

# Bet v 1 and its homologous food allergen Api g 1 stimulate dendritic cells from birch pollen-allergic individuals to induce different Th-cell polarization

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## Keywords

birch pollen allergy; birch pollen food syndrome; cross reactivity; dendritic cells; Th1/Th2.

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## Abstract

**Background:** Bet v 1 is the most relevant sensitizing protein for birch pollen (BP)-allergic individuals. Its homologues from plant foods are mainly involved in allergic reactions caused by IgE cross reactivity. We aimed to evaluate the polarizing effect of dendritic cells (DCs) pulsed with Bet v 1, Mal d 1, Api g 1 or Dau c 1 on Th-cell responses.

**Methods:** Immature DCs were generated from peripheral blood monocytes of BP-allergic and healthy donors by culture with GM-CSF and IL-4 and subsequently pulsed with allergens in combination with maturation factors. Cell surface markers were analysed by FACS. Mature DCs were co-cultured with autologous Th cells and T-cell proliferation and cytokine profiles were determined.

**Results:** In co-culture, mature allergen-pulsed DCs induced autologous Th cells of BP-allergic donors to proliferate significantly more than those of healthy individuals. Exposure of DCs from BP-allergic donors to Bet v 1 resulted in a robust Th2 skewing with significantly higher quantities of IL-5 and elevated IL-13 compared to maturation factors. In contrast, Api g 1-primed DCs from BP allergics significantly enhanced the production of the Th1 cytokine IFN- $\gamma$  and significantly down-regulated IL-13 compared to maturation factors. In healthy donors, no significant cytokine production could be detected.

**Conclusion:** Bet v 1 in contrast to homologous food allergens seems to possess distinct molecular features that enable it to condition DCs from BP-allergic donors to induce allergen-specific T-cell proliferation and Th2 polarization.

The major birch pollen (BP) allergen Bet v 1 sensitizes via the respiratory route and can also lead to BP-associated food allergy caused by its cross reactivity with homologous plant food proteins (1, 2). Bet v 1-related allergens are members of the pathogenesis-related protein family 10 (3) and are found in various plant foods, particularly in fruits of the Rosaceae (e.g. apple: Mal d 1, cherry: Pru a v 1), vegetables of the Apiaceae (e.g. celery: Api g 1, carrot: Dau c 1) and seeds of the Fabaceae (e.g. peanut: Ara h 8, soybean: Gly m 4) (4, 5).

## Abbreviations

Ag, antigen; BP, birch pollen; DC, dendritic cell; MF, maturation factors; MoDC, monocyte-derived dendritic cell; PBMC, peripheral blood mononuclear cells; Th, T helper cell.

Symptoms to Bet v 1-related plant food allergens at the humoral and cellular levels are caused primarily by sensitization against Bet v 1 (6, 7). Conservation of both surface residues and main chain conformation (8) within the Bet v 1 family is the basis for this cross reactivity, which seems to be strictly dependent on the intact tertiary fold of the protein (9–11). As a consequence, Bet v 1 homologues from plant foods were postulated to lack sensitizing capacity as they are highly labile to heat treatment and gastric digestion which leads to the destruction of their conformational epitopes (4, 12, 13). This raises the question whether Bet v 1 possesses unique immunologic attributes.

Dendritic cells (DCs) are the key players in the initial stage of the interaction of allergens and the immune system that

shapes the differentiation of naïve Th cells. DCs are regarded as the most potent antigen-presenting cells specialized in antigen capture, processing, and, in their mature state, presentation of antigenic peptides to uncommitted CD4<sup>+</sup> Th cells (14). Depending on the nature of the antigen and the maturation state of DCs (15–18), they instruct newly activated Th cells to differentiate into effector subsets of a Th1, Th2, or Th17 phenotype (14, 19, 20). In allergic individuals, the uptake of allergens by DCs results in an allergen-specific Th2-biased immune response culminating in the production of allergen-specific IgE. The initial triggers leading to the manifestation of an allergic disease are still insufficiently resolved.

In this study, we examined whether Bet v 1 and its homologues differ in their ability to activate monocyte-derived DCs (MoDCs) from BP-allergic and healthy donors as measured by up-regulation of maturation markers and induction of Th-cell proliferation and differentiation.

## Methods

### Recombinant proteins

Recombinant Bet v 1.0101 was expressed and purified as described (21). Bacterial endotoxin concentration was determined by the Limulus Amebocyte Lysate QCL-1000 assay (Cambrex, Walkersville, MD, USA) and was <3 EU/mg recombinant allergen. rMal d 1.0108 (1 EU/mg), rApi g 1.0101 (5 EU/mg) and rDau c 1.0103 (37 EU/mg) were purchased from BIOMAY AG (Vienna, Austria) and LPS from Sigma (Sigma-Aldrich, Steinheim, Germany).

### Blood samples

Heparinized or EDTA-treated blood was obtained from nine atopic donors (six women, three men, mean age 30.8) suffering from allergic rhinoconjunctivitis to BP and allergic symptoms after ingestion of BP-related foods (Table 1). Sensitization to BP was documented by case history, positive skin prick test and CAP classes  $\geq 2$  to birch. Adverse reactions to pollen-related foods were assessed by interrogation in standardized interviews. All patients stated to have an oral allergy syndrome to apple, five to hazelnut, four to peach and two to carrot. Two of these patients had experienced severe symptoms of gastrointestinal disorders after ingestion of BP-related fruits. Healthy donors (seven women, mean age 27.8) with no history of allergic symptoms were included as controls. This study was approved by the Ethical Committee of the Medical University of Vienna, and informed consent was obtained from all participants.

### Detection of IgE specific for Bet v 1-related allergens by ELISA

Maxisorp microtiter plates (Nalge Nunc International, Roskilde, Denmark) were coated with 5 µg/ml of recombinant proteins. ELISA experiments were performed as described (22). OD values were counted positive if they exceeded the mean OD of the negative controls by more than three standard deviations.

**Table 1** Characteristics of BP-allergic patients used in this study

| Patients | Total IgE<br>(kU/l) | CAP class<br>to BP | IgE as determined by ELISA (OD <sub>405 nm</sub> ) |              |              |              |              | BP-related<br>symptoms | Allergies to other<br>aeroallergens<br>determined by CAP | Food allergies<br>determined<br>by CAP | SPT positive to<br>aeroallergens/<br>food allergens |
|----------|---------------------|--------------------|----------------------------------------------------|--------------|--------------|--------------|--------------|------------------------|----------------------------------------------------------|----------------------------------------|-----------------------------------------------------|
|          |                     |                    | Bet v 1.0101                                       | Mal d 1.0108 | Api g 1.0101 | Dau c 1.0103 |              |                        |                                                          |                                        |                                                     |
| 1        | NA                  | 4                  | 2.7                                                | 0.6          | 0.0          | 0.0          | A, RC, OAS   | H, L                   | Ap, Hn, Pe                                               | NA                                     |                                                     |
| 2        | 4155                | 4                  | 1.4                                                | 0.5          | 0.3          | 0.1          | A, E, R, OAS | G, Ra, M, Al           | Ap, Hn, Pe                                               | BP, H                                  |                                                     |
| 3        | 444                 | 5                  | 3.8                                                | 0.9          | 0.2          | 0.0          | RC, OAS      | G, M, C                | Ap                                                       | BP, C, D, G, M, Ra                     |                                                     |
| 4        | 1700                | 3                  | 3.9                                                | 3.9          | 0.9          | 0.6          | RC, OAS      | G, Ra                  | Ap, Ca                                                   | BP, Ce, Ma, Mlu,<br>K, Pe, Ra          |                                                     |
| 5        | 37.5                | 3                  | 0.7                                                | 0.4          | 0.0          | 0.0          | A, RC, OAS   | Al, G                  | Ap, Hn, Pe, S                                            | Al, BP, Ce, Pe                         |                                                     |
| 6        | NA                  | NA                 | 2.3                                                | 1.4          | 0.1          | 0.1          | A, RC, OAS   | G                      | Ap, Pe                                                   | BP, G, M, Mo, Ra,                      |                                                     |
| 7        | NA                  | NA                 | 0.2                                                | 0.2          | 0.2          | 0.2          | AG, RC, OAS  | G                      | Ap, Ac, Ca,<br>Hn, Me                                    | BP, G, Mo                              |                                                     |
| 8        | 46.7                | 3                  | 1.0                                                | 0.3          | 0.0          | 0.0          | R, OAS       | G, H, Ra               | Ap                                                       | Al, BP, C, D, H,<br>Mo, Mlu, Ra, Ry    |                                                     |
| 9        | 386                 | 4                  | 3.7                                                | 2.2          | 0.2          | 0.3          | R, OAS       | M                      | Ap, Hn, Cn                                               | BP, G, Ry                              |                                                     |

NA, not available; OAS, oral allergy syndrome; A, asthma; AG, angioedema; E, eczema; R, rhinitis; RC, rhinoconjunctivitis; BP, birch pollen; G, grass; M, house dust mites; Mo, mould; Mu, mugwort; Ra, ragweed; Ry, rye; D, dog; C, cat; Al, alternaria; H, hazel; L, latex; Ap, apple; Cn, chestnut; Hn, hazelnut; K, kiwi; Pe, peach; Ca, carrot; Ce, celery; S, soy; Ac, apricot; Ma, mango; Me, melon.

### Generation of MoDCs

Immature MoDCs were generated by culture of peripheral blood monocytes as described (21). On day 7, cells were pulsed for 48 h with 10 µg/ml rBet v 1.0101, rMal d 1.0108, rApi g 1.0101, or rDau c 1.0103 in combination with 25 ng/ml TNF- $\alpha$  (Strathmann, Hamburg, Germany) and 10 ng/ml IL-1 $\beta$  (PeproTech, Rocky Hill, NJ, USA), referred to in the text as maturation-inducing factors (MFs). As controls, either the cells were treated with 20 µg/ml LPS (Sigma-Aldrich) or MFs, or left untreated (immature MoDCs).

### Expression of cell surface markers

Surface expression of DC markers was analyzed using multi-colour flow cytometry (21).  $5 \times 10^3$  events were acquired with a FACSCanto unit (BD Biosciences – Immunocytometry Systems, San Jose, CA, USA) and analyzed using the BD FACS-Diva software (BD Biosciences – Immunocytometry Systems).

### Purification of T cells

CD4<sup>+</sup> T cells were isolated from PBLs by negative selection using antibody-coated paramagnetic MicroBeads (CD4<sup>+</sup> T-cell isolation kit II, MACS; Miltenyi Biotec, Bergisch Gladbach, Germany). From the resulting CD4<sup>+</sup> population, CD45RA<sup>+</sup> T cells were enriched to >85% purity (Fig. 2A) by negative selection using a phycoerythrin (PE)-labelled anti-human CD45RO antibody (Dako, Glostrup, Denmark) and anti-PE magnetic beads (Miltenyi Biotec). T cells were frozen in Iscove's modified Dulbecco's medium (IMDM; Bio-Whittaker, Walkersville, MD, USA) containing 50% (v/v) FCS and 10% (v/v) dimethyl sulfoxide until use.

### Co-culture of T cells and autologous allergen-pulsed MoDCs

For proliferation and cytokine production assays,  $1 \times 10^5$  enriched naïve CD4<sup>+</sup> T cells were co-cultured in 96-well plates (Corning Life Sciences, Lowell, MA, USA) in triplicates with  $1 \times 10^4$  autologous MoDCs previously pulsed with MFs and/or allergen or immature MoDCs in 200 µl IMDM supplemented with 86 µg/l gentamycin and 10% (v/v) FCS. After 6 days, supernatants were collected, and cells were pulsed with 0.5 µCi of <sup>3</sup>H-thymidine per well (GE Healthcare) for 16 h. <sup>3</sup>H-thymidine incorporation was measured in a beta counter (MicroBeta TriLux; PerkinElmer, Waltham, MA, USA).

### Cytokine ELISA

IL-5, IL-10, IL-13 and IFN- $\gamma$  were assayed by sandwich ELISA (Thermo Fisher Scientific, Waltham, MA, USA) according to the instructions of the distributor. The detection limit was 6.2 pg/ml.

### Statistics

Paired analyses were performed with the non-parametric Wilcoxon signed-rank test and unpaired analyses with the

non-parametric Mann–Whitney *U*-test.  $P < 0.05$  was considered as statistically significant. Calculations were performed with SPSS (Chicago, IL, USA).

## Results

### MFs induce a mature DC phenotype in immature MoDCs from BP-allergic and healthy donors

MFs induced a mature DC phenotype similar to that induced by LPS (data not shown) characterized by the expression of the DC-specific marker CD83 and up-regulation of the costimulatory molecules CD80 and CD86 compared to immature MoDCs (Fig. 1). Upon combined administration of allergens and MFs, CD80 expression was significantly up-regulated in both groups when compared to immature MoDCs. Though not statistically significant, increase in CD80 expression was higher in the healthy donor group (Fig. 1, CD80). In case of CD86, only cells of BP-allergics pulsed with allergens plus MFs showed significantly increased marker expression upon the background level of immature MoDCs (Fig. 1, CD86, BP allergics). Compared to MFs, marker expression on MoDCs of either BP-allergic or healthy donors was not significantly modulated by addition of the allergens. CD1a and CD40 expression was kept at basal levels and did not differ between the two study groups (Fig. 1). HLA-DR expression also remained unchanged, but was higher on MoDCs of BP-allergic donors compared to MoDCs of healthy individuals (Fig. 1, HLA-DR).

### Allergen-primed MoDCs from BP-allergic patients stimulated a Th-cell proliferative response in contrast to MoDCs of healthy donors

The responsiveness of >85% pure autologous CD4<sup>+</sup> T cells (Fig. 2A) to allergen-primed MoDCs was assayed in co-culture experiments. With LPS-pulsed MoDCs as positive control, Th cells of BP-allergic donors proliferated significantly more ( $**P = 0.01$ ) than those of healthy individuals (Fig. 2B). Co-culture of mature MoDCs and autologous Th cells from BP-allergic or healthy donors (Fig. 2C, MFs) resulted in proliferation values similar to that of LPS (Fig. 2B, LPS). Allergen-specific proliferative responses of CD4<sup>+</sup> T cells of BP-allergic donors were found only for rBet v 1 with  $**P = 0.008$  to MFs values (Fig. 2C, BP allergics, Bet v 1). While rBet v 1 was capable of inducing a substantial increase in Th-cell proliferation, no significant proliferation was induced by the other allergens (Fig. 2C, BP allergics). Allergen-pulsed MoDCs of healthy donors failed to induce a proliferative response in autologous Th cells with values on a level with that induced by MFs alone (Fig. 2C, healthy donors).

### rBet v 1-pulsed MoDCs of BP-allergic donors lead to the enhancement of Th2 cytokine secretion by Th cells

rBet v 1-loaded MoDCs of BP-allergic donors demonstrated Th2 skewing with significantly higher quantities of IL-5

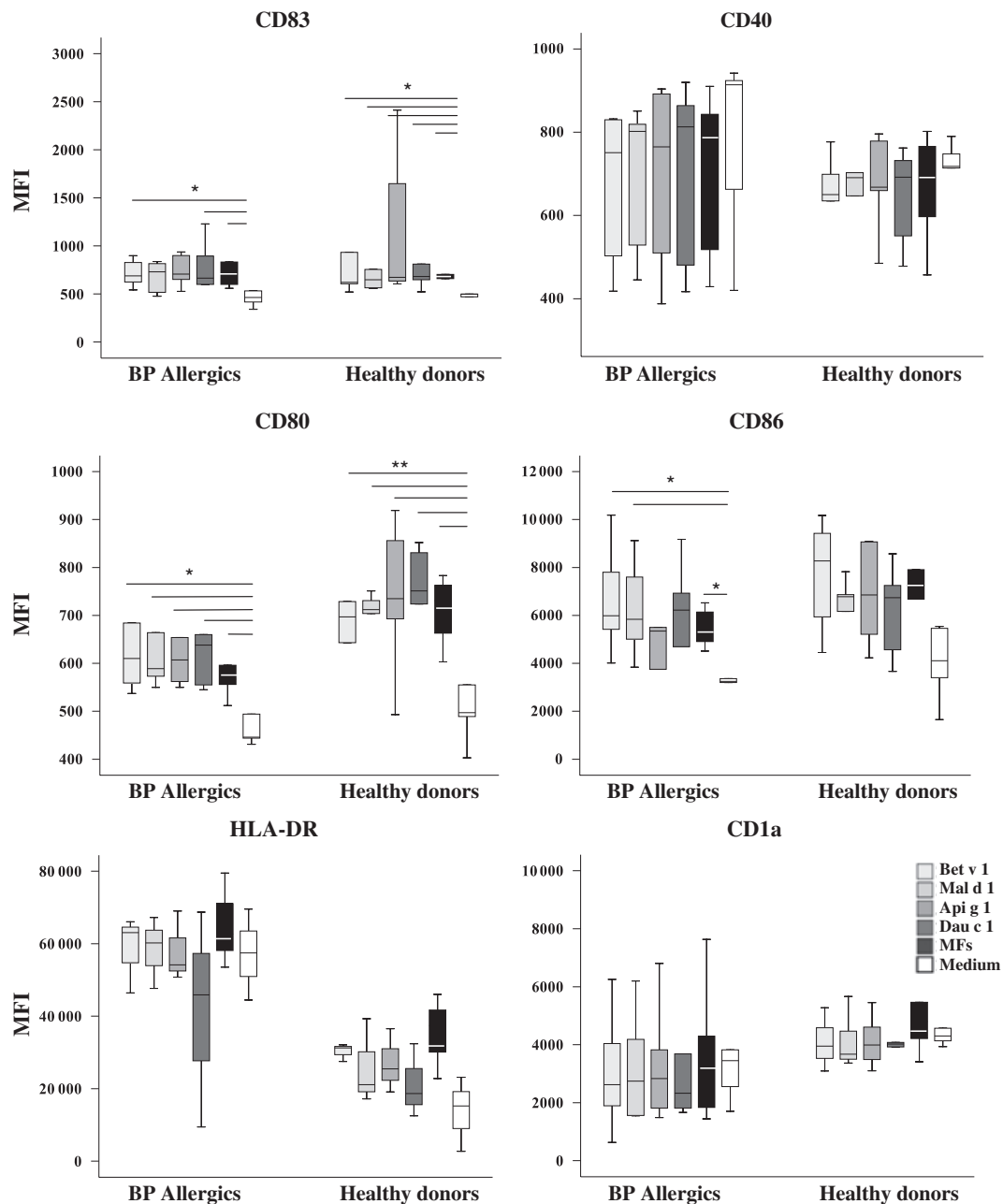


**Figure 1** Effect of Bet v 1 and Bet v 1 homologues on the expression of cell surface markers on MoDCs of BP-allergic and healthy donors. MoDCs were treated with allergens in combination with MFs for 48 h. As a control, MoDCs were either left in the immature state (medium) or treated with MFs only. Cells were analyzed

for marker expression by flow cytometry. Data, expressed as box-plot summary of the mean fluorescence intensity (MFI) of individual experiments (BP allergic  $n = 7$ ; healthy donors  $n = 5$ ), are shown for the 10  $\mu\text{g}/\text{ml}$  allergen dose. Horizontal lines represent the median. \* $P < 0.05$ ; \*\* $P < 0.01$ , paired  $t$ -test.

produced compared to MFs (\* $P = 0.017$ ), but also to rMal d 1 (\* $P = 0.043$ ), rApi g 1 (\* $P = 0.012$ ) and rDau c 1 (\* $P = 0.012$ ; Fig. 3, IL-5 BP allergics). A marked increase, although not statistically significant, could also be shown for IL-13 expression following rBet v 1 stimulation (Fig. 3, IL-13 BP allergics).

Remarkably, rApi g 1-primed MoDCs instructed autologous T cells to significantly down-regulate IL-13 production ( $P = 0.05$ ) compared to MF-induced cytokine modulation (Fig. 3, IL-13, BP allergics). rMal d 1 stimulation also led to increased production of the Th2 cytokines IL-5 and IL-13 but less pronounced than rBet v 1 (Fig. 3, IL-5 and IL-13,

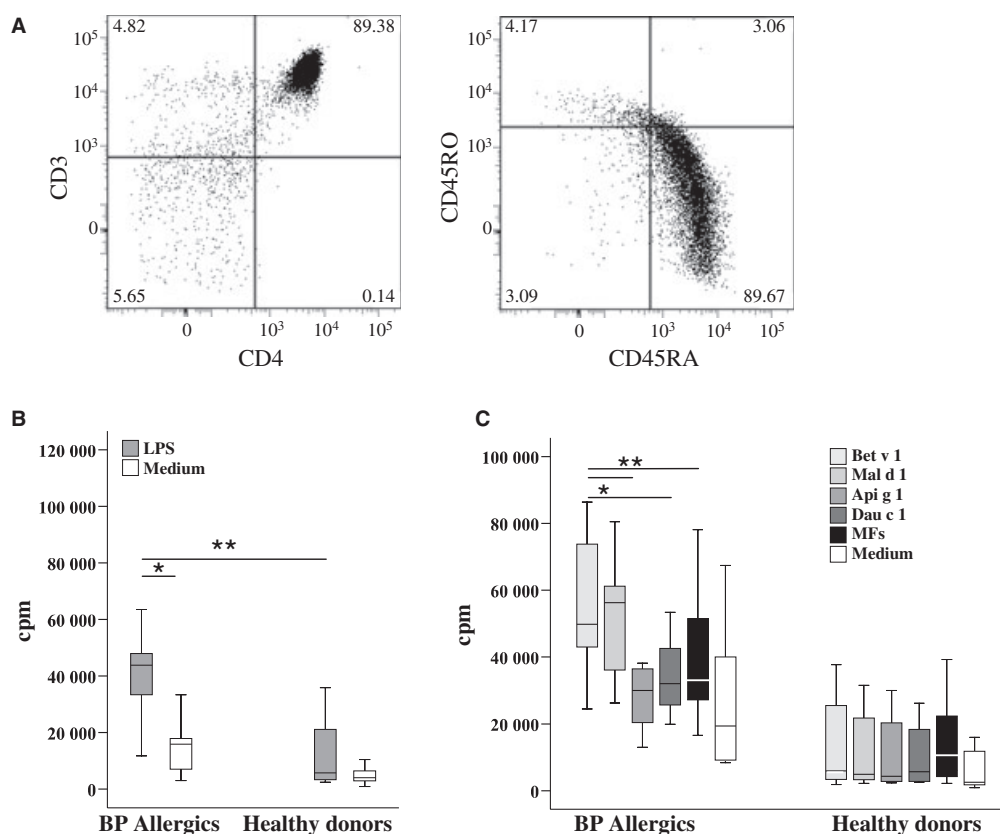


**Figure 2** Comparison of the proliferative response of enriched naïve T helper cells from BP-allergic and healthy donors after stimulation with autologous allergen-primed MoDCs. Representative depiction of enriched naïve CD4<sup>+</sup> T cells (A).  $1 \times 10^4$  MoDCs were stimulated with LPS as proliferation control and co-cultured with  $1 \times 10^5$  autologous CD4<sup>+</sup> T cells for 6 days (B). Similar experiments

were performed with MoDCs pre-exposed to  $10 \mu\text{g}$  (C) of allergens. Proliferation was assessed by measuring  $^3\text{H}$ -thymidine incorporation. Data are expressed as box-plot summary of the mean counts per minutes (cpm) derived from triplets of individual experiments (BP allergic  $n = 9$ ; healthy donors  $n = 7$ ). Horizontal lines represent the median. \* $P < 0.05$ ; \*\* $P < 0.01$ , paired  $t$ -test.

BP allergies). While the production of IL-10 in response to all allergens was kept at levels induced by MFs (Fig. 3, IL-10), the Th1 cytokine IFN- $\gamma$  was significantly enhanced by rApi g 1 (\* $P = 0.018$ ) and elevated in case of rDau c 1 stimulation ( $P = 0.063$ ; Fig. 3, IL-10 and IFN- $\gamma$ , BP allergies).

In healthy donors, addition of allergens resulted in no significant increase in IL-5 or IFN- $\gamma$  production when compared to MFs (Fig. 3, IL-5 and IFN- $\gamma$ , Healthy donors) but elevated amounts of IL-13 and IL-10 in response to rBet v 1 stimulation (Fig. 3, IL-13 and IL-10, healthy donors, Bet v 1).

In co-culture with MoDCs matured without the allergens, Th cells from BP-allergic patients produced significantly higher amounts of the Th2 cytokine IL-13 (\* $P = 0.016$ ) and also the Th1 cytokine IFN- $\gamma$  (\*\* $P = 0.001$ ) than those from healthy donors (Fig. 3, IL-13 and IFN- $\gamma$ ). IL-5 and IL-10 production was also slightly but not significantly elevated in the BP-allergic group (Fig. 3, IL-5 and IL-10).

## Discussion

The data presented in this study provide first evidence that the major birch pollen allergen Bet v 1 in contrast to Bet v 1-

related food allergens conditions human monocyte-derived dendritic cells to induce allergen-specific T-cell proliferation and Th2 polarization *in vitro*. This effect of biasing Th cells towards a Th2-directed immune response was observed only with MoDCs from atopic but not from healthy donors.

Numerous studies have shown the applicability of *in vitro* generated MoDCs to define attributes of allergenic proteins essential for driving the polarization of the immune response (17, 18, 23–25). To produce fully matured MoDCs, which are essential for allergen presentation, we used a cocktail of proinflammatory cytokines, as the allergen stimulus by itself is insufficient to induce DC maturation (26–28). MF-treated MoDCs of both BP-allergic and healthy donors displayed a mature DC phenotype as shown by up-regulation of the maturation marker CD83 and the co-stimulatory molecules CD80 and CD86 necessary for antigen presentation to T cells (Fig. 1). Exposure to the allergens, however, caused no additional significant increase in marker expression in relation to MFs (Fig. 1). Interestingly, HLA-DR expression was in general higher on MoDCs of birch pollen-allergic patients as opposed to an increased up-regulation of CD80 in the healthy donor group (Fig. 1, CD80 and HLA-DR). Higher

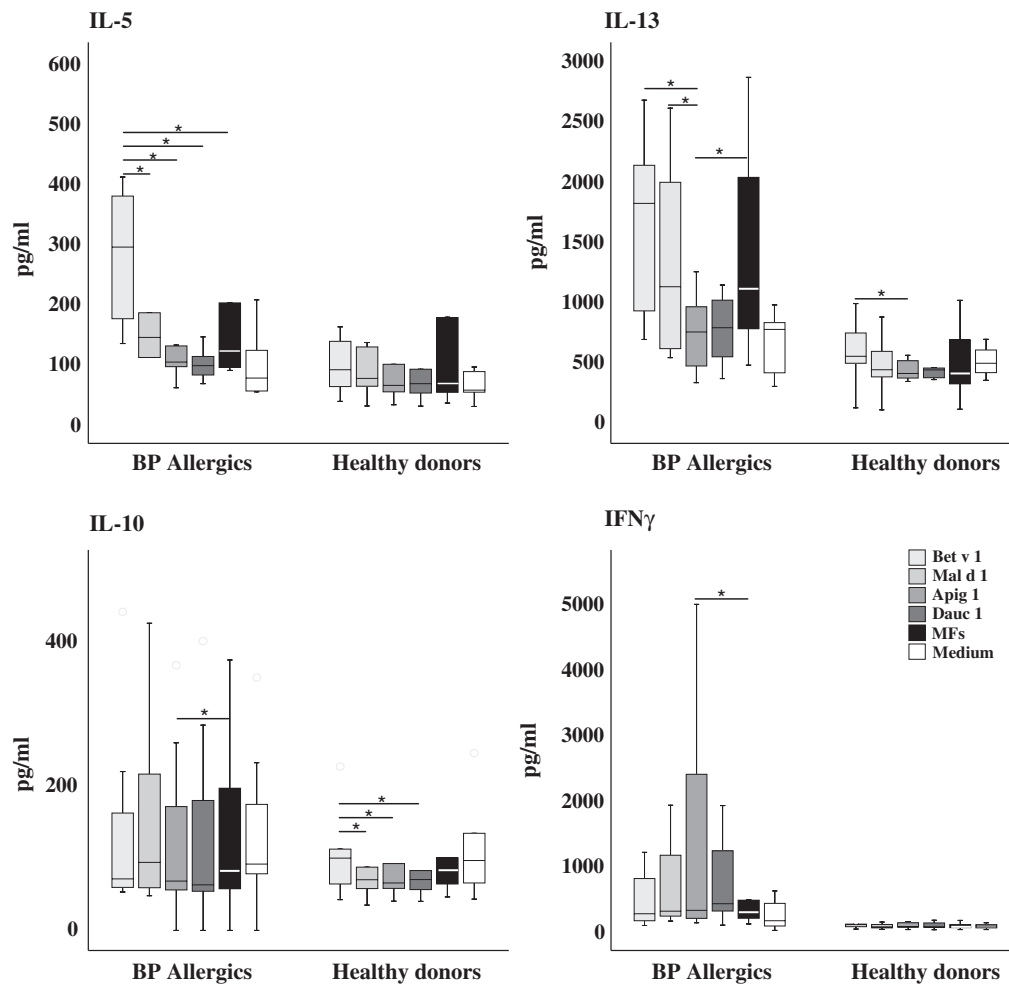


**Figure 3** Effect of allergen-pulsed autologous MoDCs on the production of Th1 and Th2 cytokines by Th cells of BP-allergic and healthy donors. Co-culture of Th cells and MoDCs from BP-allergic and healthy donors was conducted as described in the legend to Fig. 2. Supernatants were recovered after 6 days, and

cytokine levels measured by ELISA. The data are expressed as box-plot summary of the means pg/ml derived from triplets of individual experiments (BP allergic  $n = 8-9$ ; healthy donors  $n = 7$ ). Horizontal lines represent the median. \* $P < 0.05$ ; \*\* $P < 0.01$ , paired  $t$ -test.

CD80 expression on MoDCs of healthy donors compared to increased CD86 expression in sensitized individuals was also reported upon encounter with the major house dust mite allergen Der p 1 (16, 29). Although CD86 is believed to be more important than CD80 for the induction of a Th2 response (30), unresponsiveness of CD80 on DCs from sensitized donors was suggested to lead to a failure in regulatory functions of terminating specific immune responses (31).

Increased HLA-DR expression on MoDCs from atopic donors, as in our case, was proposed to result in an enhanced proliferation of T cells (31, 32). Even LPS-induced maturation of MoDCs derived from allergic donors resulted in a two-fold increased proliferation of Th cells compared to healthy donors (Fig. 2A). An allergen-specific significantly enhanced proliferative response of enriched naïve autologous Th cells was only observed for MoDCs of BP-allergic donors pulsed with rBet v 1 (\* $P = 0.008$ ; Fig. 2B, BP allergics, Bet

v 1). No significant increase could be shown for rMal d 1, rApi g 1, or rDau c 1 compared to MFs alone (Fig. 2B).

Allergen-primed MoDCs from healthy donors did not induce substantial Th-cell proliferation in our system (Fig. 2B, healthy donors). The differences observed could also be attributable to the involvement of an FcεR-mediated allergen uptake thereby altering the quality of the CD4<sup>+</sup> T-cell response induced in sensitized individuals. To address the issue of allergen-specific IgE, we performed Bet v 1-uptake studies in the presence of an anti-IgE antibody or by stripping possibly bound IgE using lactic acid. Both experiments had no impact on the uptake indicating that Bet v 1-specific IgE was not involved (data not shown). Even more convincingly, MoDCs used in our study were generated in the absence of autologous human sera excluding pre-existing allergen-specific IgE.

Exposure of MoDCs from BP-allergic donors to rBet v 1 resulted in a robust Th2 skewing with production of the Th2

cytokines IL-5 and IL-13 but also IFN- $\gamma$  (Fig. 3, Bet v 1, BP allergics). rMal d 1 induced a similar but less pronounced cytokine secretion pattern (Fig. 3, Mal d 1, BP allergics). This Th1/Th2 cytokine profile is in accordance with other *in vitro* generated data upon presentation of Der p 2, Der f 2 or peanut Ag (18, 24, 33). It seems that IFN- $\gamma$  production by naïve Th cells does not conflict with the polarization to a Th2 phenotype provided sufficient simultaneous Th2 cytokines are secreted (34). The observation that Mal d 1 mimics the polarizing ability of Bet v 1 *in vitro* to some degree is certainly a consequence of the 71% conserved surface area they share, compared to only 47% for Api g 1 or Dau c 1 (8).

In contrast, Api g 1-primed MoDCs not only significantly enhanced the production of the Th1 cytokine IFN- $\gamma$  but also down-regulated IL-13 in relation to MFs alone (Fig. 3, Api g 1, BP allergics). This finding is in contradiction to data reporting that Api g 1, similar to Bet v 1, induced the production of the Th2 cytokines IL-4 and IL-5 in Bet v 1-specific T-cell clones (35). These differences may be attributable to the cellular composition of our MoDC-T-cell system that included a mixed T-cell and not an exclusive T-cell memory population. Hence, we concluded that Api g 1 is able to drive a naïve T-cell response into a Th1 direction and possibly to convert Bet v 1-activated Th cells towards a Th1 phenotype. It has been suggested that uncommitted memory T cells maintain cytokine flexibility to adapt their cytokine expression profile to different polarizing conditions (36). Only at later stages they may become irreversibly committed memory cells as in the case of T-cell clones.

Analysis of the antibody responses in individuals allergic to birch pollen revealed that only Bet v 1 was able to induce IgE that was partly cross-reactive with its homologues from plant foods (7). Comparison of patients' data with IgE vs those without IgE to the homologues did not show differences in the ability of MoDCs to drive Th2 polarization. Similar behaviour was described for the yeast allergen Mala s 11 and its cross-reactive human homologue MnSOD (25). Thus, MoDCs of BP-allergic individuals possess the ability to discriminate between the allergenic Bet v 1 protein and its homologues despite their conserved surface areas. In healthy donors, the allergen pulse drove MoDCs to induce little or no IL-13 and IL-5, and also no significant production of the Th1 cytokine IFN- $\gamma$  in autologous Th cells compared to MFs

alone (Fig. 3, Healthy donors). Elevated IL-10 levels in response to rBet v 1 stimulation may be derived from regulatory T cells and a sign of allergen-specific tolerance in healthy donors as previously suggested (37).

Our findings are in line with recently published data on the 2S albumins Ber e 1 and SFA8 from Brazil nut and sunflower seeds, respectively (34). Although structurally very similar, the major allergen from Brazil nuts, Ber e 1, and the weak allergen SFA8 from sunflower seeds vary in their allergenic properties in terms of DC priming and Th-cell responses. Only presentation of Ber e 1 but not SFA8 could induce significant production of Th2 cytokines in addition to IFN- $\gamma$  by Th cells (34). Similar priming characteristics of MoDCs were also displayed by the major allergen Mala s 11 from *Malassezia sympodialis* and its highly homologous self antigen hMnSOD (25). Mala s 11 but not hMnSOD affected DC maturation and subsequent allogeneic lymphocyte proliferation. In addition, other studies emphasize the importance of the immanent structural features of the presented antigen. Bellinghausen et al. (23) have shown that only the hexameric native but not the monomeric form of the major allergen Per a 3 could induce Th2 cytokine production in *P. americana* sensitized donors. Unlike wild-type Der f 2, an engineered Der f 2, lacking the characteristic native  $\beta$ -barrel structure, induced an exclusive Th1-cell differentiation in atopic individuals (33).

Our data show that Bet v 1 possesses immunostimulatory properties obviously different from its related allergen Api g 1. Furthermore, birch pollen grains release not only protein allergens but also soluble factors that modulate DC function, thus providing a micro environment favouring Th2 polarization (38, 39). Taken together, the onset of Th2 responses seems to be the consequence of an interplay of DCs prone to Th2-skewing (32, 40, 41), the matrix the allergen is embedded in (38, 39), and the priming properties of the allergen itself (17, 23, 33). In future studies, it will be essential to compare the effects of Bet v 1 and allergenic/non-allergenic Bet v 1-homologues on MoDCs in detail by uptake studies and gene expression profiling.

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