

ORIGINAL ARTICLE

EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Human blood basophils do not act as antigen-presenting cells for the major birch pollen allergen Bet v 1

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To cite this article: Kitzmüller C, Nagl B, Deifl S, Walterskirchen C, Jahn-Schmid B, Zlabinger GJ, Bohle B. Human blood basophils do not act as antigen-presenting cells for the major birch pollen allergen Bet v 1. *Allergy* 2011; DOI: 10.1111/j.1398-9995.2011.02764.x

Keywords

antigen-presenting cells; basophils; Bet v 1; IgE-mediated allergy.

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Accepted for publication 12 November 2011

DOI:10.1111/j.1398-9995.2011.02764.x

Edited by: Hans-Uwe Simon

Abstract

Background: Several studies in mice have recently shown that basophils can act as antigen-presenting cells (APC) inducing Th2-mediated immune responses against parasites or protease allergens. The aim of this study was to investigate whether human basophils function as APC for the major birch pollen allergen Bet v 1.

Methods: Fluorescently labeled Bet v 1 was used to assess surface binding and internalization of allergen by basophils and different types of APC from birch pollen-allergic and nonallergic individuals. Sorted basophils were analyzed in terms of up-regulation of MHC class II and co-stimulatory molecules in the absence and presence of IL-3 and IFN- γ by flow cytometry. Expression of proteins crucial for antigen presentation, namely cathepsin S and invariant chain, was determined. Basophils were used as APC in co-culture experiments with Bet v 1-specific T-cell clones (TCCs).

Results: Basophils from birch pollen-allergic donors very efficiently bound Bet v 1 through IgE/Fc ϵ RI complexes on their surface. In contrast to professional APC, basophils did not internalize allergen and expressed marginal levels of cathepsin S and invariant chain. HLA-DP, HLA-DQ, CD80/CD86, and CD40 were absent from purified basophils even when stimulated with IL-3 plus IFN- γ . IL-3/IFN- γ marginally up-regulated HLA-DR. Bet v 1-pulsed basophils failed to induce proliferative and cytokine responses in Bet v 1-specific, HLA-DR-restricted TCCs.

Conclusion: Human basophils neither internalize, process nor present Bet v 1. Because Bet v 1 is a highly relevant allergen, we conclude that basophils play no role as APC in IgE-mediated allergy in humans.

Basophils are rare circulating granulocytes with important effector functions in helminth infection and allergic inflammation and express high levels of the high-affinity IgE receptor (Fc ϵ RI) on their surface (1). During the sensitization

phase of IgE-mediated allergy, these receptors are loaded with allergen-specific IgE antibodies. Any subsequent allergen contact induces cross-linking of IgE/Fc ϵ RI complexes resulting in the prompt release of inflammatory mediators, such as histamine and leukotrienes, which mediate the acute allergic effector phase (2).

In type I allergy, IgE to a harmless agent is produced because of a disproportional Th2 effector cell response (3). Dendritic cells (DC) are regarded as the most potent antigen-presenting cells (APC) capable of priming naïve T cells because of the high levels of MHC class II and co-stimulatory molecules on their surface (4). However, DC have never

Abbreviations

APC, antigen-presenting cells; Cat, cathepsin; DC, dendritic cells; EBV, Epstein Barr Virus; Fc ϵ RI, high-affinity IgE receptor; mDC, myeloid DC; mdDC, monocyte-derived DC; Ii, MHC class II-associated invariant chain; PBMC, peripheral blood mononuclear cells; pDC, plasmacytoid DC; SI, stimulation index; TCC, T-cell clone; TSLP, thymic stromal lymphopoietin.

been shown to produce the cytokines necessary for initiating Th2 responses, most notably IL-4 and thymic stromal lymphopoietin (TSLP) (5). The identification of cell types synthesizing these cytokines has been the subject of intense research. Basophils have been considered as early IL-4 suppliers because they rapidly produce this cytokine upon stimulation with allergen (6,7). In mice immunized with allergens, basophils are recruited to the draining lymph nodes and produce IL-4 and TSLP, thereby representing an important accessory cell type to promote Th2-like responses (8).

Recently, several studies have provided strong evidence that murine basophils may also function as APC. Kim et al. (9) demonstrated that basophils directly present peptides and cross-present antigens to CD8⁺ T cells. Yoshimoto et al. (10) showed that basophils express MHC class II, CD80, CD86, and CD62L, take-up ovalbumin, and process it into small peptides. Using the model allergen papain, Sokol et al. (11) demonstrated that basophils can initiate an allergic Th2-response even in the absence of DC. Taken together, the above-mentioned studies imply that basophils may function as APC that promote Th2 differentiation (12). However, several studies in mice have challenged this view (13–15) favoring the concept that basophils and DC cooperate.

To date, no comprehensive investigation analyzing the potential of human basophils as APC has been performed. To address this issue, we employed Bet v 1, the clinically highly relevant major allergen in birch pollen, as model allergen to investigate different key features of antigen processing and presentation in basophils from birch pollen-allergic and nonallergic individuals, namely (i) endocytosis of allergen, (ii) constitutive and IFN- γ -induced expression of MHC class II molecules and relevant molecules of the HLA class II pathway, (iii) expression of co-stimulatory molecules, and (iv) specific activation of allergen-reactive CD4⁺ effector T cells. The analyses of basophils were compared with B lymphocytes, monocytes, and different types of DC.

Material and methods

Allergens

Recombinant Bet v 1 was purchased from Biomay AG, Vienna, Austria. BM4, a non-IgE-binding variant of Bet v 1, was kindly provided by Michael Wallner, Christian Doppler Laboratory for Allergy Diagnosis and Therapy, University of Salzburg, Austria. Both proteins were conjugated to pHrodo succinimidyle ester (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Alexa 488 labeling was performed using the AlexaFluor[®] 488 Microscale Protein Labeling Kit (Invitrogen). Labeled allergen induced a similar basophil activation as unlabeled Bet v 1 (data not shown).

Allergic and nonallergic donors

All birch pollen-allergic patients suffered from rhinoconjunctivitis during spring and showed positive skin prick test responses and IgE reactivity to birch pollen (≥ 0.35 kU_A/l,

ImmunoCAP; Phadia, Uppsala, Sweden). Nonallergic controls had neither allergic symptoms nor allergen-specific IgE antibodies. The study was approved by the local ethics committee, and all patients gave informed consent.

Flow cytometry

Peripheral blood mononuclear cells (PBMC) were incubated with labeled Bet v 1, stained with anti-CD14, CD19 (BD Biosciences, San Jose, CA, USA), CD123, HLA-DR, CCR3 (Biolegend, San Diego, CA, USA), BDCA-2, CD1c, and CD141 (Milteny Biotec, Bergisch-Gladbach, Germany), and analyzed using a FACS Canto (BD Biosciences) and FlowJo software (TreeStar Inc.). To dissociate basophil-bound IgE, PBMC were first incubated in 0.14 M NaCl and 0.005 M KCl, pH 3.5, then with a serum-pool of birch pollen-allergic donors and finally with labeled allergen (16). Additionally, PBMC were incubated with titrated concentrations of human IL-3 or IFN- γ (both Peprotech, London, UK) and mixtures thereof overnight. CD14⁺BDCA-2⁺CCR3⁺CD123⁺ cells were analyzed for expression of CD80, CD86, HLA-DP, HLA-DQ (BD Biosciences), and CD40 (kindly supplied by Peter Steinberger, Institute of Immunology, Medical University Vienna, Austria). The presence of apoptotic and necrotic cells was checked by incubation with Annexin V and 7-AAD (Biolegend).

Preparation of distinct cell populations

PBMC were depleted of CD3⁺ T cells using Dynabeads[®] CD3 (Invitrogen) and stained with anti-CD19, CD14, CD123, CCR3, and BDCA-2. CD19⁺ cells were sorted as B cells, CD14⁺ cells as monocytes, and CD14⁺BDCA-2⁺CCR3⁺CD123⁺ cells as basophils on a FACSaria III (BD Biosciences) cell sorter. The human anti-CD123 clone 6H6 antibody used does not inhibit IL-3 binding to low- or high-affinity IL-3 receptors. All cell populations obtained were >98% pure (Fig. S1). Monocyte-derived DC (mdDC) and Epstein Barr Virus (EBV)-transformed B cells were generated as described previously (17).

Indirect immunofluorescence

Sorted basophils and mdDC were incubated with Alexa 488-labeled Bet v 1 at 37°C for 2 h and 15 min, respectively. Cells were fixed, permeabilized, and labeled with antibodies against EAA1 (BD Biosciences), Lamp1 (DSHB, University Iowa), and Fc ϵ RI (AbDSerotec, Oxford, UK). Analysis was performed on a Zeiss Axiovert 200 confocal microscope using Volocity software (PerkinElmer, Waltham, MA, USA) and ImageJ (NIH, Bethesda, MD, USA).

Western blotting

Cell lysates from 2.5×10^5 cells/lane were separated by 15% SDS-PAGE and immunoblotted with antibodies against cathepsin S (AbD Serotec), MHC class II-associated Ii (VICY1 kindly provided by O. Majdic, Institute of Immunology,

Table 1 Characteristics of allergen-specific T-cell clones

T-cell clone	Epitope (aa)	Restriction element	T helper subset
TCC 1	82–96	HLA-DR*1501	Th2
TCC 2	142–156	HLA-DR*0701	Th2
TCC 3	142–156	HLA-DR*0701	Th0
TCC 4	112–132	HLA-DR*1101	Th2

Medical University Vienna, Vienna, Austria), and β 2-microglobulin (Exbio, Vestec, Czech Republic).

Basophil activation test

Sorted basophils were cultured in the presence of IL-3 (300 pM) and IFN- γ (100 U/ml). Thereafter, 8×10^3 cells were stimulated with fMLP (2 μ M) (Sigma-Aldrich, St. Louis, MO, USA) or Bet v 1 (100 ng/ml) in the presence of IL-3 (120 pM) for 15 min at 37°C. Cells were stained with the LIVE/DEAD® Fixable Dead Cell Near-IR Stain Kit (Invitrogen), anti-CD63 (Biolegend), and anti-CD123.

T-cell proliferation and cytokine production

Four cryopreserved HLA-DR-restricted Bet v 1-specific CD4⁺TCR $\alpha\beta$ ⁺ T-cell clone (TCC) with determined epitope specificity (Table 1) were thawed. Autologous PBMC (5×10^4 /well), sorted B cells (2.5×10^4 /well), monocytes (2.5×10^4 /well), and mDC (10×10^3 /well) were cultured overnight in the absence or presence of Bet v 1 (5 μ g/ml). Basophils (2.5×10^4 /well) were additionally incubated with IL-3 (300 pM) and IFN- γ (100 U/ml). The next day, the populations were irradiated (60 Grey), and allergen-specific TCC (5×10^4 /well) was added and incubated for 48 h. Proliferation was assessed by methyl-[³H]-thymidine incorporation. All experiments were performed in duplicate. The stimulation index was calculated as ratio between cpm of TCC plus APC plus allergen and cpm of TCC plus APC alone. Culture supernatants were collected and analyzed for IL-4, IFN- γ , and IL-10 using the Luminex System 100 (Luminex, Austin, TX, USA).

Results

Basophils from birch pollen-allergic donors efficiently bind Bet v 1 via IgE/Fc ϵ RI complexes but do not internalize it

We compared surface binding of Bet v 1-Alexa 488 to different APC in PBMC from birch pollen-allergic and nonallergic individuals after incubation for 3 h at 4°C. Basophils (CD14⁺BDCA-2⁺CCR3⁺CD123⁺) of birch pollen-allergic patients were highly positive for Bet v 1 (Fig. 1A), whereas basophils of nonallergic individuals remained Bet v 1-negative. In contrast, monocytes (CD14⁺) from both allergic and nonallergic individuals bound Bet v 1, and myeloid DC (mDC)2 (CD141⁺CD14⁺) and plasmacytoid DC (pDC) (BDCA-2⁺) also showed minimal surface binding of the

allergen. B cells (CD19⁺) and mDC1 (CD1c⁺CD14⁺CD19⁺) (18) remained Bet v 1-negative under the conditions used. Thus, except for basophils, no difference in allergen surface binding of any cell population from allergic or nonallergic donors was observed. To assess whether the strong surface binding of Bet v 1 to basophils from allergic individuals resulted from the capture of allergen by specific IgE/Fc ϵ RI complexes (1,19), we employed Alexa 488-labeled BM4, a non-IgE-binding derivative of Bet v 1 (20). No surface binding of BM4 to basophils from birch pollen-allergic donors was detected (Fig. 1B). Additionally, we passively sensitized basophils from nonallergic donors with Bet v 1-specific IgE. These basophils showed significant binding of Bet v 1 (Fig. 1C).

As a second step, we investigated the endocytosis of allergen by basophils and other APC. PBMC from nonallergic and birch pollen-allergic individuals were pulsed for 20 h at 37°C with Bet v 1 labeled with pHrodo, a pH-dependent dye that emits bright red light only once it reaches the acidic environment in endosomes. Figure 1D shows that B cells, monocytes, mDC1, mDC2, and pDC from allergic and nonallergic individuals became Bet v 1 positive to various degrees. In contrast, no internalization of Bet v 1 was detected in basophils from allergic and nonallergic donors. We confirmed these data by indirect immunofluorescence (Fig. 2). Flow cytometric-sorted basophils (CD14⁺BDCA-2⁺CCR3⁺CD123⁺) from Bet v 1-allergic donors were analyzed regarding the co-localization of Bet v 1-Alexa 488 with Fc ϵ RI, EEA1 (a marker of early endosomes) and Lamp1 (a lysosomal marker), respectively. Bet v 1 was detected in the periphery of basophils as distinct punctae which co-localized with Fc ϵ RI (Fig. 2A). In contrast, Bet v 1 co-localized neither with Lamp1 (Fig. 2B) nor with EEA1 in basophils. A clear co-localization of Bet v 1 and EEA1 was detected in monocyte-derived (md)DC (Fig. 2C).

Human basophils show limited expression of MHC class II molecules, co-stimulatory molecules, Ii, and Cat S

For presentation to CD4⁺ T lymphocytes, APC have to load antigen-derived peptides into the binding groove of MHC class II molecules. In addition to this primary signal, naïve T cells also need a secondary signal from co-stimulatory molecules on the surface of APC. Consequently, we analyzed the expression of MHC class II and co-stimulatory molecules on sorted basophils from birch pollen-allergic individuals. We detected neither HLA-DR, -DQ, and -DP nor CD80, CD86, and CD40 on freshly isolated basophils (Fig. 3A). We then stimulated basophils with a cocktail of IL-3 and IFN- γ . This stimulation resulted in a marginal up-regulation of HLA-DR in some donors (data not shown), whereas HLA-DP, -DQ, and the co-stimulatory molecules CD80, CD86, and CD40 remained undetectable in all donors. In contrast, treating monocytes with the same cocktail led to an up-regulation of all surface markers except HLA-DQ (Fig. 3A).

We next evaluated cell lysates from different APC for the presence of MHC class II-associated invariant chain (Ii) and

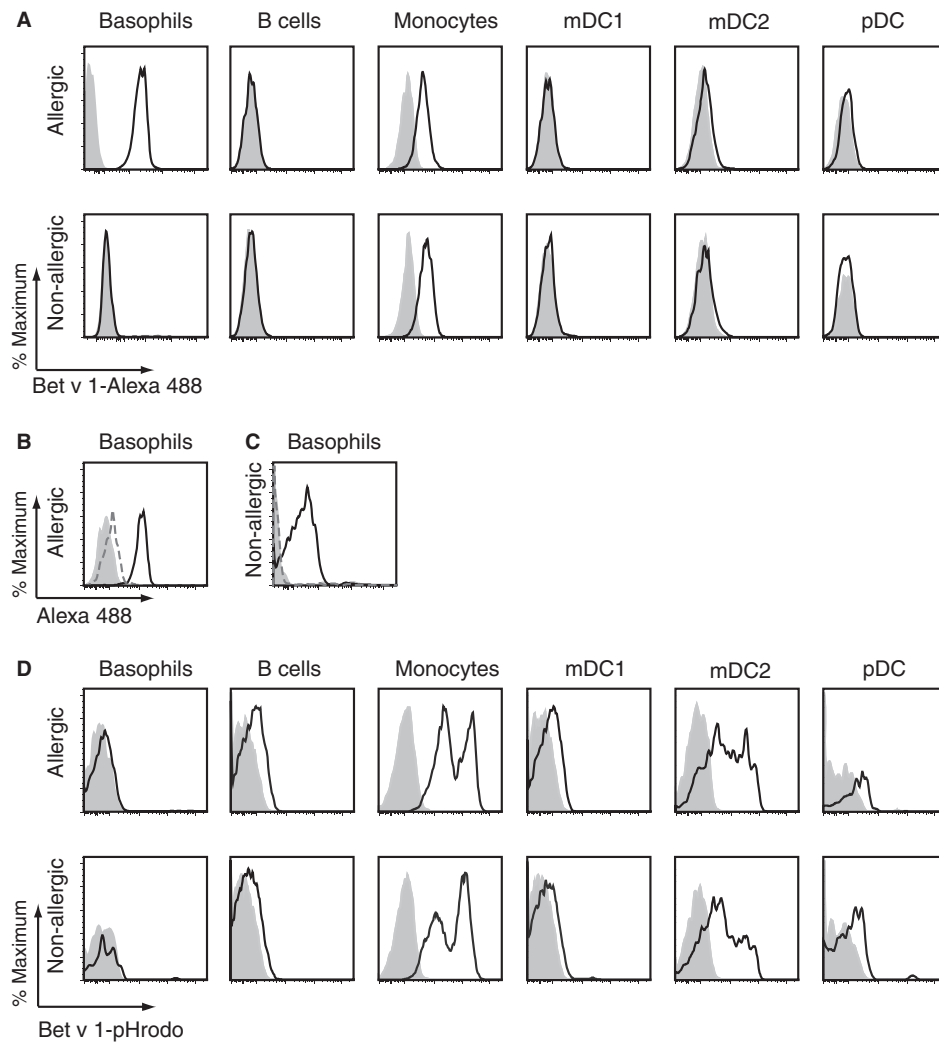


Figure 1 Surface binding and uptake of allergen by different antigen-presenting cells (APC). Different types of APC from allergic or nonallergic individuals were scored for their content of Alexa 488- (A, B, C) or pHrodo- (D) labeled allergen. Incubation with (A) Bet v 1 for 3 h at 4°C; (B) with Bet v 1 (solid line) or BM4 (dashed line) for

20 h at 37°C; (C) with Bet v 1 for 2 h at 37°C in IgE-stripped (dashed line) or passively sensitized cells; (D) with Bet v 1 for 20 h at 37°C. Shaded area, cells without labeled allergen; solid line, signal from labeled allergen. The data are representative of two separate experiments showing similar results.

the cysteine endoprotease cathepsin S (Cat S), two proteins important for protein processing, by western blotting. We detected both molecules in lysates from mdDC and EBV-transformed B cells, whereas freshly sorted basophils expressed neither Ii nor Cat S (Fig. 3B). Minimal amounts of Ii and Cat S were detectable in basophils upon stimulation with IL-3 and IFN- γ (Fig. 3B).

Human basophils do not activate allergen-specific effector T cells

Finally, we investigated whether human basophils can induce allergen-specific proliferation and cytokine production in Bet v 1-reactive, HLA-DR-restricted human TCC. We employed four different TCC specific for three distinct, frequently rec-

ognized T-cell-activating regions in Bet v 1 located in the N-terminal, C-terminal, and central region of its amino acid sequence, respectively (Table 1) (21). Prior to the addition of TCC, sorted basophils were incubated with Bet v 1, IL-3, and IFN- γ for 16 h to provide sufficient time for allergen-binding and uptake and to maximize the expression of Ii, Cat S, and HLA-DR. None of the Bet v 1-specific TCC responded to autologous allergen-pulsed basophils with substantial proliferation (Fig. 5A) or cytokine production (data not shown), whereas all TCC reacted strongly when either sorted Bet v 1-pulsed autologous B cells, monocytes, cultured mdDC or PBMC served as APC.

To rule out that basophils had been damaged by the sorting procedure, we investigated the up-regulation of CD63 expression on basophils from birch pollen-allergic donors in

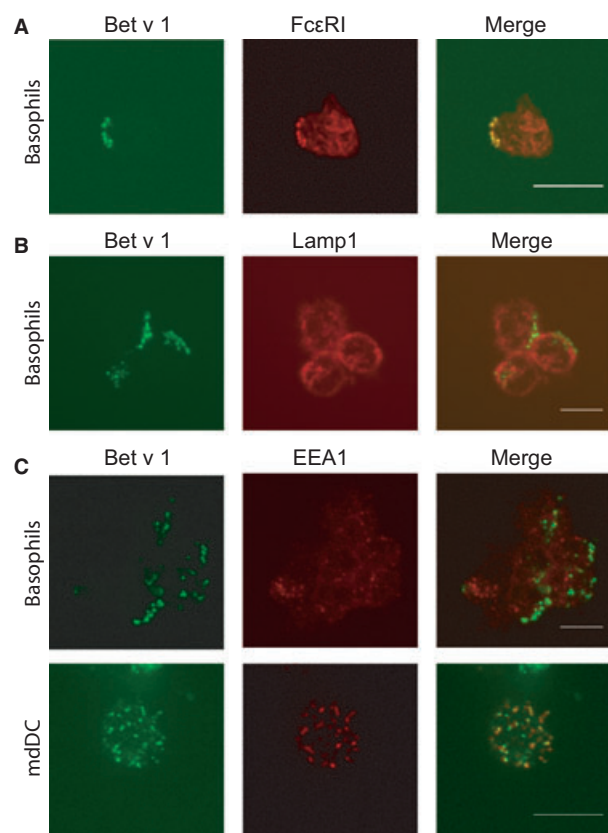


Figure 2 Localization of Bet v 1 in sorted basophils. Confocal images of sorted basophils and mdDC after pulse incubation with Alexa 488-labeled Bet v 1 at 37°C for 2 h and 15 min, respectively. Cells were fixed, permeabilized, and stained with antibodies against (A) FcεRI, (B) Lamp1, and (C) EEA1. Scale bars, 10 μm.

response to Bet v 1 or fMLP after sorting and further *in vitro* cultivation in the presence of IL-3 and IFN-γ for up to 90 h. We observed that after 18 and 42 h more than 95% of basophils became CD63 positive in response to both stimuli and detected a small loss of reactivity only after 90 h of cultivation (Fig. 4).

To exclude the possibility that basophils had died after allergen-induced activation and mediator release, we checked them by incubation with labeled Annexin V (a marker for early apoptosis) and the dye 7-AAD (a marker for cell death). After cultivation in the presence of Bet v 1, IL-3, and IFN-γ for 16 h, irradiation and incubation for another 6 h, a maximum of 11% of basophils were positive for Annexin V, and <1% had incorporated 7-AAD (data not shown).

To finally rule out that the observed nonreactivity of T cells resulted from toxic effects of the mediators released by Bet v 1-activated basophils, we stimulated two Bet v 1-specific TCC with synthetic 12mer peptides containing their respective epitopes in the presence of basophils preincubated with Bet v 1, IL-3 and IFN-γ for 16 h. Such 12mer peptides are loaded directly into functional MHC class II molecules

expressed by activated CD4⁺ T cells and induce proliferative responses in TCC albeit less strongly than in the presence of professional APC. The presence of Bet v 1-activated basophils did not reduce peptide-induced proliferation in the TCC, thereby demonstrating that basophil-derived mediators did not impair T-cell reactivity (Fig. 5B).

Discussion

To study the antigen-processing and antigen-presenting capacity of human basophils, we developed a sophisticated experimental set-up involving a clinically relevant and immunologically well-characterized allergen, highly purified basophils, and specific TCC with defined epitope reactivity and HLA-restriction. The major birch pollen allergen has high allergenic potential and is a major cause for IgE-mediated respiratory allergy in Northern and Central Europe as well as North America (22). We have extensively characterized Bet v 1 with regard to antigen-processing and T-cell reactivity by employing various types of professional APC and Bet v 1-specific TCC (17,21,23). Bet v 1 lacks the cysteine-protease activity that, in the case of the major house dust mite allergen Der p 1, has been shown to activate basophils in a nonspecific manner (24). Collectively, these features make Bet v 1 an ideal candidate to study the potential role of human basophils as APC in allergic disorders.

We took great care to obtain highly purified basophils. Human monocytes (CD14⁺) and pDC (BDCA-2⁺), two cell types with potent antigen-processing capacity, express CD123, a molecule widely used as basophil marker. Even trace amounts of these cell types in our preparations might lead to false-positive detection of molecules involved in antigen presentation and/or activation of Bet v 1-specific TCC. Using the recently identified basophil selection marker CCR3 (25), we sorted basophils from PBMC by flow cytometry as CD14[−]BDCA-2[−]CCR3⁺CD123⁺ cells. In contrast to negative selection by commercially available kits, our sorting strategy also prevented the loss of potential HLA-DR-positive basophils.

Because *in vitro* cultured basophils are considered to be rather short-lived, we supplied IL-3, an important differentiation factor for this cell type (26) that also enhances their viability (27). Indeed, sorted basophils remained viable and responsive in basophil activation assays for up to 90 h (Fig. 4). As we could detect neither HLA-DR, -DQ nor -DP on the surface of freshly sorted basophils, we added IFN-γ, a pro-inflammatory cytokine, known to up-regulate the expression of MHC class II molecules on different types of nonprofessional APC, for example, keratinocytes (28), mast cells (29), or intestinal epithelial cells (30). IFN-γ-activated basophils were additionally studied for the expression of Ii and Cat S (Fig. 3B). Ii is crucial for the association of the α and β chain of MHC class II molecules and thus for proper formation of the peptide-binding groove (31). Furthermore, Ii targets MHC class II molecules to the endosomal pathway (32,33), where Cat S processes it to class II-associated Ii peptide (34). It has been demonstrated that Cat S-dependent Ii-processing is required for IgE/FcεRI-mediated antigen

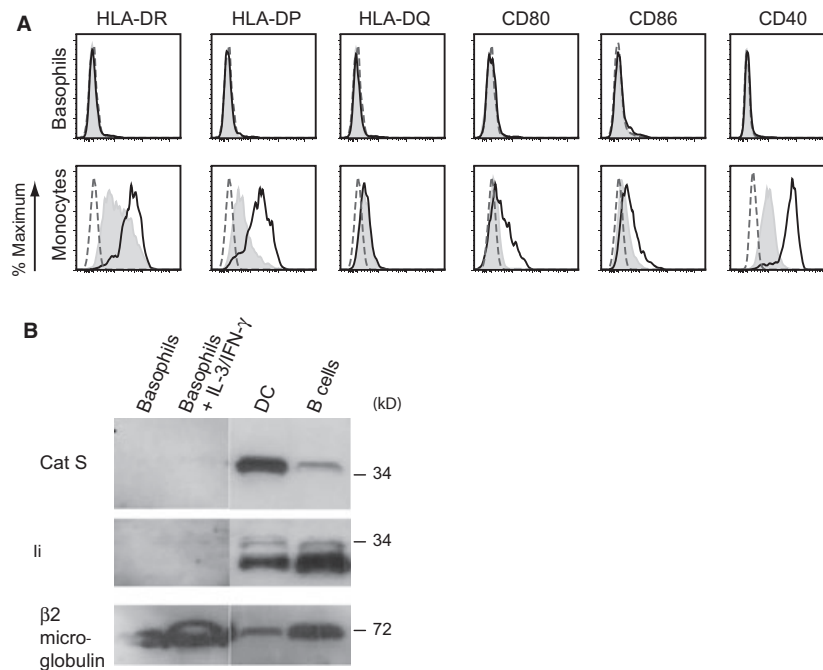


Figure 3 Expression of molecules of the protein presentation pathway and co-stimulatory molecules on different antigen-presenting cells. (A) Flow cytometric analysis of HLA-DR, -DP, -DQ, CD80, CD86, and CD40 expression on sorted basophils (CD14⁺BDCA-2⁺CCR3⁺CD123⁺) or monocytes from allergic donors incubated for 18 h in the absence (shaded histogram) or presence of IL-3 and IFN-γ (solid line) compared with isotype-matched

controls (dashed line). Data are representative of two independent experiments showing similar results. (B) Analysis of the presence of Cat S and Ii in cell lysates from sorted basophils of allergic donors incubated in the absence or presence of IL-3 and IFN-γ for 14 h, from mdDC- or EBV-transformed B cells. β2 Microglobulin was detected as loading control. DC, dendritic cells.

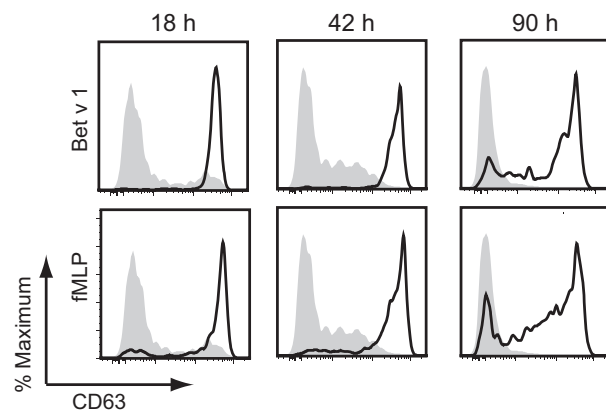


Figure 4 Functional integrity of basophils after sorting from PBMC. Sorted basophils from allergic donors were incubated with IL-3/IFN-γ for 18, 42, and 90 h and analyzed for CD63 expression in response to stimulation with Bet v 1 or fMLP. Shaded histogram, unstimulated; solid line, stimulated basophils. Representative example of three independent experiments.

presentation by human DC (35). However, even IFN-γ-stimulated basophils showed only marginal expression of MHC class II molecules, Ii, or Cat S (Fig. 3).

Finally, allergen-pulsed basophils did not induce substantial proliferative and cytokine responses in Bet v 1-specific

TCC (Fig. 5; data not shown). By employing TCC specific for three epitopes dispersed over the entire sequence of Bet v 1 (Table 1), we sought to prevent false-negative results because of a possibly limited fragmentation of certain parts of the allergen during antigen processing by basophils. In addition, we carefully excluded that poor survival rates of either basophils or TCC might explain our negative results. We also confirmed that our Bet v 1-specific TCC proliferated upon stimulation with the respective epitope-containing peptides without the addition of professional APC (Fig. 5B). Activated CD4⁺ T cells express functional MHC class II molecules but are devoid of co-stimulatory molecules. Thus, our clones responded to peptide/HLA-DR complexes in the absence of co-stimulatory molecules expressed by professional APC. Consequently, they should also have reacted to Bet v 1-derived peptides bound to HLA-DR molecules present on the surface of basophils lacking CD80, CD86, and CD40 (Fig. 3).

Basophils from birch pollen-allergic donors efficiently captured Bet v 1 via FcεRI-bound allergen-specific IgE on their surface. The delivery of allergens into the Cat S-dependent pathway of MHC class II presentation by FcεRI has been demonstrated for DC (35). However, we found no evidence for FcεRI-mediated antigen presentation in basophils from allergic donors. Together, human basophils did neither endocytose allergen, express detectable levels of MHC class II molecules, co-stimulatory molecules, and relevant molecules

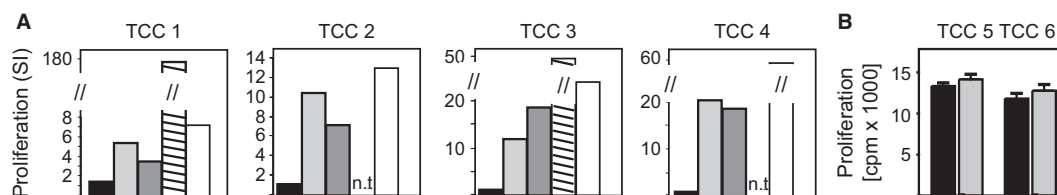


Figure 5 Proliferation of allergen-specific effector T cells. Autologous IL-3/IFN- γ -activated sorted basophils (black), B cells (gray), monocytes (light gray), mdDC (hatched), and PBMC (white) from allergic donors were incubated with Bet v 1 for 16 h, irradiated and subsequently used as antigen-presenting cells for four different Bet v 1-specific T-cell clone (TCC). Proliferation was assessed after 48 h by ^3H -thymidine incorporation. Stimulation indexes (SI)

are shown, n.t. not tested. (B) Functional integrity of TCC. Two Bet v 1-specific TCC with different epitope specificity were activated with the 12mer peptides containing their epitope in the presence of sorted basophils previously incubated without (black) or with Bet v 1 (gray) in the presence of IL-3/IFN- γ for 16 h. Proliferation was assessed after 48 h by ^3H -thymidine incorporation. DC, dendritic cells.

of the antigen-processing pathway nor substantially activate allergen-reactive CD4 $^{+}$ effector T cells. Thus, human basophils play no role as APC in allergic disorders.

Acknowledgments

The work was supported by the Austrian Science Fund, project SFB F1807-B14, Biomay AG and the Christian Doppler Research Association, Vienna, Austria.

Conflict-of-interest

The authors declare no competing financial interests.

Author contributions

C.K. and B.B. designed the experiments; C.K., B.N., S.D., and C.W. performed the experiments and analyzed the data;

G.Z. helped with cytokine analyses, B.S. contributed to the experimental design and writing of the paper. C.K. and B.B. wrote the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

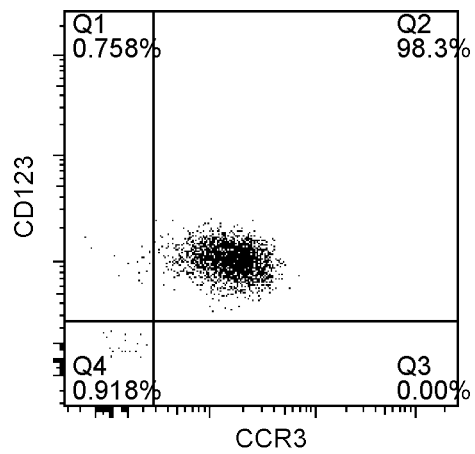
Figure S1. Purity of basophils after flow cytometric sorting as CD14 $^{-}$ BDCA-2 $^{-}$ CCR3 $^{+}$ CD123 $^{+}$ cells from T cell depleted PBMC.

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Supplementary figure 1: Purity of basophils after flow cytometric sorting as CD14⁻BDCA-2⁻ CCR3⁺CD123⁺ cells from T cell depleted PBMC.