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Disturbed Cytokine Production at the Systemic Level in Difficult-to-Control Atopic Asthma: Evidence for Raised Interleukin-4 and Decreased Interferon-γ Release following Lipopolysaccharide Stimulation

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Abstract

Background: Disturbed cytokine production is thought to govern inflammation in asthma, which, in its turn, may lead to uncontrolled disease. The aim of this study was to assess the relationship between cytokine production from blood leucocytes and the level of asthma control.

Methods: We compared the production of interleukin (IL)-4, IL-6, IL-10, interferon (IFN)-γ and tumour necrosis factor-α from peripheral blood leucocytes in non-atopic healthy subjects (n = 22), atopic non-asthmatics (n = 10), well-controlled asthmatics [Juniper asthma control questionnaire (ACQ) score <1.5; n = 20] and patients with uncontrolled asthma despite inhaled or oral corticoids (ACQ score >1.5; n = 20). Fifty microlitres of peripheral blood was incubated for 24 h with RPMIc, lipopolysaccharide (LPS; 1 ng/ml) or phytohaemagglutinin (1 μg/ml), and cytokines were measured by immunotrapping (ELISA).

Results: Both controlled and uncontrolled asthmatics as well as atopic non-asthmatics spontaneously produced more IL-4 than non-atopic healthy subjects ($p < 0.001$). IL-4 production induced by LPS was significantly greater ($p < 0.05$) in both asthma groups compared to atopic non-asthmatics and non-atopic healthy subjects. By contrast, IFN-γ release induced by LPS was lower in uncontrolled asthmatics than in non-atopic healthy subjects ($p < 0.05$) and controlled asthmatics ($p < 0.05$). IL-10 release after LPS was greater in uncontrolled asthmatics than in atopic non-asthmatics ($p < 0.05$). No difference was observed regarding other cytokines.

Conclusion: Blood cells from patients with difficult-to-control atopic asthma display highly skewed Th2 cytokine release following LPS stimulation.

Keywords : Interleukin-4 ; Interferon-γ ; Endotoxin ; Asthma control

Introduction

Human studies suggest that exposure to lipopolysaccharide (LPS) can influence the development and severity of asthma. Endotoxin is considered to have a dual role in asthma. While it may prevent the development of atopy when exposure occurs in early life [1], this bacterial product may worsen asthma control when inhaled by adult asthmatics in whom the disease is already well established [2].

Airway exposure to endotoxin is known to promote airway [3] and systemic [4] neutrophilic inflammation, but this bacterial compound has a broad range of activities in vitro. Endotoxin is a potent stimulus for the innate immune system and is able to activate both the mononuclear [5] and granulocyte fraction from blood leucocytes [6, 7]. Some studies have suggested that persistent, difficult-to-treat asthma may be linked to an impaired innate immunity favouring chronic infection [8].

Although pathological heterogeneity of the disease has been highlighted over the past years [9], asthma often features an airway eosinophilic inflammation [10,11] orchestrated by Th2 cytokines [12, 13]. Our recent study showed that uncontrolled asthma encountered in daily practice is associated with increased airway eosinophilic inflammation as compared to well-controlled asthma [14]. Whether this relationship is also observed at the systemic level has not been investigated.

The purpose of our study was to determine if there was any relationship between asthma control and cytokine production from blood leucocytes in response to endotoxin. Interleukin (IL)-4 and interferon (IFN)-γ were chosen as markers of the Th2/Th1 balance, tumour necrosis factor (TNF)-α and IL-10 as pro- and anti-inflammatory cytokines, respectively [15], and IL-6 as a cytokine playing a role in the transition from innate towards adaptive immunity [16].

The present study was performed on atopic asthmatics recruited from our asthma clinic and classified into two subgroups according to their level of asthma control [controlled asthma, i.e. asthma control questionnaire (ACQ) score <1.5, and uncontrolled asthma, i.e. ACQ score ≥1.5].

In order to clarify the role of asthma versus atopy in cytokine production, asthmatics were compared to atopic non-asthmatics and non-atopic healthy subjects.

Materials and Methods

Study Design and Subject Characteristics

Patient demographics and functional and treatment characteristics are given in table 1. The Juniper ACQ is known to have strong evaluative and discriminative properties and can be used with confidence to measure asthma control [17].

In this study, 20 controlled atopic asthmatics (ACQ score <1.5) were compared with 20 uncontrolled atopic asthmatics (ACQ score ≥1.5), 10 atopic non-asthmatics [grass pollen rhinitis studied out of season with a provocative concentration of methacholine producing a 20% fall in forced expiratory volume in 1 s (FEV_1) (PC20M) >16 mg/ml] and 22 non-atopic healthy subjects. Patients were recruited from our asthma clinic at CHU Liege Sart-Tilman between January 2006 and June 2009, and the group of atopic non-asthmatics comprised subjects with asymptomatic rhinitis recruited from a database for a clinical trial on immunotherapy.

All asthmatics were diagnosed on the basis of significant FEV_1 reversibility (≥12% from baseline) with β_2-agonists or bronchial hyperresponsiveness to methacholine (PC20M <16 mg/ml). Atopy was defined as a positive skin prick test reaction (wheal ≥3 mm compared with control) to common aeroallergens, including house dust mites, cat and dog dander, grass, tree, pollen and moulds.

The protocol was approved by the local ethics committee, and every subject gave written informed consent.

Peripheral Blood Sampling and Cell Count

Peripheral blood samples were collected in apyrogenic, heparinized tubes (Venosafe, Terumo®, Belgium). Total and differential blood cell counts were obtained with an Advia 210 automatic counter (USA). Counting and cell typing were based on flow cytometry with bidimensional volume distribution, peroxidase concentration and lobularity of leucocytes as parameters.

Blood Cell Culture and Cytokine Assay

Cytokines (IL-4, IL-6, IL-10, TNF-α, IFN-γ) were measured by a two-step sandwich-type immunoassay. The antibodies and standards were purchased from Biosource (Cytosets, Biosource, Invitrogen, Belgium). Fifty microlitres of standards or whole blood (diluted twice) was incubated at 37°C with 200 µl of Roswell Park Memorial Institute medium (RPMI)-1640 supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin (Cambrex, Verviers, Belgium) and 2% of inactivated fetal calf serum (Cambrex), or LPS (*Salmonella enteridis*, Sigma, St. Louis, Mo., USA; 1 ng/ml) or phytohaemagglutinin (PHA; Biochrom AG, Berlin, Germany; 1 µg/ml) in apyrogen microwells which had previously been coated with specific antibodies directed towards the chosen cytokines.

After 24 h, the wells were washed and 100 µl of a solution containing biotinylated detection antibodies specific to the cytokines was added for 2 h at room temperature. The wells were washed again and filled with a solution containing streptavidin horseradish peroxidase for 45 min at room temperature. Then, 100 µl of tetramethylbenzidine chromogen solution was added for 10-20 min in the dark. The reaction was stopped by adding 50 µl of 1 M H_2SO_4. The amount of substrate converted to products was thereafter detected as the optical density at 450 nm in an ELISA reader (Multiscan Ascent, Thermo Labsystems, Helsinki, Finland). The sensitivities of our assays were 6 pg/ml for IL-4, 6 pg/ml for IL-6,4 pg/ml for IL-10, 6 pg/ml for TNF-α and 7

pg/ml for IFN-γ.

Statistical Analysis

Blood cell counts as well as cytokine levels were expressed as medians (range), unless otherwise stated. Comparisons between the four groups were performed by Kruskal-Wallis test (non-parametric ANOVA) followed, in the case of significance, by Dunn's multiple-comparison test. A p value <0.05 was considered statistically significant.

Table 1. *Demographic, functional, airway inflammatory and treatment characteristics according to ACQ scores*

	Non-atopic healthy subjects (n = 22)	Atopic non-asthmatics (n = 10)	Asthmatics with ACQ <1.5 (n = 20)	Asthmatics with ACQ ≥1.5 (n = 20)
Age, years	42 ± 13	33 ± 10	43 ± 19	40 ± 16
Males/females	13/9	5/5	13/7	7/13
Tobacco status				
Never smoked	12	1	17	12
Ex-smoker	5	6	2	2
Current smoker	5	3	1	6
BMI	24 ± 3	23 ± 3	25 ± 5	28 ± 7[c]
Positive skin prick test	0	10	20	20
NO, ppb	-	11 (6-27)	20 (5-222)	55 (8-165)[c]
IgE, kU/l	-	63 (21-303)	331 (56-1,670)	356 (37-2,532)[c]
Sputum eosinophils, %	0 (0-3.6)	0 (0-4)	2.2 (0.4-19.4)[a]	8.8 (0-80.4)[b,c]
Sputum neutrophils, %	38 (0-87)	23 (2-52)	40 (3-93)	40 (0-99)
FEV_b %	108 ± 16	103 ± 12	87 ± 19[a]	65 ± 17[b,e,f]
FVC, %	111 ± 17	103 ± 12	97 ± 18	85 ± 16[b]
FEV_1/FVC, %	83 ± 8	85 ± 8	76 ± 15	65 ± 13[b,d]
Reversibility, %	-	-	6.8 ± 4.5	21 ± 18[d]
ACQ score	-	-	0.86 (0.49-1.28)	3.21 (2.07-4.58)[e]
PC20M, mg/ml	-	-	3.09 (0.62-16)	0.83 (0.2-3.4)
Oral CS	0	0	0	6
Inhaled CS	0/22	0/10	9/20	19/20
Inhaled CS, eq budesonide/day	-	-	0 (0-1,600)	2,000 (l,600-2,800)[e]
LABA	-	-	9/20	17/20
LTRA	-	-	3	11
Theophylline	-	-	-	6

Results are expressed as means ± SD or numbers of patients, except PC20M, which is expressed as the geometric mean (range), and NO, IgE, ACQ score, inhaled corticosteroids and sputum neutrophils and eosinophils, which are expressed as medians (range). FVC = Forced vital capacity; BMI = body mass index; LABA = long acting β_2-agonist; LTRA = leucotriene receptor antagonist; CS = corticosteroids. [a] $p < 0.01$, [b] $p < 0.001$ versus non-atopic healthy subjects; [c] $p < 0.05$, [d] $p < 0.01$, [e] $p < 0.001$ versus atopic non-asthmatics; [f] $p < 0.05$ versus asthmatics with ACQ <1.5.

Results

Demographic, Lung Function, Airway Inflammation and Treatment Characteristics according to Asthma Control

The subjects were well matched for their age and tobacco consumption. As expected, FEV_1 values were clearly different (65 ± 17% pred.) in the group with ACQ score ≥1.5 when compared to the group with ACQ score <1.5 (87 ± 19% pred.; $p < 0.05$) and to atopic non-asthmatics and non-atopic healthy subjects ($p < 0.001$ for both). Similarly forced vital capacity was also significantly decreased in the uncontrolled asthma group as compared to non-atopic healthy subjects, and the ratio of FEV_1 to forced vital capacity was also significantly lower in uncontrolled asthmatics (65 ± 13%) than in non-atopic healthy subjects (83 ± 8%; $p < 0.001$) and atopic non-asthmatics (85 ± 8%; $p < 0.01$).

It is also of interest to note that patients with an ACQ score ≥1.5 were taking higher doses of inhaled corticosteroids (2,000 eq budesonide/day range 1,600-2,800) in comparison with patients with an ACQ score <1.5 (0 eq budesonide/day range 0-1,600). Nine out of 20 controlled asthmatics and 17 out of 20 uncontrolled asthmatics were receiving inhaled long-acting β_2-agonists. Some uncontrolled asthmatics were also taking oral corticosteroids (6/20), leucotriene receptor antagonists (11/20) or theophylline (6/20).

Controlled and uncontrolled asthmatics exhibited higher sputum eosinophil counts than non-atopic healthy subjects ($p < 0.01$ and $p < 0.001$, respectively), while uncontrolled patients also had a greater sputum eosinophil count than atopic non-asthmatics ($p < 0.05$; table 1).

Table 2. *Blood cell counts*

	Non-atopic healthy subjects	Atopic non-asthmatics	Asthmatics with ACQ <1.5	Asthmatics with ACQ ≥1.5
Leucocytes, /µl	6,410 (4,200-12,200)	6,390 (4,000-7,490)	7,770 (5,430-13,280)[d]	8,690 (5,860-18,130)[b,f]
Neutrophils, %	52 (41-73)	49 (42-75)	55 (41-84)	58 (33-90)
Neutrophils, /µl	3,564 (2,088-5,914)	3,300 (1,760-5,230)	4,025 (2,850-11,100)	4,720 (2,380-15,190)[b,d]
Lymphocytes, %	35 (19-47)	40 (19-47)	33 (9-41)[c,f]	26 (9-42)[g]
Lymphocytes, /µl	1,918 (598-3,546)	1,940 (1,270-2,930)	2,250 (1,230-3,950)	2,360 (810-3,670)
Monocytes, %	6.1 (4.7-11.5)	8.5 (4.3-11.2)	5.6 (3.9-10.6)	5.7 (0.7-9)
Monocytes, /µl	404 (256-1,014)	460 (300-810)	430 (300-790)	445 (60-1,190)
Eosinophils, %	1.7 (0.7-6.3)	1.7 (0.4-4.3)	4.3 (0.6-8.8)	3.8 (0.1-21)
Eosinophils, /µl	120 (30-370)	90 (30-190)	315 (70-650)[a,d]	350 (9-l,350)[a,d]
Basophils, %	0.7 (0.4-1.5)	0.5 (0.1-0.9)	0.7 (0.2-1.6)	0.6 (0-1.9)
Basophils, µl	45 (21-98)	30 (10-60)	55 (10-150)	75 (20-150)[e]

Results are expressed as medians (range). [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ versus non-atopic healthy subjects; [d] $p < 0.05$, [e] $p < 0.01$, [f] $p < 0.001$ versus atopic non-asthmatics; [g] $p < 0.001$ versus asthmatics with ACQ <1.5.

Table 3. *Cytokine production from a standardized blood volume*

Cytokine	Stimulant	Non-atopic healthy subjects	Atopic non-asthmatics	Asthmatics with ACQ <1.5	Asthmatics with ACQ ≥1.5
IL-4, pg/ml	RPMI	0 (0-9)	32 (0-169)[b]	16 (0-57)[b]	12 (0-349)[b]
	PHA	61 (6-176)	172 (11-276)[a]	70 (21-171)	120 (7-761)
	LPS	0 (0-24)	17 (0-171)[a]	28 (0-98)[b]	43 (0-541)[b]
IL-6, pg/ml	RPMI	0 (0-152)	0 (0-24)	0 (0-264)	0 (0-1,421)
	PHA	81 (0-492)	36 (0-471)	86 (0-2,362)	58 (0-1,754)
	LPS	307 (14-1,472)	184 (96-649)	311 (20-1,123)	167 (1-1,760)
IL-10, pg/ml	RPMI	0 (0-6)	9 (0-138)	1 (0-45)	0 (0-430)
	PHA	321 (72-696)	172 (90-404)	212 (57-657)	328 (35-1,681)
	LPS	487 (83-832)	289 (98-842)	470 (187-1,346)	684 (14-2,388)[c]
IFN-γ, pg/ml	RPMI	0 (0-239)	0 (0-39)	0 (0-57)	7 (0-89)
	PHA	684 (25-2,972)	1,001 (124-2,503)	413 (31-1,630)	398 (123-1,407)
	LPS	54 (0-936)	60 (0-1,145)	53 (7-398)	31 (0-238)[a,d]
TNF-α, pg/ml	RPMI	42 (0-793)	0 (0-110)	64 (0-1,403)	50 (0-2,733)
	PHA	1,993 (666-7,552)	1,160 (739-2,338)	1,610 (928-7,536)	2,269 (267-4,654)
	LPS	2,350 (1,140-5,755)	1,649 (1,370-2,754)	2,509 (592-5,995)	2,571 (80-5,052)

Results are expressed as medians (range). Values for LPS and PHA represent the raw data as they were measured from ELISA without subtracting RPMI results. [a] $p < 0.05$, [b] $p < 0.001$ versus non-atopic healthy subjects; [c] $p < 0.05$ versus atopic non-asthmatics; [d] $p = 0.06$.

Blood Cell Counts

Asthmatics with an ACQ score > 1.5 had a greater total blood cell count compared to non-atopic healthy subjects ($p < 0.01$) and atopic non-asthmatics ($p < 0.001$).

Both groups of asthmatics exhibited significantly raised systemic absolute eosinophil counts when compared to atopic non-asthmatics and non-atopic healthy subjects ($p < 0.05$ for both).

The absolute neutrophil count was significantly increased in the uncontrolled group when compared to non-atopic healthy subjects and atopic non-asthmatics ($p < 0.01$ and $p < 0.05$, respectively).

There was no difference between controlled and uncontrolled asthmatics apart from the percentage of lymphocytes, which was lower in uncontrolled asthmatics ($p < 0.001$; table 2).

Cytokine Production from Blood Cell Culture

The results regarding cytokine production from blood cells are given in table 3.

Both groups of asthmatics and the atopic non-asthmatics were characterized by a significantly raised spontaneous IL-4 production ($p < 0.001$ for the three groups). Likewise, controlled and uncontrolled asthmatics also exhibited raised IL-4 production after stimulation by LPS when compared to atopic non-asthmatics ($p < 0.05$ for both) and non-atopic healthy subjects ($p < 0.05$ and $p < 0.01$, respectively; fig. 1).

IL-4 measured after LPS stimulation in atopic non-asthmatics was also greater than in non-atopic healthy subjects ($p < 0.05$), although there was no evidence of a real response to LPS in this case, the production of IL-4 being quite similar to that seen with RPMI alone (table 3). After stimulation by PHA, only atopic non-asthmatics exhibited raised IL-4 production when compared to non-atopic healthy subjects ($p < 0.05$).

Fig. 1. *LPS-induced IL-4 (a) and IFN-γ (b) production from blood leucocytes in non-atopic healthy subjects (NAHS), atopic non-asthmatics (ANA), controlled asthmatics (CA; ACQ score <1.5) and uncontrolled asthmatics (UA; ACQ score ≥1.5). Each point represents the subtraction of LPS - RPMI for IL-4 and IFN-γ. Zero values were transformed to 1 for graphic representation. The bars represent the median.*

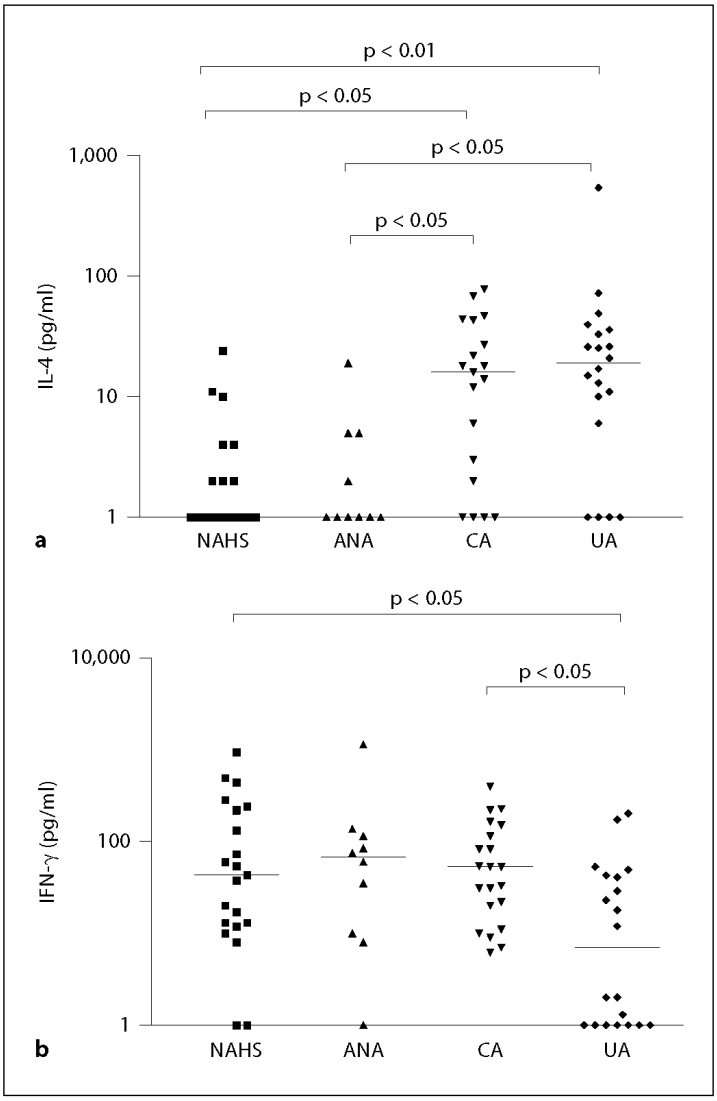
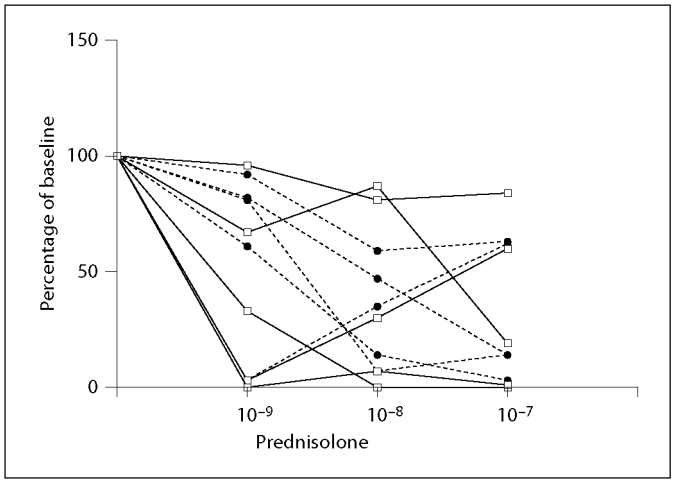


Fig. 2. *Effect of prednisolone (in M) on IFN-γ from blood leucocytes in healthy subjects and patients with difficult-to-control asthma. Each line represents 1 patient; healthy subjects are represented by squares and continuous lines and patients with difficult-to-control asthma by circles and dashed lines. Results are expressed as a percentage of baseline.*



The group with uncontrolled asthma showed lower release of IFN-γ following LPS exposure when compared to non-atopic healthy subjects ($p < 0.05$; fig. 1) and controlled asthmatics ($p < 0.05$). Uncontrolled asthmatics also differed from atopic healthy subjects in terms of increased IL-10 production (table 3). There was no significant difference between the groups with regard to the production of other cytokines.

In the controlled asthma group, there were no significant differences regarding cytokine production between those who were steroid naïve ($n = 12$) and those regularly receiving inhaled corticoids ($n = 8$). We did not find any relationship between the dose of inhaled corticosteroids received by the patients and the level of IFN-γ production ($r = -0.2$, $p = 0.21$).

The effect of prednisolone on IFN-γ production from blood cells was assessed in vitro in a pool of healthy subjects and patients with difficult-to-control asthma ($n = 10$). At 10^{-9}, 10^{-8} and 10^{-7} M, it produced significant inhibition of 48 ± 12% ($p < 0.01$), 63 ± 10% ($p < 0.001$) and 68 ± 10% ($p < 0.0001$), respectively (fig. 2).

Discussion

Our study shows that endotoxin-induced cytokine release from blood leucocytes reveals a clear Th2 pattern in asthmatics and atopic non-asthmatics as compared to non-atopic healthy subjects. In particular, LPS-induced IL-4 release was clearly increased in controlled and uncontrolled asthmatics when compared to non-atopic healthy subjects. By contrast, uncontrolled asthmatics displayed a strikingly decreased production of IFN-γ in response to endotoxin as compared to non-atopic healthy subjects.

Although greater in asthmatics than in healthy subjects, the blood eosinophil count was not associated with uncontrolled asthma, which is different from results we recently reported for sputum eosinophilia [12]. Blood neutrophilia was raised in uncontrolled asthmatics as compared to atopic non-asthmatics and non-atopic healthy subjects but did not distinguish controlled from uncontrolled asthmatics.

The raised spontaneous production of IL-4 seen in asthmatics and atopic non-asthmatics supports the pivotal role of Th2-driven inflammation in atopic diseases [18]. It has been clearly demonstrated that stimulation of peripheral blood monuclear cells (PBMC) in vitro with an allergen resulted in greater release of IL-4 in sensitized subjects [19]. Our study expands this finding by using endotoxin as another type of environmental stimulus. Although endotoxin is rather considered to favour a Th1 pathway accompanied by neutrophilic inflammation [7], our results show that endotoxin enhances IL-4 release from circulating leucocytes in asthmatics but not in atopic non-asthmatics nor in non-atopic healthy subjects.

This finding indicates that amplification of Th2 cytokine release from leucocytes following endotoxin exposure is restricted to atopic asthmatics but did not distinguish controlled from uncontrolled asthmatics. Remarkably,

there was no relationship between the level of asthma control and spontaneous IL-4, which is a hallmark of atopy rather than of asthma.

Our results are in keeping with those of Magnan et al. [20], who, using the same whole-blood model, found that IL-4 release was more dependent on atopy than on asthma. Interestingly, circulating leucocytes from non-atopic healthy subjects, the large majority of whom failed to spontaneously release IL-4, were also largely unable to release this cytokine after stimulation with LPS. In this respect, IL-4 behaves differently from other cytokines like IL-6, IL-10 or IFN-γ, which, although not spontaneously produced by the majority of healthy subjects, are clearly released following exposure to LPS. In contrast to LPS, PHA induces IL-4 production by leucocytes from healthy subjects. This shows that healthy subjects are perfectly capable of producing this Th2 cytokine under certain circumstances. In our study, atopic non-asthmatics were particularly prompt in releasing IL-4 in response to PHA.

As we worked on a whole-blood model including all types of leucocytes, cells involved in IL-4 release may differ according to the type of stimulus. While T lymphocytes are recognized to be strongly activated by the polyclonal activator PHA and are probably the main source of IL-4 after PHA stimulation [21], release of IL-4 following LPS is perhaps more dependent on the granulocyte fraction, as eosinophils [22] and basophils [23] are also able to release this cytokine. Whichever the mechanisms, it is clear that asthmatics, and in particular patients with difficult-to-control asthma, still exhibit raised IL-4 release despite heavy treatment with inhaled and sometimes oral corticoids, a class of drug that shows a convincing inhibitory effect on IL-4 production both in vitro [24, 25] and in vivo [24, 26].

In contrast to what was found for IL-4, uncontrolled asthmatics differed from non-atopic healthy subjects in terms of a diminution of IFN-γ production following LPS exposure, which points to a deficiency of the Th1 pathway in response to this bacterial product in the more severe types of asthma. Treatment with a high dose of inhaled corticoids or oral corticoids may play a role in this reduction of IFN-γ release, as we found that prednisolone inhibited IFN-γ release from blood leucocytes in vitro in a similar way to that reported by Braun et al. [24] from PBMC. However, the impact of treatment with corticoids on IFN-γ production in vivo is highly controversial [26, 27]. In addition, the fact that, in the group with well-controlled asthma, patients taking inhaled corticoids failed to differ from their steroid-naive counterparts suggests that inhaled corticoids might not be the main reason for the reduced production of IFN-γ seen in our patients with difficult-to-control asthma. Moreover we did not find a relationship between the dose of inhaled corticoids and the level of IFN-γ produced in asthmatics. Our finding is in keeping with the literature, although the methodology used may differ between studies. Peripheral blood cells from children with both mild and moderate-to-severe atopic asthma were found to release less IFN-γ than those of healthy children when stimulated by lectins like concanavalin A or PHA [28,29]. Furthermore, Leonard et al. [19] found that, in adult subjects, IFN-γ release from PBMC following allergen stimulation in vitro was lower than in atopic non-asthmatics and healthy subjects. Additionally, in that study, IFN-γ release was inversely related to symptom score in asthmatics [19]. As for the consequences of impaired IFN-γ production, it is important to mention that IFN-γ is a type 2 IFN involved in host defence against micro-organisms [30]. It is believed that some difficult-to-control asthma may be linked to persistent infection [31]. In view of this, impaired IFN-γ production in response to LPS may be an immunological feature that can make asthmatics prone to chronic infection [8, 32].

In conclusion, stimulation of blood leucocytes by endotoxin enhances IL-4 release in controlled and uncontrolled atopic asthmatics, which differentiates atopic asthmatics from atopic non-asthmatics and non-atopic healthy subjects. In addition, it reveals an impairment of IFN-γ production selectively observed in uncontrolled asthmatics. This impairment of IFN-γ release combined with increased secretion of IL-4 highlights the strongly skewed immune response towards the Th2 pattern following endotoxin stimulation in difficult-to-control asthma.

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