

Involvement of the mannose receptor in the uptake of Der p 1, a major mite allergen, by human dendritic cells

Gaëtan Deslée, MD,^{a,*} Anne-Sophie Charbonnier, PhD,^{a,*} Hamida Hammad, PhD,^a Gerhild Angyalosi, PhD,^b Isabelle Tillie-Leblond, MD, PhD,^{a,c} Alberto Mantovani, PhD,^d André-Bernard Tonnel, MD, PhD,^{a,c} and Joël Pestel, PhD^a
Lille, France, and Milan, Italy

Background: Immature dendritic cells (DCs) take up antigens in peripheral tissues and, after antigen processing, mature to efficiently stimulate T cells in secondary lymph nodes. In allergic airway diseases DCs have been shown to be involved in the induction and maintenance of a T_H2-type profile.

Objective: The present study was undertaken to determine pathways of Der p 1 (a house dust mite allergen) uptake by human DCs and to compare Der p 1 uptake between DCs from patients with house dust mite allergy and DCs from healthy donors.

Methods: Monocyte-derived DCs (MD-DCs) were obtained from patients with house dust mite allergy (n = 13) and healthy donors (n = 11). Der p 1 was labeled with rhodamine. Der p 1 uptake by MD-DCs was analyzed by means of flow cytometry and confocal microscopy.

Results: Rhodamine-labeled Der p 1 was demonstrated to be taken up by MD-DCs in a dose-, time-, and temperature-dependent manner. The involvement of the mannose receptor (MR) in the Der p 1 uptake was demonstrated by using (1) inhibitors of the MR-mediated endocytosis (mannan and blocking anti-MR mAb), which inhibited the Der p 1 uptake from 40% to 50%, and (2) confocal microscopy showing the colocalization of rhodamine-labeled Der p 1 with FITC-dextran. Interestingly, compared with DCs from healthy donors, DCs from allergic patients expressed more MR and were more efficient in Der p 1 uptake.

Conclusion: These results suggest that the MR could play a key role in the Der p 1 allergen uptake by DCs and in the pathogenesis of allergic diseases in dust mite-sensitive patients. (J Allergy Clin Immunol 2002;110:763-70.)

Key words: Dendritic cells, mannose receptor, uptake, house dust mite, allergy

Abbreviations used

CRD:	Carbohydrate recognition domain
DC:	Dendritic cell
DC-SIGN:	DC-specific intercellular adhesion molecule 3-grabbing nonintegrin
GMFI:	Geometric mean fluorescence intensity
LY:	Lucifer Yellow
MD-DC:	Monocyte-derived dendritic cell
MR:	Mannose receptor
PE:	Phycocerythrin
RPMI-FP:	Mixture of RPMI 1640–10% FCS and PBS

Dendritic cells (DCs) are the most potent antigen-presenting cells of the immune system. They play a central role in the initiation of the primary immune response and in the enhancement of the secondary immune response. Immature DCs localized in peripheral tissues are able to take up antigens and subsequently to migrate through afferent lymphatics to the T cell-rich zone of secondary lymph nodes. During migration, immature DCs undergo an additional maturation step and become able to present processed antigens to naive T cells.¹ Some observations suggest that DCs play a critical role in allergic respiratory pathology characterized by a T_H2 profile²: (1) their number increases in the lungs of atopic patients^{3,4}; (2) administration of ovalbumin-pulsed DCs into the trachea of naive mice induces T_H2-dependent sensitization, leading to eosinophilic airway inflammation⁵; and (3) a selective DC depletion in ovalbumin-sensitized mice leads to an abolition of the T_H2 response, a decrease in IgE production, and a lack of eosinophil recruitment, 3 specific features of the pulmonary allergic response.⁶

The house dust mite is a common allergen involved in allergic airway diseases, such as allergic rhinitis and asthma. Among many species of mites in house dust, *Dermatophagoides pteronyssinus* is reported to be one of the most prevalent in the Western world.⁷ Der p 1, a 25-kd glycoprotein found in mite feces, is an immunodominant allergen of *D pteronyssinus*. Der p 1 is involved in the polarization of the immune response toward a T_H2 response.⁸⁻¹¹ Recently, we showed that the DC reactivity

From ^aUnité INSERM U-416, Institut Pasteur de Lille, Lille; ^bInstitut de Biologie de Lille, Lille; ^cDépartement de Pneumologie, Hôpital A. Calmette, CHRU de Lille, Lille; and ^dthe Department of Immunology and Cell Biology, "Mario Negri" Institute, Milan.

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*G.D. and A.-S.C. have equally contributed to this work

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Reprint requests: Joël Pestel, PhD, INSERM U416-Institut Pasteur de Lille, 1 rue du Professeur Calmette, BP 245, 59019 Lille Cedex, France.

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toward Der p 1 depended on the allergic status of the donor. Indeed, monocyte-derived DCs (MD-DCs) from allergic patients pulsed with Der p 1 secrete a restricted panel of cytokines (high levels of IL-10 and IL-6 and an absence of IL-12) and express high levels of the costimulatory molecule CD86, thus amplifying the type 2 specific T-cell response. In contrast, MD-DCs from healthy donors secrete a different panel of cytokines (low levels of IL-10 and IL-6 and high levels of IL-12) and express low levels of CD86, thus favoring a T_H1 profile.¹²

Immature DCs use several pathways for antigen uptake: (1) macropinocytosis, a cytoskeleton-dependent endocytosis mediated by membrane ruffling¹³; (2) receptor-mediated endocytosis through C-type lectin receptors, such as the mannose receptor (MR),¹³ DC receptor (DEC-205),¹⁴ langerin,¹⁵ and DC-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN),¹⁶ or through Fc receptors, such as FcεRI¹⁷ and FcγRI¹⁸; and (3) phagocytosis for particulate antigens.¹⁹⁻²¹ The used pathway depends on the type of antigen and on the type of DC, and for allergens, the uptake pathways used by DCs remain to be evaluated. Only 2 recent studies have focused on this subject, using DCs from healthy donors: (1) allergenic components of the yeast *Malassezia furfur* are taken up by MD-DCs through the MR,²² and (2) the recombinant pollen allergens Bet v 1 and Phl p 1 are taken up by Langerhans-like DCs by means of macropinocytosis.²³

The aim of this report was to determine pathways of Der p 1 uptake by MD-DCs and to compare Der p 1 uptake between MD-DCs from allergic patients and MD-DCs from healthy donors. First, results obtained suggest that the MR is involved in the uptake of Der p 1. Second, we showed that MD-DCs from allergic patients are more efficient in Der p 1 uptake through the MR than MD-DCs from healthy donors.

METHODS

Patients

Blood was collected from 13 healthy donors (7 female and 6 male subjects; mean age, 33 years; age range, 24-54 years) and from 11 patients with house dust mite allergy (6 female and 5 male subjects; mean age, 29 years; age range, 21-50 years). All allergic patients presented the usual features of house dust mite allergy: symptomatic allergic rhinitis, asthma, or both; total IgE concentrations of greater than 150 IU/mL (range, 150-1200 IU/mL; CAP system, Pharmacia, Uppsala, Sweden); specific IgE antibodies for *D pteronyssinus* of greater than 3.5 IU/mL (CAP system); and positive skin test responses to *D pteronyssinus* (Stallergènes SA, Antony, France). A positive skin test response to *D pteronyssinus* was defined by a wheal of 3 mm or greater in size than that produced by the saline control. Histamine was used as a positive control. Allergic patients were not treated with antiallergic medication, such as antihistamines or corticosteroids, and had not experienced any desensitization. Healthy donors had no history of allergic disease, asthma, or rhinitis.

This study was performed according to the recommendations of the local institutional ethics committee. Informed consent was obtained from each subject before the study.

Generation of DCs

MD-DCs were generated in vitro according to a previously described protocol.^{24,25} Briefly, after platelet-rich plasma depletion,

blood cells were diluted in RPMI 1640 (Life Technologies, Paisley, Scotland) and layered over Ficoll gradient (Pharmacia). After centrifugation (400g for 30 minutes), PBMCs were harvested and washed. Purified PBMCs were incubated with mouse anti-human CD14 mAb conjugated to magnetic beads (Miltenyi Biotec, Paris, France) at 4°C for 30 minutes. Cells were then washed and applied onto a column placed in the magnetic field of a MACSR separator (Miltenyi Biotec). Negative cells were eliminated, and the column was removed from the separator and eluted. CD14⁺ cells (monocytes) were collected and washed twice with RPMI 1640. CD14⁺ cells were cultured (3×10^6 cells per well) into 6-well flat-bottomed culture plates in RPMI 1640–10% FCS (Life Technologies) supplemented with 1% antibiotics, 2 mmol/L L-glutamine, 20 ng/mL GM-CSF (Peprotech, London, United Kingdom), and 200 IU/mL IL-4 (Tebu, Le Perray en Yvelines, France) at 37°C at 5% CO₂ in air. MD-DCs were collected after 7 days and used for phenotype analysis and functional assays.

Phenotype analysis

Cell staining was performed with either FITC-, phycoerythrin (PE)-, or Cy-chrome-conjugated mAbs. The following mAbs were used: anti-CD80 FITC, anti-CD1a FITC, anti-CD86 PE, anti-MR PE, anti-HLA-DR Cy-chrome, and irrelevant mAbs of the same isotypes (all from Becton Dickinson, Mountain View, Calif). Events (5×10^4) were acquired with a FACScalibur unit (Becton Dickinson).

Rhodamine labeling of Der p 1

Der p 1 (relative molecular mass = 25,000 d) was immunopurified from *D pteronyssinus* extracts as previously described²⁶ and kindly provided by G. A. Stewart (University of Western Australia, Perth, Australia). Der p 1 was labeled with rhodamine by using the Fluoreporter conjugated Tetramethylrhodamine Protein Labeling Kit (Molecular Probes, Eugene, Ore), according to the manufacturer's instructions. Two hundred micrograms of Der p 1 was labeled at each labeling reaction. Briefly, the labeling solution containing rhodamine (relative molecular mass = 500 d) was added to the Der p 1 solution (1 mg/mL) and incubated for 1 hour at room temperature in the dark. The labeling reaction was stopped by adding a hydroxylamine solution (1.5 mol/L), and the solution was further extensively dialyzed against PBS with a dialyzer system (FastDialyser Interbiotech, Molecular Probes). The rhodamine-labeled Der p 1 solution was then collected and stored at 4°C, protected from light, until use.

Antigen uptake and inhibition assays

FITC-dextran (relative molecular mass = 40,000 d) and Lucifer Yellow (LY) were purchased from Molecular Probes. Collected MD-DCs were washed twice with RPMI 1640 and were resuspended (3×10^5 cells per sample) in 95 μL of a medium containing 45 μL of RPMI 1640–10% FCS and 50 μL of PBS (RPMI-FP). Before use, the labeled molecules (rhodamine-labeled Der p 1, FITC-dextran, or LY) were spun in a microfuge to eliminate aggregates. MD-DCs were prewarmed for 20 minutes at 37°C and subsequently incubated with rhodamine-labeled Der p 1 (5, 10, 20, 30, 40, or 50 μg/mL), FITC-dextran (50 μg/mL), LY (200 μg/mL), or rhodamine alone dialyzed against PBS (rhodamine). MD-DCs were collected at various time points (4, 10, 30, 60, and 120 minutes) and washed 3 times with ice-cold PBS. The quantitative uptake of rhodamine-labeled Der p 1, FITC-dextran, LY, or rhodamine was then immediately determined with a FACScalibur unit (Becton Dickinson) and expressed as geometric mean fluorescence intensity (GMFI). At least 5000 cells per sample were analyzed. Similar experiments were also performed at 4°C.

Inhibition of labeled molecule uptake was carried out either with the previously used concentration of mannan (3 mg/mL)¹³ from

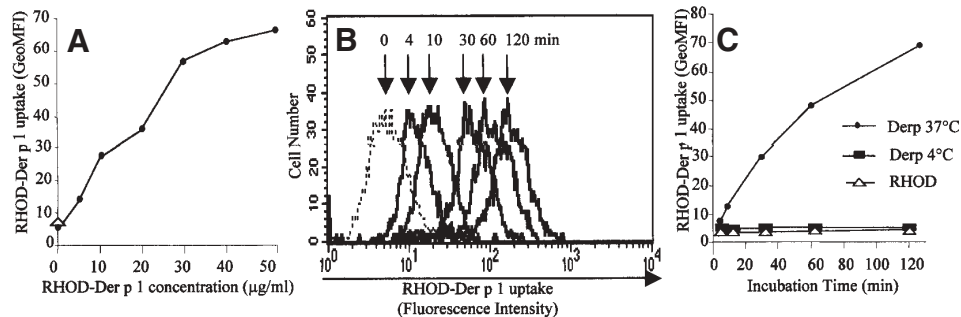


FIG 1. Der p 1 uptake by MD-DCs. **A**, MD-DCs were incubated with various concentrations of rhodamine-labeled Der p 1 (filled circles) or with rhodamine alone (open triangles) for 120 minutes at 37°C. Der p 1 uptake was measured by means of flow cytometry and expressed as GMFI. Data represent one experiment of a set of 3 (healthy donors). **B**, MD-DCs were incubated with 50 µg/mL rhodamine-labeled Der p 1. Fluorescence was measured at various time points (0, 4, 10, 30, 60, and 120 minutes). This graph is representative from a set of 9 experiments carried out with cells from patients allergic to house dust mite. **C**, MD-DCs were incubated with 50 µg/mL rhodamine-labeled Der p 1 at 37°C (Derp 37°C) or at 4°C (Derp 4°C) or with rhodamine alone at 37°C (RHOD), and fluorescence was measured at various time points (0, 4, 10, 30, 60, and 120 minutes). This graph is representative of 6 experiments carried out with cells from healthy donors.

Saccharomyces cerevisiae (Sigma Immunochemicals, St Louis, Mo), with 10 µg/mL blocking anti-MR mAb (PAM-1; A. Mantovani, Mario Negri Institute, Milan, Italy),²⁴ or with 5 µg/mL cytochalasin D (Sigma). Cells were incubated with inhibitors for 20 minutes at 37°C before addition of labeled molecules.

Confocal microscopy

MD-DCs were incubated with rhodamine-labeled Der p 1 (50 µg/mL) or FITC-dextran (50 µg/mL) for 15 minutes at 37°C in RPMI-FP, as described above. Some coincubation experiments were performed with both tracers, rhodamine-labeled Der p 1 and FITC-dextran. After a 15-minute incubation period, cells were washed 3 times in ice-cold PBS and collected immediately or after a further 60-minute chase at 37°C in tracer-free RPMI-FP. The collected cells were fixed in 2% paraformaldehyde before adhesion to glass slides. A total of 5×10^4 cells was adhered to individual fields of glass slides coated with 10 µg/mL poly-L-lysine (Sigma). After embedding in Fluoprep medium (BioMerieux, Marcy l'Etoile, France), the slides were examined with a confocal laser scanning microscope system (Leica NS-CT; Leica Lasertechnik, Heidelberg, Germany) fitted with lasers emitting light at 488 and 555 nm for the excitation of FITC and rhodamine, respectively. Appropriate controls were always included, and bleed-through corrections were done according to the manufacturer's recommendations and tested in single-labeling experiments. Images corresponding to fluorescence signals originating from 0.5-µm sections of individual cells were acquired. The images collected in the 2 channels were merged to visualize the relative positional distribution of the 2 fluorochromes.

Statistical analysis

Data were expressed as the mean of GMFI $\pm$ SEM. Comparisons were performed with the Student *t* test, and *P* values of less than .05 were considered statistically significant.

RESULTS

MD-DCs take up Der p 1 in a dose-, time-, and temperature-dependent manner

MD-DCs from allergic patients or healthy donors were first incubated with various concentrations of Der p 1 conjugated with rhodamine for 120 minutes at 37°C, and Der

p 1 uptake by MD-DCs was studied by means of flow cytometry. As shown for healthy donors (Fig 1, A), fluorescence intensity increased from 5 to 50 µg/mL rhodamine-labeled Der p 1, demonstrating that Der p 1 uptake was dose dependent. When MD-DCs were incubated with rhodamine alone, fluorescence was close to the specific background autofluorescence of control cells (cells that had not been exposed to rhodamine-labeled Der p 1), suggesting that the signal was specific to Der p 1 (Fig 1, A). Similar dose-dependent curves were obtained for allergic patients but with higher fluorescence intensity (not shown). The concentration of 50 µg/mL rhodamine-labeled Der p 1 was chosen for further experiments to detect sufficient amounts of fluorescence in MD-DCs for short incubation periods. MD-DCs were then incubated with 50 µg/mL rhodamine-labeled Der p 1 at 37°C, and the fluorescence intensity was measured at various time points. Fluorescence intensity increased progressively from 4 to 120 minutes, thus demonstrating that Der p 1 uptake was time dependent, as shown for MD-DCs from allergic patients (Fig 1, B) and for healthy donors (*n* = 9, data not shown). Finally, Der p 1 uptake was demonstrated to be temperature dependent for healthy donors (Fig 1, C) and for allergic patients (*n* = 6, data not shown). Indeed, when MD-DCs were incubated with rhodamine-labeled Der p 1 at 4°C, fluorescence intensity did not increase from 4 to 120 minutes and was close to the background autofluorescence of control cells.

MR is involved in the uptake of Der p 1 by MD-DCs

The 2 main pathways of antigen uptake by MD-DCs have been investigated: receptor-mediated endocytosis and macropinocytosis. Two MR inhibitors were used to investigate the involvement of the MR in Der p 1 uptake: mannan and a blocking anti-MR mAb (PAM-1). FITC-dextran, which is mainly taken up through the MR, was used as a positive control. LY, which is taken up through

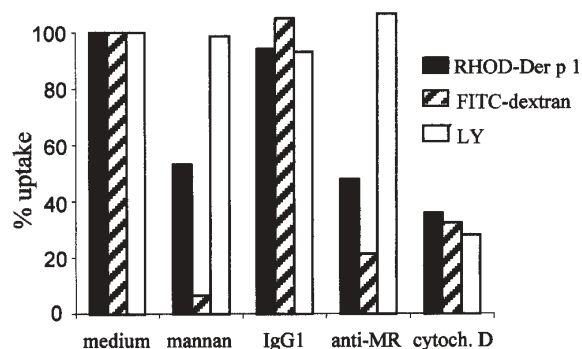


FIG 2. The MR is involved in Der p 1 uptake. MD-DCs were incubated at 37°C in normal medium (RPMI-FP) or in medium containing mannan, anti-MR antibody (PAM-1), irrelevant IgG1, or cytochalasin D. After 20 minutes, rhodamine-labeled Der p 1 (filled bars), FITC-dextran (hatched bars), or LY (white bars) was added. Uptake was measured after 120 minutes and expressed as a percentage of the uptake in medium alone. This graph is representative of a set of 5 experiments carried out with MD-DCs from patients allergic to house dust mite.

macropinocytosis, was used as a negative control. Uptake of labeled molecules in the presence of inhibitors was measured by means of flow cytometry after 120 minutes and compared with uptake in an inhibitor-free medium. Der p 1 uptake was inhibited at least at 40% by mannan or at least 50% by PAM-1, as shown for allergic patients (Fig 2). As expected, the FITC-dextran uptake was strongly inhibited by mannan or PAM-1, although the LY uptake was not inhibited. The percentage of inhibition of the Der p 1 uptake by MR inhibitors was compared between MD-DCs from allergic patients ($n = 5$) and MD-DCs from healthy individuals ($n = 5$). No difference was found (data not shown), suggesting that the MR might be equally involved in the uptake of Der p 1 by MD-DCs from allergic patients and MD-DCs from healthy donors.

Cytochalasin D, an inhibitor of membrane ruffling, was used to investigate the involvement of macropinocytosis. Der p 1 uptake was strongly inhibited by cytochalasin D (Fig 2). Cytochalasin D strongly inhibited LY uptake, as well as FITC-dextran uptake, showing that membrane ruffling is involved both in macropinocytosis and MR-mediated endocytosis.

A confocal analysis was performed to confirm the involvement of the MR in Der p 1 uptake by MD-DCs. Rhodamine-labeled Der p 1 and FITC-dextran internalizations were analyzed after a 15-minute incubation period and after a further 60-minute chase in a marker-free medium. Single-labeling (rhodamine-labeled Der p 1 or FITC-dextran) and coincubation experiments (rhodamine-labeled Der p 1 and FITC-dextran) were performed at 37°C. Rhodamine-labeled Der p 1 and FITC-dextran were internalized by MD-DCs. As shown in the case of allergic patients (Fig 3, A-D) or healthy donors (data not shown), they were localized in peripheral vesicles after a 15-minute incubation period (Fig 3, A and D) and mainly in central vesicles after a 60-minute chase in marker-free medium (Fig 3, B and E). Furthermore, rho-

damine-labeled Der p 1 and FITC-dextran were partially colocalized in both conditions (Fig 3, C and F), suggesting that Der p 1 and FITC-dextran use the same route of internalization in MD-DCs.

MD-DCs from allergic patients are more efficient in Der p 1 uptake and express more MR than MD-DCs from healthy individuals

The Der p 1 uptake was compared between MD-DCs from allergic patients ($n = 9$) and MD-DCs from healthy donors ($n = 9$). MD-DCs were incubated with 50 μ g/mL rhodamine-labeled Der p 1 for various times at 37°C and analyzed for Der p 1 uptake by means of flow cytometry. MD-DCs from allergic patients were more efficient in Der p 1 uptake than MD-DCs from healthy donors (GMFI index for Der p 1 uptake: 30 minutes, 40.49 ± 5.32 vs 28.39 ± 3.75 [$P < .01$]; 60 minutes, 63.94 ± 9.48 vs 42.03 ± 7.46 [$P < .001$]; and 120 minutes, 106.13 ± 15.01 vs 60.44 ± 9.53 [$P < .001$]; Fig 4, A). Similar results were obtained for FITC-dextran uptake by MD-DCs from allergic patients and MD-DCs from healthy donors (GMFI index for FITC-dextran uptake: 30 minutes, 18.17 ± 2.47 vs 11.21 ± 2.8 [$P < .01$]; 60 minutes, 35.1 ± 5.82 vs 18.48 ± 3.94 [$P < .001$]; and 120 minutes, 50.84 ± 7.15 vs 23.35 ± 5.42 [$P < .001$]; Fig 4, B). A surface phenotype analysis was performed on MD-DCs from allergic patients and healthy donors, and the presence of MR was evaluated to explain this difference. No difference was found in the expression of CD1a, HLA-DR, and the costimulatory molecules CD80 and CD86 on MD-DCs from both groups. The low expression of HLA-DR, CD80, and CD86 shows that MD-DCs are at an immature state in both groups (Fig 4, C and D). However MD-DCs from allergic patients express more MR than MD-DCs from healthy donors (GMFI index for MR expression: 31.15 ± 7.6 vs 11.43 ± 3.24 [$P < .001$]; Fig 4, C and D). These results suggest that the uptake through the MR is more efficient in MD-DCs from allergic patients than in MD-DCs from healthy donors.

DISCUSSION

Our study indicates that the MR is involved in Der p 1 uptake by DCs. Indeed, inhibitors of the MR pathway (mannan and PAM-1) reduce the Der p 1 uptake by MD-DCs from 40% to 50%. Furthermore, Der p 1 colocalizes in MD-DCs with FITC-dextran, which is known to be taken up by the MR. The MR is a 175-kD type I transmembrane glycoprotein and a member of the C-type lectin receptor family²⁷ and belongs to the pattern-recognition receptor family.²⁸ The MR is expressed on the surface of various cells, such as macrophages and DCs.¹³ It contains 8 carbohydrate recognition domains (CRDs) with broad specificity for various sugars.^{27,28} The N-terminal cysteine-rich domain preferentially mediates Ca^{2+} independent binding to sulphated N-linked sugars; CRDs 4 to 8 are sufficient to bind mannose-terminated glycoconjugates in a Ca^{2+} -dependent manner but can also bind to fucose, N-

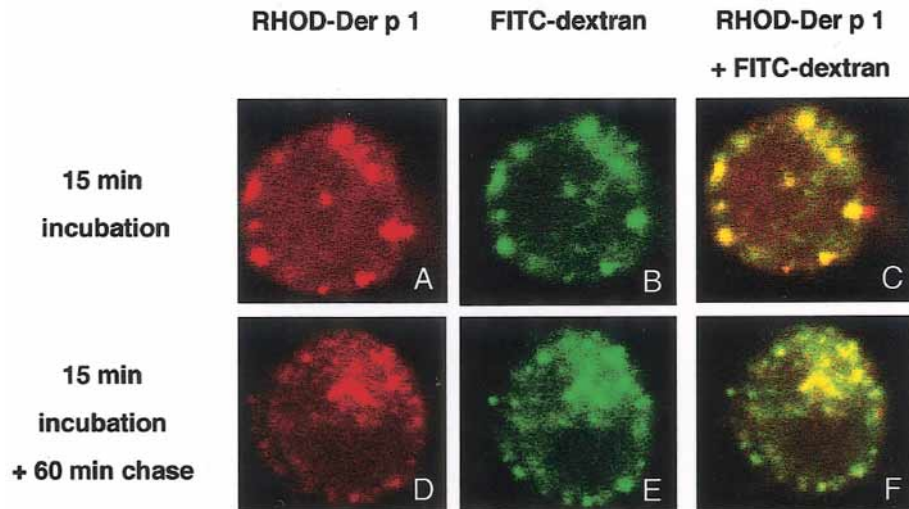


FIG 3. Intracellular localization of Der p 1 in MD-DCs. **A-C**, MD-DCs were incubated for a 15-minute period at 37°C with 50 µg/mL rhodamine-labeled Der p 1 (RHOD-Der p 1) (red; Fig 3, A), 50 µg/mL FITC-dextran (green; Fig 3, B), or both tracers (Fig 3, C). **D-E**, A further 60-minute chase at 37°C in tracer-free medium (RPMI-FP) was performed after a 15-minute incubation period with 50 µg/mL rhodamine-labeled Der p 1 (red; Fig 3, D), 50 µg/mL FITC-dextran (green; Fig 3, E), or both tracers (Fig 3, F). Cells were analyzed by means of confocal laser scanning microscopy. Colocalization is indicated in yellow. The images shown are representative of 3 experiments performed with MD-DCs from allergic patients. Similar observations were done with MD-DCs from 2 healthy donors.

acetyl-glucosamine, or galactose residues. The MR plays a key role in host defense against pathogens by mediating internalization and by delivering foreign antigens to MHC class II compartments for efficient subsequent antigen presentation.^{29,30} Because terminal sugars, such as mannose, fucose, and N-acetylglucosamine, are rarely found in mammalian cell-surface or serum proteins, the MR could be responsible for recognition of modified self and nonself antigens.²⁸ In allergic diseases the MR is involved in the uptake by MD-DCs of the allergenic components of *M furfur*, which is involved in atopic dermatitis.²² In our study, used Der p 1 is immunopurified from native house dust mite extracts and has an N-glycosylation site,³¹ the sugar moiety of which can be recognized by carbohydrate recognition receptors, such as the MR. Indeed, either by using concanavalin A or peanut agglutinin, native immunopurified Der p 1 was demonstrated to be to some extent glycosylated,³² as previously anticipated.³³ The presence of oligomers having a core disaccharide galactose $\beta(1-3)$ N-acetyl galactosamine was also suggested.³² The study of the interaction between native Der p 1 with human lung surfactant proteins A and D, both of which are collagenous C-type lectins, indicated the presence of carbohydrates on Der p 1 allergen.³⁴ The binding was demonstrated to be calcium dependent, to be inhibited by mannose for surfactant protein A and maltose for surfactant protein D, and to be abolished after deglycosylation of Der p 1. Moreover, the partial involvement of the MR in Der p 1 uptake by MD-DCs compared with its complete involvement in FITC-dextran uptake could then be explained by the difference in the degree of glycosylation on Der p 1 compared with that seen in dextran.³⁵ In fact, in our experimental conditions

Der p 1 and mannan were added at the same time, and because the involvement of CRDs 4, 5, and 7 are required for the high-affinity binding of complex sugars with terminal mannose residues as mannan, Der p 1, which can interact with less CRDs than mannan, might reduce the accessibility to the MR, the capacity of which to bind to highly mannosylated sugars was reported to be dependent on various parameters, such as steric conformation.²⁷

The partial inhibition of Der p 1 uptake by mannan and a blocking anti-MR mAb indicate that other pathways than the MR are probably used for Der p 1 uptake. First, macropinocytosis is probably involved, as suggested by the inhibition of Der p 1 uptake by cytochalasin D, which affects the membrane ruffling. However, as shown in our study, cytochalasin D is not a specific inhibitor of macropinocytosis, and our study does not provide sufficient evidence to prove its involvement of this uptake pathway.

Second, other receptors, such as the newly described C-lectin receptors (DEC-205 and DC-SIGN), have not been studied in this report but could be involved as additional receptors with the MR in Der p 1 uptake. However, DEC-205 is mainly expressed on mature DCs rather than on immature DCs, and the uptake of FITC-dextran by this receptor does not appear to be effectively inhibited by mannose-rich sugars, such as mannan,³⁵ which is in contrast with our present observations. The potential involvement of DC-SIGN is limited because although it is expressed on immature DCs, the margin of its specificity is narrower than those of other C-type lectins, and higher binding appears restricted to multiple high-mannose oligosaccharides instead of glycoproteins containing few sugars.³⁶

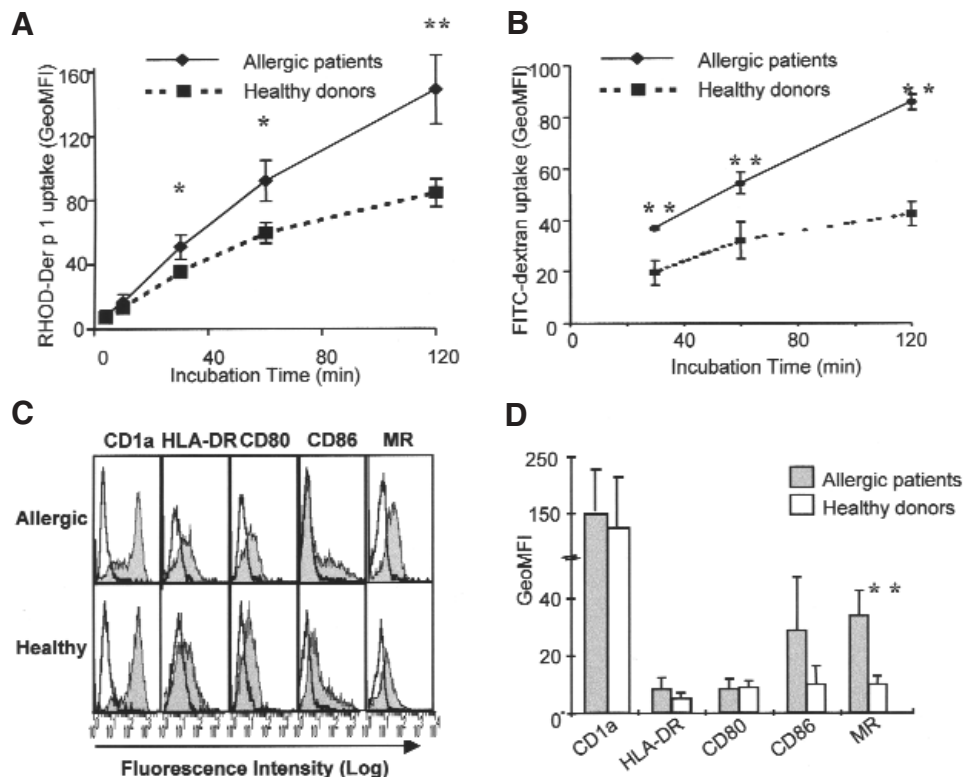


FIG 4. Comparison of the uptake of Der p 1 by MD-DCs from allergic patients and MD-DCs from healthy donors: involvement of the MR. **A**, MD-DCs were incubated with 50 μ g/mL rhodamine-labeled Der p 1 (RHOD Der p 1) at 37°C. The level of fluorescence was measured by means of flow cytometry at various time points and expressed as GMFI. Mean $\pm$ SEM values were obtained from 9 allergic patients (filled diamonds) and 9 healthy donors (filled squares). * $P < .01$; ** $P < .001$. **B**, MD-DCs were incubated with 50 μ g/mL FITC-dextran at 37°C. The level of fluorescence was measured by means of flow cytometry at various time points and expressed as GMFI. Mean $\pm$ SEM values were obtained from 9 allergic patients (filled diamonds) and 9 healthy donors (filled squares). * $P < .01$; ** $P < .001$. **C**, Representative phenotype of MD-DCs from an allergic patient or a healthy donor. Monocytes were cultured in the presence of GM-CSF and IL-4 for 7 days. The cells were then collected and analyzed by means of flow cytometry for CD1a, HLA-DR, CD80, CD86, and MR expression (filled histograms). Open histograms represent the reactivity of fluorochrome-matched isotype. **D**, Comparison of the CD1a, HLA-DR, CD80, CD86, and MR expression between MD-DCs from allergic patients and MD-DCs from healthy donors. MD-DCs were analyzed by means of flow cytometry and fluorescence intensity expressed as GMFI. Mean $\pm$ SEM values were obtained from 9 allergic patients and 9 healthy donors. ** $P < .001$.

Third, concerning the potential involvement of FcRs, some experimental arguments rule out the possibility of an FcR-mediated Der p 1 uptake in our study. Concerning Fc ϵ RI, no cell surface-bound IgE was detected on immature MD-DCs in a recent report,²² and no significant expression of Fc ϵ RI was detected on the surface of MD-DCs in our study (data not shown). Concerning Fc γ RI, the involvement of Fc γ RI in antigen uptake has been mainly shown for blood DCs but not for nonstimulated MD-DCs,¹⁸ and no significant expression of Fc γ RI was detected on MD-DCs in our study (data not shown). Our study indicates that MD-DCs from allergic patients are more efficient in Der p 1 uptake, express more MR, and are more efficient in antigen uptake through the MR than MD-DCs from healthy individuals. However, no difference was found in the expression of CD1a, HLA-DR, and the costimulatory molecules CD80 and CD86 on MD-DCs from both groups, suggesting that differences

in antigen uptake and MR expression were not due to a different state of maturation of MD-DCs. The higher Der p 1 uptake by MD-DCs from allergic patients could be involved in the difference of DC reactivity toward Der p 1 that we have described previously.¹²

Moreover, the MR-mediated endocytosis of antigen has been demonstrated to strongly enhance HLA class II-restricted antigen presentation by DCs.^{29,30} Because of their higher potency to take up Der p 1 through the MR, DCs from allergic patients could then have an enhanced potency to stimulate T lymphocytes and to promote a T_H2 response, as was reported with carbohydrates.^{37,38} Indeed, whereas the expression of MRs could be reduced in the presence of IFN- γ (a pro-T_H1 cytokine), its surface expression is increased in the presence of IL-4. However, further studies are needed to determine the effect of inhibition of the MR on T-cell response in the context of house dust mite allergy.

In vivo inhaled Der p 1 could interact with DCs. Indeed, the cysteine protease activity of Der p 1 causes disruption of epithelial architecture,³⁹ and inhaled Der p 1 could then pass across the bronchial epithelium to gain access to immune cells, such as DCs. Interestingly, lung DCs have been shown to express efficient MR.⁴⁰ Taken together, these results suggest that the MR might be involved in vivo in the uptake of some inhaled allergens by human DCs.

In conclusion, our study provides evidence that the MR is involved in the Der p 1 uptake by MD-DCs and that the MR pathway is more efficient in MD-DCs from allergic patients than in MD-DCs from healthy donors. These results suggest that the interaction between Der p 1 and the MR could play a key role in the pathogenesis of allergic diseases in dust mite-sensitive patients.

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