

Expression of B7 co-stimulatory molecules and CD1a antigen by alveolar macrophages in allergic bronchial asthma

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Summary

Background Lung allergen recognition that takes place in the airways of asthmatic subjects is still a controversial matter.

Objective We hypothesized that a rapid allergen recognition process requires the presence, at the mucosal surface, of professional APC, such as B7⁺ alveolar macrophages (AM) and/or CD1⁺ dendritic cells, which usually have a lower expression in the normal human lung.

Methods Studies were performed on bronchoalveolar lavage (BAL) fluid collected from 10 untreated allergic subjects and 10 adult normal volunteers. Further controls consisted of five untreated pulmonary sarcoidosis (PS) and four extrinsic allergic alveolitis (EAA) individuals. To ascertain whether T helper 2-type cytokines or allergen influence B7 and CD1 antigen expression, *in vitro* studies were carried out using unprimed (naive) cord blood plastic-adherent monocytes.

Results Cytofluorimetric analysis revealed that AM from asthmatics, unlike those from normal subjects or patients with PS or EAA, overexpressed B7-2, CD1a and, to a lesser extent, B7-1 surface molecules. Immunohistochemical studies confirmed the presence of CD1⁺ dendritic cells in the BAL fluid from asthmatic subjects. On *in vitro* cultured naive cord blood monocytes both purified *Dermatophagoides pteronyssinus* allergen and T-cell cytokines, i.e. IL-4 and granulocyte macrophage colony-stimulating factor, induced surface expression of B7-2 and CD1a receptors, whereas they had no appreciable effect on that of B7-1 membrane molecule.

Conclusions Taken together, these findings support the proposal that airways of atopic individuals are equipped with professional APC that synergize with allergen-specific T cells for the recognition of intact allergens. When the recognition process takes place, asthmatic symptoms could develop in genetically susceptible individuals.

Keywords: asthma, allergens, dendritic cells, co-stimulatory molecules, cytokines, TH₂ monocyte/macrophages

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Introduction

Normal human lung is exposed to large amounts of contaminants and aerodispersed antigen (Ag) and, despite

having normal levels of class II MHC Ag [1] and bactericidal activity, alveolar macrophages (AM) have been shown to be defective for activating resting T lymphocytes and initiating an inflammatory immune response [2]. If this were due to a lack of expression of B7-1 and B7-2/B70 surface molecules [3], which normally recognize CD28 glycoproteins on the T-cell membrane, the co-stimulatory signals

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required for complete T-cell activation would not be provided [4]. Rather than unfavourable, the failure of professional mucosal APC to express co-stimulatory molecules may be advantageous, since the apparent defect limits otherwise harmful immune processes in normal lungs continuously exposed to a wide spectrum of foreign Ag [3]. It might also explain why Ag exposure in the normal airways or gastrointestinal tracts has been shown to cause immunological tolerance [5]. In fact, Ag presentation *in vitro* in the absence of B7-CD28 co-stimulation results in T-cell unresponsiveness or tolerance [5,6]. If the hypothesis holds, then the B7-1 and B7-2/B70 molecules may be overexpressed on the surface of AM in classical pulmonary inflammatory diseases, i.e. sarcoidosis (PS), extrinsic allergic alveolitis (EAA) and, to a greater extent, allergic bronchial asthma (ABA). We have recently demonstrated that, in the latter condition, there is an expansion of allergen-specific $\gamma\delta$ T lymphocytes [7,8], a cell population that identifies allo-antigens either directly, in a manner akin to that of immunoglobulins [9], or in the context of other MHC-related gene products, such as CD1 [10]. Interestingly, CD1-restricted Ag have now been detected in lipid and glycolipid structures [11] that are the major external biochemical component of several aerodispersed allergens.

The aim of the present investigation was, therefore, to search for CD1, B7-1, and B7-2/B-70 Ag expression on the surface of AM isolated from bronchoalveolar lavage (BAL) fluid of untreated subjects with ABA. AM from healthy volunteers and patients with PS or EAA served as controls. In addition, to ascertain whether certain cytokines are potent inducers of CD1 and B7 Ag expression, experiments were carried out using human adherent cord blood monocytes as a source of naive APC. Our results indicate that AM from subjects suffering from ABA, unlike those from normal persons or patients with PS or EAA, overexpress CD1 and B7 co-stimulatory Ag. Moreover, allergens and cytokines, such as IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) upregulate the *in vitro* expression of both CD1 and B7 co-receptors on the surface of naive APC.

Methods

Patients and controls

Ten subjects (five males and five females, age range from 5 to 35 years) with mildly symptomatic chronic asthma were included in the study. At the time of enrolment, all the asthmatic subjects had stable pulmonary function with a FEV₁ 70% of that predicted for their age and height. None were being treated with inhaled or oral corticosteroids, sodium cromoglycate, theophylline or β_2 -agonists. All had increased airway responsiveness to methacholine

(concentration producing a decrease of 20% from baseline in FEV₁ <8 mg/mL) and were positive (monosensitive) at the skin prick tests with purified *Dermatophagoides pteronyssinus* (DP1) allergen extract (Neo Abellò, Madrid, Spain) and for circulating allergen-specific IgE (ELISA assay, DPC Corp., Los Angeles, CA, USA). All patients were non-smokers and were free from upper respiratory tract infection within 8 weeks of investigations. BAL from 10 age- and sex-matched healthy non-atopic, non-smoking volunteers, and adult subjects diagnosed as having PS ($n=5$) or EAA ($n=4$), two pathological conditions known to be associated with increased BAL mononuclear cellular component, overexpanded lung macrophages and pulmonary T-cell populations which display the CD4⁺ and CD8⁺ phenotype, respectively, were also assessed. Informed consent was given by the children's relatives or directly by the adult patients and the clinical research conducted in accord with the local ethical committee guidelines.

BAL fluid collection

Premedication and fiberoptic bronchoscopy were performed as previously reported [12]. Lavage of the medial right lobe was done with 20 mL aliquots of pre-warmed physiological saline. The BAL fluid was chilled on ice until processing, at which time the BAL fluid was pooled and centrifuged at 200 *g* for 20 min. The cell pellet was washed once with PBS and resuspended in RPMI 1640 (Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated FCS, 4 mmol/L L-glutamine, 100 U/mL penicillin and 100 μ g/mL streptomycin (Gibco BRL) (complete medium). The cell pellet was then layered over Percoll (45%) (Pharmacia, Uppsala, Sweden) to remove contaminating lymphocytes and red cells, and centrifuged for 45 min at 800 *g* at 4°C. The cells at the interface were collected, washed twice in PBS, and resuspended in complete medium. This method yielded an 80–85% recovery of cells in the BAL fluid, and >95% of the harvested cells were large granular macrophages. Some of the recovered BAL cells were spun immediately at 200 *g* for 10 min on a Shandon cytocentrifuge, the cytopins were frozen at –20°C and prepared for the immunohistochemical staining procedure after 10 min acetone fixation at room temperature. Binding of MoAb (anti-CD1a, OKT6, IgG1, Ortho, Raritan, NJ, USA) on cytocentrifuge preparations was detected by a three-stage immuno-alkaline phosphatase (APAAP) technique, as previously described [13].

Reagents and flow cytometry

Immunophenotyping was carried out using the following mouse MoAb: fluorescein-conjugated anti-B7-1 and anti-B7-2 (T cell Sciences, Cambridge, MA, USA), purified anti-B-70 MoAb (IgG1, provided by Dr M. Azuma,

Juntendo University, Tokyo, Japan) which recognizes two major, non-overlapping epitopes of the CD28 ligand on the surface of APC and purified anti-OKT6 (Ortho) that identify the 49 kDa CD1a Ag on the surface of human macrophages. Isotype specific goat anti-mouse Ab (Southern, Biotechnology Ass., Birmingham, AL, USA) conjugated with either fluorescein isothiocyanate or phycoerythrin were used as developing reagent for some MoAbs. For staining, 5000–10 000 cells were resuspended in 50 μ L of saline, incubated at 4°C for 30 min, washed and analysed by flow cytometry (FACScan®, Becton-Dickinson, Mountain View, CA, USA). The Lysis II program (Becton-Dickinson) was used to optimize gating of AM or purified cord blood monocytes, and to provide an objective means of excluding debris (non-cellular events due to particulate matter).

Preparation of naive monocyte containing population

Cord blood samples were collected paying attention in choosing donors amongst families in which atopy and/or respiratory allergy history was not present. APC were prepared from heparinized umbilical cord blood (CB, five different samples) by incubating fresh mononuclear cells ($5\text{--}10 \times 10^6$ cells/mL) in 60 mm plastic tissue culture dishes in complete medium for 2 h at 37°C in 7% air humidified CO₂. Non-adherent cells were removed by repeated gentle washings with warm RPMI 1640 containing 5% FCS. The remaining adherent cells (monocytes with small numbers of dendritic cells) were layered over a Percoll density gradient (Pharmacia, Uppsala, Sweden), as previously mentioned, then extensively washed with iced PBS, harvested and resuspended in complete medium at a final concentration of 1×10^6 /mL.

Cell cultures

Purified endotoxin-free DP1 extract was obtained from DPC Corp. (Los Angeles, CA, USA) and used in culture at 10 μ g/mL concentration. In addition, adherent cord blood APC were cultured in the presence of cytokines (rIL-4: 100 U/mL, Amersham Int., Amersham, UK; rGM-CSF: 50 ng/mL, Genzyme, Cambridge, MA, USA) in separate plastic culture plates (Costar, Cambridge, MA, USA) at the concentration of 1×10^6 /mL. Cell phenotype analysis was performed at regular 24 h intervals for 5 days. CD1, B7-1 or B7-2/B70 surface Ag expression was assayed by flow cytometry.

Statistical analyses

Due to the non-normal distribution of samples a non-parametric one-way analysis of variance test was adopted to calculate the differences in B7-1, B7-2/B70 and CD1a

distribution among the various cell populations. Differences in the mean percentages and SEM were calculated by the Mann-Whitney *U*-test. Values of *P* below 0.05 were considered to be significant.

Results

Clinical characteristics and disease history for study participants are given in Table 1. With respect to age dispersion in patients' population, we tried to enrol a comparable set (age-matched) of normal controls. Patients with sarcoid alveolitis or EAA were chosen on the basis of their 'off therapy' status (at least 2 months). AM from healthy control subjects and patients with PS or EAA did not express B7-1 and B7-2/B70 Ag on their surface (Figs 1 and 2). In contrast, untreated asthmatic subjects displayed raised numbers of activated macrophages that expressed both these molecules (B7-1: mean $12.2 \pm 1.9\%$, $P < 0.005$ vs NC, EAA or sarcoidosis; B7-2 mean: 34.6 ± 2.4 , $P < 0.001$ vs NC, EAA or sarcoidosis). Values displayed a certain degree of dispersion that, however, did not correlate with age or sex. The percentage of lung macrophages reactive with the OKT6 (CD1a) MoAb was significantly higher in asthma patients ($42.5 \pm 8.4\%$) than in controls or subjects with PS or EAA (Fig. 3). Data derived from flow cytometric analysis were confirmed by an immunohistochemical staining technique (Fig. 4). This procedure was adopted to verify by morphological criteria if CD1a⁺ cells actually corresponded with dendritic cells. The procedure revealed a positive stain in

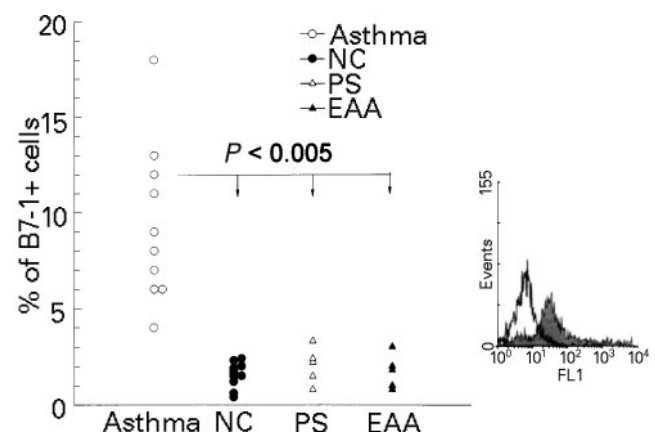


Fig. 1. B7-1 expression on alveolar macrophages (AM) from patients with asthma ($n = 10$) compared with normal volunteers ($n = 10$), as well as with five pulmonary sarcoidosis (PS) and four extrinsic allergic alveolitis (EAA) subjects. Cells were stained with fluorescein conjugated (FTIC) anti-B7-1 MoAb. A FITC goat anti-mouse IgG served as negative control on gated macrophages. Histograms showing the overlay between negative control (line) and a positive sample derived from asthmatic patient (shaded area) are also depicted on the right.

Table 1. Clinical characteristics of study population

Case number	Age	Sex	Skin prick tests*	Diagnosis	Age of onset	Previous therapy**	Disease status
1	35	Male	DP	Mild asthma	28	Sodium cromoglycate	
2	19	Male	DP and DF	Mild asthma	18	none	
3	22	Female	DP	Mild asthma	21	none	
4	5	Female	DP	Mild asthma	5	none	
5	12	Male	DP and DF	Mild asthma	12	none	
6	8	Male	DP	Mild asthma	8	none	
7	18	Female	DP Pariet.	Mild asthma	14	β_2 -agonists	
8	15	Male	DP Pariet.	Mild asthma	14	none	
9	30	Male	DP and DF	Mild asthma	18	β_2 -agonists + steroids	
10	29	Female	DP	Mild asthma	25	β_2 -agonists	
Control 1	29	Female	Negative				
Control 2	5	Female	Negative				
Control 3	8	Male	Negative				
Control 4	12	Male	Negative				
Control 5	18	Female	Negative				
Control 6	15	Male	Negative				
Control 7	30	Male	Negative				
Control 8	35	Male	Negative				
Control 9	19	Male	Negative				
Control 10	22	Female	Negative				
PS 1	65	Female		Sarcoidosis	55	Steroids	Chronic-progressive
PS 2	48	Male		Sarcoidosis	45	Steroids	Chronic-progressive
PS 3	50	Male		Sarcoidosis	40	Steroids	Chronic-progressive
PS 4	36	Female		Sarcoidosis	35	none	
PS 5	42	Female		Sarcoidosis	40	none	
EAA 1	36	Male		EAA	34	None	Subacute
EAA 2	40	Male		EAA	38	None	Chronic
EAA 3	50	Male		EAA	45	None	Chronic
EAA 4	29	Female		EAA	28	None	Subacute

* Skin prick tests were done for *Parietaria officinalis* (Pariet.), *Graminaceae*, *Olea europea*, *Dermatophagoides pteronyssinus* (DP) and *farinae* (DF), *Alternaria tenuis*. Positive results are considered with a weal-and-flare reaction > 10 mm. ** All subjects were off therapy for up 2 months at the time of study enrolment. PS, pulmonary sarcoidosis; EAA, extrinsic allergic alveolitis.

medium-sized cells morphologically resembling dendritic cells, whereas a few true AM from sarcoidosis or EAA patients exhibited a weak intracytoplasmic stain. All samples were examined independently by two observers unaware of the type of monoclonal antibody used. Reported percentages were evaluated in parallel with those derived from cytofluorimetric technique, and no significant discordances in the number of positive cells were recorded. We could not apply the same procedure to the study of B7-1 and B7-2 molecules because they were purchased in the fluorescein-conjugated preparation.

Based on recent evidence which suggests that B7-2 co-signalling preferentially induces naive T cells to develop

into T helper 2 (TH₂) cells [14], we postulated that in genetically susceptible individuals the cytokine-conditioned milieu and/or environmental allergen exposure play a fundamental role in determining the surface phenotype of AM in respiratory allergic disorders. To test the hypothesis that allergens and/or cytokines are able to induce membrane expression of B7-2 and CD1a Ag, we adopted an *in vitro* model based on the use of naive adherent cells collected from human cord blood (CB). Five independent CB samples were used, for this purpose, as a source of naive monocytes. We first evaluated the levels of B7-2 and CD1a on resting adherent CB mononuclear cells and found that $12.1 \pm 1.9\%$ expressed B7-2, while $8.2 \pm 0.9\%$ displayed CD1a molecule.

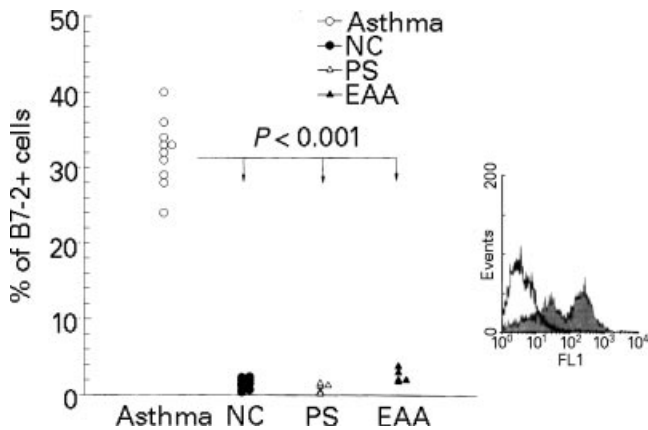


Fig. 2. B7-2 expression on alveolar macrophages (AM) from patients with asthma ($n = 10$) compared with normal volunteers ($n = 10$), as well as with five pulmonary sarcoidosis (PS) and four extrinsic allergic alveolitis (EAA) subjects. Cells were stained with fluorescein-conjugated (FTIC) anti-B7-2 MoAb. A FITC goat anti-mouse IgG served as negative control on gated macrophages. Histograms showing the overlay between negative control (line) and a positive sample derived from asthmatic patient (shaded area) are also depicted on the right.

No significant modifications were noted in the intensity of B7-2/B70 and CD1a Ag expression in unstimulated 5-day cultures (data not shown). In another set of experiments, adherent CB cells were cultured in the presence of 100 U/mL IL-4, 50 ng/mL GM-CSF or 10 μ g/mL purified DP1 extract

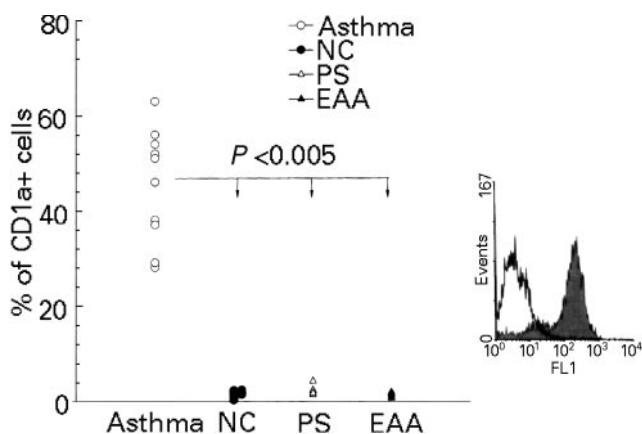


Fig. 3. CD1a expression on alveolar macrophages (AM) from patients with asthma ($n = 10$) compared with normal volunteers ($n = 10$), as well as with five pulmonary sarcoidosis (PS) and four extrinsic allergic alveolitis (EAA) subjects. Cells were stained with anti-OKT6 MoAb, which recognizes group 1 CD1 proteins (i.e. CD1a), and developed with FITC-conjugated goat anti-mouse IgG as second-step antibody. The latter antibody, used alone, served also as negative control (line). Histograms showing the overlay between negative control and a positive sample derived from asthmatic patient (shaded area) are also depicted on the right.

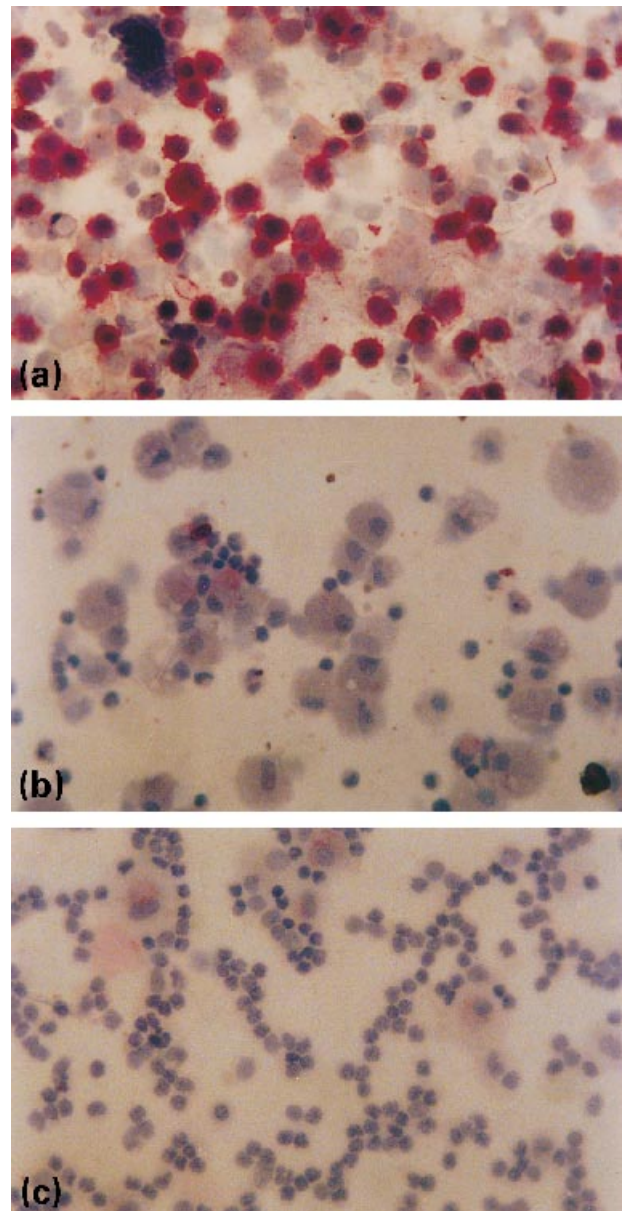


Fig. 4. Immunohistochemical staining of CD1a⁺ dendritic cells. (a) BAL mononuclear cells from a *Dermatophagoides pteronyssinus*-sensitive asthmatic child, were cytocentrifuged, incubated with purified anti-OKT6 (CD1a) mouse MoAb, and further developed with APAAP technique. A significant proportion of alveolar macrophages with the morphology of large granular dendritic cells are positively stained (original magnification $\times 40$). (b) BAL mononuclear cells from a sarcoidosis patient, stained with anti-CD1a MoAb and developed with APAAP technique. As can be seen, very few positive cells (intracellular staining) are present. Original magnification $\times 20$. (c) BAL mononuclear cells from a patient with Extrinsic Allergic Alveolitis stained with anti-CD1a MoAb and developed with APAAP technique. Only a small number of macrophages show a faint positive reaction, whereas the vast majority displays a negative pattern. Original magnification $\times 20$.

and the surface expression of B7-1, B7-2 and CD1a analysed. Time-course experiments (Fig. 5) demonstrated that IL-4 ($P < 0.005$ from basal values at 24 h; $P < 0.05$ at 48 h) and GM-CSF ($P < 0.001$ at 24 h; $P = \text{NS}$ at 48 h) were potent inducers of the B7-2/B70 molecule. Moreover, incubation with DP1 allergen alone boosted B7-2/B70 Ag expression transiently, with a peak at 24 h of culturing ($P < 0.005$). Surface expression of CD1a was also positively modulated by incubation with GM-CSF ($P < 0.005$ at 24 h; $P = \text{NS}$ at 48 h), IL-4 ($P < 0.05$ at 24 h; $P < 0.05$ at 48 h) and DP1 ($P < 0.001$ at 24 h; $P < 0.05$ at 48 h). The molecular modulations were reflected by both a rise in the number of stained cells, and an increase in the logarithm of fluorescence intensity, particularly evident in cells stained with B7-2/B70 MoAb (data not shown). Finally, it is worth noting that B7-1 Ag expression was not appreciably modified by either allergen or TH₂-type cytokines incubation: the percentage of positively stained cells ranged from 0.8 to 5.9%, which did not differ from that seen in basal conditions ($P = \text{NS}$).

Discussion

The mechanisms responsible for the induction of immunological tolerance at the mucosal surfaces have never been fully elucidated. The fact that Ag presentation *in vitro* in the absence of B7/CD28 interaction results in T-cell unresponsiveness or tolerance [15,16], together with the demonstration that expression of the B7-1 and B7-2/B-70 molecules are reduced on the membrane of AM from normal subjects [3], supports the hypothesis that *in vivo* the absence of B7/CD28 interactions prevents the local immune response to inhaled Ag. Thus, Ag-specific responsiveness can be elicited only by lung dendritic cells, which constitutively express surface B7-2 molecules [17] and are present in the peribronchial connective tissue, but not at the epithelial surfaces or in the BAL fluid [3]. In other words, despite their very efficient phagocytic activity, AM seem to mediate a down-regulatory effect on Ag-specific immune processes. In this context, presumably only pulmonary dendritic cells can activate immuno-mediated local inflammation. However, not only do the results presented here fail to sustain this proposal [3], but also dissect between lung inflammatory diseases with different aetiology. Overexpression of B7-1, B7-2/B70 and CD1a glycoproteins seems to be a distinctive feature of macrophages recovered from the BAL of patients with ABA. Sarcoid alveolitis and EAA, which have the clinical and immunological aspects of inflammatory lung disorders and an absolute increase in the number of BAL AM, never displayed these *in vivo* phenotypical characteristics. These data, however, disagree from those recently reported in patients with pulmonary tuberculosis, idiopathic pulmonary fibrosis, *Pneumocystis carinii* pneumonia or sarcoidosis, in which raised B7-2⁺ AM have been reported

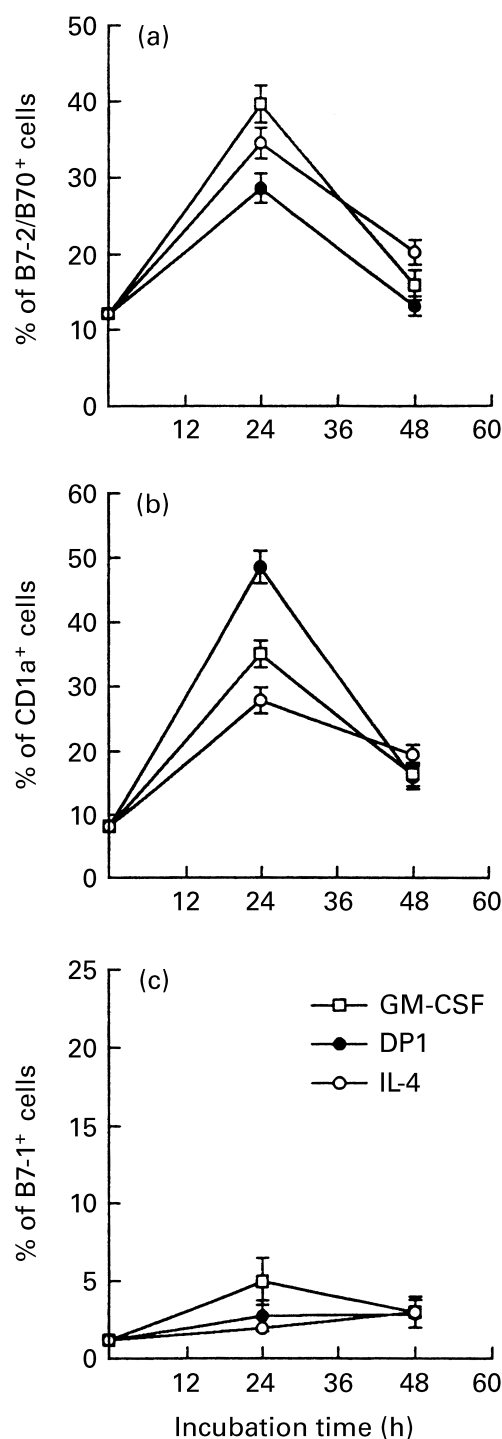


Fig. 5. Modulation of surface expression of B7-2, CD1a and B7-1 Ag on purified adherent cells from naive cord blood mononuclear cells. The incubation with cytokines (IL-4: 100 U/mL; rGM-CSF 50 ng/mL) or DP1 allergen (10 µg/mL) determined the induction of B7-2 and CD1a surface molecules [(a) and (b)] with a peak at 24 h. The lower panel shows that neither allergen, nor cytokines were able to influence the membrane expression of B7-1 Ag. Data, representative of all performed in triplicate on the five different cord blood samples, are depicted as mean $\pm$ sd.

[18]. Undoubtedly, these pathological conditions, particularly sarcoidosis, differ from ABA, where a polarized expansion of T lymphocytes producing TH₂-type cytokines has been reported [19,20]. However, some of the discrepancies may be explained by the small number of sarcoidosis patients included in our study ($n=5$) and in the reported study ($n=4$), as well as by their clinical status, that in our group was represented by relatively short-term followed-up subjects (1–3 years in three out five studied). Another bias may also be represented by therapeutic intervention (steroids) given in three patients, although all drugs were withdrawn at least 2 months prior to study enrollment. Future studies, possibly involving large series of selected patients, are needed to best characterize the activation state of AM in sarcoidosis.

As CD28/B7 co-stimulation has been recently demonstrated to play a key role in the generation of TH₂ immune responses [14,21], the presence of B7⁺ APC in the bronchial mucosa of asthmatic subjects is likely to be of functional relevance [22]. In fact, the recent demonstration, in the murine model of allergic asthma, that blockade of co-stimulation with CTLA4-Ig, a fusion protein known to prevent co-stimulation by blocking CD28:B7 interactions, inhibits airway hyperresponsiveness, inflammatory infiltration and allergen-specific responsiveness of thoracic T cells seems to strongly support this concept [23,24]. Despite these evidences, a few studies exist in humans on the role of B7 co-stimulatory molecules in TH cell development. Using peripheral blood mononuclear cells and T-cell clones, Bashian *et al.* have recently demonstrated that blocking B7-2 molecules (with an anti-CD86 MoAb) down-regulates both proliferation and cytokine gene expression induced by ragweed and tetanus toxoid antigens [25]. These data, although on *in vitro*-stimulated peripheral blood mononuclear cells, argue against a definite role of these molecules in influencing the functional cytokine secretion by differentiated, antigen-specific T cells. Lung APCs differ substantially from those of peripheral blood. We have previously demonstrated the functional effectiveness of pulmonary APC in asthmatics to present specific allergen to locally expanded T lymphocytes [8]. Experiments are in progress in our laboratory in order to assess the functional capacity of CTLA4-Ig fusion protein at inhibiting BAL T-cell/APC interactions *in vitro* following stimulation with specific allergen.

The presence of pulmonary CD1⁺ dendritic cells in asthmatic subjects, but to a lesser extent in normal controls [26] or in patients with PS or EAA, is intriguing. Group 1 CD1 proteins (CD1a, -b and -c) are expressed on a variety of specialized APC, including major populations of dendritic cells in lymphoid and non-lymphoid tissues [27]. They are also inducible *in vitro* on virtually all circulating human monocytes following exposure to GM-CSF. These data

suggest that such surface molecules could be up-regulated on tissue macrophages in many inflammatory lesions. CD1 proteins may be Ag-presenting molecules that have evolved the ability to present non-peptide antigens to T cells [28]. It is worth noting that many of the airborne dispersed pollens responsible for allergic rhinitis and/or asthma exhibit an external glycolipidic monolayer enriched in phospholipids (Spinozzi & Corazzi, unpublished observations). If CD1 proteins are ligands for complex lipidic or glycolipidic Ag, then allergic subjects are equipped with mucosal resident cells which selectively bind and present the intact airborne pollen to allergen-specific T cells. Our results support these conclusions since both allergen and TH₂-type cytokines up-regulated the *in vitro* expression of CD1a and B7-2 Ag on the surface of naive CB APC, whereas they had little or no effects on B7-1 expression. On the other hand, recent data from experimental animal models suggest that there is a transient but significant increases (up to threefold those of resting tissue) in intraepithelial airway DC that follow the local triggering with bacterial cell wall (i.e. glycolipidic) extracts [29]. There is only one population among the resident mucosal T lymphocytes which is able to recognize allergen non-peptide external structures and that is the $\gamma\delta$ T lymphocytes, which *in vivo* express a TH₂-type cytokine profile (e.g. IL-5, IL-6 [30] and IL-4 [18], but not TH₁ cytokines) and *in vitro* respond to specific allergen stimulation [8,18]. Activated (B7-2/B70⁺) CD1⁺ dendritic cells, together with allergen-specific $\gamma\delta$ T lymphocytes may, therefore, represent a distinctive pattern of reactive cells present at the mucosal surfaces of allergic subjects [31]. In this context activated macrophages only stimulate allergen-specific T cells, since no response could be obtained with unrelated allergens in our *in vitro* experimental conditions [18]. Another group has recently reported a cytokine-conditioned modulation of B7 co-stimulatory molecule expression on adult normal monocytes that differs from the one we describe on naive adherent cultured mononuclear cells. In particular, IL-4 down-modulated B7-2 and moderately up-regulated B7-1 expression on adult CD14⁺ monocytes [32]. However, the experimental system we used in this study (culturing naive adherent monocytes with cytokines or allergen) may be a more reliable tool for investigating the effects that both cytokines and allergens exert on B7 surface expression. In addition, since they are presumably activated *in vivo*, results from adult monocytes are more difficult to interpret than those obtained from naive CB counterparts. On the other hand, since the source of our naive APC were cord blood MC from non-atopic parents, results obtained in experiments with allergen incubation deserve additional comments. In fact, the observed rise in B7-2⁺ cells in cultures triggered with DP1 alone was only transient and the percentages of positively stained cells decreased at 48 h near to basal values. Apart from the functional

differences among circulating and resident APC [33], it seems that allergen *per se* is a weak stimulus in the up-regulation of these molecules, but otherwise in parallel with the cytokine milieu at priming (a fact that is undoubtedly genetically determined [34]) allergen can positively and long-lastingly up-regulate surface B7-2 expression. Classical experiments performed on peripheral blood DC precursors have demonstrated the synergistic positive effects of GM-CSF and IL-4 in favouring the fully functional DC maturation *in vitro* [35].

In conclusion, the data presented here may reflect the dynamics of the interactions between airborne allergens, TH₂-type cytokines and APC which occur *in vivo* in the respiratory tract of patients with allergic asthma. The recognition of intact specific allergen at mucosal surfaces, rather than complex intracellular processing in the regional lymph nodes, may play an important role in the exacerbation of asthma.

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