$ SD	24.9 $\pm$ 13.7	1.7 $\pm$ 1.5
Control subjects		
1	1.5	1.0
2	0.5	1.0
3	1.0	2.2
4	0.5	1.0
5	3.5	0.5
Mean $\pm$ SD	1.4 $\pm$ 1.2	1.1 $\pm$ 0.6

ND, Not done.

proportions of AMs expressing CD80 ($1.4\% \pm 1.2\%$) and CD86 ($1.1\% \pm 0.6\%$) were detected in control subjects, whereas the asthmatic subjects had a higher proportion of CD80⁺ AMs ($24.9\% \pm 13.7\%$; $P = .02$) but a comparable proportion of CD86⁺ AMs ($1.7\% \pm 1.5\%$; $P > .05$; Table II). No significant correlations were found between the percentage of either CD80⁺ or CD86⁺ AMs and that of any of the inflammatory cells in BAL fluid, either in control or asthmatic subjects ($P > .05$ for each comparison; data not shown).

Antigen presentation to autologous T lymphocytes

The relative efficiency of AMs versus blood monocytes as APCs (APC ratio) was calculated as the percent proliferation of primary cultures of CD4-enriched T lymphocytes. AMs from asthmatic subjects presented soluble antigens (TT and S-O) significantly better than AMs from nonatopic control subjects ($P < .05$ for each antigen). Moreover, in some asthmatic individuals (nos. 2, 3, 7, 10, and 11) the AMs were equally or more effective than monocytes in presenting single soluble antigens to blood CD4 T lymphocytes (APC ratio, >1). This was never observed in any of the control subjects, as expected⁸ (Table III). The APC ratio to *Dermatophagoides* antigens could be calculated only in 4 of 11 asthmatic subjects (nos. 3, 5, 7, and 9) who showed significant proliferation (ie, more than twice than that observed in the control subjects) of primary CD4 T lymphocytes to this allergen (Table III). There was no correlation between the APC ratio for any antigen and the percentage of either CD80⁺ or CD86⁺ AMs ($P > .05$ for each comparison).

TABLE III. Relative efficiency of AMs versus blood monocyte as APCs (APC ratio)

	APC ratio		
	S-O	TT	DER
Asthmatic subjects			
1	0.062	0.035	NC
2	1.254	0.065	NC
3	1.634	0.987	1.290
4	0.045	0.024	NC
5	0.050	0.021	0.370
6	0.039	0.018	NC
7	0.870	0.049	1.231
8	0.081	0.054	NC
9	0.980	0.530	0.791
10	1.210	0.679	NC
11	1.021	0.650	NC
Mean $\pm$ SD	0.658 $\pm$ 0.609	0.282 $\pm$ 0.356	
Control subjects			
1	0.051	0.0212	—
2	0.051	0.024	—
3	0.022	0.014	—
4	0.094	0.024	—
5	0.124	0.081	—
Mean $\pm$ SD	0.068 $\pm$ 0.040	0.032 $\pm$ 0.027	

DER, *Dermatophagoides* antigen; NC, Not calculated (ie, when the proliferation of T cells to the indicated antigen presented by monocytes was less than twice the background proliferation).

The APC ratio was calculated according to the formula reported in the Methods section and indicates the ability to present the indicated soluble antigen to CD4 T lymphocytes of alveolar AMs in comparison with peripheral blood monocytes.

Involvement of the CD80 and CD86 molecules in antigen presentation to autologous T lymphocytes and T_{H2} cytokine production

Inhibition experiments were performed to study the functional relevance of the CD80 molecule expressed on AMs in relation to the effector function on T cells. Preincubation of the T-cell cultures with the B7-blocking reagent CTLA4-Ig completely inhibited the proliferation to both T_{H1} (S-O, TT) and T_{H2} (*Dermatophagoides* spp) antigens presented by AMs (Fig 1) or by monocytes (not shown). In contrast, no changes in ³H-TdR incorporation were observed with the control soluble recombinant molecule CD4-Ig, thus suggesting the specific engagement of B7 costimulatory molecules in the response of T cells to these antigens (Fig 1).

Allergen (*Dermatophagoides* spp)-specific T-cell lines were derived from patients 5 and 9. As expected, these cell lines were T_{H2} polarized and produced consistently higher amounts of IL-4 than IFN- γ when stimulated for 48 hours with the specific allergen presented by peripheral monocytes (not shown). T_{H2} cytokines were also released in the presence of AMs. Indeed, when stimulated with allergen in the presence of autologous AMs as APCs, the *Dermatophagoides*-specific T cells produced significant amounts of both IL-4 and IL-5 (not shown). The inactivation of the CD80 molecule on AMs by the

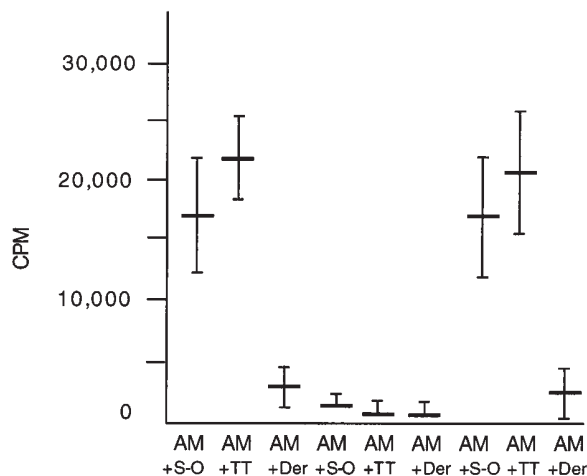


FIG 1. Proliferation of freshly isolated peripheral blood and CD4-enriched T lymphocytes from patient 3 to *Dermatophagoides* (Der) antigen presented by autologous AMs. Results are expressed as mean values of thymidine incorporation by quadruplicate microcultures. SDs are indicated. The T cell:APC ratio was 1:5. CTLA4-Ig (a chimeric recombinant molecule binding to both CD80 and CD86) was used as a specific inhibitor of costimulation-dependent antigen presentation. CD4-Ig, an irrelevant chimeric recombinant molecule containing the same Ig domains as CTLA4-Ig, was used as a control for the specificity of CTLA4-Ig inhibition. Background proliferation was not subtracted out. CPM, Counts per minute.

addition of different amounts of a blocking mAb resulted in a dose-dependent inhibition of the T-cell proliferation (Fig 2, C) and in a dose-dependent downregulation of IL-4 and IL-5 production (Fig 2, A and B). On the contrary, neither an isotype-matched (IgM) irrelevant mouse mAb (not shown) nor an mAb to CD86 (Fig 2) or its isotype-matched (IgG1) control (not shown) interfered with T-cell proliferation or with IL-4 or IL-5 production in this assay.

DISCUSSION

The results of this study indicate that the following apply to allergic asthmatic individuals as compared with nonatopic control subjects: (1) The subset of AMs expressing the CD80 costimulatory molecule is expanded; (2) the capability to present T_H1 and T_H2 antigens by AMs in a large proportion of the individuals we studied is increased and can support T-lymphocyte proliferation and lymphokine production; and (3) by blocking CD80 with a recombinant high-affinity ligand, the proliferative response of T lymphocytes to antigens and the release of IL-4 and IL-5 cytokines are significantly inhibited. Taken together, these results suggest that the increased antigen-presenting capability of AMs may play a pathogenic role in asthma, at least in some individuals, and that B7 molecules are at least partially involved in this phenomenon.

The ability of mononuclear cells to internalize protein antigens and present them as small peptides to T cells in the context of HLA surface molecules forms the basis of

the initiation of the immune response. AMs can provide this function, although they are not as effective of APCs as other mononuclear phagocytes.²⁰ This physiologically impaired APC function can, at least partially, result from the lack of costimulation.

The recognition of processed antigen by the T-cell receptor complex can lead to different outcomes (activation, tolerance, and polarization toward a given cytokine phenotype), depending on the associated interactions between an array of molecules on the T cells (eg, CD80, CD86, and lymphocyte function-associated antigen-1 [LFA-1]) and their counterreceptors on the APCs.²¹ CD80 and CD86 are the ligands for the counterreceptors (CD28 and CTLA-4) expressed on T lymphocytes constitutively and on activation. The B7 molecules (CD80 and CD86) are absent on normal human AMs.⁸

Lung disease can influence the capacity of macrophages to serve as accessory cells, and an alteration in the ability of AMs to interact with lymphocytes and deliver inhibitory signals has been described in various pathologic conditions. For example, AMs serve as efficient APCs (measured as T-lymphocyte proliferation) in patients with sarcoidosis,²² asthma,²³ and tuberculosis.²⁴ The possibility that activation of AMs could imply an enhanced accessory cell function and influence the proportion and the pathogenic role of T lymphocytes in the lower respiratory tract is suggested by several reports.^{12,23,25,26} Here we show that the CD80 costimulatory molecule is expressed on a subset of AMs from atopic asthmatic subjects, which is significantly more expanded than in nonatopic control subjects.

We subsequently performed experiments aimed at studying whether the increased CD80 expression was associated with an increased antigen-presenting capability of AMs, expressed as the APC ratio. We found that in allergic patients, the APC ratio for both TT and S-O was significantly higher than that found in nonatopic control subjects. Moreover, in some asthmatic individuals the AMs were equally or more effective than monocytes in presenting single soluble antigens to blood CD4 T lymphocytes (APC ratio, >1). This was never observed in any of the nonatopic control subjects, as expected.⁸

The ability to present antigens did not correlate with the percentage of AMs expressing the CD80 molecule. This discrepancy may depend on the complexity of the parameters affecting the antigen-presentation mechanism (eg, the expression on the APC of a complex array of adhesion molecules involved in costimulation, the influence of the ratio of lymphocytes to AMs, individual variability in T-lymphocyte response to the antigens, and the activation/differentiation stage of AMs). However, by blocking CD80 and CD86 with a recombinant high-affinity ligand for both molecules, the primary proliferative response of T lymphocytes to all of the tested antigens was blocked.

We could measure the efficiency of antigen presentation to freshly isolated T cells (ie, in an experimental set-up closely related to the in vivo situation) by using typical T_H1 antigens, such as TT and S-O. In 4 patients a measurable T-cell proliferation to *Dermatophagoides* antigen

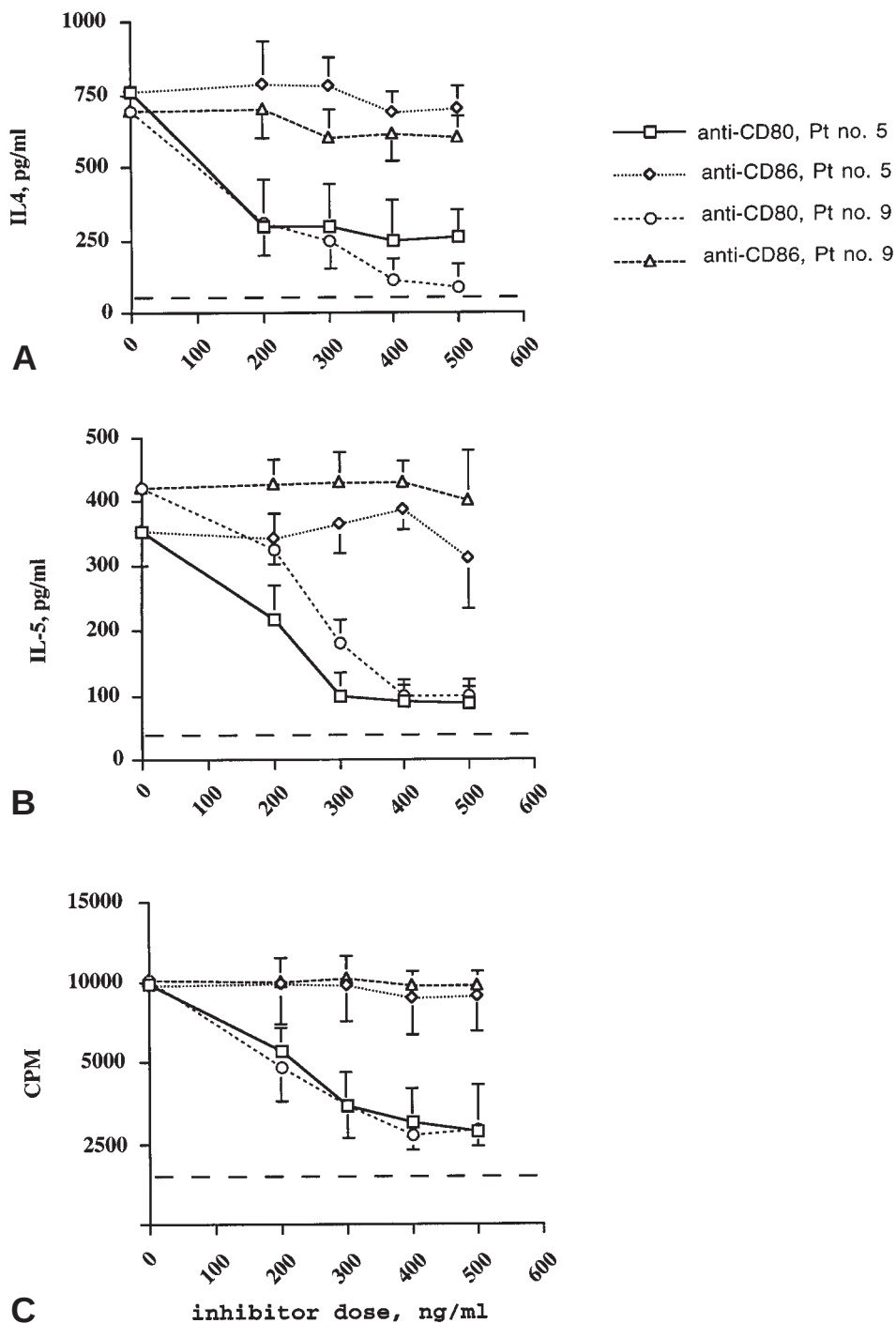


FIG 2. CD80-dependent inhibition of IL-4 and IL-5 production (**A** and **B**) and cell proliferation (**C**) by *Der-matophagoides*-specific CD4 T_{H2} lymphocytes stimulated with antigen presented by AMs from 2 asthmatic individuals (nos. 5 and 9). Monoclonal antibodies to CD80 or CD86 were used on APCs (autologous AMs) in 48-hour assays at the indicated concentrations. Isotype-matched (IgM and IgG1, respectively) mAbs of irrelevant specificity (10 μ g/mL) were used as controls. Each dot represents the mean value of quadruplicate micro-cultures. SDs are indicated. Dotted lines indicate background values for each assay.

allowed the APC ratio to be calculated also with this T_{H2} antigen. Nevertheless, it is likely that in vivo the expression of CD80 on AMs could promote T-cell response to allergens in the lungs of atopic asthmatic subjects.

Harris et al²⁷ have recently reported that in a mouse model CD80 expressed on AMs contributed to eosinophil recruitment by means of IL-5 production, although CD80 was not involved in the priming of the

T_{H2} immune response. Similarly, in a different murine model of asthma, Tsuyuki et al²⁸ showed that the IL-5-dependent recruitment of eosinophils to the lung can be inhibited by CTLA4-Ig. In humans Agea et al²⁹ recently reported an increase in CD86-expressing AMs in asthma. However, they also found a minor increase of CD80⁺ AMs. This difference may depend on several factors, including the possible influence by the allergen exposure on B7 molecule expression. Further studies on a larger cohort of patients are needed to elucidate this issue.

In our experimental system we dissected the relative role of CD80 and CD86 on antigen presentation by measuring the effect of CD80- and CD86-specific inhibitory ligands on the proliferation of autologous allergen-specific T lymphocytes. These cells likely mirror the repertoire of the resident (memory) T-cell population in the lungs of atopic asthmatic subjects. We show that T lymphocytes are indeed strictly dependent on CD80 expression for accomplishing their effector function, as measured by antigen-specific proliferation and production of IL-4 and IL-5. We cannot exclude the possibility that CD80 blockade by a specific mAb could impair antigen presentation by downregulated or altered expression of other costimulatory molecules.

Moreover, CTLA4-Ig was more effective than each single anti-B7-blocking mAb, thus leaving the possibility that both CD80 and CD86 on AMs could be involved in costimulation. However, CD80, but not CD86, engagement produced the loss of most of the effector functions measured in our experimental system.

In conclusion, taken together our results support the hypothesis that costimulatory molecules, such as CD80, may be involved in the maintenance and/or amplification of the lung allergic response in asthma. These molecules may represent an attractive therapeutic potential for immunosuppressive strategies designed to interfere with the interaction between T cells and APCs.³⁰

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