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Dendritic cell-derived exosomes carry the major cat allergen Fel d 1 and induce an allergic immune response

H. Vallhov¹, C. Gutzeit¹, K. Hultenby², R. Valenta³, H. Grönlund⁴ & A. Scheynius¹

¹Department of Medicine Solna, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden; ²Department of Laboratory Medicine, Karolinska Institutet, Huddinge, Sweden; ³Department of Pathophysiology and Allergy Research, Medical University of Vienna, Austria; ⁴Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

Correspondence

Helen Vallhov, The Translational Immunology Unit, Department of Medicine Solna, Karolinska Institutet and Karolinska University Hospital L2:04, 171 76 Stockholm, Sweden.

Tel.: + 46(0)8 517 759 34

Fax: +46(0)8 33 57 24

E-mail: helen.vallhov@ki.se

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Short title

Exosomes carry Fel d 1 and induce immune responses

Keywords

aeroallergens; allergy; dendritic cells; exosomes; Fel d 1

Abstract

Exosomes are nano-sized membrane vesicles (50-120 nm), which are released from a wide variety of cells. Depending on their cellular origin, they can induce immune stimulatory-, inhibitory-, or tolerance-inducing effects. However, it is still unclear what role exosomes play during human inflammatory diseases. It has not been studied whether exosomes derived from human dendritic cells (DCs), the first cells to encounter allergens in the mucosa, can carry aeroallergens and contribute to allergic immune responses. We therefore explored whether DC-derived exosomes can present the major cat allergen Fel d 1, and if they thereby contribute to the pathogenesis of allergic disease. Our results demonstrate that exosomes are able to present aeroallergens and thereby induce T-cell T(H)2-like cytokine production in allergic donors. Thus, these exosomes may be important immune-stimulatory factors in allergic immune responses and important targets or engineered tools in immunotherapy.

Exosomes are nano-sized membrane vesicles (50-120 nm) derived from the endosomal compartment, released from a wide variety of cells, and they can be found in several body fluids like plasma, breast milk and bronchoalveolar lavage (BAL) (1, 2). They have been

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demonstrated to have immune stimulatory-, inhibitory-, or tolerance-inducing effects, depending on their cellular origin (3). Especially exosomes from antigen-presenting cells have gained interest, since they carry immunorelevant molecules like MHC class I and II and co-stimulatory molecules, and thus are investigated for use in vaccine and immune therapeutic strategies. Previous studies have demonstrated that exosomes are able to transfer functional MHC I/II/peptide complexes to bystander antigen-presenting cells (APCs) (2). It has also been demonstrated that exosomes isolated from B cells can present T-cell-activating peptides from the major birch allergen Bet v 1 and thereby induce T-cell proliferation and T(H)2-like cytokine production (4). However, it has not been studied whether exosomes derived from human dendritic cells (DCs), the first cells to encounter allergens in the mucosa, can carry aeroallergens and contribute to allergic inflammation. We therefore explored whether DC-derived exosomes can present aeroallergens, such as the major cat allergen Fel d 1, and if they thereby contribute to the allergic immune response.

Material and methods

Detailed experimental methods are given in Supporting Information.

Results and discussion

Peripheral blood mononuclear cells (PBMCs) were isolated from cat allergic or healthy blood donors, who's plasma had been analyzed for IgE and IgG₄ to Fel d 1 (Table S1). The concentration of IgE was in the range of 0.49-37 kAU /L to Fel d 1 in the allergic donors

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and below $<0.10 \text{ kAU/L}$ in each of the healthy blood donors, and the IgG₄ levels were above 0.05 mg/L in only two of the cat allergic donors (Table S1). We started to investigate exosomes isolated from healthy blood donors. The exosomes were either generated from monocyte-derived dendritic cells (MDDCs) cultured alone (Exos), from MDDCs co-cultured with rFel d 1 (10 $\mu\text{g/mL}$; Exos-rFel d 1) or from MDDCs cultured with similar amount of LPS (0.08 ng/mL; Sigma-Aldrich, Steinheim, Germany) found in the rFel d 1 preparation (Exos/LPS) for 48 h. To prove that Exos-rFel d 1 carries Fel d 1 which first has been taken up by MDDCs, another control was added where MDDCs were first cultured in the presence of rFel d 1 for 15 h, thereafter the MDDCs were washed to remove any rFel d 1 not endocytosed by the MDDCs. After additional culture for 48 h in fresh media without rFel d 1 the supernatant was collected for isolation of exosomes (Exos-rFel d 1 15 h). To address whether Fel d 1 is able to bind directly to exosomes without the involvement of cells, rFel d 1 (10 $\mu\text{g/mL}$) was added for 30 min to one part of the supernatant from MDDCs cultured alone (Exos+rFel d 1). The phenotype of MDDCs was not affected by the LPS amount found in rFel d 1 or by rFel d 1 itself as seen by flow cytometry (Fig. S1). The exosomes from the various supernatants were isolated by ultracentrifugation. Flow cytometry showed that both Exos and Exos-Fel d 1 had a similar surface expression of HLA-DR, CD63 and CD81 (Fig. 1A, B), which corresponds to a typical phenotype of MDDC derived exosomes (3). Immuno-negative electron microscopy staining (iEM) showed the characteristic morphology of exosomes and the presence of the exosome marker CD81 on the surface of Exos-rFel d 1 (Fig. 1C) (3), which was not seen when using the isotype control (Fig. 1C). Nanoparticle tracking analysis indicated that Exos, Exos/LPS, Exos-rFel d 1 and Exos+rFel d 1 exhibited relatively similar sizes with a diameter ranging from 135 to 150 nm (Fig. 1D). To investigate the presence of rFel d 1 on exosomes, ELISA was

performed, indicating that Exos-rFel d 1, Exos+rFel d 1 and Exos-rFel d 1 15 h are able to carry rFel d 1 (Fig. 2A). Similar amounts of rFel d 1 were found on the different exosomes, within the range of 3-390 ng rFel d 1/mL (n=15) in 10 µg exosome preparations (Fig. 2A). The wide range reflects the individual variations between the different blood donors. Since rFel d 1 is detected on all types of rFel d 1-exosomes, Fel d 1 seems to be able to be endocytosed by MDDCs, loaded onto exosomes and released on exosomes (Exos+rFel d 1 15 h), and also to bind directly to exosomes isolated from MDDCs cultured alone (Exos+rFel d 1). The presence of rFel d 1 on rFel d 1-exosomes were further supported by TEM, which visualizes how rFel d 1-exosomes (Fig. 2B), but not Exos (Fig. 2C), bind to latex beads covered with anti-Fel d 1 Abs, representing the presence of rFel d 1 on the surface of exosomes. Beads covered with the isotype control showed no binding to Exos-rFel d 1 (Fig. 2D).

To investigate whether rFel d 1 on the exosomes have immunogenic effects on PBMCs, cultures of PBMCs from healthy or cat allergic donors were set up pairwise and co-cultured with either Exos, Exos/LPS control, Exos-rFel d 1, Exos+rFel d 1, Exos-rFel d 1 15 h or only rFel d 1. Cytokine analysis was performed using ELISpot. Results showed that Exos-rFel d 1 and Exos+rFel d 1 did not induce IL-4 production in PBMCs from healthy donors, whereas in the cat allergic donors Exos-rFel d 1 and Exos+rFel d 1 were able to induce IL-4 responses (Fig. 2E). IL-4 responses were also detected in control experiments with Exos-rFel d 1 15 h, where we had a response of 10 and 47 IL-4 spots/ 2.5×10^5 PBMCs, respectively, from two cat allergic donors, whereas no spots were detected by PBMCs from two healthy donors. Despite the low amount of rFel d 1 found on the different rFel d 1-exosomes (Fig. 2A) they are still able to induce similar IL-4 responses as 10 µg/mL pure rFel d 1 (Fig. 2E and

Fig. S2). Thus, these data suggest that exosomes are able to enhance the IL-4 stimulatory capacity of rFel d 1 in cat allergic individuals.

Additional cytokine analysis (IL-6, IL-10, IL-17A, IFN- γ , and TNF) was performed with CBA, but no significant differences were observed between PBMCs from cat allergic and healthy donors co-cultured with the different exosome preparations (data not shown). Similar results for IL-10 and IFN- γ has been previously observed for PBMCs treated with only Fel d 1 (5).

Our results demonstrate a novel route for distribution of aeroallergens via exosomes derived from DCs. These exosomes are able to present allergens and thereby induce T-cell T(H)2-like cytokine production in allergic donors, and may therefore be important immunostimulatory factors in allergic immune responses. Thus, exosomes carrying allergens may be important targets in immunotherapy. Our novel finding also shows the ability to engineer exosomes by adding allergens, which may be useful for future exosome-based vaccines.

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Author contributions

AS and HV conceived and designed the study; HV, CG, and KH performed experiments and analysis. RV and HG contributed with reagents. All authors participated in interpretation of data. HV and AS drafted the manuscript and all co-authors approved the final version.

Conflict of interest

RV has received research grants from Biomay AG, Vienna, Austria and Thermo Fisher, Uppsala, Sweden, and serves as a consultant for these companies.

Supporting Information

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

Data S1. Materials and methods.

Table S1. Subject characteristics.

Figure S1. Expression of MDDC surface markers.

Figure S2. IL-4 production in PBMCs from cat allergic donors and healthy controls when cultured with only rFel d 1.

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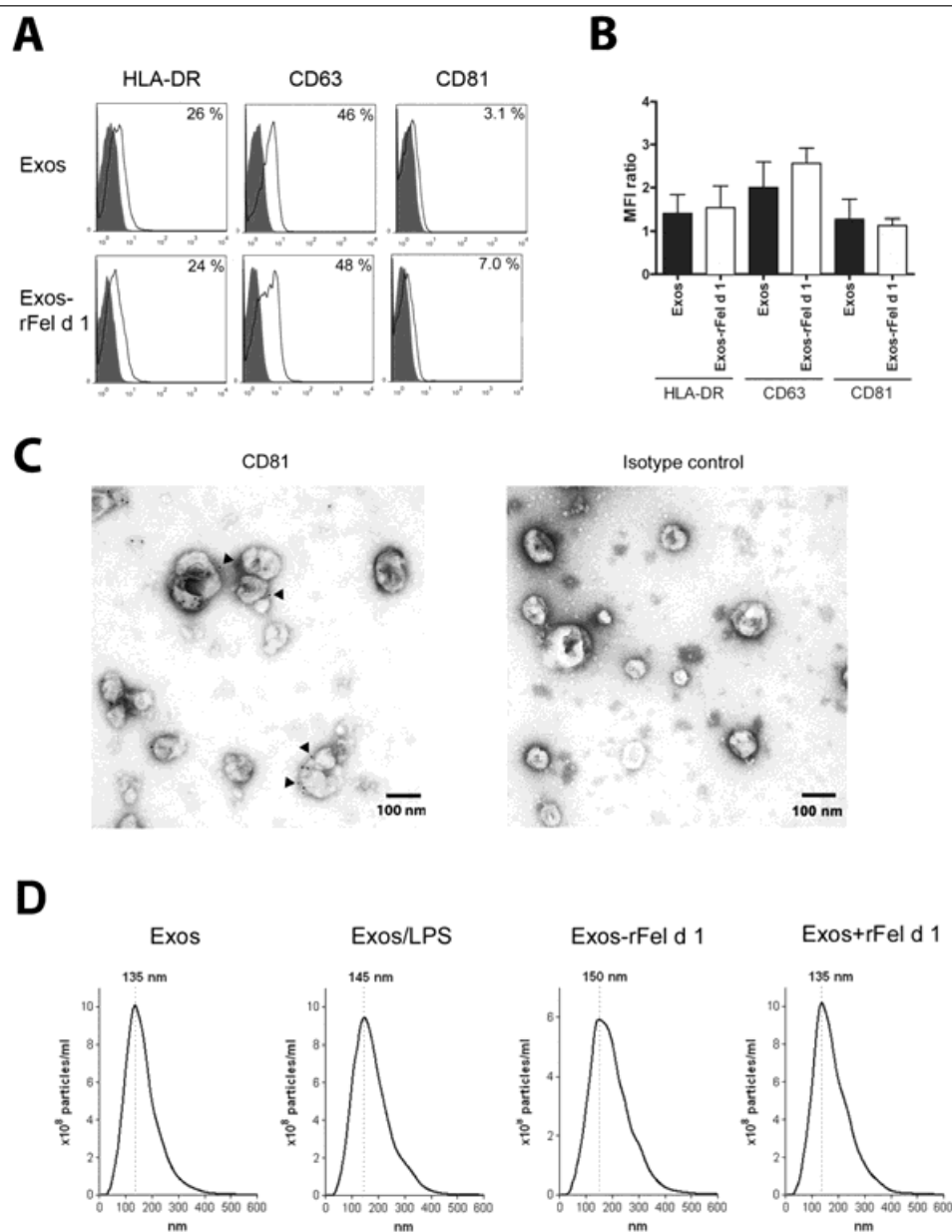


Figure 1 Characterization of Exos-rFel d 1. Expression of the surface markers HLA-DR, CD63 and CD81 on Exos and Exos-rFel d 1 were measured by flow cytometry and are shown as (A) histograms and % positive cells from one representative experiment of three independent experiments using exosomes from different healthy blood donors (isotype control in black), or as (B) the ratio of the mean fluorescence intensity (MFI). Results represent the mean $\pm$ SEM from 3 independent experiments. (C) The morphology of Exos-rFel d 1 was visualized by performing iEM on pools of Exos-rFel d 1 from 5 healthy blood donors using a mAb against CD81 (detected with a 10 nm gold-particle-conjugated anti-rabbit Ab; indicated by arrows) and corresponding isotype control. (D) The mean particle size distribution from five different exosome batches for Exos, Exos/LPS, Exos-rFel d 1 and Exos+rFel d 1 analyzed by nanoparticle tracking analysis (NanoSight).

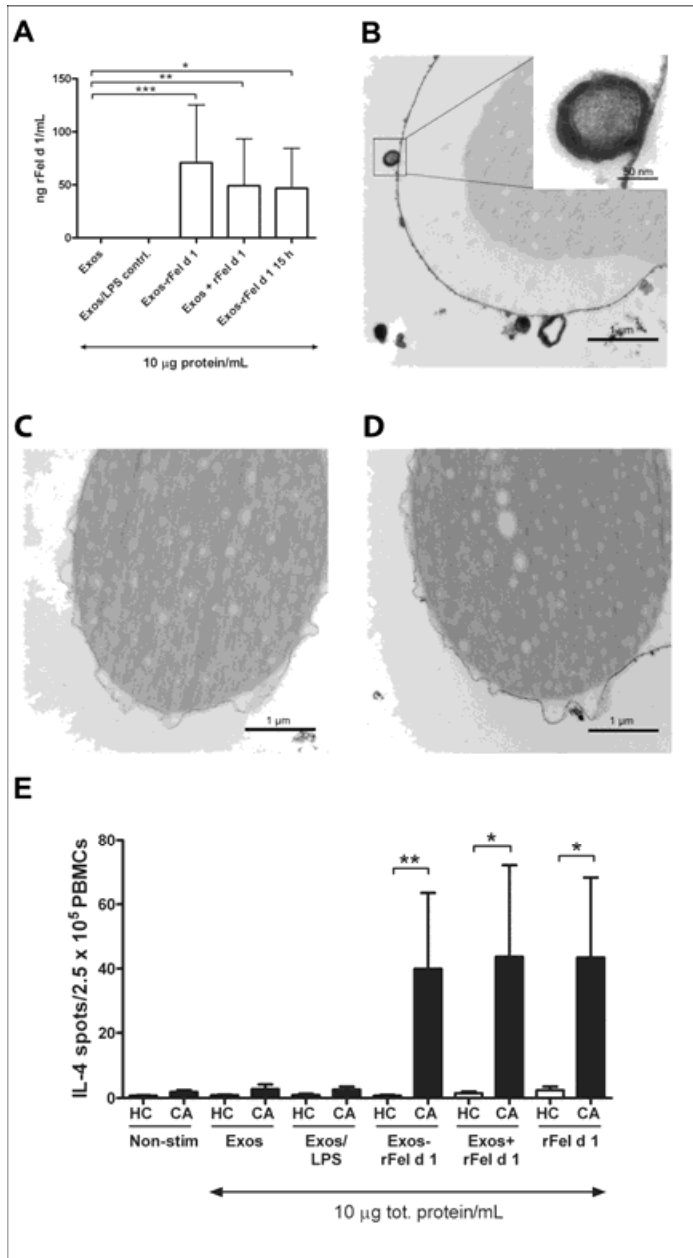
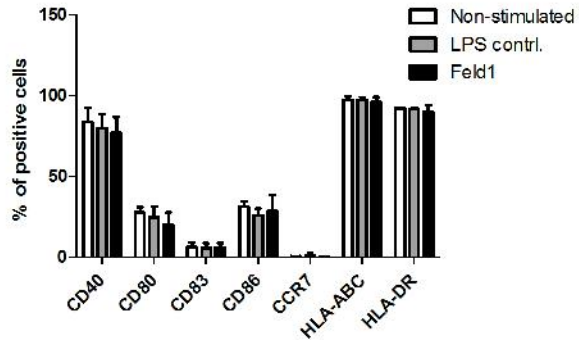
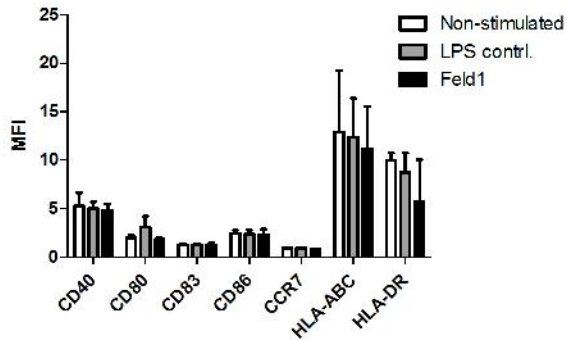


Figure 2 Exosomes generated from MDDCs carry rFel d 1 and induce IL-4 production in PBMCs from cat allergic donors. (A) The presence of rFel d 1 on Exos-rFel d 1 (n=7), Exos+rFel d 1 (n=5) and Exos-Fel d 1 15 h (n=3) generated from MDDCs from healthy non-allergic blood donors was analyzed by using ELISA and a mouse mAb against Fel d 1. Exos and Exos/LPS were used as controls (n=7). (B) Exos-Fel d 1, but not (C) Exos bind to latex beads coupled with anti-Fel d 1 rabbit Abs, as visualized by using TEM on a pool of Exos-rFel d 1 from 2 healthy blood donors. (D) No binding is seen with latex beads coupled with an isotype control Ab. (E) IL-4 production in PBMCs from cat allergic (CA) donors and healthy controls (HC) measured by ELISpot when cultured alone (non-stimulated; n=7), with Exos (n=7), Exos/LPS (n=7), Exos-rFel d 1 (n=7), Exos+rFel d 1 (n=5) or only rFel d 1 (10 μ g; n=7) for 48 h. Data represent mean $\pm$ SEM. * P < 0.05, ** P < 0.01 and *** P < 0.001, Mann-Whitney U test.

A**B**

IL-4 spots/ 2.5×10^5 PBMCs

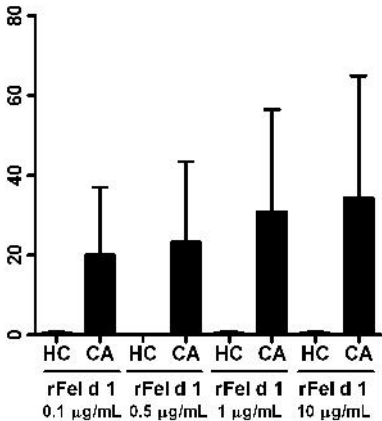


Table S1. Subject characteristics.

Individual	Sex	Age (years)	Plasma Fel d 1 IgE (k _A U/L) ¹⁾	Plasma Fel d 1 IgG ₄ (mg/L) ²⁾
CA*	F	32	2.2	0.07
CA	F	33	0.49	<0.05
CA	M	29	2.9	0.19
CA	F	20	37	<0.05
CA	M	22	28	0.05
CA	F	47	13	<0.05
CA	F	28	1.2	<0.05
CA	F	36	2.9	n.d. [§]
CA	M	24	16	n.d.
Median (range)		30 (20-47)	12 (0.49-37)	0.07 (<0.05-0.19)
HC [†]	n.k. [‡]	n.k.	<0.10	<0.05
HC	n.k.	n.k.	<0.10	<0.05
HC	n.k.	n.k.	<0.10	<0.05
HC	n.k.	n.k.	<0.10	<0.05
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HC	n.k.	n.k.	<0.10	<0.05
HC	n.k.	n.k.	<0.10	<0.05
HC	n.k.	n.k.	<0.10	n.d.
HC	n.k.	n.k.	<0.10	n.d.
Median (range)		n.k.	<0.10	<0.05

*CA = Cat allergic donor

[†]HC = Healthy blood donor

[‡]n.k. = not known

[§]n.d. = not done

¹⁾ Specific plasma IgE to Fel d 1 analyzed with ImmunoCAP 1000™ (Thermo Fisher Scientific, Uppsala, Sweden), allergy to cat was defined by ≥0.35 (kU/L)

²⁾ Specific plasma IgG₄ to Fel d 1 analyzed with ImmunoCAP 250™ (Thermo Fisher Scientific)

F = female

M = male

Supporting Materials and Methods

Cat allergic donors and control subjects

Nine individuals in the age of 20-47 years diagnosed with allergy to cat and with plasma IgE reactivity ≥ 0.49 (k_AU/L) to Fel d 1 (ImmunoCAP 1000TM, Thermo Fischer Scientific, Uppsala, Sweden) were recruited from the Stockholm area (Table 1). Healthy non-allergic blood donors (n = 9) were used as controls (Karolinska University Hospital Blood Bank, Stockholm, Sweden) (Table S1). The study was approved by the local ethical committee.

Isolation of PBMCs and generation of human monocyte-derived dendritic cells (MDDCs)

Human peripheral blood mononuclear cells (PBMCs) from cat allergic donors were separated from peripheral blood and from buffy coats from healthy blood donors by standard gradient centrifugation with Ficoll Paque (Amersham Pharmacia Biotech AB, Uppsala, Sweden). To generate MDDCs the PBMCs were harvested, and washed three times with PBS, followed by resuspension in MACS-buffer (80 μ L/10⁷ cells) containing 0.5 % w/v BSA, 2 mM EDTA in PBS. Anti-CD14 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) were added according to the manufacturer's instructions. After 30 min at 4 °C, the CD14⁺ cells were separated from the solution by autoMACS (Miltenyi Biotec). Immature MDDCs were generated as described before (S1) by culturing CD14⁺ monocytes in RPMI 1640 medium (HyClone, Logan, UT, USA), supplemented with 25 μ g/mL gentamicin (Gibco Invitrogen Corporation, Paisley, UK), 2 mM L-glutamine (Gibco Invitrogen Corporation), 100 IU/mL penicillin (Gibco Invitrogen Corporation), 100 μ g/ml streptomycin (Gibco Invitrogen Corporation), 50 μ M β -mercaptoethanol (KEBO-lab, Spånga, Sweden), 10 % heat inactivated

fetal calf serum (HyClone, South Logan, UT, USA; depleted of exosomes by centrifugation (S2)) and with the recombinant (r) cytokines GM-CSF (550 IU/mL; Invitrogen/GIBCO, Paisley, UK), and IL-4 (800 IU/mL; Biosource International, Camarillo, CA, USA), at a density of 4×10^5 cells/mL, in 37 °C with a 6 % CO₂ atmosphere. After 6 days, the cell surface molecules CD1a, CD11c, CD14 and CD83 were analyzed by flow cytometry (see below) to confirm a phenotype of immature MDDC with low CD83 expression (always below 6 %).

Preparation of Fel d 1

Monomeric, folded and >95% pure rFel d 1 (2+1) was produced and verified as previously described (S3). rFel d 1 (2+1) consists of chain 2 and chain 1 fused together without additional linker and has immunological properties indistinguishable from the natural heterodimeric protein. Each batch of rFel d 1 produced was controlled for lipopolysaccharide (LPS) concentrations by the endpoint chromogenic LAL test method (Limulus Amebocyte Lysate endochrome, Charles River Endosafe, Charleston, SC, USA) according to the manufacturer's instructions. LPS concentrations measured were always below 0.3 ng/mL which was further reduced to 0.08 ng LPS/mL when diluted in cell cultures.

Antibodies to Fel d 1

Anti-Fel d 1 rabbit Abs from hyperimmune serum (a kind gift from Rudolf Valenta) was affinity purified over Fel d 1 covalently linked to a N-hydroxysuccinimide (NHS)-activated HiTrap column according to instructions (GE Healthcare, Wauwatosa, WI, USA). Unbound material was washed by 10 column volumes of 10 mM phosphate buffered saline, pH 7.4. Allergen-specific antibodies were eluted by 0.1 M glycine-HCl, pH 2.5, and neutralized by 1 M carbonate buffer, pH 9.6. A monoclonal anti-Fel d 1 Ab (IgG1) was produced by a mouse hybridoma (clone 5) in serum free media and concentrated by filtration. The concentration of

the rabbit Ab was 0.42 µg/mL and of the mouse Ab 200 µg/mL, as determined by an ELISA coated with rFel d 1.

Preparation of exosomes

Either exosomes were isolated from MDDCs cultured alone (Exos), from MDDCs co-cultured with rFel d 1 (10 µg/mL) or with similar amount of LPS (0.08 ng/mL; Sigma-Aldrich, Steinheim, Germany) found in rFel d 1 at a concentration of 10 µg/mL (Exos/LPS) for 48 h. To prove that Exos-rFel d 1 carries Fel d 1 which first has been taken up by DCs, another control was added where MDDCs were first cultured in the presence of rFel d 1 for 15 h, thereafter the MDDCs were washed to remove any rFel d 1 not taken up by the MDDCs. After additional culture for 48 h in fresh media without rFel d 1 the supernatant was collected for isolation of exosomes (Exos-rFel d 1 15 h). The supernatants from all co-cultures were centrifuged at 3000 g, 30 min to remove cells. rFel d 1 (10 µg/mL) was added for 30 min to one part of the supernatant from MDDCs cultured alone, to address whether Fel d 1 is able to bind directly to exosomes without the involvement of cells (Exos+rFel d 1). Exosomes from the various supernatants were further isolated and prepared by serial ultracentrifugation as previously described (S4). Pellets were resuspended in PBS, and the protein content was measured using a Bradford assay (BioRad, Hercules, CA, USA). Exosomes preparations were stored at -80°C until further use.

Immuno-negative electrone microscopy staining (iEM)

Exos-rFel d 1 coated grids were blocked from unspecific binding by floating grids face down on drops of 10 % normal goat serum (NGS; Dako, Glostrup, Denmark) in 0.1 M phosphate buffer (PB) for 10 min followed by incubation with a mAb against CD81 (BD Biosciences, CA, USA) or corresponding isotype control for 2 h. Grids were washed several times in phosphate buffer containing 0.1 % NGS and bound antibodies were detected by secondary

gold-particle conjugated goat anti-rabbit Abs (10 nm; Biocell, BBInternational, Cardiff, England).

Nanoparticle tracking analysis

Size distribution of MDDC-derived exosome preparations were analyzed by measuring the rate of Brownian motion using a NanoSight LM10 system, equipped with a fast video capture and particle-tracking software NTA 2.2 (NanoSight, Amesbury, UK). Exosome preparations were diluted in PBS and measured for 1 min in triplicates at a concentration of $\sim 5 \times 10^8$ particles/mL.

Phenotypic analysis of MDDCs and exosomes by flow cytometry

Non-stimulated MDDC or MDDC co-cultured with rFel d 1 or LPS control were labeled with fluorescent phycoerythrin (PE) conjugated rat monoclonal antibody (mAb) specific for CCR7 (BD Biosciences); and with fluorescein isothiocyanate (FITC) conjugated mouse mAbs specific for: CD40, CD80, CD83, CD86 and HLA-ABC and HLA-DR (Biolegend, CA, USA) according to the manufacturer's instructions. Control samples were labeled with isotype-matched Abs conjugated with the same fluorochrome. Fluorescence was measured with a FACSCalibur flow cytometer (BD Biosciences). Ten thousand viable cells were counted and analyzed by the program CellQuestPro. Sulfate-aldehyde latex microspheres (4 μ m; Invitrogen) were coated with Exos or Exos-rFel d 1 at a ratio of 5 μ g protein per μ L beads and rotated overnight at room temperature. Latex beads with exosomes were labeled with a panel of fluorescein isothiocyanate (FITC)-conjugated mAbs specific for HLA-DR, CD63 and CD81 (BD Biosciences) and the corresponding isotype-matched antibodies (S5). Data were acquired using a FACSCalibur (BD Biosciences). 5000 beads were counted and analyzed by using FlowJo software (Tree Star Inc., Ashland, OR, USA).

ELISA analysis

Exos, Exos/LPS control, Exos-rFel d 1, Exos+rFel d 1 and Exos-rFel d 1 15 h were analyzed in triplicates by ELISA for the presence of rFel d 1. Microtiter plates (96 wells; Nunc, Roskilde, Denmark) were coated with various exosome preparations (10 µg/mL) or with only rFel d 1 (10 µg/mL) in 0.1 M carbonate buffer, pH 9.6. After overnight adsorption at 4 °C, the plates were emptied, and the remaining protein binding sites were blocked with 200 µl of PBS containing 1 % w/v bovine serum albumin for 1 h at RT. The monoclonal mouse anti-rFel d 1 Ab was diluted to 20 µg/mL in PBS containing 0.05 % Tween 20 (PBS-T) and added to each well. After 1 h 50 µL of goat anti-mouse IgG horseradish peroxidase (HRP; Miab, Uppsala, Sweden, diluted 1:1000 (v/v)) was added, and incubation was continued for 1 h. Tetramethylbenzidine (TMB) substrate (Biolegend) was used, and the color reaction was monitored at 450 nm in an ELISA reader (Multiskan Ascent; Thermo Fisher Scientific).

Negative electron microscopy staining (TEM)

Latex beads (Invitrogen) were either coated with anti-Fel d 1 rabbit Abs or as a control with normal rabbit IgG (1 µg/1 µL beads), according to the manufacturer's instructions. Thereafter, both types of coated beads were coated with Exos or Exos-rFel d 1 at a ratio of 10 µg protein per µL beads and rotated overnight at room temperature. Beads were fixed in 2.5 % glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 at room temperature and further fixed over the night in the refrigerator. After fixation beads were rinsed in 0.1 M phosphate buffer and centrifuged. The pellets were then postfixed in 2 % osmium tetroxide in 0.1 M phosphate buffer, pH 7.4 at 4 °C for 2 h, dehydrated in ethanol followed by acetone and embedded in LX-112 (Ladd, Burlington, Vermont, USA). Ultrathin sections (approximately 40-50 nm) were cut by a Leica ultracut UCT (Leica, Vienna, Austria). Sections were contrasted with uranyl acetate followed by lead citrate and examined in a Tecnai 12 Spirit Bio TWIN transmission electron microscope (FEI Company, Eindhoven, the Netherlands) at 100 kV.

Digital images were taken by using a Veleta camera (Olympus Soft Imaging Solutions, GmbH, Münster, Germany).

Cytokine analysis

PBMCs were cultured alone (non-stimulated), or with various exosome/Fel d 1 samples, or with only rFel d 1 at a concentration of 0.1, 0.5, 1 and 10 µg/mL. Experiments were performed pairwise with one cat allergic donor and one healthy control (Table S1). IL-4 ELISpot assays were performed in duplicates for 48 h according to the manufacturer's instructions (Mabtech, Stockholm, Sweden) and analyzed by an ELISPOT automated reader (AID Diagnostika, Straßberg, Germany). Culture supernatants were harvested after 3 days and kept at – 20 °C until analyzed. Production of IL-6, IL-10, IFN-γ, TNF and IL-17A by PBMCs was assessed by Cytometric Bead Array (CBA, Human Th1/Th2/Th17 kit, Becton Dickinson) according to the manufacturer's instruction. Samples were run in duplicates with FACSCalibur flow cytometer (Becton Dickinson). Lowest detection limits were 2.4 pg/mL for IL-6, 4.5 pg/mL for IL-10, 18.9 pg/mL for IL-17A, and 3.8 pg/ml for IFN- γ and TNF.

Statistics

Mann-Whitney U test was used to determine differences between groups. A *P*-value less than 0.05 was considered significant.

Ethics

The study was approved by the local ethical committee.

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HC	n.k.	n.k.	<0.10	<0.05
HC	n.k.	n.k.	<0.10	n.d.
HC	n.k.	n.k.	<0.10	n.d.
Median (range)		n.k.	<0.10	<0.05

*CA = Cat allergic donor

[†]HC = Healthy blood donor[‡]n.k. = not known[§]n.d. = not done¹⁾ Specific plasma IgE to Fel d 1 analyzed with ImmunoCAP 1000™ (Thermo Fisher Scientific, Uppsala, Sweden), allergy to cat was defined by ≥0.35 (kU/L)²⁾ Specific plasma IgG₄ to Fel d 1 analyzed with ImmunoCAP 250™ (Thermo Fisher Scientific)

F = female

M = male

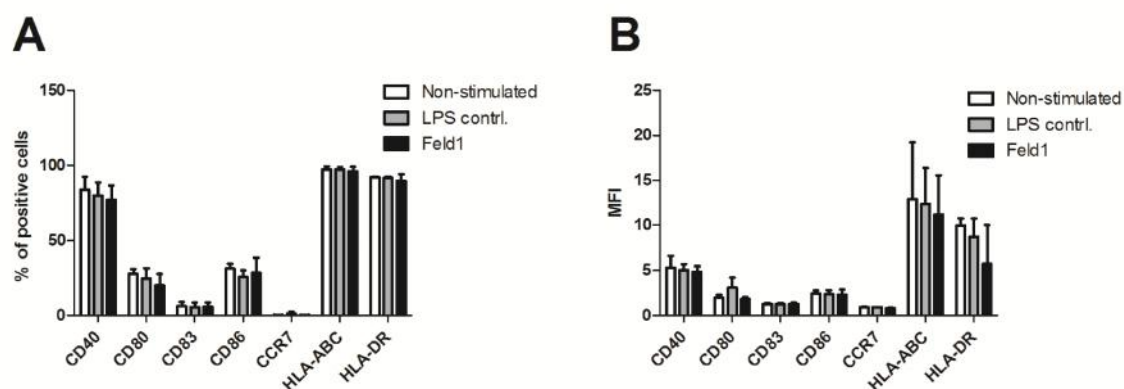


Figure S1 rFel d 1 and the LPS amount found in rFel d 1 does not affect the expression of MDDC surface markers. MDDCs treated with LPS (0.08 ng/mL) or with rFel d 1 (10 μ g/mL) were analyzed for their expression of surface molecules CD40, CD80, CD86, CD83, CCR7, HLA-ABC and HLA-DR by flow cytometry after 48 h and are presented as percentage of positive cells (A) and as MFI (B). Results represent the mean $\pm$ SEM of 4 healthy donors, except of HLA-ABC and HLA-DR where 2 individual experiments were performed.

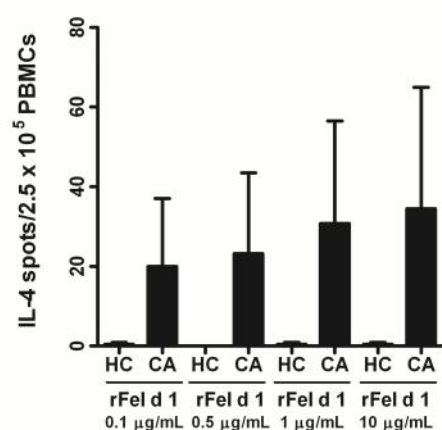


Figure S2 IL-4 production in PBMCs from cat allergic (CA) donors and healthy controls (HC) measured by ELISpot when cultured with only rFel d 1 at 0.1, 0.5, 1 and 10 μ g/mL for 48 h. Data represent mean $\pm$ SEM of two healthy non-allergic controls and two cat allergic donors.