



CD80 and CD86 Expression on LPS-Stimulated Monocytes and the Effect of CD80 and CD86 Blockade on IL-4 and IFN- γ Production in Nonatopic Bronchial Asthma

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Abstract. CD80 and CD86 seem to play an important role in the allergen-induced secretion of interleukin (IL)-5 and IL-13. Up to now, the expressions of CD80 (B7.1) and CD86 (B7.2) on monocytes and the kinetics of the expression of these molecules on lipopolysaccharide-stimulated monocytes in nonatopic asthma have not been defined. Using monoclonal antibodies, we have compared the expressions of CD80 (B7.1) and CD86 (B7.2) on the monocytes of healthy persons and nonatopic asthmatic patients. We have also assessed the effect of CD80 and CD86 inactivation on IL-4 and interferon (IFN)- γ production in nonatopic asthmatics and healthy subjects. We found that a low expression of CD80 (1.64 ± 0.65 vs. $3.53 \pm 1.43\%$) and a moderate expression of CD86 (41.25 ± 13.4 vs. $49.46 \pm 11.49\%$) on the studied monocytes were characteristic for asthma. In nonatopic asthma patients inactivation of CD80 or CD86 blockade significantly reduced IFN- γ production by T lymphocytes ($p < 0.02$; $p < 0.03$). In both the studied groups, anti-CD80 antibodies did not diminish T lymphocyte production of IL-4. However, anti-CD86 antibodies significantly ($p < 0.04$) reduced the IL-4 concentration in culture supernatants. Our results confirm that both the CD80 and CD86 molecules play an important role in the maintenance and amplification of the inflammatory process. It suggests that in the inflammatory process that occurs in nonatopic bronchial asthma, Th1 as well as Th2 lymphocytes are equally important.

Key words: CD80 (B7.1); CD86 (B7.2); IL-4; IFN- γ ; monocytes; lymphocytes; nonatopic bronchial asthma.

Introduction

In two different studies, marked increases in the number of CD68⁺ macrophages and granulocyte-macrophage colony-stimulating factor receptor α (GM-CSFR α) mRNA-positive cells were observed in biopsies taken from nonatopic compared with atopic asthma patients^{20, 29}. This may suggest some form of macrophage dysfunction in nonatopic bronchial as-

thma^{19, 20, 29}. Macrophages, as monocytes, dendritic cells and B cells, are known to be professional antigen-presenting cells (APCs)^{25, 39}. The important role of the co-stimulatory molecules CD80 (B7.1) and CD86 (B7.2) expressed on alveolar macrophages in allergen presentation to T helper (Th)2 lymphocytes in allergic bronchial asthma, hypersensitivity pneumonitis, and interstitial lung diseases is well established^{2, 6, 22, 27}. Similarly, expression of CD86 on monocytes is significantly

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increased on cells cultured in the presence of antigen in allergic rhinitis subjects and Crohn's disease^{31, 40}. The process of T lymphocyte activation, proliferation and cytokine production requires the engagement of the T cell receptor (TCR) with a major histocompatibility complex II/peptide complex and cross-linking of CD80 (B7.1) and CD86 (B7.2) co-stimulatory molecules, on APCs with CD28 or cytotoxic T lymphocyte antigen 4 (CTLA-4) on the T cell surface^{4, 8, 11, 14}.

CD80 and/or CD86 are important in allergen-induced secretion of interleukin (IL)-5 and IL-13^{23, 24, 30}. Both CD80 and CD86 are able to co-stimulate IL-4, IL-5 and interferon (IFN)- γ production in the murine model of allergic pulmonary inflammation^{33, 34}. CD80 and CD86 play important roles in the differentiation of Th1 or Th2 phenotypes^{32, 38}. Both molecules act complementary in inducing development of allergic pulmonary infiltration, but CD86 seems to play a more important role in allergic reactions and atopic asthma^{24, 32, 33}.

One cannot rule out the possibility that abnormal monocytes/macrophages activation is connected with impaired expression of CD80 and CD86. Unfortunately there is no evidence of the role of these co-stimulatory molecules in nonatopic (intrinsic) asthma. The expression of CD80 and CD86 on monocytes freshly isolated from peripheral blood and the kinetics of these co-stimulatory molecules' expression on lipopolysaccharide (LPS)-stimulated monocytes in nonatopic asthma have not yet been defined. We wanted to know whether IL-4 and IFN- γ production in blood mononuclear cell cultures changed in the presence of anti-CD80 or/and anti-CD86 antibodies. To conduct our studies we have used peripheral blood monocytes or cultures of monocytes and/or lymphocytes from nonatopic asthmatics and healthy subjects supplemented with anti-CD80 or anti-CD86 monoclonal antibodies.

Materials and Methods

Subjects. The study was performed on 42 patients with moderate nonatopic bronchial asthma (20 females, 22 males, mean age 43 ± 11.2 years). All of the patients were treated in the Department of Allergology and Internal Medicine of the University Hospital, Białystok, Poland. Each patient had suffered from bronchial asthma for from 12 to 26 years before the study. The diagnosis of nonatopic bronchial asthma was established in each case according the NHLBI/WHO criteria²¹. Spirometry measurements were performed according to American Thoracic Society/European Respiratory Society (ATS/ERS) criteria with commercially available Pneumoscreen II (Erich Jaeger GmbH, Wurzburg, Germany). Mean resting spirometric parameters in the bronchial asthma group indicated moderate obstruction: $FEV_1 = 62.7 \pm 6.6\%$, $FEF_{25-75} = 54.7 \pm 9.1\%$, $FVC = 80.4 \pm 9.1\%$ of predicted values. At least 20% reversibility of FEV_1 was achieved in each patient after salbutamol inhalation (400 μ g). All patients were non-smokers, had no history of atopy, had serum immunoglobulin (Ig)E within the normal range (34.1 ± 18.6 IU/ml), and negative skin-prick tests to a panel of common allergens (house dust mite, grass pollen, tree pollen, cat and dog). Patients who had received any systemic corticosteroid therapy within the preceeding 4 weeks were excluded from the study. Other medications such as inhaled long-acting β -mimetics, inhaled corticosteroids or oral slow-released theophylline were stopped 24 h before blood sampling. At the time of the study the patients presented no physical signs of asthma exacerbation and did not suffer from any infection (controlled by total leukocyte count, ESR and serum CRP levels).

The control group consisted of 22 healthy individuals, non-smokers (12 females, 10 males, mean age

Table 1. Flow cytometric evaluation of the peripheral blood components in healthy persons and nonatopic asthmatic patients (mean value $\pm$ SD)

	Healthy persons (n=22)	Nonatopic asthmatics (n=42)
WBC ($\times 10^9/l$)	5.90 ± 1.39	6.67 ± 1.52
Monocytes (%)	6.48 ± 1.39	7.04 ± 1.76
Lymphocytes (%)	38.40 ± 4.77	30.40 ± 5.72
CD3 ⁺ T lymphocytes (%)	70.84 ± 4.04	68.38 ± 5.83
CD4 ⁺ Th lymphocytes (%)	44.15 ± 13.89	49.60 ± 7.59
CD8 ⁺ T suppressor lymphocytes (%)	26.10 ± 6.09	16.70 ± 6.08
CD19 ⁺ B lymphocytes (%)	9.82 ± 2.83	7.86 ± 2.98
CD3 ⁺ -HLA-DR ⁺ activated T lymphocytes (%)	5.64 ± 1.89	$8.65 \pm 3.03^*$
CD3 ⁺ CD16 ⁺ CD56 ⁺ NK cells (%)	12.65 ± 4.94	11.56 ± 4.43

* $p < 0.001$.

40.8±11.9 years). All of them had no history of asthma, allergy or other systemic disease within the last 2 years, had normal serum total IgE concentrations (27.3±15.3 IU/ml), negative skin-prick tests and normal FEV₁ (mean 97.5±8.19%), FEF_{25–75} (mean 106.3±12.45%) and FVC (mean 96.45±8.8%) of predicted values.

All of the studied individuals had no significant changes in morphological blood parameters, except for the CD3⁺-HLA-DR⁺ T cell subpopulation (Table 1). A full blood cell count, including an automated differential count, was performed with a Coulter MAXM counter. Informed written consent was obtained from all the subjects before participation. The study was approved by the Ethics Committee of the Medical University in Białystok.

Peripheral blood mononuclear cell preparation. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by density gradient centrifugation on Lymphoprep (Nycomed, Birmingham, UK) according to BOYUM⁵.

CD80 and CD86 expression in monocyte cultures. Fresh CD14⁺ monocytes were separated by negative magnetic depletion using hapten-conjugated CD3, CD7, CD19, CD45RA, CD56, and anti-IgE monoclonal antibodies (mAbs) (Monocyte Isolation Kit; cat. no. 130053301) in an LS Column (cat. no. 13004042401; Miltenyi Biotec, Germany)³⁵ for the magnetic cell separator (MACS) according to the manufacturer's instructions, routinely resulting in >95% purity of CD14⁺ cells. The viability of these cells, assessed with trypan blue exclusion, was equal to or greater than 95%. Monocytes at a cell density of 1 × 10⁶/ml were centrifuged and suspended in culture medium enriched with 10 µg/ml LPS *Escherichia coli* 0111: B4 serotype (Sigma Chemicals, USA). The cells were cultured in tissue culture plates (Falcon, USA). CD80 and CD86 expression was cytometrically assessed on freshly isolated (0 h) and activated monocytes after 6, 24 and 48 h of culture.

Effect of CD80 (B7.1) or CD86 (B7.2) blockade on the production of IL-4 and IFN-γ. Negative magnetic depletion using a cocktail of hapten-modified CD8, CD11b, CD16, CD19, CD36 and CD56 antibodies (CD4⁺T Cell Isolation Kit; cat. no. 130053101; Miltenyi Biotec, Germany) in an LS Column for the MACS was used to obtain CD4⁺ T lymphocytes³⁵.

The effects of CD80 or CD86 blockade on the production of IL-4 and IFN-γ were examined in lymphocyte (cell density 1 × 10⁶/ml) and 24 h LPS-preincubated monocytes (2 × 10⁵/ml) cultures. The cells were supplemented with 10 µl of anti-CD3 mAbs (R&D Systems Inc., Germany) or 10 µg PHA (Difco, USA), and

10 µg/ml anti-CD80 (hB7.1) mAbs (R&D Systems Inc., Germany) or 5 µg/ml anti-CD86 (hB7.2) mAbs (R&D Systems Inc., Germany). A 5:1 ratio of CD4⁺ T cells and monocytes was used on the basis of published data^{6,13}. The cells were cultured for 24 h. Supernatants were collected and stored at -70°C.

Flow cytometry analysis of cultured monocytes. The 200 µl of cultured monocytes was stained with 10 µl of two-color mAbs anti-CD14 (PE)/anti-CD80 (FITC) or anti-CD14 (PE)/anti-CD86 (FITC). After 30 min of incubation at 4°C, the samples were washed and suspended in PBS enriched with 2% fetal calf serum and 0.2% sodium azide (NaN₃). An minimum of 10⁴ monocytes was analyzed by EPICS XL flow cytometer (Coulter, USA). As a negative isotypic control, mouse IgM and IgG₁ mAbs supplied by the same manufacturer were used.

Quantitation of cytokine production. Culture supernatants were stored at -70°C for all subjects until cytokine measurement. The lymphocyte production of IL-4 and IFN-γ were measured using commercially available immunoassay kits from ELISA Quantikine (R&D System Inc., USA). The procedure was performed strictly according to the manufacturer's instructions. Measurements were done in triplicate and mean values were used for further statistical evaluation. The sensitivities of IL-4 and IFN-γ detection were respectively 3 pg/ml and 5 pg/ml.

Statistical analysis. Statistical analysis was performed using the U-Mann-Whitney and Wilcoxon paired test when appropriate; p values less than 0.05 were considered statistically significant. All statistical analysis was performed using Statistica 5.0 for MS Windows.

Results

Freshly isolated peripheral blood monocytes from patients with nonatopic bronchial asthma and healthy controls were analyzed by flow cytometry for the expression of CD80 (B7.1) and CD86 (B7.2). As shown in Table 2, CD80 expression on monocytes was equal 1.64±0.65% in the control group, and 3.53±1.43% in asthmatics. CD80 expression on freshly isolated monocytes in the group of nonatopic asthma patients was significantly higher compared to healthy volunteers (p<0.001), which suggests stronger activation of monocytes in asthma.

CD86 co-stimulatory molecules were present on 41.25±13.41% of the monocytes received from healthy individuals and 49.46±11.49% of the cells from nona-

	CD80 (B7.1)							
	healthy persons (n=10)				nonatopic asthmatics (n=10)			
	0 h	6 h	24 h	48 h	0 h	6 h	24 h	48 h
	1	2	3	4	5	6	7	8
Mean value ± SD (% CD80 ⁺ cells)	1.79±0.46	37.1±11.48	60.39±11.88	42.48±16.1	3.83±1.21	21.58±8.5	43.23±17.3	35.75±12.9
Increase of CD80 mean values	–	21 ×	34 ×	24 ×	–	6 ×	11 ×	9 ×
Wilcoxon test		p ₁₋₂ <0.006,	p ₁₋₃ <0.006,	p ₁₋₄ <0.006,	p ₅₋₆ <0.006,	p ₅₋₇ <0.006,	p ₅₋₈ <0.006	
U-Mann-Whitney test		p ₁₋₅ <0.002,	p ₂₋₆ <0.005,	p ₃₋₇ <0.04,	p ₄₋₈ >0.05			

Table 4. Kinetics of CD86 (B7.2) expression on LPS-stimulated human monocytes

	CD86 (B7.2)							
	healthy persons (n=10)				nonatopic asthmatics (n=10)			
	0 h	6 h	24 h	48 h	0 h	6 h	24 h	48 h
	1	2	3	4	5	6	7	8
Mean value $\pm$ SD (% CD86 ⁺ cells)	43.57 $\pm$ 8.97	82.55 $\pm$ 13.01	73.53 $\pm$ 12.74	61.04 $\pm$ 13.70	47.32 $\pm$ 12.42	87.26 $\pm$ 9.95	76.62 $\pm$ 14.56	57.32 $\pm$ 12.36
Increase of CD86 mean values	–	1.9 $\times$	1.7 $\times$	1.4 $\times$	–	1.8 $\times$	1.6 $\times$	1.2 $\times$
Wilcoxon test	$p_{1-2}<0.006$, $p_{1-3}<0.006$, $p_{1-4}<0.02$, $p_{5-6}<0.006$, $p_{5-7}<0.006$, $p_{5-8}<0.02$							
U-Mann-Whitney test	$p_{1-5}>0.6$, $p_{2-6}>0.7$, $p_{3-7}>0.5$, $p_{4-8}>0.4$							

Table 5. The effect of CD80 (B7.1) and CD86 (B7.2) inactivation on IL-4 production by monocyte/lymphocyte cultures

	IL-4 concentration in cultures of monocytes and lymphocytes					
	healthy persons (n=22)			nonatopic asthmatics (n=42)		
	anti-CD3	anti-CD3 + anti-CD80	anti-CD3 + anti-CD86	anti-CD3	anti-CD3 + anti-CD80	anti-CD3 + anti-CD86
	1	2	3	4	5	6
Mean value $\pm$ SD (pg/ml)	630 $\pm$ 171	725 $\pm$ 148	523 $\pm$ 136	718 $\pm$ 178	742 $\pm$ 143	659 $\pm$ 138
U-Mann-Whitney test	$p_{1-2}<0.07$, $p_{1-3}<0.04$, $p_{1-4}<0.06$, $p_{2-5}<0.8$, $p_{4-5}<0.07$, $p_{3-6}<0.002$, $p_{4-6}<0.04$					

Table 6. The effect of CD80 (B7.1) and CD86 (B7.2) inactivation on IFN- γ production by monocyte/lymphocyte cultures

	IFN- γ concentration in cultures of monocytes and lymphocytes					
	healthy persons (n=22)			nonatopic asthmatics (n=42)		
	anti-CD3	anti-CD3 + anti-CD80	anti-CD3 + anti-CD86	anti-CD3	anti-CD3 + anti-CD80	anti-CD3 + anti-CD80
	1	2	3	4	5	6
Mean value $\pm$ SD (pg/ml)	2926 $\pm$ 1122	2368 $\pm$ 908	3317 $\pm$ 1465	2172 $\pm$ 875	1795 $\pm$ 896	2858 $\pm$ 1381
U-Mann-Whitney test	$p_{1-2}<0.09$, $p_{1-3}<0.6$, $p_{1-4}<0.01$, $p_{2-5}<0.02$, $p_{3-6}<0.3$, $p_{4-5}<0.04$, $p_{4-6}<0.03$					

As illustrated in Table 6, inactivation of the CD86 molecule on monocytes in PBMC (lymphocyte/monocyte ratio 5:1) cultures by the addition of saturated amounts of a blocking mAb resulted in inhibition of IFN- γ production in both healthy (2926 $\pm$ 1122 vs. 2368 $\pm$ 908 pg/ml) and nonatopic asthmatic individuals (2172 $\pm$ 875 vs. 1795 $\pm$ 896 pg/ml). IFN- γ production was significantly diminished only in cultures of asthmatics' lymphocytes ($p_{4-5}<0.04$). Treatment with anti-CD80 mAb led to a significant ($p_{2-5}<0.02$) decrease in IFN- γ concentration of nonatopic asthma patient culture supernatants (1795 $\pm$ 896 pg/ml). In contrast to IFN- γ concentration in the asthma group, lymphocytes of healthy persons still produced high amounts of IFN- γ (2368 $\pm$ 908 pg/ml). Finally, IFN- γ secretion was significantly increased only in cultures of nonatopic asthma lymphocytes affected by added anti-CD86 antibodies ($p_{4-6}<0.03$).

Discussion

In bronchial asthma, cells of monocyte/macrophage lineage participate in the production of proinflammatory cytokines and chemokines, especially in exacerbation of the disease^{20, 36, 37, 47}. POULTER and BURKE⁴² conclude that the dysregulation of the immune response in asthma is related to a dysfunction in T cell/macrophage interaction. Monocytes and macrophages are necessary for the generation of cell-mediated responses by processing and presenting antigen to antigen-specific lymphocytes. Antigen presentation and T cell activation requires a second signal, which is given by co-stimulatory molecules^{11, 14, 39}. It is believed that higher expression of co-stimulatory molecules on activated monocytes/macrophages occurs as a result of endogenous stimulation^{6, 42}.

We have confirmed that in nonatopic bronchial asthma, the CD80 and CD86 co-stimulatory molecules on

freshly isolated and LPS-stimulated monocytes are over-expressed in comparison with the control group. Our data show similarly low CD80 expression and high numbers of CD86-positive resting monocytes in all our subjects when compared with other studies conducted on healthy persons^{9, 10, 12, 43}. The proportion of CD80/CD86-positive monocytes in our study is very similar to results of investigations in which macrophages or B, or T cells received from patients with allergic asthma, allergic rhinitis or atopic dermatitis were assessed^{2, 6, 39, 40, 41}.

In our study, expression of both co-stimulatory molecules on freshly isolated peripheral blood monocytes from nonatopic asthma patients (in contrast to healthy persons) was significantly increased ($p > 0.001$). The higher expression of these molecules on APC increases the ability of monocytes to present antigen to lymphocytes and suggests that both CD80 and CD86 may upregulate the inflammatory process in nonatopic bronchial asthma.

CD86 expression on resting monocytes in our study was equal to about 40% and was a few times higher than CD80 molecules expression on these cells. CD86 were induced on LPS-stimulated monocytes earlier than were CD80 molecules and reached maximal values after 6 h of culture.

This is in accordance with results of other investigators, which show that CD86 plays an important role in the early inflammatory reactions affecting the production of Th2 cytokines IL-4, IL-5, IL-13, IL-10 and Th2 proliferation^{18, 24, 26, 32, 41}. HACZKU et al.¹⁶ suggested that CD86 molecules dominate not only at the early phase of allergic inflammatory reaction but can also modulate and chronically maintain this process.

It is generally accepted, except by BURASTERO et al.⁶ and HARRIS et al.¹⁷, that the role of CD80 (B7.1) is less important in the induction of the allergic inflammatory process^{28, 32, 44, 46}.

In our opinion, in nonatopic bronchial asthma both CD80 and CD86 could be involved in the pathomechanism of CD4⁺ lymphocyte dysfunction. The kinetics of CD80 expression on LPS-stimulated monocytes confirms that these co-stimulatory molecules are probably more important in the late period of inflammation, because we could observe maximal expression not before 24 h after stimulation. The later appearance of CD80 may prolong or increase the intensity of co-stimulatory signals delivered by the APC to the CD4⁺ T cells.

CD80 induction on APCs mainly affects Th1 lymphocyte proliferation and activity^{1, 3, 15, 33}. Th1 cells are important in cellular response, in the late phase of inflammatory reactions. These CD4⁺ lymphocytes pro-

duce IFN- γ , a proinflammatory cytokine, which can diminish the activity of Th2 cells by biofeedback mechanism^{1, 7, 45}. The increased ratio of CD80-positive non-stimulated monocytes in nonatopic asthmatics and the lower CD80 expression on LPS-stimulated monocytes are further evidence that in nonatopic bronchial asthma both the CD80 and CD86 molecules have a specific role in the induction and maintenance of the disease.

CD80 blockade in our experiments upregulated IL-4 concentrations in lymphocyte culture supernatants in both the healthy and nonatopic asthma groups, but the increase was not statistically significant ($p_{1-2} > 0.05$, $p_{4-5} > 0.05$, respectively). The treatment of these cultures with anti-CD80 mAbs also significantly diminished IFN- γ production in the nonatopic asthma group ($p_{4-5} < 0.04$). As the IL-4 concentration did not increase significantly and the IFN- γ production was reduced, we suggest that CD80 blockade mainly affected Th1 lymphocytes.

In conclusion, our results confirm that monocytes are involved in the pathogenesis of nonatopic bronchial asthma and both the CD80 and CD86 co-stimulatory molecules play an important role in the maintenance and amplification of the inflammatory process observed in airways. This suggests that in the inflammatory process that occurs in nonatopic bronchial asthma, Th1 as well as Th2 lymphocytes are equally important.

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