

Original article

Cat allergen induces proinflammatory responses by human monocyte-derived macrophages but not by dendritic cells

Background: The upper airway mucosa of healthy humans contains a dense network of cells with dendritic morphology of which the majority express a macrophage-like phenotype (CD14 + CD64 + CD68 +), whereas the smaller population are immature dendritic cells (DC; CD11c + CD14–). Our aim was to study the proinflammatory response of human monocytes and *in vitro*-generated macrophages and DC after contact with cat allergens.

Methods: Monocyte-derived DC and monocyte-derived macrophages were exposed to cat allergen extract or *Escherichia coli*. Purified monocytes were stimulated with allergen extracts from cat or house dust mite (HDM) or the major allergenic protein Fel d 1 and induction of proinflammatory cytokines by monocytes was analyzed before and after blocking CD14.

Results: We show that cat allergen extract induced tumor necrosis factor (TNF) and interleukin (IL)-6 production by CD14-positive macrophages but not by CD14-negative DC. Moreover, monocytes produced significantly higher levels of TNF in response to cat allergens than in response to HDM allergens. We observed no differences in levels of TNF and IL-6 from either macrophages or monocytes after exposure to cat allergen when comparing healthy and cat-allergic individuals. Finally, the proinflammatory cytokine production from monocytes in response to cat allergen extract but not to HDM allergen was significantly reduced by blocking CD14.

Conclusion: These results indicate that closely related innate immune cells from the myeloid lineage respond differentially to cat allergen extract and that the pattern-recognition receptor CD14 might be one of the mediators involved in the inflammatory responses to inhalant allergens.

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The human upper airway mucosa is constantly exposed to a wide variety of exogenous antigens, which are sampled by antigen-presenting cells (APC) and presented to T cells. In humans, it has recently been described that the mucosa of the upper respiratory tract contains two main APC populations with a dendritic morphology. The majority of these cells express a macrophage-like phenotype (CD14 + CD64 + CD68 +), whereas the smaller population is predominantly constituted by immature dendritic cells (DC; CD11c + CD14–; 1). The DC are generally considered to be the principal APC involved in presenting microbial structures to naive T cells. Immature DC can be differentiated from CD14-positive monocytes *in vitro* by incubation with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin 4 (IL-4; 2), and studies from animal models indicate that monocytes may also differentiate into DC *in vivo* (3). Recognition of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs) expressed on DC leads to up-regulation of activation

markers, induction of cytokine production and migration to lymph nodes, which are crucial events for generation of adaptive immune responses (4). In contrast to DC, which transfer information from the periphery to the regional lymph nodes, tissue macrophages appear to exert their function *in situ*. These highly phagocytic cells also express PRRs, such as CD14 and Toll-like receptors (TLRs), and play an important role in innate immunity, for example, by producing proinflammatory cytokines (5). CD14 has been described to play a key role in initiating cell activation induced by a group of cell wall components from both gram-negative and gram-positive bacteria (6). CD14, however, lacks an intracellular domain and therefore has no intracellular signaling capacity, but has been suggested to function as a co-receptor for TLRs (7).

The role of different APC in the induction of allergic disease is not fully understood. Airway DC are essential for inducing Th2 responses and sensitization to inhaled allergen as shown in a murine model of asthma (8). In contrast, alveolar macrophages appear to be important in

prevention of allergic sensitization in the healthy lung, as depletion of these cells can lead to vigorous immune responses with influx of T cells and immunoglobulin E (IgE) production in allergic mice (9). However, the reactivity to allergens by tissue macrophages, or their *in vitro* correlates, has not been studied although they are present in large numbers in the nasal human mucosa.

Proteins from domestic cats (*Felis domesticus*) are some of the most potent elicitors of allergic disease, particularly asthma, affecting people worldwide. The most prominent allergen in cat dander is a glycoprotein, termed Fel d 1, which elicits IgE responses in 90–95% of patients with cat allergy and accounts for 60–90% of the total allergenic activity (10). There are several differences between the major allergens derived from cats and dust mites (Der p 1 and Der f 1). In contrast to Fel d 1, both Der p 1 and Der f 1 have an enzymatic activity that can cleave CD25 as well as open up epithelial junctions, while Fel d 1 is heavily glycosylated (11, 12). Moreover, cat allergens are small continuously air-borne particles, whereas mite allergens are larger in size and are air-borne only with disturbance. This results in a 10- to 100-fold higher exposure to cat allergens compared with house dust mite (HDM) allergens (13). However, the immunologic outcome of the interaction between cat and HDM allergens and various innate immune cells present in the upper airway mucosa has not been studied. Therefore, we set out to investigate the ability of cat and HDM allergen extract to induce proinflammatory cytokine responses by human monocyte-derived DC, monocyte-derived macrophages, and monocytes.

Materials and methods

Reagents

Dried defatted allergen extracts from cat (*F. domesticus*) and HDM (*Dermatophagoides pteronyssinus*) were reconstituted according to the protein content. Cat allergen extract and recombinant cat allergen (Fel d 1) expressed in a baculovirus expression system were obtained from ALK-Abelló (Hørsholm, Denmark) (14). The HDM allergen extract was purchased from Greer Laboratories (Lenoir, NC, USA). The endotoxins were depleted from cat allergen extract by use of END-X B15 endotoxin affinity resin (Associates of Cape Cod, Falmouth, MA, USA) (15). The cat allergen extract contained 9.5 µg lipopolysaccharide (LPS)/µg, Fel d 1 contained $<1.3 \times 10^{-8}$ pg LPS/µg and HDM allergen extract contained 0.2 pg LPS/µg tested by *Limulus amoebocyte* lysate end-point test (LAL-test; Bio-Whittaker, Walkersville, MD, USA). *Escherichia coli* was obtained from the Culture Collection of the University of Göteborg (CCUG; Göteborg, Sweden) and LPS from *E. coli* (serotype 055:B5) were purchased from Sigma-Aldrich (St Louis, MO, USA).

Cell separation

Blood samples were obtained from adults who were either sensitized [skin prick test (SPT)+; $n = 8$] or not sensitized (SPT-; $n = 5$) to cat allergen. A wheal diameter of >3 mm was used as the criterion

to select the SPT+ subjects. All patients had a history of cat allergen-induced moderate or severe rhinoconjunctivitis with duration of at least 4 years. The volunteers gave informed consent to participate in the study, which was approved by the local Human Research Ethical Committee of the Medical Faculty, Göteborg University, Göteborg, Sweden. For blocking experiments on monocytes, buffy coats from blood donors were obtained from the blood donor center at Sahlgrenska University Hospital. Mononuclear cells were separated over Lymphoprep™ (Nycomed, Oslo, Norway) and CD14-positive monocytes were purified by magnetic cell sorting using a positive selection technique (MACS® system™, Miltenyi Biotech, Bergisch Gladbach, Germany). Analysis with flow cytometry showed that more than 95% of the purified cells expressed CD14. To exclude that positive selection for CD14-expressing cells would interfere with blocking experiments using anti-CD14 antibodies, we also purified monocytes by the counter-current centrifugal elutriation technique (16) and similar results were obtained in experiments blocking CD14 with either purification method.

Generation of monocyte-derived macrophages and dendritic cells

For generation of macrophages, monocytes (5×10^5 /ml) were cultured with rM-CSF (2 ng/ml; Sigma) in macrophage-SFM medium (Invitrogen, San Diego, CA, USA). For generation of DC, monocytes (5×10^5 /ml) were cultured with rGM-CSF (300 U/ml) and rIL-4 (500 U/ml; R&D systems, Minneapolis, MN, USA) in RPMI-1640 supplemented with 2% heat-inactivated autologous serum, L-glutamine (1 mM) and gentamycin (50 µg/ml). The cells were cultured in 5% CO₂ at 37°C for 6–7 days and were re-fed with M-CSF or GM-CSF and IL-4 every second day. This resulted in generation of macrophages positive for CD14, and in DC positive for CD11c but negative for CD14.

Cell cultures

Monocyte-derived macrophages (5×10^5 /ml) and monocyte-derived DC (5×10^5 /ml) were stimulated with cat allergen extract (10 µg/ml) or *E. coli* (5×10^6 /ml). Monocytes (5×10^5 /ml) were stimulated with allergen extract from cat and HDM (100 µg/ml), Fel d 1 (10 µg/ml) or LPS. During stimulation, all cell cultures were kept in 5% CO₂ at 37°C for 24 h in serum-free media. In blocking experiments, monocytes (5×10^5 cells/ml) were incubated with monoclonal antihuman TLR4 (HTA125), a-CD14 (UCHM-1) or the IgG2a isotype control antibody (10 µg/ml) for 45 min at 4°C prior to stimulation with allergen extracts from cat or HDM (100 µg/ml) for 24 h. All antibodies were purchased from Serotec (Oxford, UK). To exclude that cytokine production was induced by LPS contamination we added various concentrations of polymyxin B (an antibiotic that neutralizes LPS; Sigma-Aldrich) together with the allergens to the cultures. One microgram per ml of polymyxin B neutralizes 2 ng/ml LPS.

Cytokine analysis

A standard enzyme-linked immunosorbent assay (ELISA) procedure was performed as described in detail elsewhere (17). Briefly, Costar plates (Invitrogen) were coated with the antihuman capture monoclonal antibodies (mAbs): tumor necrosis factor (a-TNF; MAb1), a-IL-6 (MQ2-13A5) and standard curves were generated using recombinant human TNF and IL-6. The following biotinylated detector antibodies were used: a-TNF (MAb11) and a-IL-6 (MQ2-39C3). All antibodies and standards

were purchased from Pharmingen (Pharmingen, San Diego, CA, USA). Samples, standards, biotinylated antibodies, and strept-
 avidin-horse radish peroxidase (HRP) were diluted in high-per-
 formance ELISA dilution buffer Sanquin (Amsterdam, the
 Netherlands).

Statistical analysis

The data were analyzed by the Wilcoxon matched pairs test
 (GraphPad Prism, San Diego, CA, USA) or Mann–Whitney *U*-test
 as described in figure legends.

Results

Cat allergen induces proinflammatory cytokine production from
 monocyte-derived macrophages but not from monocyte-derived DC

As the human upper airway mucosa contains not only
 CD14-negative immature DC but also a majority of
 CD14-positive macrophages, we set out to study the
 interactions between these cell types and environmental
 allergens by generating human monocyte-derived DC
 and monocyte-derived macrophages *in vitro*. As shown
 in Fig. 1A, approximately 90% of the monocyte-derived
 macrophages expressed CD14 on the surface, whereas
 monocyte-derived DC did not express this molecule. We
 then analyzed the cytokine production from the CD14-
 negative DC and the CD14-positive macrophages in
 response to cat allergen extract (cat) or the positive
 control UV-killed *E. coli*. As shown in Fig. 1B, we
 found that DC produced very low levels of TNF and no
 IL-6 in response to cat. Similar results were obtained
 when the dose of cat was increased from 10 to 100 $\mu\text{g/ml}$
 (data not shown). In contrast to DC, macrophages
 produced considerably higher levels of both TNF and
 IL-6 when exposed to cat. In response to *E. coli* bacteria,
 TNF and IL-6 was produced in high amounts by both
 DC and macrophages (Fig. 1C). However, the proin-
 flammatory cytokine production was significantly higher
 from macrophages compared with DC. Unstimulated
 DC or macrophages did not produce any proinflammato-
 ry cytokines. We observed no differences in levels of
 cytokines from either DC or macrophages when compar-
 ing healthy and allergic individuals after stimulation
 with cat or *E. coli* (Table 1). Thus, cat allergen extract
 induces proinflammatory responses only from the CD14-
 positive macrophages, whereas *E. coli* is able to induce
 TNF and IL-6 from both macrophages and CD14-
 negative DC.

Next, we investigated how CD14-positive monocytes
 respond to stimulation with different allergens (Fig. 2A),
 as they in contrast to macrophages do not require to be
 generated *in vitro*. As shown in Fig. 2B, we found that
 allergen extracts from both cat and HDM induced TNF
 production from monocytes in a dose-dependent manner.
 Similar results were obtained for IL-6 production (data
 not shown). However, when comparing cat and HDM

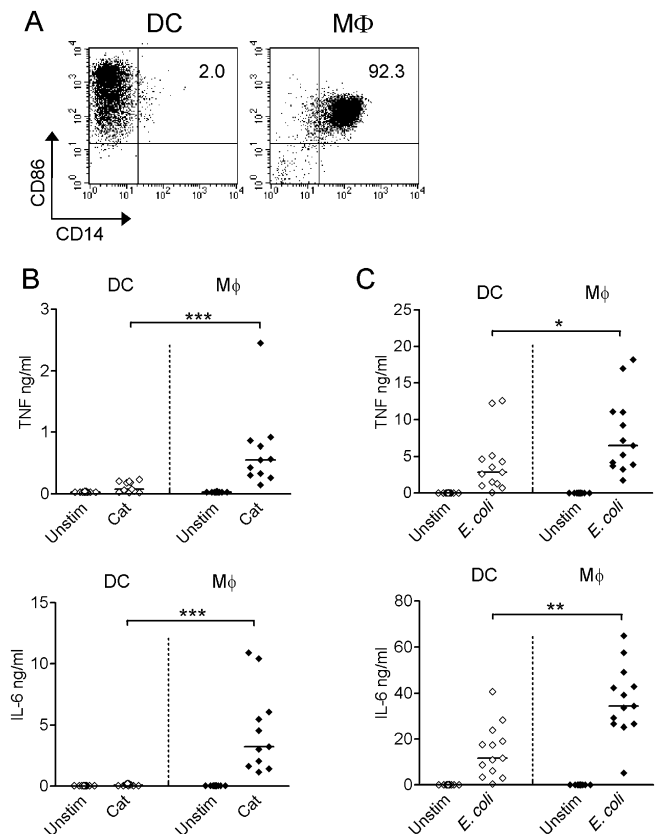


Figure 1. (A) Expression of CD14 and CD86 on unstimulated human monocyte-derived dendritic cells (DC) and macrophages. The percentages of positive cells are indicated in the upper right quadrants of the panels. (B and C) tumor necrosis factor (TNF) and interleukin (IL)-6 production by monocyte-derived DC and macrophages ($5 \times 10^5/\text{ml}$) in response to (B) cat allergen extract (10 $\mu\text{g/ml}$) or (C) *Escherichia coli* (5×10^6) for 24 h. Each symbol represents one individual and the horizontal bars represent the median value. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ (Mann–Whitney *U*-test).

allergen-induced proinflammatory cytokine production we found that the TNF-production induced in response to cat allergen was approximately fivefold higher than that induced by the HDM allergen, whereas both allergens induced high levels of IL-6 (Fig. 2C,D). These data indicate that the induction of TNF and IL-6 does not correlate with the LPS contamination of the allergens used because the cat allergen contained 50-fold higher LPS levels relative to the HDM allergen. Interestingly, we did not observe any proinflammatory cytokine production by monocytes after exposure to LPS-free recombinant Fel d 1, suggesting that other components than the major allergenic protein in the cat allergen extract are responsible for triggering TNF and IL-6 production. As for DC and macrophages, we did not observe any difference in cytokine production by cat allergen-stimulated monocytes from sensitized relative to nonsensitized subjects (Table 2).

Table 1. Cytokine production by dendritic cells (DC) and macrophages from non-allergic and cat-allergic individuals stimulated with cat allergen or *Escherichia coli**

	Nonallergic (n = 5)	Cat-allergic (n = 6)	P-value
Cat allergen			
DC			
TNF (ng/ml)	0.07 (0.03–0.24)†	0.11 (0.02–0.20)	NS
IL-6 (ng/ml)	0.09 (0.04–0.21)	0.09 (0.04–0.20)	NS
MØ			
TNF (ng/ml)	0.56 (0.33–2.45)	0.36 (0.14–0.87)	NS
IL-6 (ng/ml)	2.04 (1.15–6.05)	3.88 (1.42–10.91)	NS
	(n = 5)	(n = 8)	
<i>E. coli</i>			
DC			
TNF (ng/ml)	2.85 (1.10–4.30)	3.10 (0.092–12.59)	NS
IL-6 (ng/ml)	8.75 (3.36–17.60)	15.40 (0.41–40.72)	NS
MØ			
TNF (ng/ml)	9.24 (1.74–18.18)	4.71 (3.28–17.02)	NS
IL-6 (ng/ml)	42.35 (5.34–57.53)	33.95 (26.55–65.06)	NS

*Stimulation of monocyte-derived DC and macrophages (5×10^5 /ml) from non-allergic and cat-allergic subjects after stimulation with cat allergen extract (10 µg/ml) or *E. coli* (5×10^6) for 24 h.

†Data represent the median value and the range is shown in brackets.

Blocking of CD14 leads to abrogation of cat allergen-triggered cytokine production

To further investigate the role of CD14 in cat allergen-induced cytokine production, monocytes were preincubated with CD14-specific antibodies prior to stimulation with cat. As shown in Fig. 3A, blocking CD14 reduced the allergen-induced cytokine production by approximately 60% (TNF: 61% and IL-6: 60%) compared with the isotype control antibody. The TNF and IL-6 production induced by HDM, on the other hand, was not inhibited by blocking CD14 (Fig. 3B). To exclude that the cytokine production and the blocking effect was induced by contamination of the allergen with LPS, we added polymyxin B to the cultures. We found that the blocking effect of anti-CD14 on the production of TNF and IL-6 after cat stimulation remained stable after neutralization of LPS (Fig. 3A). In Fig. 3C, we show that polymyxin B (1 µg/ml) inhibited LPS-induced (100 ng/ml) TNF production by 85% and IL-6 by 88%, indicating that polymyxin B is able to neutralize LPS efficiently in our serum-free culture system. Moreover, the fact that the addition of LPS (1, 10 or 100 ng/ml) to the cat allergen extract did not increase the TNF production (Fig. 3D) further supports that the allergen rather than the LPS contamination was responsible for the cellular activation. Similar results were obtained for IL-6 production (data not shown). Finally, 10 ng of LPS, which is approximately 10-fold higher levels than that present in the cat allergen, did not induce any production of TNF by monocytes under serum-free conditions (Fig. 3E). We also investigated the role of TLR4 in cat allergen recognition. However, no inhibitory effect on proinflammatory cytokine production was observed after blocking

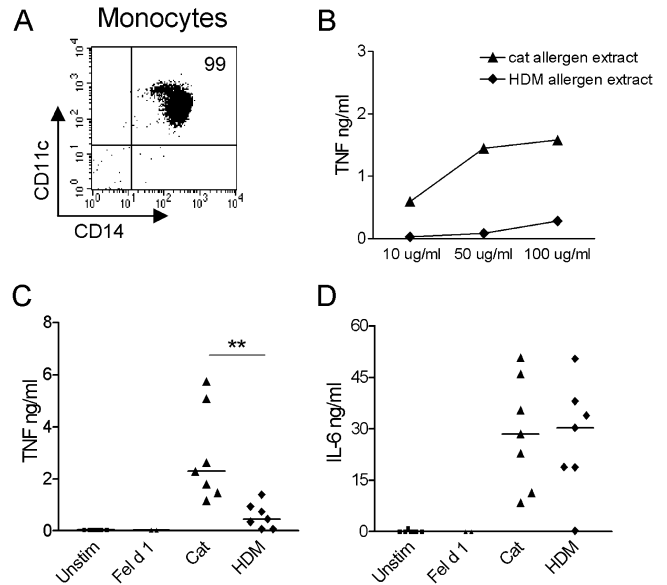


Figure 2. (A) Expression of CD14 and CD11c on freshly isolated human monocytes. The percentage of positive cells is indicated in the upper right quadrant of the panel. (B) Tumor necrosis factor (TNF) production by monocytes (5×10^5 /ml) in response to cat and house dust mite (HDM) allergen extracts (10, 50 or 100 µg/ml) for 24 h. (C and D) TNF and interleukin (IL)-6 production by monocytes (5×10^5 /ml) in response to Fel d 1 (10 µg/ml) or allergen extracts from cat or HDM (100 µg/ml) for 24 h. Each symbol represents one individual (atopic status unknown) and the horizontal bars represent the median value. ** $P < 0.01$ (Mann–Whitney U-test).

Table 2. Cytokine production by monocytes from non-allergic and cat-allergic individuals stimulated with cat allergen.*

Cat allergen	Non-allergic (n = 4)	Cat-allergic (n = 4)	P-value
Mo			
TNF (ng/ml)	0.81 (0.32–2.71)†	1.11 (0.45–1.91)	ns
IL-6 (ng/ml)	15.28 (8.48–28.04)	18.04 (11.46–19.16)	ns

*Stimulation of monocytes (5×10^5 per ml) with cat allergen extract (100 µg/ml) for 24 h.

†Data represent the median value and the range is shown in brackets.

this receptor (data not shown). Moreover, no additive inhibitory effect was seen when CD14 antibodies were combined with a-TLR4 (data not shown). In summary, these data suggest that CD14 might be involved in cat allergen extract-induced proinflammatory cytokine production from CD14-positive cells.

Discussion

The upper airway mucosa of humans contains a dense network of heterogeneous populations of APC with dendritic morphology (1). These innate immune cells have developed to recognize microbial antigens through PRRs in order to mount a proinflammatory innate response and induce a subsequent acquired immune

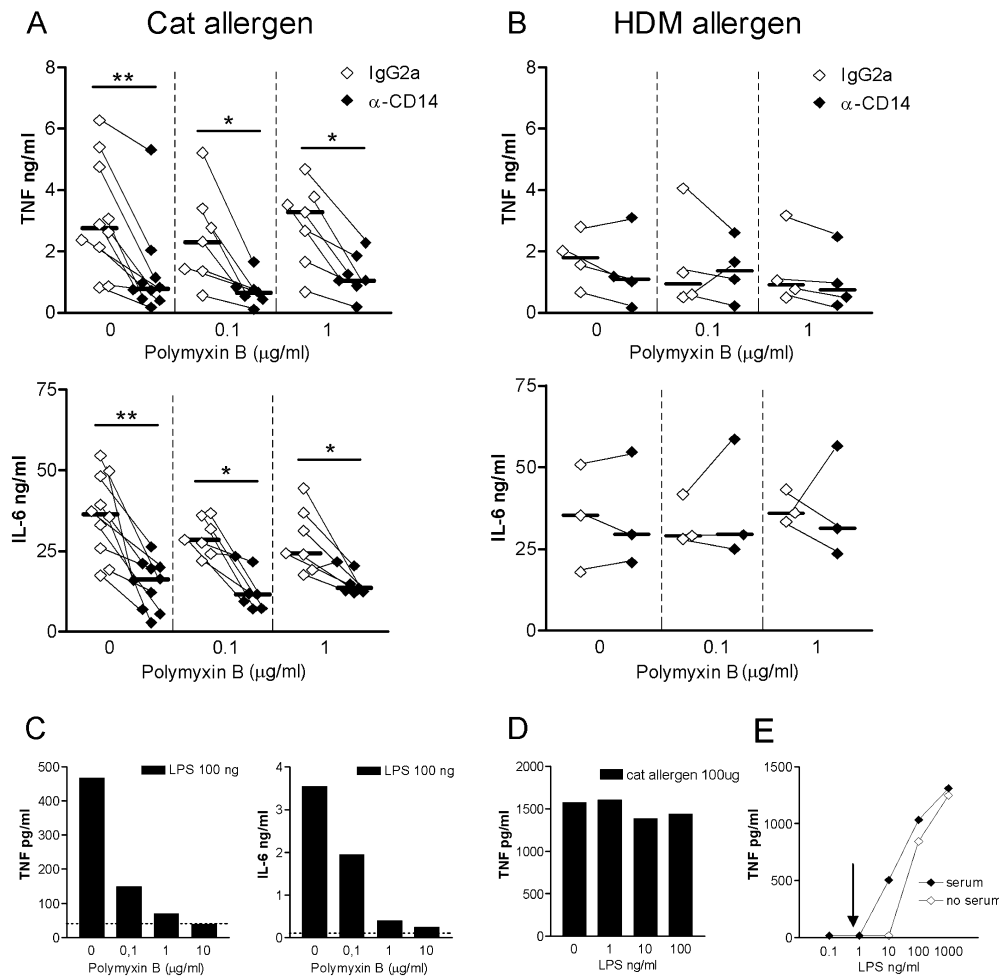


Figure 3. Tumor necrosis factor (TNF) and interleukin (IL)-6 production by human monocytes ($5 \times 10^5/\text{ml}$) after incubation with α -CD14 monoclonal antibodies (mAbs) or IgG2a isotype control antibody ($10 \mu\text{g/ml}$) for 45 min prior to stimulation with cat (A) or house dust mite (HDM) (B) allergen extract ($100 \mu\text{g/ml}$) in absence or presence of polymyxin B (0.1 or $1 \mu\text{g/ml}$). Each symbol represents one individual and the horizontal bars represent the median value. (C) Cytokine production by monocytes after stimulation with lipopolysaccharide (LPS; 100 ng/ml) in the presence of polymyxin B (0.1 , 1 or $10 \mu\text{g/ml}$; dotted lines represent the detection limit). (D) TNF production by monocytes ($5 \times 10^5/\text{ml}$) after stimulation with cat allergen extract in combination with various doses of LPS (1 , 10 or 100 ng/ml). (E) TNF production by human monocytes ($5 \times 10^5/\text{ml}$) in response to *Escherichia coli* LPS (0.1 , 1 , 10 , 100 and 1000 ng/ml) in presence (filled diamonds) or absence (open diamonds) of serum. Arrows indicate the LPS content in $100 \mu\text{g/ml}$ of cat allergen extract. * $P < 0.05$ and ** $P < 0.01$ (Wilcoxon matched pairs test).

response. The role of APC in the induction of allergic sensitization and disease is being explored. In mouse models, airway mucosal DC have been shown to have a central role in the induction of Th2-mediated immune responses to environmental allergens and also in the inflammatory allergic airway response to allergen challenge (8, 18). The maturation of DC is central for T cell activation and differentiation. Human immature DC that are exposed to inflammatory stimuli such as TNF and IL-1 β , no longer endocytose but instead up-regulate adhesion and costimulatory molecules and thereby develop into mature DC (19). In the present study, we found that human monocyte-derived CD14-positive macrophages produced high levels of proinflammatory

cytokines after cat allergen exposure, whereas CD14-negative monocyte-derived DC were virtually unresponsive. Therefore, proinflammatory cytokines from macrophages of the upper airway mucosa might provide maturation signals to DC which subsequently may migrate to the regional lymph nodes and induce a T cell response.

Recent studies have shown that ovalbumin does not activate DC *in vivo* in the absence of PAMPs, such as LPS, and is thereby unable to induce Th2-sensitization in mouse models (20). Thus, signals provided by the innate immune system seem to be required not only for Th1 but also for Th2 differentiation. The fact that individuals react with antigen-specific Th2 polarized immune

responses to inhaled allergens may result from concomitant exposure to low levels of PAMPs or that allergens contains structures that are recognized by receptors expressed on innate immune cells. We have previously found that exposure of DC to birch allergen extract together with high levels of LPS leads to production of TNF and IL-6 by DC themselves, while birch allergen alone did not induce proinflammatory cytokines. Using LPS + birch allergen-exposed DC as APC resulted in inhibition of Th2 differentiation of naive autologous T cells from newborn children (21). Thus, exposure of the mucosa with an allergen in combination with low levels of PAMPs might induce production of maturation signals by macrophages, which may indirectly activate DC and enable them to migrate to the lymph nodes and induce a Th2 response. In contrast, concomitant exposure to high levels of PAMPs might instead induce a direct maturation of DC, possibly leading to a Th1 immune response (20).

The fact that macrophages and immature DC differ in their ability to respond to cat allergen indicate that macrophages express receptors not found on DC, which have the capacity to recognize structures in the cat allergen extract. One such candidate receptor is CD14, which is expressed on monocyte-derived macrophages but not on monocyte-derived immature DC. *Escherichia coli* bacteria, on the other hand, which are likely to be recognized by a complex combination of receptors expressed on most innate immune cells induced high levels of TNF and IL-6 from both macrophages and DC (22, 23). CD14-positive monocytes represent a large pool of circulating precursors that can differentiate into macrophages or DC (2, 24). We found that cat and HDM allergen-induced comparable levels of IL-6 by monocytes. However, cat allergen induced significantly higher levels of TNF relative to HDM allergen, suggesting that structures recognized by monocytes differ among allergens. We also observed that recombinant LPS-free Fel d 1 did not induce proinflammatory cytokines by human monocytes, indicating that other components than the major allergenic protein in the cat allergen extract elicit TNF and IL-6 production. Chen et al. propose that HDM allergen-proteins stimulate mouse alveolar macrophages to release proinflammatory mediators via recognition of carbohydrate moieties (25). Thus, carbohydrate structures in the cat allergen extract might be responsible for the induction of TNF and IL-6 production by macrophages and monocytes.

In the present study, blocking CD14 but not TLR4 on monocytes reduced cat allergen-induced proinflammatory cytokine production. However, as CD14 does not have an intracellular signaling capacity it remains to be elucidated which other cooperating receptors that are involved. In contrast to cat allergen, the cytokine response to HDM allergen was not reduced when blocking CD14, which indicates that the inhibition is not unspecific. It could be argued that the effect of the cat allergen on the monocytes

was caused by contamination of LPS. However, several lines of evidence speak against this assumption. First, the cat allergen extract contained very low levels of LPS (9.5 pg LPS/ μ g extract). Secondly, when the LPS-neutralizing agent polymyxin B was added to the cultures the inhibitory effect remained. Moreover, adding LPS to the cat allergen extract did not increase the proinflammatory cytokine production. Finally, using 10-fold higher levels of LPS than present in the cat allergen cultures did not induce any production of TNF or IL-6 by monocytes when stimulation was performed in absence of serum. In accordance with our observations, it has been demonstrated that LPS-binding protein (LBP) and soluble CD14, which both are present at high concentrations in human serum, represent key elements in monocyte activation by LPS and that the presence of LBP is prerequisite for cell activation using low LPS concentrations (26, 27).

Our study indicates that APC such as monocytes, macrophages, and DC from healthy and allergic subjects do not differ in their ability to induce proinflammatory responses in response to cat allergen. It has previously been shown that monocyte-derived DC from HDM-sensitized patients pulsed with the immunodominant allergen protein Der p 1 produced higher levels of IL-6 than those of healthy donors (28). The fact that we did not observe any differences regarding cytokine responses from allergic and nonallergic subjects might be due to that HDM and cat allergen activate APC via distinct receptors, and that only some of these receptors are differentially expressed on cells from allergic and healthy individuals (29).

The present study shows that human monocyte-derived macrophages produce high levels of TNF and IL-6 in response to cat allergen, whereas monocyte-derived DC do not. The PRR CD14, which also recognizes endotoxin, might be one of the mediators in the inflammatory response as blocking this receptor on monocytes significantly reduced the cat allergen-induced cytokine production. Epidemiologic studies suggest that endotoxin exposure during infancy may induce tolerance to environmental allergens and thus reduce the risk of allergy (30, 31). Moreover, several studies seem to have demonstrated a protective effect of cat ownership with respect to development of allergy (32–35). The protective effect was only found when children were exposed to high levels of cat allergen, whereas exposure to medium doses of cat allergen appeared to promote allergic sensitization (33). However, future prospective studies with well-characterized populations are needed to confirm these findings as well as exploring other measures of prevention (36). Also, the mechanisms for the possible protective effect of exposure to both bacterial components and cat allergens are presently unknown and need to be investigated. These findings may also play an important role in allergen desensitization.

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References

- Jahnsen FL, Gran E, Haye R, Brandtzaeg P. Human nasal mucosa contains antigen-presenting cells of strikingly different functional phenotypes. *Am J Respir Cell Mol Biol* 2004;**30**:31–37.
- Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* 1994;**179**:1109–1118.
- Randolph GJ, Inaba K, Robbiani DF, Steinman RM, Muller WA. Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo. *Immunity* 1999;**11**:753–761.
- Medzhitov R. Toll-like receptors and innate immunity. *Nat Rev Immunol* 2001;**1**:135–145.
- Aderem A, Underhill DM. Mechanisms of phagocytosis in macrophages. *Annu Rev Immunol* 1999;**17**:593–623.
- Dziarski R, Tapping RI, Tobias PS. Binding of bacterial peptidoglycan to CD14. *J Biol Chem* 1998;**273**:8680–8690.
- Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2001;**2**:675–680.
- Lambrecht BN, De Veerman M, Coyle AJ, Gutierrez-Ramos JC, Thielemans K, Pauwels RA. Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. *J Clin Invest* 2000;**106**:551–559.
- Thepen T, Kraal G, Holt PG. The role of alveolar macrophages in regulation of lung inflammation. *Ann N Y Acad Sci* 1994;**725**:200–206.
- Kleine-Tebbe J, Kleine-Tebbe A, Jeep S, Schou C, Lowenstein H, Kunkel G. Role of the major allergen (Fel d 1) in patients sensitized to cat allergens. *Int Arch Allergy Immunol* 1993;**100**:256–262.
- Schulz O, Sewell HF, Shakib F. Proteolytic cleavage of CD25, the alpha subunit of the human T cell interleukin 2 receptor, by Der p 1, a major mite allergen with cysteine protease activity. *J Exp Med* 1998;**187**:271–275.
- Wan H, Winton HL, Soeller C, Tovey ER, Gruenert DC, Thompson PJ et al. Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *J Clin Invest* 1999;**104**:123–133.
- Custis NJ, Woodfolk JA, Vaughan JW, Platts-Mills TA. Quantitative measurement of airborne allergens from dust mites, dogs, and cats using an ion-charging device. *Clin Exp Allergy* 2003;**33**:986–991.
- Seppala U, Hagglund P, Wurtzen PA, Ipsen H, Thorsted P, Lenhard T et al. Molecular characterization of major cat allergen Fel d 1: expression of heterodimer by use of a baculovirus expression system. *J Biol Chem* 2005;**280**:3208–3216.
- Trivedi B, Valerio C, Slater JE. Endotoxin content of standardized allergen vaccines. *J Allergy Clin Immunol* 2003;**111**:777–783.
- Hansson M, Asea A, Ersson U, Hermodsson S, Hellstrand K. Induction of apoptosis in NK cells by monocyte-derived reactive oxygen metabolites. *J Immunol* 1996;**156**:42–47.
- Karlsson H, Hessel C, Rudin A. Innate immune responses of human neonatal cells to bacteria from the normal gastrointestinal flora. *Infect Immun* 2002;**70**:6688–6696.
- Huh JC, Strickland DH, Jahnsen FL, Turner DJ, Thomas JA, Napoli S et al. Bidirectional interactions between antigen-bearing respiratory tract dendritic cells (DCs) and T cells precede the late phase reaction in experimental asthma: DC activation occurs in the airway mucosa but not in the lung parenchyma. *J Exp Med* 2003;**198**:19–30.
- Sallusto F, Cella M, Danieli C, Lanzavecchia A. Dendritic cells use macrophinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J Exp Med* 1995;**182**:389–400.
- Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, Toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002;**196**:1645–1651.
- Andersson AC, Seppala U, Rudin A. Activation of human neonatal monocyte-derived dendritic cells by lipopolysaccharide down-regulates birch allergen-induced Th2 differentiation. *Eur J Immunol* 2004;**34**:3516–3524.
- Basu S, Fenton MJ. Toll-like receptors: function and roles in lung disease. *Am J Physiol Lung Cell Mol Physiol* 2004;**286**:L887–892.
- Karlsson H, Larsson P, Wold AE, Rudin A. Pattern of cytokine responses to gram-positive and gram-negative commensal bacteria is profoundly changed when monocytes differentiate into dendritic cells. *Infect Immun* 2004;**72**:2671–2678.
- Chomarat P, Banchereau J, Davoust J, Palucka AK. IL-6 switches the differentiation of monocytes from dendritic cells to macrophages. *Nat Immunol* 2000;**1**:510–514.
- Chen CL, Lee CT, Liu YC, Wang JY, Lei HY, Yu CK. House dust mite *Dermatophagoides farinae* augments proinflammatory mediator productions and accessory function of alveolar macrophages: implications for allergic sensitization and inflammation. *J Immunol* 2003;**170**:528–536.
- Gallay P, Barras C, Tobias PS, Calandra T, Glauser MP, Heumann D. Lipopolysaccharide (LPS)-binding protein in human serum determines the tumor necrosis factor response of monocytes to LPS. *J Infect Dis* 1994;**170**:1319–1322.
- Dunzendorfer S, Lee HK, Soldau K, Tobias PS. Toll-like receptor 4 functions intracellularly in human coronary artery endothelial cells: roles of LBP and sCD14 in mediating LPS responses. *FASEB J* 2004;**18**:1117–1119.
- Hammad H, Charbonnier AS, Duez C, Jacques A, Stewart GA, Tonnel AB et al. Th2 polarization by Der p 1-pulsed monocyte-derived dendritic cells is due to the allergic status of the donors. *Blood* 2001;**98**:1135–1141.
- von Bubnoff D, Scheler M, Hinz T, Matz H, Koch S, Bieber T. Comparative immunophenotyping of monocytes from symptomatic and asymptomatic atopic individuals. *Allergy* 2004;**59**:933–939.
- Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002;**347**:869–877.

31. Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001;**358**:1129–1133.
32. Hesselmar B, Aberg N, Aberg B, Eriksson B, Bjorksten B. Does early exposure to cat or dog protect against later allergy development? *Clin Exp Allergy* 1999;**29**:611–617.
33. Platts-Mills T, Vaughan J, Squillace S, Woodfolk J, Sporik R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. *Lancet* 2001;**357**:752–756.
34. Ronmark E, Perzanowski M, Platts-Mills T, Lundback B. Four-year incidence of allergic sensitization among schoolchildren in a community where allergy to cat and dog dominates sensitization: report from the Obstructive Lung Disease in Northern Sweden Study Group. *J Allergy Clin Immunol* 2003;**112**:747–754.
35. Erwin EA, Wickens K, Custis NJ, Siebers R, Woodfolk J, Barry D et al. Cat and dust mite sensitivity and tolerance in relation to wheezing among children raised with high exposure to both allergens. *J Allergy Clin Immunol* 2005;**115**:74–79.
36. Gore C, Custovic A. Can we prevent allergy? *Allergy* 2004;**59**:151–161.