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Cytokine regulation of low-affinity IgE receptor (CD23) on monocytes from asthmatic subjects

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SUMMARY

The regulation of CD23 expression (FcεRII) by cytokines on monocytes from normal subjects, asymptomatic and acute asthmatics was investigated. CD23 was weakly expressed on cells from controls, but was significantly enhanced in the two groups of asthmatics. The addition of IL-4 on monocytes induced an increase of CD23 expression in cells from controls and asthmatics. Interferon-gamma (IFN-γ) did not modulate CD23 expression in asthmatics or control subjects, while high doses of IL-6 (2000 U/ml) enhanced CD23 expression on cells from asthmatics or controls. *In vitro* stimulation of monocytes with Timothy grass pollen allergen did not enhance CD23 receptor in asthmatics with a positive skin test to this pollen. We speculate that CD23 expression in asthmatics is markedly enhanced by Th2-dependent cytokines, such as IL-4 and IL-6. Thus, the regulation of Th2 cell activation by anti-cytokine therapy could have an important effect on the down-regulation of CD23 on monocytes, and in shifting a Th2 subpopulation into a Th1 subpopulation by blocking Th2-dependent cytokines.

Keywords macrophages monocytes asthma low affinity IgE receptor cytokines

INTRODUCTION

The low-affinity receptor for IgE and its soluble fragment IgE binding factor (IgE-BF) are multifunctional molecules. They regulate IgE synthesis [1,2], autocrine growth of B cells [3] and thymocyte maturation [4]. This receptor, FcεRII, has recently been cloned and its structure has been defined [5,6]. CD23 is a 45-kD glycoprotein that cleaves into soluble fragments. Two molecular forms of CD23, FcεRIIa and FcεRIIb, have been previously described and are known to differ only in the N-terminal cytoplasmic region. FcεRIIa is expressed by B cells and FcεRIIb by monocytes [7].

In allergic disease the immediate reaction is related to antigen interaction with FcεRI present on mast cells [8]. This event is characterized by release in the microenvironment of various inflammatory mediators such as histamine, leucotriene B_4, and platelet-activating factor, which contributes to acute bronchoconstriction. Yet the events involved in the late-phase response to the allergen [8,9], in which inflammatory cells such as B lymphocytes, macrophages and eosinophils seem to be involved, are unclear. These cells play an important role after allergen-specific activation, through the expression of low-affinity IgE receptors [10–13]. Several studies have been performed to investigate the expression of CD23 on B lymphocytes [14,15], mononuclear cells [16,17] and alveolar macrophages [10], from normal and asthmatic subjects. It has been previously reported that CD23 expression on B cells and monocytes is modulated by IL-4, which also regulates CD23 synthesis [14,15]. Conflicting data have been reported on modulation of CD23 by granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-gamma (IFN-γ) or IFN-α. Some authors have shown that GM-CSF is not able to modulate CD23 [17] on monocytes from normal subjects, but more recently regulation of CD23 by this cytokine has been demonstrated [18]. Modulation by IFN-γ is also controversial; some studies show that IFN-γ induces CD23 expression on monocytes [19], while Vercelli *et al.* failed to induce CD23 receptor with either IFN-γ or IFN-α [17,20]. However, it has been well established that IL-4 is a potent inducer of CD23 on monocytes [16,17]. Major studies in this field have employed monocytes from normal subjects, but little is known about modulation of this receptor in allergic asthma.

Recent data show that CD23 expression on monocytes and alveolar macrophages from asthmatic subjects constitutively express high levels of CD23 [21], but in these patients cytokine regulation of this receptor is unclear. To clarify this point, we investigated CD23 expression on monocytes in healthy donors,

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Table 1. Clinical details

	Subjects (*n*)	M/F	Age (years)	Duration of asthma (years)	FEV_1 (% predicted)
Asthmatic subjects					
with respiratory symptoms	9	4/5	31·1 ± 18·6*	9·7 ± 7·7	61·3 ± 24·2
without respiratory symptoms	10	5/5	24·5 ± 10·2	6·8 ± 6·7	117·0 ± 12·0
Normal subjects	10	5/5	32·4 ± 9·8	—	113·0 ± 15·8

* Mean ± s.d.

asymptomatic asthmatics and asthmatics with acute asthma, to understand whether this receptor is regulated by cytokine treatment, since a comprehensive understanding of CD23 modulation could be achieved with more appropriate immunotherapeutic protocols in the bronchial asthma syndrome.

SUBJECTS AND METHODS

Study population

Our study included 10 healthy donors and 19 asthmatic subjects. All asthmatics, nine of whom were males, had strongly positive skin tests for grass pollen. Asthmatic symptoms were seasonal, occurring during spring. Nine asthmatics had respiratory symptoms and a variable degree of airway obstruction, the other 10 were observed during an asymptomatic period and had normal spirometric values. The nine asthmatic subjects with acute asthma had a mean percentage predicted FEV_1 of 61·3 (s.d. 24·2). The characteristics of the study population are reported in Table 1.

Reagents and media

RPMI 1640 medium and fetal bovine serum (FBS) were obtained from Eurobio Laboratories (Paris, France). Endotoxin lipopolysaccharide from *Escherichia coli* 055 : 135 (LPS) was obtained from Difco Laboratories (Detroit, MI). Human recombinant IL-4 and human recombinant IL-6 were purchased from GIBCO BRL (Grand Island, NY) and mouse monoclonal anti-human low-affinity Fc-IgE receptor (CD23) FITC conjugate was purchased from Biosource International (Camarillo, CA). Human recombinant IFN-γ and mouse anti-human IL-6 were purchased from Boehringer Mannheim Biochemica (Milan, Italy). Tumour necrosis factor-alpha (TNF-α) and rabbit anti-human TNF polyclonal antibodies were purchased from Genzyme Corp. (Boston, MA). Ficoll–Hypaque solution was purchased from Pharmacia Fine Chemicals (Piscataway, NJ). Timothy grass (*Phleum pratense*) pollen was purchased from Balfer (Garbagnate, Italy).

Preparation of peripheral blood monocytes

Peripheral blood monocytes (PBM) were obtained as previously described [22]. Briefly, heparinized venous blood, obtained from healthy donors or from asthmatic subjects, was diluted with RPMI 1640 plus 5% FBS (hereafter referred to as cRPMI). Mononuclear cells were separated by Ficoll–Hypaque density gradient centrifugation. Cells were washed twice in cRPMI, plated in cell culture Petri dishes (Nunc, Naperville, IL) at a concentration ranging between 2×10^6 and 3×10^6/ml in cRPMI and incubated at 37°C in 5% CO_2 for 1 h. Non-adherent cells were removed by washing the dishes three to five times with warm RPMI 1640 medium, and the adherent cells were carefully removed using a rubber policeman. The latter cells were 95–98% positive to esterase staining, and cell viability was assessed by trypan blue dye exclusion test (more than 98% viable). Cells were harvested by low speed centrifugation (100 *g*) and diluted to 1×10^6/ml for flow cytometric analysis.

Flow cytometry analysis

Human monocytes (1×10^6) in cRPMI, untreated or treated with cytokines or Timothy grass pollen, were incubated for 72 h at 37°C in 5% CO_2. The cultured cells were washed twice in PBS containing 0·5% bovine serum albumin (BSA) and 0·4% sodium azide. Cells (1×10^6) in 50 μl were mixed with 10 μl of CD23-FITC conjugate. After 45 min of incubation on ice, the cells were washed three times and fluorescence intensity was analysed on a FACS (Becton Dickinson, San Jose, CA).

Production of PBM culture supernatants and assaying for TNF activity

Supernatants from PBM were obtained according to a previously described method [22]. Briefly, PBM were seeded at a concentration of 2×10^6/ml of cRPMI for 48 h without or with LPS (10 μg/ml) or Timothy grass pollen. Supernatants were harvested, filtered and stored at −80°C until assayed. TNF was determined as described [22], using actinomycin D-treated L-929 cells as target. Serial dilutions (0·1 ml) of assay supernatants were added to 4×10^4 L-929 cells seeded in 0·1 ml in flat-bottomed microtitre plates. The determination of TNF activity was made in comparison to commercially available preparations with known titres, and the results were expressed as U/ml. Selected samples were incubated with MoAbs anti-TNF-α before performing the cytokine neutralization assay.

Statistical analysis

CD23 expression is a binomial variable. In tables and figures the original data are presented. Therefore, for the statistical analysis the empirical logistic transformation was used in order to stabilize the variance of the dependent variable CD23. Standard methods of statistical analysis were used. The unpaired *t*-test was used to examine differences in CD23 expression between asthmatics and controls. The paired *t*-test was used to examine differences in CD23 expression before and after stimulation of cytokines (IL-4, IL-6, IFN-γ) or Timothy grass pollen.

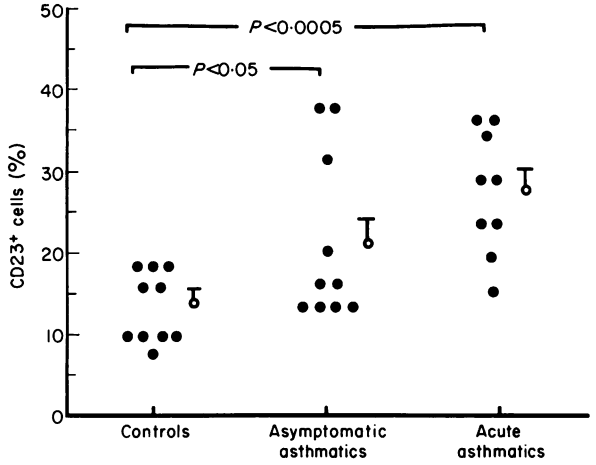

Fig. 1. Per cent of CD23 expression on monocytes from healthy donors (controls), asymptomatic asthmatics or acute asthmatics. The cells were cultured for 3 days and the percentage of $CD23^+$ cells was evaluated.

RESULTS

Previous studies have demonstrated that monocytes from asthmatic subjects constitutively express CD23 [21]. To evaluate whether CD23 was expressed differently in asthmatic patients during an asthmatic attack or in asymptomatic periods, we performed experiments in three groups of subjects: healthy donors, asymptomatic asthmatics and asthmatics with acute asthma.

In preliminary experiments, CD23 expression was evaluated on freshly isolated cells or after various days of culture (1, 2 or 3 days). The expression of CD23 receptor showed a slight increase during the culture period, but results were not statistically different; subsequently, cells cultured for 3 days were used.

As shown in Fig. 1, CD23 expression on monocytes after 3 days of culture was significantly higher in the two groups of asthmatics with respect to control subjects. The mean percentage values of $CD23^+$ cells were 13·9 (s.d. 4·0) in 10 normal subjects, and 25·5 (s.d. 9·4) in 19 asthmatic subjects ($P < 0·0001$). There was no significant difference between the two groups of asthmatics.

IL-4 has been described as a potent inducer of CD23 expression in monocytes from healthy donors [16,17]. For this purpose, experiments were performed to verify the modulation of IL-4 in cells from asthmatics, in which CD23 was previously expressed. In preliminary experiments the time course of cytokine-induced CD23 expression was evaluated; optimal stimulation was obtained after 2–3 days of culture.

As shown in Fig. 2, IL-4 up-regulated CD23 in monocytes from control subjects and from both groups of asthmatics. After IL-4 stimulation, CD23 in monocytes from controls reached levels similar to those of unstimulated asthmatic cells.

It has been reported that IL-6 and IFN-γ are not effective in stimulating CD23 on monocytes from normal subjects [16,17]; thus, we examined the effect of IL-6 and IFN-γ on cells from asymptomatic or acute asthmatics. Since the results between the two groups of asthmatics were not statistically different, the results have been pooled and reported in Fig. 3. IFN-γ was unable to modulate CD23 expression on cells from asthmatics in our experimental conditions; on the contrary, high doses of IL-6 (1000 U/ml) led to a significant increase of CD23 expression only when monocytes from asthmatic subjects were used. When mouse anti-human IL-6 (Biosource) antibodies were

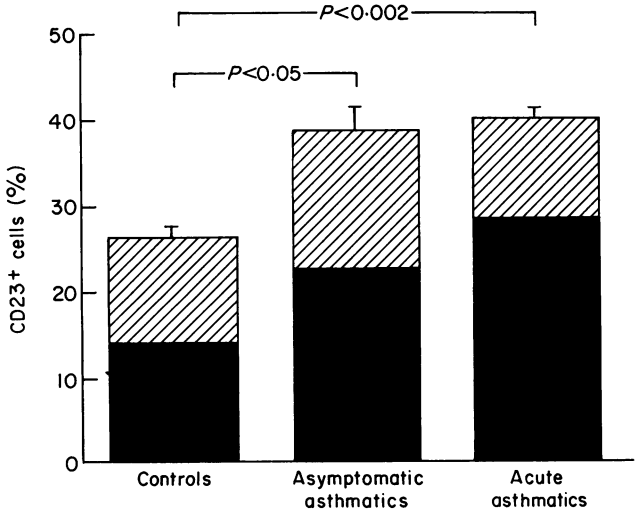

Fig. 2. Per cent of CD23 expression on monocytes from healthy donors (controls), asymptomatic asthmatics and acute asthmatics. The cells were cultured for 3 days without or with IL-4 (100 U/ml). Data represent the mean ± s.e.m. of five single determinations from five different donors. ▨, IL-4-induced CD23; ■, CD23.

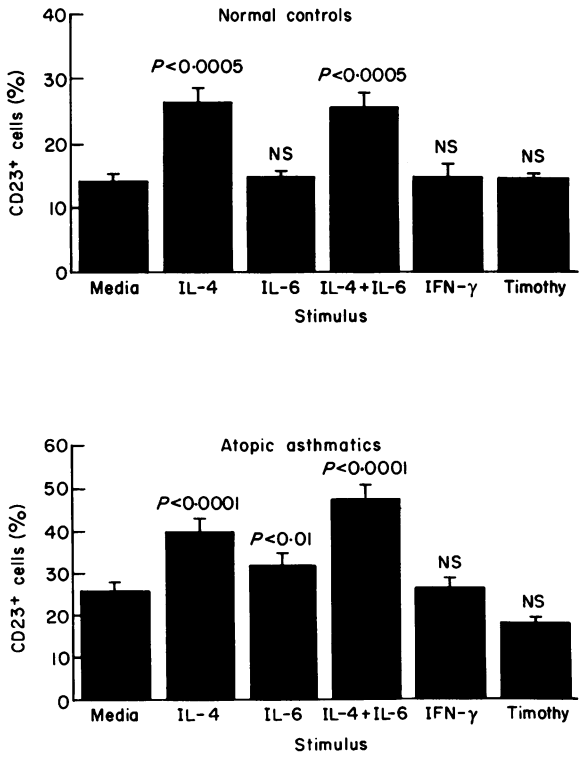

Fig. 3. Per cent of CD23 expression on monocytes from healthy donors (controls), asymptomatic asthmatics and acute asthmatics. The cells were cultured for 3 days without or with IL-4 (100 U/ml), IL-6 (1000 U/ml), IFN-γ (100 U/ml), Timothy grass pollen (1 μg/ml). Data represent the mean ± s.e.m. of four single determinations from five different donors.

Table 2. Dose response of IL-4- and IL-6-induced CD23 expression on monocytes from control subjects or asthmatic patients

Cytokine treatment*	Doses (U/ml)	Per cent CD23 expression from Controls	Asthmatics
IL-4	0	12·7 ± 1·7	25·6 ± 3·0
	10	18·8 ± 0·4	32·4 ± 3·0
	100	30·1 ± 2·9†	42·4 ± 1·2†
	1000	42·3 ± 4·8†	54·2 ± 2·2†
IL-6	0	13·9 ± 2·0	26·6 ± 3·4
	10	11·4 ± 1·8	26·5 ± 4·0
	100	12·9 ± 2·2	29·7 ± 5·0
	1000	18·4 ± 4·2	34·2 ± 2·8†
	2000	22·6 ± 2·6†	45·5 ± 3·1†

* IL-4 and IL-6 were added to culture monocytes for 3 days before the test. The results represent the mean of four separate experiments from four different donors ± s.e.m.

† $P < 0{\cdot}01$ (cytokine-treated *versus* untreated cells).

added to IL-6 the effect was abrogated, suggesting that IL-6, but not other molecules, was responsible for the observed phenomenon. Moreover, IL-6 did not affect IL-4 up-regulation of CD23 in both groups of asthmatics when combined stimulation of these cytokines was employed. The effect of various doses of IL-4 or IL-6 on CD23 expression was evaluated in monocytes from normal and asthmatic subjects. The results in Table 2 showed that IL-4 up-regulated the receptor on cells from both groups in a dose-dependent manner, and a similar percentage of increase was observed in the two groups.

The up-regulation by IL-6 was evidenced when high doses of this cytokine were employed; cells from asthmatics were more susceptible than those from healthy donors. In fact, 1000 U/ml was able to increase CD23 in asthmatics but not in normal subjects. However, 2000 U/ml of IL-6 was able to increase CD23 also in cells from controls.

Furthermore, the *in vitro* addition of Timothy grass pollen was not effective at the dose used to regulate CD23 expression on monocytes from asthmatics with a positive skin test to this pollen as well as normal subjects. On the contrary, an induction of TNF production was observed in monocytes stimulated with Timothy grass pollen (Table 3).

Table 3. Tumour necrosis factor (TNF) production by monocytes from asthmatic subjects or normal controls after *in vitro* stimulation with specific antigen (Timothy grass pollen)

In vitro stimulation	TNF (U/ml) asthmatics ($n = 6$)	TNF (U/ml) controls ($n = 5$)
—	8·2 ± 1·5	4·2 ± 1·0
LPS (10 μg/ml)	97·5 ± 7·2	100 ± 8·6*
Timothy grass pollen (1 μg/ml)	18·7 ± 3·7*	9·5 ± 3·1
Timothy grass pollen (10 μg/ml)	37·5 ± 7·2*	22·5 ± 3·2*

Monocytes (2×10^6/ml) were cultured for 18 h and then TNF levels in supernatants were evaluated as described in Subjects and Methods. The test was performed on monocytes from six asthmatics with strongly positive skin tests for grass pollen.

* $P < 0{\cdot}01$ (*in vitro* treated *versus* untreated cells).

LPS, Lipopolysaccharide.

DISCUSSION

The data presented here show that monocytes from asymptomatic asthmatics as well as asthmatics with acute asthma presented an increase of CD23 expression compared with healthy donors. Cytokine treatments regulated this receptor. IL-4 induced an increase of CD23 on monocytes from both asthmatics and normal subjects, and after IL-4 treatment the expression of this receptor in the latter group reached levels comparable to those of unstimulated cells from asthmatics.

IFN-γ did not play a role in the regulation of CD23 in our experimental conditions, while the addition of IL-6 was able to up-regulate this receptor on monocytes from asthmatics or controls. Moreover, *in vitro* challenge with the specific allergen, Timothy grass pollen, was not able to modulate CD23 expression on monocytes from asthmatics. It is possible that the up-regulation of CD23 expression by the specific allergen was mediated by T cells or their soluble factor, thus the presence of T cells may be required for this phenomenon.

In our experimental conditions monocytes from normal subjects cultured for 3 days expressed appreciable levels of CD23 receptor. These data are in contrast to Te Velde *et al.* [20], but in agreement with other authors [12,23,24]. Recent data show that PBM from asthmatics constitutively express high levels of CD23 [21]. Our results confirm these data; moreover, we demonstrate that monocytes from asymptomatic asthmatics show levels of CD23 expression comparable to those of acute asthmatics. The expression of CD23 seems to be associated with hypersensitivity to one or more allergens than with the airway obstruction of acute asthma. Positive skin tests in asthma have an analogous meaning [25].

Macrophages are a candidate as primary cells in asthma, because they possess low-affinity IgE receptor and can be activated in an IgE-dependent manner [26,27] to secrete a wide range of inflammatory mediators, including tromboxane A_2, prostaglandin D_2, leucotriene B_4 and cytokines such as TNF and IL-1. In our opinion, regulation of CD23 receptor could be a useful therapeutical approach in the asthmatic syndrome through neutralization of the mediators which up-regulate the expression of this receptor.

It is known that helper lymphocytes ($CD4^+$) are activated in asthmatic subjects [28,29] and elaborate proinflammatory cytokines; this event leads to the improvement of lung function [18]. In allergic disease, the subpopulation of $CD4^+$ lymphocytes mainly involved is Th2 [30–32] that produces IL-4, IL-5, IL-6; and it has been reported that mRNA for IL-4 and IL-5 is expressed predominantly by T lymphocytes [30] even if IL-4 is also derived from $CD8^+$ T cells [33]. Monocytes are the predominant source of IL-6, although high levels of 'complexed' IL-6 are found in human blood [34]. Hence, it is possible that in asthmatics activated monocytes are the source of IL-6 in addition to Th2 cells. The involvement of IL-4 in allergic disease has been established; in fact, it plays an important role in IgE regulation synthesis of B cells [35], and could be responsible, together with high doses of IL-6, for the induction of CD23 expression in a large percentage of circulating monocytes from asthmatics.

Some studies report the inability of IL-6 to modulate CD23 expression on monocytes from normal subjects [24]; on the contrary, a modulating effect on a monocytic cell line, such as U937 and THP-1 [16], was observed. This phenomenon may be ascribed to different regulatory pathways, cell differentiation or activation dependence. Our results show differences in the capability of monocytes to respond to IL-6 depending upon basal conditions of CD23 expression. It seems possible that the high levels of CD23 expression in asthmatic cells could favour IL-6 efficacy. In fact, this phenomenon is similar to that observed in the cell line which constitutively expresses CD23 in large amounts, suggesting that cells from asthmatics are primed for an increased response; when activated they are more susceptible to cytokines with respect to controls. IL-4 and IL-6 CD23 regulation on cells from asthmatics and controls allows a comparative analysis, and shows a different capability in responding to IL-6. It seems that asthmatics require lower doses than controls. Hence, an important role in allergic disease is played by IL-4, but the simultaneous presence of other cytokines, in particular Th2-dependent, contributes to maintaining high levels of CD23 receptor IgE ligand, which is essential for the secretion of macrophage proinflammatory mediators in allergic disease.

In our experiments the allergen-specific challenge on monocytes from asthmatics does not affect CD23, suggesting that an increase of CD23 expression is mediated by cytokine secretion from T lymphocytes activated by specific allergen. Moreover, *in vitro* addition of specific allergen on monocytes includes TNF secretion, which appears to be a major mediator in the inflammatory process [36,37], including the asthmatic syndrome [38].

The monitoring of CD23 on monocytes could be a marker for asymptomatics and acute asthmatics. We speculate that the cytokine-driven CD23 in the asthmatic syndrome could suggest a therapeutical approach with anti-cytokine therapy, particularly in the lung, in patients non-responsive to corticosteroid therapy.

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