Monocyte-derived dendritic cells exposed to Der p 1 allergen enhance the recruitment of Th2 cells: major involvement of the chemokines TARC/CCL17 and MDC/CCL22

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ABSTRACT. Dendritic cells (DC) are potent antigen – presenting cells that can orientate the immune response towards a Th1 or a Th2 type. DC produce chemokines that are involved in the recruitment of either Th1 cells, such as IP10 (CXCL10), Th2 cells such as TARC (CCL17) and MDC (CCL22), or non-polarized T cells such as RANTES (CCL5) and MIP-1α (CCL3). We investigated whether monocyte-derived DC (MD-DC) generated from healthy donors or from patients sensitive to *Dermatophagoides pteronyssinus* (Dpt) and exposed to the cysteine-protease Der p 1(allergen of Dpt), could upregulate the expression of chemokines involved in type 1 or type 2 T cell recruitment. MD-DC were pulsed with either Der p 1 or with LPS as the control and the chemokines produced were evaluated using ELISA and chemotaxis assays. Der p 1-pulsed DC from allergic patients showed increased TARC (CCL17) and MDC (CCL22) production without modifying IP-10 (CXCL10) release. Der p 1-pulsed DC from healthy donors showed only increased IP-10 (CXCL10) secretion. RANTES (CCL5) and MIP-1α (CCL3) production were similarly increased when DC were from healthy or allergic donors. The selective Th2 clone recruitment activity of supernatants from Der p 1-pulsed DC of allergic patients was inhibited by anti-TARC (CCL17) and anti-MDC (CCL22) neutralizing Abs. By using anti-IP10 (CXCL10) blocking Abs, supernatants of Der p 1-pulsed DC from healthy donors were shown to be involved in the recruitment of Th1 cells. These results suggest that in allergic patients exposed to house dust mites, DC may favour the exacerbation of the Th2 response via the increase in type 2 chemokine production.

Keywords: dendritic cells, chemokines, Th2, allergy

INTRODUCTION

Close interactions between antigen-presenting cells and T cells are necessary to initiate and establish an immune response. Immature dendritic cells (DC) that have captured antigens in the tissues migrate into lymphoid organs where they differentiate into mature DC to stimulate naive T cells [1]. Thus stimulated, lymphocytes can differentiate into two subsets of effector T cells: Th1 and Th2 cells. Th1 cells produce lymphotoxin and IFN-γ, which both promote cellular responses. In contrast, Th2 cells release IL-4 and IL-5, which are involved in the humoral responses and in the allergic reaction [2]. These two subsets are differentially attracted depending on the nature of the inflammatory reaction and the chemokines produced.

Chemokines are small, secreted polypeptides that play a key-role in various inflammatory and immunological processes by recruiting different subsets of leukocytes [3]. Chemokines are divided into two major families. The CXC chemokines, determined by the presence of one amino acid between the first two conserved cysteines, include IL-8 and IP-10 (CXCL10). The CC family, in which the two cysteines are adjacent, include RANTES (CCL5), MCP-1α (CCL3), MDC (monocyte-derived chemokine) (CCL22) and TARC (thymus- and activation-regulated chemokine) (CCL17). The CXC chemokines are involved in the recruitment and activation of neutrophils, whereas CC chemokines preferentially act on monocytes, basophils, eosinophils or lymphocytes [4].

Some chemokines have been demonstrated to be specifically involved in the recruitment of Th1 and Th2 cells into inflammed tissues. For example, TARC (CCL17) and MDC (CCL22), both CC chemokines produced by DC, attracts CD4+ CD45RO+ cells polarized to produce Th2 cytokines [5]. In contrast, IP-10 (CXCL10) preferentially attracts Th1 cells [6]. The differential expression of chemokine receptors between Th1 and Th2 cells is related to this effect [7]. Indeed CXCR3 and CCR5 [8] are preferentially expressed by Th1 cells, whereas Th2 cells preferentially express CCR4 [5] and CCR3 [9].

In mice, TARC (CCL17) was shown to be produced by thymic and lymph node DC as well as by CD11c + cells in the lung. Murine TARC (CCL17) had no chemoattractant effect on naive CD4+ cells, but favoured the migration of ovalbumin-specific CD4+ cells Th2 cells [10]. In a mouse model of atopic dermatitis, where NC/Nga mice displayed a high susceptibility to IgE-dependent anaphylactic shock induced by ovalbumin, a leukocyte infiltration mainly composed of CD4+, CD8+ cells and macrophages [11] was detected in the skin lesions, as well as an overproduction of both TARC (CCL17) and MDC (CCL22). The overproduction of type-2 chemokines would be responsible for the recruitment of Th2 lymphocytes to the atopic dermatitis-like skin lesions [12].

In previous work [13], we have compared the effect of the cysteine protease Der p 1, (one major allergen of the house dust mite Dermatophagoides pteronyssinus (Dpt)), on monocyte-derived DC obtained from healthy donors, from pollen-sensitive patients, or from patients sensitive to Dpt. Results showed that in the presence of Der p 1, DC from Dpt-sensitive patients produced more IL-6 and IL-10, but less IL-12 than DC from healthy donors. Moreover, purified T cells from Dpt-sensitive patients stimulated by autologous Der p 1-pulsed DC, preferentially produced IL-4 rather than interferon-γ. These effects were abolished in the presence of the inactive precursor of Der p 1 (ProDer p 1). As these data suggested that DC from Dpt-sensitive patients, in contrast to DC from healthy donors, might play a pivotal role in the enhancement of the Th2 response-dependent allergic reaction developed in response to house dust mite exposure, it appeared necessary to verify whether Der p 1-pulsed DC from either allergic patients or healthy donors might produce different Th1- or Th2-attracting chemokines, known to be involved in the polarisation of the immune response. In this report, the capacity of Der p 1-pulsed DC from both healthy donors and allergic patients to produce type 2-chemokines (TARC (CCL17) and MDC (CCL22)) or type 1 chemokine (IP-10) was comparatively evaluated.

PATIENTS AND METHODS

Patients

Blood was collected from 12 patients sensitive to the house dust mite, 6 patients sensitive to birch pollen and from 10 healthy donors. All allergic patients had a history of asthma and presented the usual features of house dust mite or pollen sensitization: specific IgE antibodies and positive skin prick tests towards Dpt or birch pollen. Total IgE concentrations were 421 ± 162 IU/ml. Healthy donors did not display any of these characteristics and their total IgE concentrations were below 150 IU/ml.

Peripheral blood mononuclear cell isolation

After platelet-rich plasma depletion, blood cells were then diluted in RPMI 1640 (Life Technologies Paisley Scotland), and layered over a Ficoll gradient (Pharmacia, Uppsala Sweden). After centrifugation (400 g, 30 min), PBMC were harvested and washed. Der p 1 was isolated from spent growth medium and immunopurified. Endotoxin levels of Der p 1 were checked using the LAL assay and were below the limits of detection of the assay.

Dendritic cell differentiation

PBMC were incubated with magnetic beads conjugated with monoclonal mouse anti-human CD14 on ice for 30 min. After washing, the cells were applied onto a column placed in the magnetic field of a MACS separator (Miltenyi Biotec). After elimination of negative cells, the column was removed from the separator and the $CD14^+$ cells (monocytes) were collected and washed twice in RPMI 1640 medium. Cells were cultured (2×10^6 cells per well) in 6-well, flat-bottomed culture plates in RPMI 1640 supplemented with 1% antibiotics and 10% fetal calf serum (Life Technologies) for 7 days, in the presence of GM-CSF (Peprotech, London, UK) (20 ng/ml) and IL-4 (R&D systems, Oxon, UK) (200 IU/ml). As previously described [13], the immature DC obtained under these experimental conditions displayed a high expression of CD1a and CD11c and a low expression of HLA-DR, of the costimulatory molecules CD80 and CD86, and CD83.

Antigen loading of DC

Monocyte-derived DC were pulsed for 6 and 24 hours with either Der p 1 (0, 10, 100 and 1000 ng/ml) or the proenzymatic, inactive form of Der p 1, ProDer p 1 [14]. Some experiments were also carried out in the presence of increasing doses of Bet v 1, one major allergen of birch pollen (kindly provided by A. Didierlaurent and C.André, Stallergènes SA, Antony, France). A control of DC maturation was performed by pulsing DC with 1 μg/ml of LPS (Sigma-Aldrich).

Chemokine assay

Supernatants of 2×10^6 generated DC, pulsed or not with Der p 1, ProDer p 1 or LPS, were harvested 6 and 24 hours following stimulation. They were centrifuged (400 g for 7 min) and assayed for the presence TARC (CCL17), MDC (CCL22), IP-10 (CXCL10), RANTES (CCL5) and MIP-1α (CCL3) using specific ELISA and using Eli-pairs (R&D systems). The sensitivity of detection of the chemokines was 10 pg/ml.

Human Th1 and Th2 clones

The Th1 and Th2 clones were generated as described [15], and polarised cytokine production was confirmed by intracellular staining of IL-4 and IFN-γ, as described elsewhere [16].

Chemotaxis assay

Following a 7-day restimulation with IL-2, and in the presence (for Th2 clones) or not (for Th1 clones) of IL-4 (1000 IU/ml) and neutralizing anti-IL-12 antibodies (10 μg/ml), T cells clones were harvested and resuspended in RPMI at a concentration of 1×10^6 cells/ml. The chemotaxis protocol was performed with a 48-well, microchemotaxis chamber (Neuro Probe Cabin John, MD, USA), using a 5 μm pore polycarbonate filter (Nucleopore Corp, Pleasanton, CA, USA) for 2 hours at 37 °C, in 5% CO_2 using supernatants of DC pulsed or not with 100 ng/ml of Der p 1. T cells that migrated through the filter and reached the bottom well were enumerated under a light microscope, with a × 500 magnification, using a Thomas cell. Each condition was performed in triplicate, and at least four fields were counted for each well. The

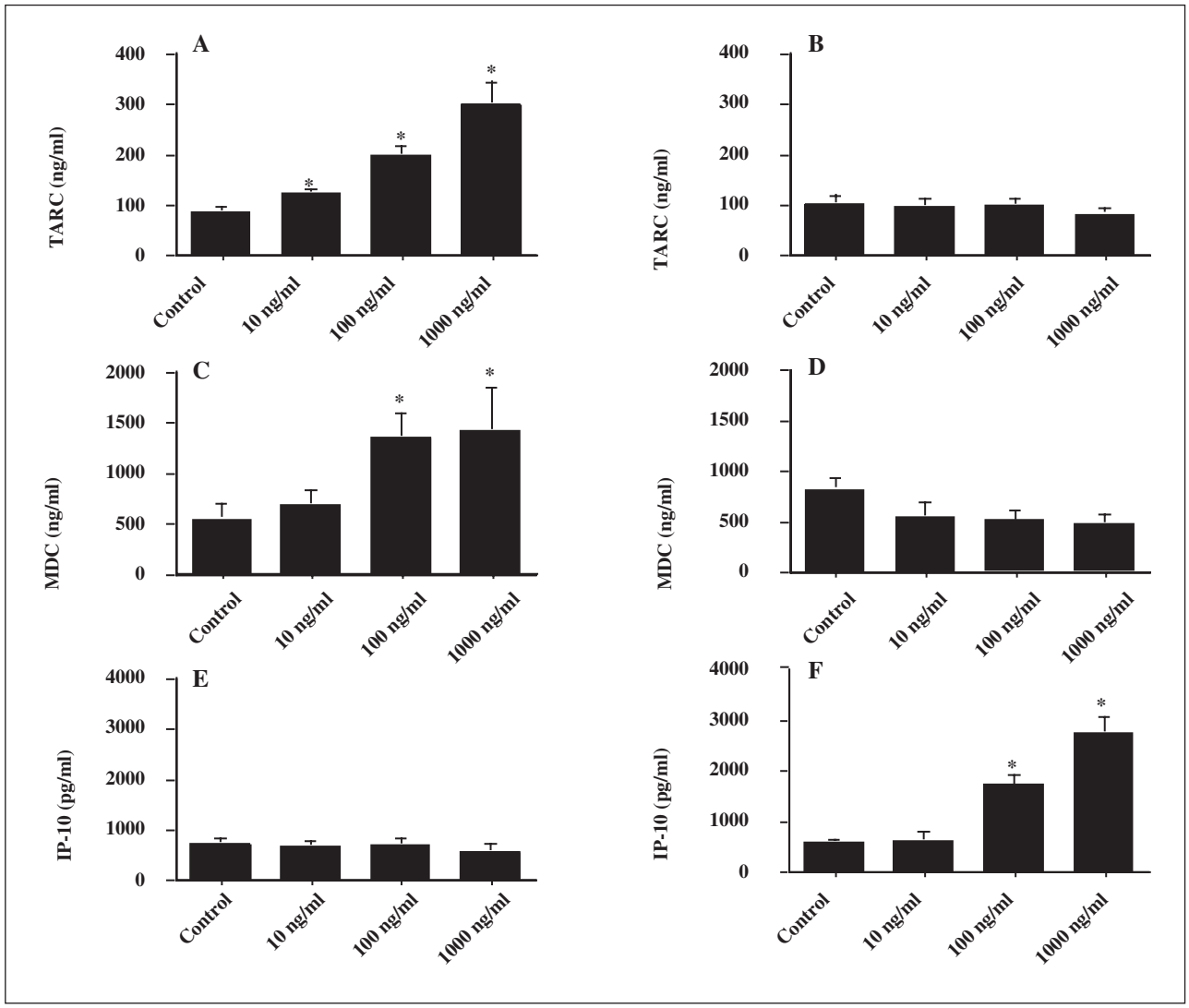

Figure 1

Effect of Der p 1 on TARC (CCL17), MDC (CCL22) and IP-10 (CXCL10) production by monocyte-derived dendritic cells. DC obtained from house dust mite-allergic patients (left panels) or from healthy donors (right panels) were incubated for 24 hours with different concentrations of Der p 1. Supernatants were collected and assayed for the presence of TARC (CCL17), MDC (CCL22) and IP-10 (CXCL10). Results are expressed as Mean ± SEM. *: p values < 0.05.

results were expressed as the subtraction of the mean number of migrating cells T cells with RPMI medium alone (negative control) from the mean number of migrating T cells with the samples. For positive controls, recombinant human MDC (CCL22) and TARC (CCL17) for Th2 clones, and IP-10 (CXCL10) for Th1 clones were used at the optimal dose of 6.10^{-7} M (data not shown) (Tebu, Le Perray-en-Yvelines France). For neutralization assays, samples were incubated for 2 hours with anti-human TARC (CCL17), anti-human MDC (CCL22) or anti-human IP10 (CXCL10) neutralizing antibodies, at a concentration of 50 µg/ml before the chemotaxis assay. To distinguish chemotaxis from chemokinesis, a T cell migration assay was performed with the test solution in the upper compartment and negative control (RPMI alone) in the lower compartment. The test solutions used were those inducing the maximal migration of clones in the standard chemotaxis assay (i.e. supernatants of DC incubated with Der p 1).

RESULTS

Der p 1 acts in a dose-dependent manner to induce the chemokine production

As shown in Figure 1, when incubated with a low dose of Der p 1 (10 ng/ml) for 24 hours, DC from patients sensitive to Dpt produced more TARC (CCL17) than unstimulated cells (Figure 1A). When DC from allergic patients were incubated with increasing doses of Der p 1 (100 and 1000 ng/ml), both TARC (CCL17) and MDC (CCL22) production was significantly increased in a dose-dependent manner at least up to 24 hours after Der p 1 incubation (Figures 1A and 1C). In contrast, IP-10 (CXCL10) production was not altered by the allergen, whatever the dose of Der p 1 used (Figure 1E).

In contrast to DC from allergic patients, when incubated with the same doses of Der p 1, DC generated from healthy donors did not modify TARC (CCL17) or MDC (CCL22) production (Figures 1B and 1D). However, IP-10

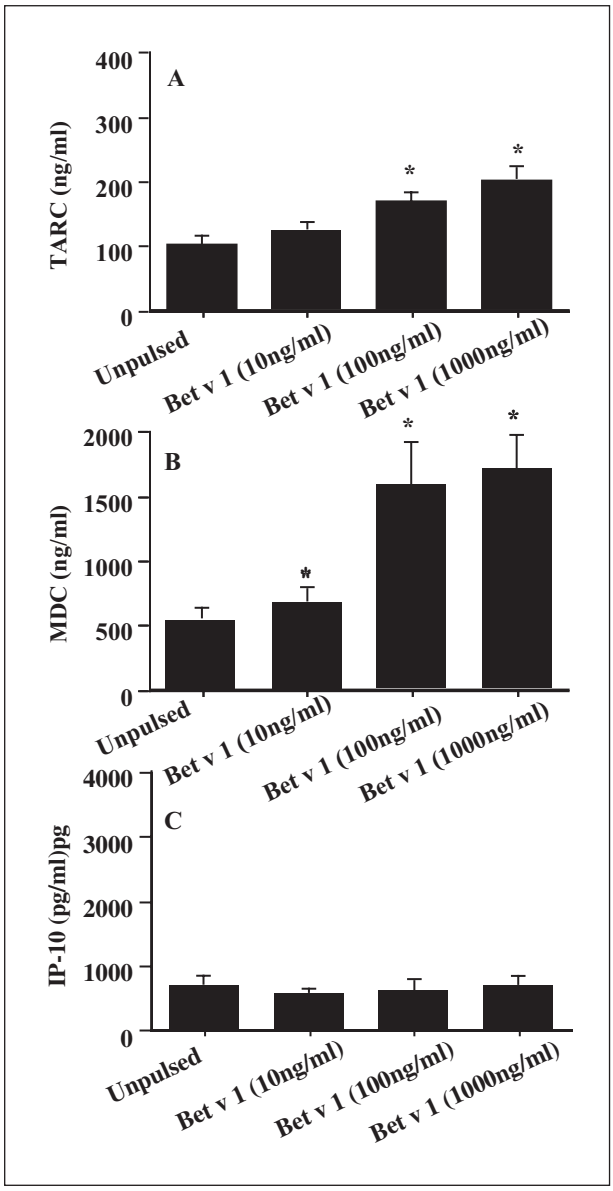

Figure 2
Effect of Bet v 1 on the production of TARC (CCL17), MDC (CCL22) and IP-10 (CXCL10) by dendritic cells of birch pollen-sensitive patients. Generated DC were incubated for 24 hours with increasing doses of Der p 1. Supernatants were then collected and assayed for the presence of TARC (CCL17), MDC (CCL22) and IP-10 (CXCL10). Results are expressed as Mean ± SEM. *: p values < 0.05.

(CXCL10) secretion was increased in a dose-dependent manner (Figure 1F).

Interestingly, when incubated with increasing doses of the irrelevant allergen Bet v 1 (a major allergen of birch pollen), DC from patients sensitive to Dpt did not not modify TARC (CCL17) and MDC (CCL22) production (data not shown). Nevertheless, as shown in Figure 2, monocyte-derived DC from patients sensitive to birch pollen, after incubation with Bet v 1, did produce increased amounts of TARC (CCL17) (Figure 2A) and MDC (CCL22) (Figure 2B), but IP-10 (CXCL10) production was not affected (Figure 2C).

Taken together, these results show that the increase in Th2-attracting chemokine production appeared to be related to the allergen used, as it required the stimulation of DC from allergic patients with the relevant allergen.

Moreover, as the dose of 100 ng/ml of Der p 1 was sufficient to induce a statistically significant increase in chemokine production by DC from allergic patients or from healthy donors, the following experiments were performed only with this dose.

The effect induced by Der p 1 is dependent on its enzymatic activity.

DC from both allergic patients and from healthy donors produced TARC (CCL17), MDC (CCL22) and IP10 (CXCL10), under baseline conditions (i.e without stimulation). However, as shown in Figure 3, unpulsed DC from allergic patients produced higher amounts of TARC (CCL17) than DC from healthy donors. Following stimulation with Der p 1 at the dose of 100 ng/ml for 6 or 24 hours, DC from allergic patients significantly increased production of TARC and MDC (CCL22) ($p < 0.05$) as compared to unpulsed DC (Figures 3A and 3C). However, in the presence of the enzymatic, inactive form of Der p 1 (ProDer p 1), no increase in TARC (CCL17) or MDC (CCL22) production was observed. Moreover, ProDer p 1 did not show any effect on IP10 (CXCL10) production by DC from allergic patients (Figure 3E). Preincubation of Der p 1 with E64, the specific inhibitor of cysteine protease led to effects similar to those observed with Pro Der p 1, the enzymatic, inactive form of Der p 1 (data not shown).

The stimulation of DC from healthy donors with Der p 1 for 6 or 24 hours did not affect TARC (CCL17) or MDC (CCL22) production (Figures 3B and 3D), but it did increase IP-10 (CXCL10) production (Figure 3F). Interestingly, this Der p 1-induced increase in IP10 (CXCL10) release was also dependent on the enzymatic activity of Der p 1 as assessed by the inhibitory effect observed with ProDer p 1-pulsed DC.

In order to determine whether Der p 1 could specifically induce the increase in the production of type 1 or type 2 chemokines by DC from healthy donors and from allergic patients, the effect of Der p 1 was investigated on RANTES (CCL5) and MIP-1α (CCL3) secretion, which is required for recruiting both Th1 and Th2 cells.

Der p 1-pulsed DC from both allergic patients and healthy donors showed a significant increase in the production of MIP-1α (CCL3) (Figures 4A and 4B respectively) and RANTES (CCL5) (Figures 4C and 4D respectively). The effect induced by Der p 1 was reduced in the presence of ProDer p 1. LPS also induced the increase of both chemokines. No statistically significant differences were observed between DC from allergic patients and DC from healthy donors.

Der p 1 favours the attraction of pre-established Th2 cells by monocyte-derived DC from allergic patients through TARC (CCL17) and MDC (CCL22) production.

The involvement of TARC (CCL17), MDC (CCL22) and IP10 was addressed in chemotaxis assays on IFN-γ-producing Th1 and IL-4-producing Th2 clones (Figure 5), and by specific chemokine blockade experiments. Super-

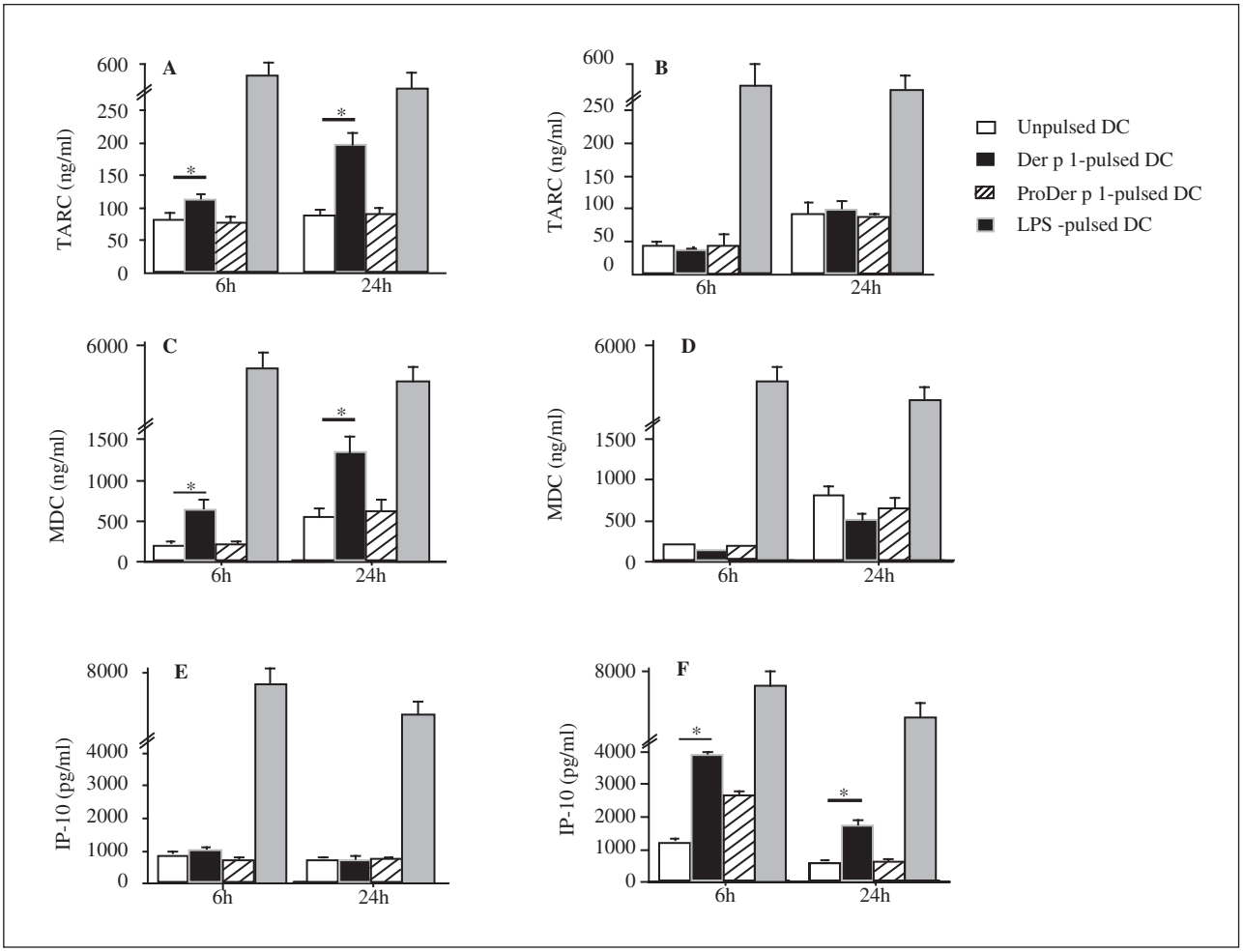

Figure 3

The effect induced by Der p 1 is dependent on its enzymatic activity. DC obtained from allergic patients (left panels) or from healthy donors (right panels) were pulsed or not for 6 and 24 hours with 100 ng/ml of Der p 1 100 ng/ml of ProDer p 1 or with 1 µg/ml of LPS. Supernatants were collected and assayed for the presence of TARC (CCL17), MDC (CCL22) and IP-10 (CXCL10). Results are expressed as Mean ± SEM. *: p values < 0.05.

natants of DC generated from allergic patients and incubated with Der p 1, showed a high capacity to recruit Th2, as compared to unpulsed DC but no enhanced capacity to recruit Th1 clones (Figure 6A). The addition of neutralizing anti-TARC (CCL17) or anti-MDC (CCL22) neutralizing antibodies to the supernatants of Der p 1-pulsed DC prior to the assay greatly reduced the chemotactic activity towards Th2 clones (68% and 51% respectively). Anti-IP10 blocking antibodies did not affect Th2 clone recruitment and showed only a slight effect on Der p 1-induced Th1 clone recruitment.

In contrast, supernatants of Der p 1-pulsed DC from healthy donors showed a high capacity to recruit Th1 clones, as compared to unpulsed DC, but no enhanced capacity to recruit Th2 clones (Figure 6B). The effect, Der p 1-pulsed DC-induced recruitment of Th1 clones was greatly reduced only in the presence of neutralizing anti-IP10 antibodies.

DISCUSSION

The prevalence of atopic diseases has increased rapidly during recent years. These diseases represent complex and chronic inflammation disorders that involve the recruitment and activation of many inflammatory cells releasing many inflammatory mediators. Chemokines and their receptors are shown to be critical in the leukocyte trafficking from blood to the inflammation sites. Among the chemokines, only a few of them are specifically involved in the recruitment of defined subsets of T cells. Whereas IP-10 (CXCL10) has been described as attracting Th1 cells [17], TARC (CCL17) and MDC (CCL22) chemokines have been more recently associated with the recruitment of Th2 cells [18].

Allergic diseases are characterized by a Th2 profile. DC can favour this Th2 response in the absence of IL-12 production [19], and through the production of chemokines specifically involved in the recruitment of Th2 effector cells at the inflammation sites. In a previous work, we have shown that DC from allergic patients exposed to the allergen Der p 1 enhanced the Th2 response through an increase in IL-4 production by autologous T cells [13].

In this study, we have shown that DC from allergic patients exposed in vitro to Der p 1, rapidly increase their TARC (CCL17) and MDC (CCL22) production, whereas IP-10 (CXCL10) production was not modified by Der p 1. In contrast, DC from healthy donors incubated with Der p 1 preferentially increased IP-10 (CXCL10) production,

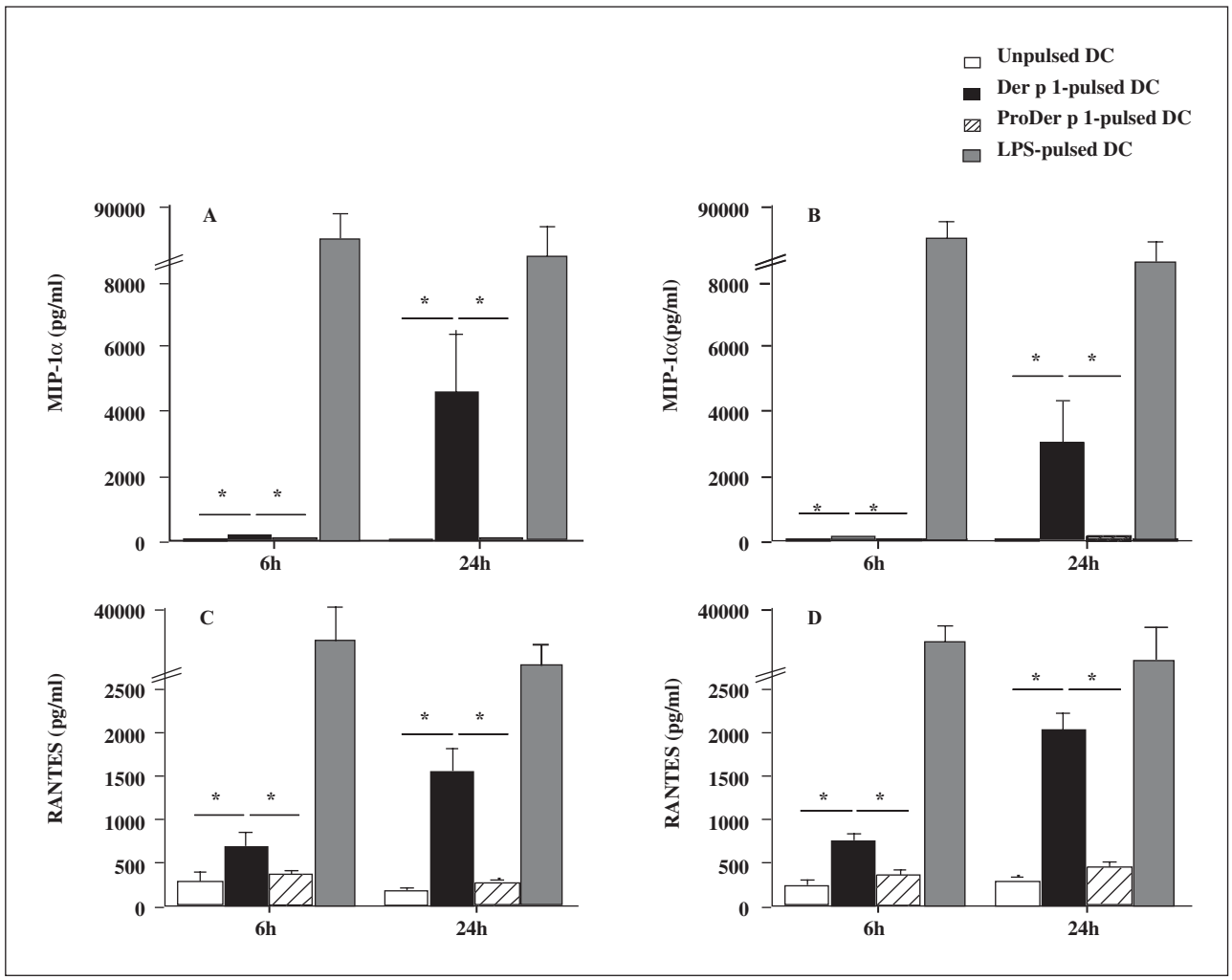

Figure 4

Effect of Der p 1 on the production of MIP-1α (CCL3) and RANTES (CCL5) by dendritic cells. DC obtained from allergic patients (left panels) or from healthy donors (right panels) were pulsed or not for 6 and 24 hours with 100 ng/ml of Der p 1 100 ng/ml of ProDer p 1 or with 1 µg/ml of LPS. Supernatants were collected and assayed for the presence of TARC (CCL17), MDC (CCL22) and IP-10 (CXCL10). Results are expressed as Mean ± SEM. *: p values < 0.05.

but TARC (CCL17) and MDC (CCL22) release remained unchanged in the presence of Der p 1. DC have the capacity to constitutively produce chemokines such as MDC (CCL22) [20] or TARC (CCL17). In these cells, only TARC (CCL17) and MDC (CCL22) were increased following exposure to Der p 1. TARC (CCL17) and MDC (CCL22) have been shown to be associated with type 2 disorders. MDC (CCL22) was reported to be more expressed by DC in skin biopsies of subjects with atopic dermatitis. In addition, very low amounts of MDC (CCL22) were detected in sera of patients with Crohn's disease characterized by a Th1 predominance [21]. In mice and in humans, both TARC (CCL17) and MDC (CCL22) were associated with atopic dermatitis and were expressed in skin DC [12, 22]. They were also expressed in a murine model of pulmonary allergic inflammation [23] and in bronchial biopsies of asthmatic patients [24]. Interestingly, in our study, both TARC (CCL17) and MDC (CCL22) were highly expressed by DC from allergic patients, and were increased after stimulation with Der p 1. The up-regulation of TARC (CCL17) and MDC (CCL22) release suggest that DC from allergic patients are highly efficient at attracting Th2 cells.

IP-10 (CXCL10) is constitutively produced by DC and can be up-regulated *in vitro*, particularly after a stimulation with LPS (i.e. Th1 conditions) [25], or *in vivo* as observed in a mouse model of acute lung inflammation [26]. In our study, an antigenic stimulation of DC with tetanus toxoid (leading to a Th1 response), showed an increase in IP-10 (CXCL10) production (data not shown). This had already been shown after incubation of DC with the C-fragment of tetanus toxin, which induced an increase in maturing cytokines TNF-α and IL-1β associated with an up-regulation of IP-10 (CXCL10) production in a dose-dependent manner [27]. Interestingly, in our case, only DC from healthy donors increased IP-10 (CXCL10) release after Der p 1 exposure. As IP-10 (CXCL10) is considered to be a Th1-specific chemokine [16], these results suggest that DC from healthy donors exposed to an allergen or to an antigen, would preferentially attract Th1 cells via IP-10 (CXCL10) production. In contrast, in DC from allergic

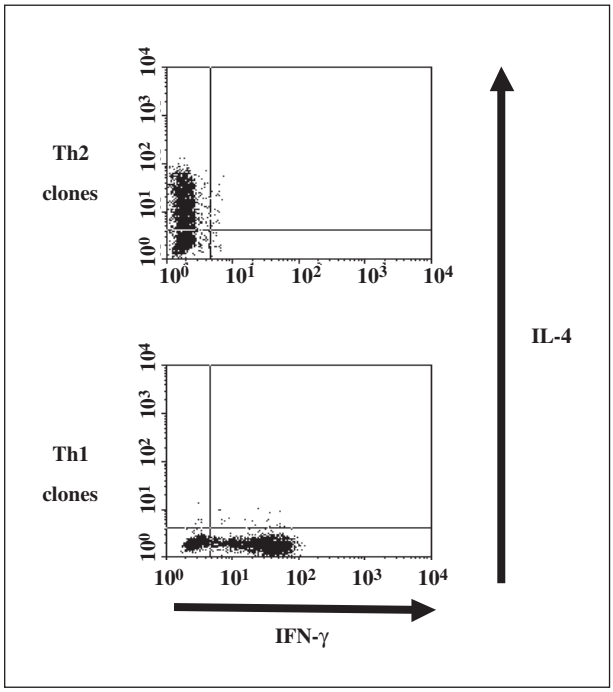

Figure 5
Cytokine profile of Th1 and Th2 clones. The polarised cytokine profiles of the Th1 and Th2 clones were routinely analysed by intracellular staining after every restimulation. Therefore, resting Th cells were stimulated with PMA (10 ng/ml) and ionomycin (1 µg/ml) for 6 hours, the last 5 hours in the presence of Brefeldin A (10 µg/ml). After fixation, the cells were stained with anti-human IFN-γ-FITC and anti-human IL-4-PE in the presence of 0.5% saponin. The data shown here for one Th1 and one Th2 clone are representative for all Th clones used.

patients exposed to Der p 1, IP-10 (CXCL10) production was not modified. Surprisingly, IP-10 (CXCL10) production was not modified by Der p 1 or Bet v1 suggesting DC from allergic patients are not very efficient at recruiting Th1 cells.

We have already reported that DC from patients sensitive to Dpt react to Der p 1 (IL-6 and IL-10 production) differently to DC from healthy donors (IL-12 production), not only *in vivo* [13], but also *in vivo* [28]. To explain the difference observed in chemokine production, several parameters must be taken into account. Firstly, it must be underlined that DC were derived from monocytes, and in allergic patients these cells have already been in contact with Th2 cytokines (IL-4, IL-5, IL-13), which are known to favour the MDC and TARC production [29, 30]. Dysregulation between the different Der p 1-pulsed dendritic cell populations from allergic and non-allergic donors was recently reported [31]. Secondly, PGE2 and cyclic AMP-elevating agents were shown to enhance the MDC and TARC production [32]. In addition, prostaglandins such PGE2 can inhibit 5-lipoxygenase-activating protein expression and leukotriene B4 production from dendritic cells via an IL-10-dependent mechanism [33], and also suppress the production of IFN-inducible protein-10 (IP-10), the ligand for CXC chemokine receptor 3 expressed on Th1 cells [34]. As, under our conditions, IL-10 production was observed only with Der p 1-pulsed DC from patients sensitive to Dpt, a regulatory function via prostaglandins can be hypothesized. A recent study has emphasized the potential effect of prostaglandins and their derivatives on the peroxisome proliferator-activated receptor gamma and the polarisation of the immune response towards a Th2 profile [35].

Moreover, to assess the potential involvement of TLRs [36] (such as TLR4, which is known to be implicated in the IL12 p70 and IP-10 production associated with Th1 responses), and the STAT factors or NF-kappa B family of transcription factors in the fine regulation of chemokine production, further experiments are required.

Der p 1 is known to display a cysteine protease activity, which is important in type 2 responses both *in vivo* [27, 37] and *in vitro* [38]. Another work also reported the importance of this enzymatic activity in the production by epithelial cells of many cytokines [39]. In this report, we have shown that Der p 1-induced TARC (CCL17) and MDC (CCL22) production was also highly dependent on its cysteine protease activity, as type 2 chemokine production was reduced after incubation either with E64 (a specific inhibitor of cysteine protease) or with the proenzymatic and inactive form of Der p 1, ProDer p1. In a previous report [13], we have shown that Der p 1-pulsed DC from Dpt-sensitive patients produced more IL-6 and IL-10, but less IL-12 than DC from healthy donors, and favoured autologous purified T cells to preferentially produce IL-4 rather than interferon-γ. Interestingly, this DC reactivity was shown to be associated with the enzyme activity of Der p 1 and with the costimulatory molecule (CD80/CD86) expression [13]. One potential mechanism by which Der p 1 could modify some DC functions may be due to the effect of Der p 1 on CD23 marker. In preliminary experiments (n = 4) carried out on patients sensitive to Dpt, a significant increase in CD86 was observed when DC were incubated either with Der p 1, anti-CD23 IgG antibodies or with soluble CD23 (which mimicked the effect of Der p 1); this increase was abolished if blocking anti-CD23 Fab was added before Der p 1 allergen. Some reports have underlined the capacity of Der p 1 to cleave CD23 and to favour the development of Th2 profile [37, 38]. However, CD23 is also expressed by DC from patients sensitive to the pollen Bet v 1, and Der p 1 can increase CD80 expression on DC from healthy donors. Thus the "specificity" of the stimulation of DC mainly by the related allergen might involve other regulatory pathways. The involvement of protease-activated receptors (whose PAR-2 is associated with allergy), the Toll-like receptors [36], and other receptors required for antigen uptake [40] are currently being investigated. Nevertheless, as DC were derived from blood monocytes, the potential participation of residual IgE at the cell surface in the selective uptake of the allergen can not be totally excluded.

In the last part of this work, the capacity of supernatants of DC, from both allergic patients and healthy donors, to recruit Th2 clones (Th1/Th2) was tested. At baseline, DC from allergic patients showed a greater capacity than DC from healthy donors to attract Th2 cells. After stimulation with Der p 1, the number of Th2 cells recruited by DC from allergic patients was further increased. Conversely, after Der p 1 exposure, DC from healthy donors showed a poor capacity to recruit Th2 cells. These chemotaxis data fit well with the profile of chemokines produced in response to Der p 1 by both DC from allergic patients and healthy donors, and with the known effects of TARC (CCL17) and MDC (CCL22) in the recruitment of Th2 cells [25, 29]. The involvement of both chemokines in allergen-induced recruitment of Th2 cells was furthermore confirmed using

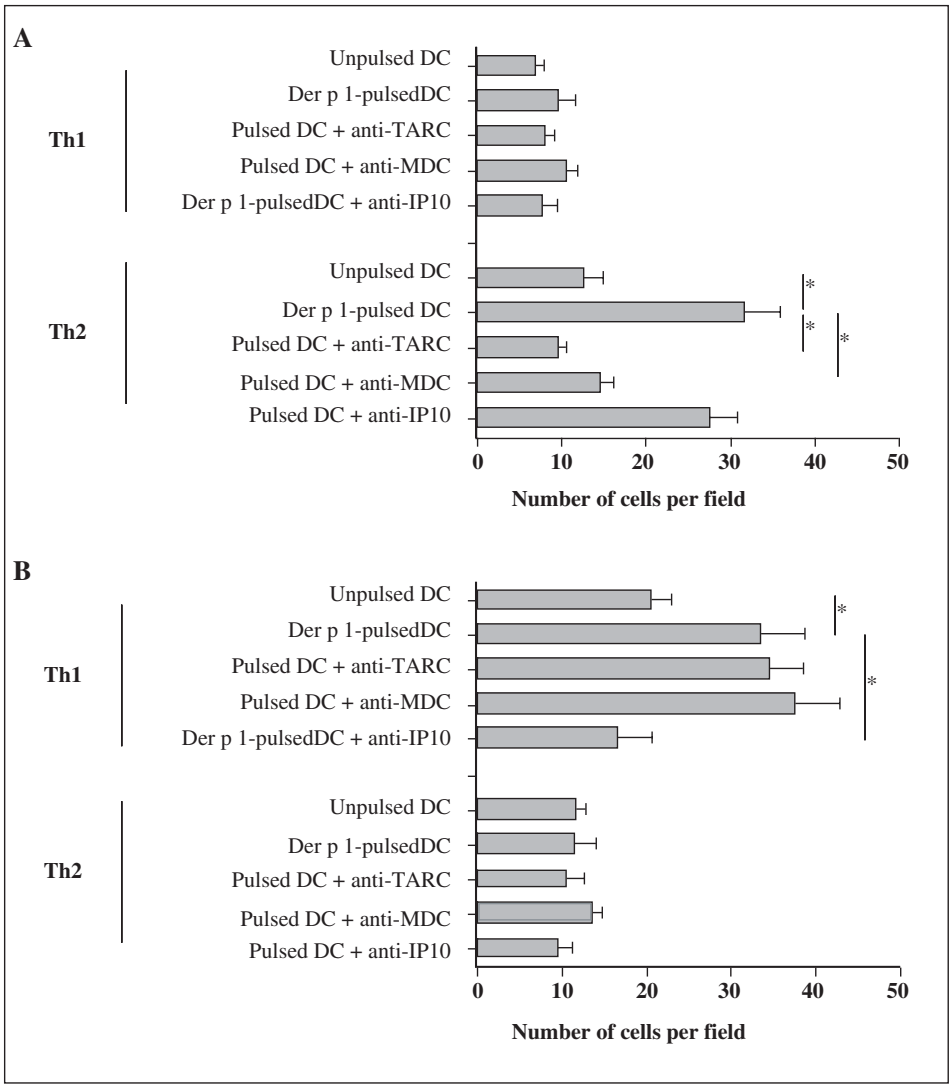

Figure 6

Capacity of dendritic cells exposed or not to Der p 1 to induce chemotaxis of Th1 and Th2 clones. Supernatants of dendritic cells from allergic patients (A) and from healthy donors (B) incubated or not with 100 ng/ml of Der p 1, were harvested after 24 hours and used in an *in vitro* chemotaxis assay with either Th1 or Th2 clones. Where indicated, culture supernatants had been pre-incubated with neutralizing anti-TARC (CCL17) anti-MDC (CCL22) or anti-IP-10 (CXCL10) mAb at a final concentration of 50 µg/ml. Results are expressed as Mean ± SEM. *: p values < 0.05.

neutralizing anti-TARC (CCL17) and anti-MDC (CCL22) antibodies. However, the inhibition observed with both of these antibodies was not complete, suggesting the involvement of other Th2 chemokines. Allergen-induced IP10 production by DC from healthy donors was shown to induce the preferential recruitment of Th1 clones, as assessed by the use of neutralizing anti-IP10 antibodies. IP10 has been suggested to have a role in the maintenance of protective Th1 responses in healthy donors [16].

In allergic patients, the up-regulation of TARC (CCL17) and MDC (CCL22) production by DC exposed to Der p 1, may contribute to the recruitment of early activated Th2 cells expressing CCR4 [10, 17, 41]. In this way, DC ensure that recently activated Th2 cells are attracted, retained in the airways and do not migrate back to the draining lymph nodes [42]. This would mean that DC, through the production of the type 2 chemokines closely associated with the specific recruitment of Th2 cells, such as TARC (CCL17) and MDC (CCL22), may participate in the enhancement of the allergic reaction.

Acknowledgements. The authors wish to thank Pr. B. Wallaert and Dr. A. Tsicopoulos and the personnel of the Calmette Hospital for the selection of patients and for the blood collection. We also wish to thank Drs A.Didierlaurent and C. André (Stallergènes SA, Antony, France) for providing Bet v 1.

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