hDectin-1 is involved in uptake and cross-presentation of cellular antigens

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Human Dectin-1 (hDectin-1) is a member of the C-type lectin–like receptor family that was shown to be the major receptor for fungal beta-glucans and to play an important role in the cellular responses mediated by these carbohydrates. In this study, we demonstrate that hDectin-1 is involved in the uptake and cross-presentation of cellular antigens. Furthermore, activation of monocyte-derived dendritic cells (MDCs) with toll-like receptor 3 (TLR3) ligand but not with TLR2 ligand or TLR7 ligand resulted in down-regulation of hDectin-1 expression and reduced phagocytosis of apoptotic tumor cells as well as presentation of pp65-derived T-cell epitopes upon engulfment of cytomegalovirus (CMV)–infected human foreskin fibroblasts. (Blood. 2008;111:4264-4272) © 2008 by The American Society of Hematology

Introduction

Apoptosis, in contrast to necrosis, is a well-regulated process of controlled cell death and plays a pivotal role throughout the lifespan of each organism starting from morphological changes in embryogenesis to the maintenance of adult tissue homeostasis. Under physiological conditions, these apoptotic cells or cell fragments have to be cleared rapidly by phagocytes or neighboring cells to avoid loss of membrane integrity and the release of intracellular contents. This process, called secondary (postapoptotic) necrosis, can result in harmful inflammation and induce clinically relevant autoimmune reactions and diseases.1

Dendritic cells (DCs) are professional antigen-presenting cells that were shown to be involved in the uptake and clearance of such cell corpses.3 DCs can engulf both apoptotic and necrotic cells and the kind of incorporated remnant directly influences their immunologic behavior. Antigens delivered to DCs via apoptotic cells normally induce tolerance, but under certain circumstances, may also induce DC maturation and immunity like necrotic cell material.4

The removal of these dying cells is initiated through the recognition of special molecules, so-called eat-me signals, on the cell’s surface.5 The best characterized and probably most important one is phosphatidylserine (PS) that is translocated from the inner to the outer leaflet of the plasma membrane.6 It is recognized by the recently cloned PS receptor whose blockade abolishes the engulfment of apoptotic cells by macrophages.7 In addition, there are other molecules described to function as eat-me signals, including oxidized low-density lipoprotein-like sites,8 thrombospondin-1–binding sites,9 and sites for binding the complement proteins C1q or C3b.10,11 Furthermore, other C-type lectins were shown to be involved in this process and several binding sites for different collectins such as the mannose-binding lectin (MBL) or the lung surfactant proteins A and D (SP-A, SP-D) were described.11

Human Dectin-1 (hDectin-1; GenBank accession number AY009090), a recently identified member of the C-type lectin receptor family, has been the focus of intensive research for the last several years.12-15 It is a small type II transmembrane protein that consists of the characteristic single extracellular carbohydrate recognition domain, a stalk and a transmembrane region, and an intracellular cytoplasmic tail that harbors an immunoreceptor tyrosine-activation motif (ITAM)–like motif. It is alternatively spliced in several isoforms of which only the hDectin-1a and -b are functional in binding to zymosan, a β-glucan–rich particle derived from the cell wall of Saccharomyces cerevisiae.14 These 2 splice variants differ in the presence or absence of the stalk region, a membrane proximal part of the extracellular domain (ECD) between the carbohydrate recognition domain and the transmembrane domain.

hDectin-1 is expressed mainly on human DCs and macrophages and to a lesser extent in some other hematopoietic cell types.12,15,16 It was demonstrated that hDectin-1 triggers nonopsonic phagocytosis of yeast and synergizes with TLR2 to increase proinflammatory cytokine responses to the pathogens.17-19 Furthermore, using a whole-cell binding assay, it could be shown that hDectin-1 binds to T lymphocytes in a carbohydrate-independent manner, indicating that additional receptors or binding sites may exist on mammalian cells.14 We and others12,20 found that it exhibits stimulatory properties, as hDectin-1b–transfected HeLa cells are able to induce proliferation of cocultured T lymphocytes. In this study, we analyzed the possible involvement of hDectin-1 in recognition and clearing of human cells.

Methods

Cells

All cells were cultured in RP10 medium (RPMI 1640 with glutamax I, supplemented with 10% inactivated FCS and antibiotics [Invitrogen, Carlsbad, CA]).

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The cell lines used in the experiments were HEK-293 (embryonic kidney), K562 (chronic myeloid leukemia [CML] in blast crisis), CCRF-CEM (T-cell acute lymphoblastic leukemia [T-ALL]), and HeLa (cervix carcinoma), all from the DSMZ (Braunschweig, Germany).

**Generation of MDCs**

Monocyte-derived dendritic cells (MDCs) were generated from peripheral blood monocytes by magnetic cell sorting (MACS) as described previously. In brief, peripheral blood mononuclear cells were isolated by Ficoll/Paque (Biochrom, Berlin, Germany) density gradient centrifugation of blood obtained from buffy coats of healthy volunteers from the blood bank of the University of Tübingen. For MACS, CD14 magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) were used and purities of more than 95% were achieved. Monocytes were plated (10^6 cells/3 mL per well) into 6-well plates (BD-Falcon, Heidelberg, Germany) in RPMI 10 medium supplemented with granulocyte-macrophage colony-stimulating factor (GM-CSF, 100 ng/mL; sargamostim; Berlex, Richmond, VA) and IL-4 (20.0 ng/mL; R&D Systems, Wiesbaden, Germany). Cytokines were added to differentiating MDCs every 2 to 3 days. In some experiments, MDC maturation was induced on day 6 by adding one of the following toll-like receptor (TLR) ligands: Pam3CSK4 (TLR2L, 5.0 μg/mL; EMC Microcollection, Tübingen, Germany), poly-I:C (TLR3L, 50.0 μg/mL; Sigma-Aldrich, Deisenhofen, Germany), and R848 (TLR7/8L, 2.0 μg/mL; Invivogen, San Diego, CA), respectively. Phenotypic analyses were done by flow cytometry (fluorescence-activated cell sorting [FACS]).

**PAGE and Western blotting**

For the preparation of whole-cell lysates, cells were lysed in a buffer containing 1% Igepal, 0.5% sodium deoxycholate, 0.1% SDS, 2.0 mM EDTA, 1.0 mM phenylmethylsulfonyl fluoride, and 2.0 μg/mL aprotinin. Protein concentrations were determined using a BCA assay (Pierce, Perbio Science, Bonn, Germany). Protein extracts (20.0 μg) were separated on a 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred onto nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany). The blot was probed with antibodies recognizing hDectin-1 (rabbit polyclonal, generated against a synthetic peptide representing the amino terminus of hDectin-1) or GAPDH (6C5, mouse monoclonal; HyTest, Turku, Finland), which was followed by incubation with an appropriate horseradish peroxidase–conjugated secondary antibody. Bands were visualized using the enhanced chemiluminescence (ECL) staining system (Amersham Biosciences, Freiburg, Germany).

**Quantitative reverse transcriptase–polymerase chain reaction**

Quantification of hDectin-1 transcripts was done on a Taqman device (Applied Biosystems, Darmstadt, Germany). RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany) and subjected to cDNA synthesis with the Superscript II cDNA synthesis system (Invitrogen). For amplification of hDectin-1a-d, the following primers were used in different combinations: FP hDectin Ex3 nt 5’-ctcaacagactgaaacac-3’; RP hDectin Ex3 nt 5’-ctgggctagcaggggttc-3’. For amplification of hDectin-1b ECD (nt 275–677) was amplified by reverse transcription (RT)-PCR using MDC cDNA and inserted in the pCR2.1 TOPO vector (Invitrogen). For subcloning, the insert was excised and ligated into the BamHI and SalI site of the expression vector pQE-30 (Qiagen) that codes for an additional N-terminal 6xHis tag. The protein was expressed in *Escherichia coli* and after cell lysis inclusion bodies were solubilized in 8.0 M urea/PBS and purified using Ni-NTA-Agarose (Qiagen). After several rounds of dialysis against 50 mM Tris (pH 8.0), 19.0 mM NaCl, 0.8 mM KCl, 2.0 mM GSH, 0.2 mM GSSG with decreasing concentrations of urea, refolding was performed and purity was examined by SDS-PAGE and following silver staining. Dithiofproolate reductase (DHFR) was expressed in an analogous manner from the plasmid pQE-40 (Qiagen).

**ECD staining and induction of apoptosis**

Cell staining with the hDectin-1b ECD was performed by incubating the cells using approximately 10.0 μg/mL ECD, followed by incubation with 2.0 μg/mL anti-His antibody (His-1, mouse monoclonal; Sigma) and an appropriate FITC-conjugated polyclonal antimouse antibody (BD-Biosciences, Heidelberg, Germany). All incubation steps were done for 1 hour at 4°C in PBS (In vitro containing) 0.5% BSA (Roche Diagnostics, Mannheim, Germany). For precipitation experiments, hDectin-1 ECD was mixed with zymosan particles and incubated for 5 minutes at 4°C. Afterward the suspension was cleared and the supernatant was used for cell staining. For characterization studies of the ligand of hDectin-1, K562 cells were treated with N-glycosidase F (10.0 U/mL; Roche Diagnostics), pronase (5.0 mg/mL; Calbiochem, Merck Biosciences, Schwalbach, Germany), or proteinase K (5.0 mg/mL; Calbiochem, Merck Biosciences), respectively, for 10 minutes at 37°C in PBS. Apoptosis was induced by resuspending HEK-293 cells in PBS followed by UV irradiation in Petri dishes with 100.0 mL on a Stratalinker 2400 (Stratagene, Amsterdam, The Netherlands). Cells were immediately transferred in RPMI medium for overnight cultivation and were harvested the next day for staining and phagocytosis experiments. For annexin V–FITC and propidium iodide (PI) staining, the Annexin-V-Fluos Staining Kit (Roche Diagnostics) was used according to the manufacturer’s instructions. The mean of annexin V–positive apoptotic cells was 59.3% (+SD = 2.4) throughout all experiments.

**Phagocytosis assay**

For FACS analysis in phagocytosis experiments, MDCs and HEK-293 cells or human foreskin fibroblasts (HFFs) were labeled with PKH67 (green) or PKH26 (red) membrane dyes, respectively, according to the manufacturer’s instructions (Sigma). In the case of HEK-293 cells, staining was done after UV irradiation. For phagocytosis, 10^6 MDCs and 10^6 HEK-293 cells or HFF were cocultivated in 24-well plates in 1 mL RPMI medium. In some experiments, 10.0 μg/mL hDectin-1b-ECD, 10.0 μg/mL recombinant DHFR, 10^6 zymosan particles, or 100.0 μg/mL curdlan was added. After incubation for 24 hours at 37°C, cells were harvested and either analyzed by FACS or used as stimulators in IFN-γ enzyme-linked immunosorbent spot (ELISPOT) assays.

**CMV infection of human foreskin fibroblasts (HFFs)**

HFF-A2^−^ HFFs were cultured in MEM medium (Invitrogen) containing 2.4 μM glutamine, 100.0 μg/mL gentamycin, and 5% FCS. HFFs were used for experiments between passages 10 and 25. For preparation of virus stocks, HFFs were infected at a multiplicity of infection (moi) of 0.1 with human cytomegalovirus (CMV) strain AD169. Supernatants of infected cultures were harvested 6 days after infection, cells and cell debris were removed by centrifugation, and cell-free virus preparations were used for infection of HFF cultures. The medium was removed and replaced by virus preparations for 120 minutes at 37°C. Subsequently, virus preparations were removed, and cells were washed with fresh medium and used for immunologic assays.

**IFN-γ ELISPOT assay**

A CMV-specific HLA-A2^+^ cytotoxic T lymphocyte (CTL) line was generated in vitro using autologous MDCs loaded with the HLA-A2^+^–specific pp65-peptide NLVPVMVATV (amino acids 495-503; A*0201), as described previously. After one restimulation, cells were collected and incubated at a concentration of 10^5 or 5 × 10^5 cells/well in an antihuman IFN-γ-antibody (antihuman IFN-γ, 10 μg/mL; Hölzel Diagnostika, Köln, Germany)--coated 96-well plate with 10^5 autologous MDCs. As positive or negative controls, MDCs were pulsed with the specific pp65-CMV-peptide
or an irrelevant HLA-A2-binding peptide derived from human immunodeficiency virus (HIV; ILKEPVHG; amino acids: 476-484; pol HIV-1 reverse transcriptase), respectively. Furthermore, MDCs that were cocultivated with irradiated (96 Gy, GammaCell 1000; Atomic Energy of Canada, Mississauga, ON) CMV-uninfected or -infected HFFs in the presence or absence of zymosan, curdlan, or the hDectin-1b ECD were used as stimulators, respectively. After 48 hours of incubation, visualization of IFN-γ release was done using a biotin-labeled antihuman IFN-γ antibody (antihuman IFN-γ biotin, 2.0 μg/mL; Hölzel Diagnostika). Spots were counted using an automated ELIspot reader (Immunospot Analyzer; CTL Analyzers, Cleveland, OH).

**Standard 51Cr-release assays (CTL assays)**

A CMV-specific HLA-A2 + CTL line was generated in vitro as described in the preceding section. CTL assays were performed as described previously. In brief, target cells were generated by loading autologous MDCs with the specific pp65-CMV-peptide, an irrelevant HLA-A2–binding HIV peptide or by coculture of MDCs with UV-irradiated CMV-uninfected or -infected HFFs. In addition, MDCs were incubated with HFFs electroporated with in vitro transcript (IVT) coding for pp65 or EGFP protein and used as targets in the assay.

**Statistical analysis**

Paired t test was performed to compare means of measured data (GraphPad Prism V 4.0; GraphPad Prism Software, San Diego, CA). Results were considered statistically significant with P values of less than .05.

**Results**

**Quantitative analysis of hDectin-1 mRNA expression in different DC populations**

CD14 + peripheral blood monocytes from healthy donors were cultured with GM-CSF and IL-4 to generate MDCs. On day 7, the cells had the typical DC morphology with its dendrite shape. These cells were analyzed by FACS and showed the characteristic phenotype with CD1a and HLA-DR expression and the absence of CD14 (data not shown). Stimulation of these cells on day 6 with TLR ligands led to an up-regulation of CD54, CD80, CD83, CD86, CD40, and HLA-A, HLA-B, HLA-C, and CCR7 (Figure 1 and data not shown).

By analyzing expression kinetics (Figure 2A) of the hDectin-1 isoforms a to d in monocytes differentiating into immature MDCs (without TLR agonists) by quantitative RT-PCR, it became clear that hDectin-1 transcripts were already detectable after the first hour of differentiation and reached expression levels of immature MDCs after 18 hours. Furthermore, it revealed that hDectin-1b is the major isoform expressed in these in vitro–cultured cells and that hDectin-1a and -1d were found to be expressed at a much lower extent, while hDectin-1c was hardly detectable.

To analyze hDectin-1 expression in matured MDCs, immature MDCs were additionally activated with different compounds for another 24 hours (Figure 2B). Surprisingly, stimulation with the TLR ligand poly I:C (TLR3L) led to a dramatic down-regulation of hDectin-1 mRNA expression. In contrast, maturation with the other TLR ligands pam3cys (TLR2L) or R848 (TLR7/8L) did not substantially affect hDectin-1 transcription, thus indicating that TLR ligand–mediated stimuli may evoke distinct activation signals that result in different functional properties of DCs.

**Figure 1. Phenotypic analysis of in vitro–generated MDCs.** MDCs were generated from CD14 + monocytes with GM-CSF and IL-4. Maturation was induced on day 6 by adding pam3cys (TLR2L), poly I:C (TLR3L), or R848 (TLR7/8L). DMSO was used as the solvent control. Overlay diagrams show expression of indicated surface molecules. Solid histograms: labeling with isotype-matched irrelevant MoAb. Numbers represent mean fluorescence intensity.
To further extend and confirm the expression of hDectin-1 in different DC subpopulations, we performed Western blot analysis using a polyclonal antibody generated against a synthetic peptide derived from the N-terminus of the protein. As shown in Figure 3 in line with the results from quantitative RT-PCR, stimulation of immature MDCs with the TLR ligand poly I:C led to a concentration-dependent complete down-regulation of hDectin-1b, while the level of protein expression was not affected by other stimuli.

Recombinant expression of the ECD of hDectin-1b

Some of the known C-type lectins such as DC-SIGN were shown to be involved in the uptake of cells or cellular material. To analyze the possible involvement of hDectin-1 in this process, we recombinantly expressed the ECD of hDectin-1b fused to a 6xHis-tag in E. coli and used it to stain tumor cells. The ECD was purified through immobilized metal chelate affinity chromatography, renatured, and analyzed for purity in SDS-PAGE followed by silver staining (Figure 4A). As demonstrated in Figure 4B, the recombinant ECD of hDectin-1b bound not only to HEK-293 cells that were demonstrated to have a very low binding capacity of hDectin-1 but also to other cell lines, which suggests the possibility of hDectin-1 in the phagocytic activity of immature and mature MDCs and its regulation by different TLR ligands.

Recognition of apoptotic cells by hDectin-1

In our experiments analyzing the binding of the hDectin-1b ECD to tumor cell lines, we observed that the staining of some lines depended on the cell culture conditions. This suggested that the cell viability might affect the binding properties of the ECD. We therefore examined the ability of hDectin-1 to bind to apoptotic cells. To accomplish this, we used HEK-293 cells that were induced apoptotic death in these cells by UV irradiation in a reproducible manner. As shown in Figure 5, binding of the ECD to HEK-293 cells is increased upon induction of apoptosis. To confirm recognition of the apoptotic cell population, staining with annexin V and PI was performed to exclude secondary necrosis as described recently (data not shown).

Phagocytosis of apoptotic cells

Based on the results derived from the expression data of hDectin-1 and the “staining” of apoptotic cells with the ECD, we sought to determine a possible involvement of hDectin-1 in the phagocytic activity of immature and mature MDCs and its regulation by different TLR ligands.

Initially, we analyzed the phenotype of MDCs incubated with HEK-293 cells. Although untreated or UV-irradiated apoptotic HEK-293 cells to immature MDCs had no effect on their phenotype (Figure S1, available on the Blood website; see the Supplemental Materials link at the top of the online article).
In the next series of experiments, we labeled MDCs and the apoptotic cells with different membrane dyes for FACS analyses and coincubated them for 24 hours in the presence or absence of zymosan or the hDectin-1b ECD, respectively (Figure 6). Immature MDCs showed a strong phagocytic activity and uptake of large amounts of apoptotic cells could be observed (Figure 6 dot blots; shift to red, upper right) as expected. Surprisingly, pam3cys, an irrelevant protein produced in an analogous manner as the ECD, diminished uptake of apoptotic cells, which correlated with the reduced hDectin-1 expression (Figures 2,3). Furthermore, the addition of the ECD of hDectin-1b, and to a stronger extent zymosan (Figure 6A,B) or the more hDectin-1-specific linear (1-3)-β-D-glucan polymer curdlan (Figure 6C,D), to the cocultures led to a significant decrease of the uptake of apoptotic cells. As controls, phagocytosis was performed in the presence of DHFR as an irrelevant protein produced in an analogous manner as the ECD (Figure 6C,D). In addition, labeled cells were coincubated at 4°C or were mixed shortly before FACS analysis to exclude unspecific diffusion of membrane dyes (data not shown).

hDectin-1 mediates cross-presentation of antigens derived from engulfed infected cells

Uptake of cellular material was shown to result in cross-presentation of epitopes derived from engulfed proteins and stimulation of MHC class I-restricted CTLs. We therefore analyzed the involvement of hDectin-1-mediated uptake of cells in the presentation of peptide antigens to human CTLs. To test this, we used a CTL line generated from an HLA-A2- and CMV-positive donor by performing serial restimulations with an HLA-A2-binding peptide derived from the pp65 CMV antigen. As shown in Figure 7A, this in vitro–generated CTL line specifically recognized the cognate pp65-peptide. Stimulation of T cells with autologous MDCs pulsed with an irrelevant HIV peptide resulted only in background IFN-γ production as analyzed in an ELISpot assay. In the next set of experiments, autologous MDCs were incubated with HLA-A2-negative HFFs either infected with CMV or left untreated and were used as stimulators in ELISpot assays. As shown in Figure 7B, immature MDCs efficiently stimulated IFN-γ secretion by CMV-specific autologous CTLs when they were cocultured overnight with CMV-infected HFFs. In contrast, addition of untreated HFFs to the MDC cultures had no effect as was the case if phagocytosis was performed at 4°C (data not shown). Likewise, the uptake of apoptotic cells and in succession stimulation of the CTLs was reduced significantly upon addition of zymosan (Figure 7B), curdlan (Figure 7D), or the ECD of hDectin-1b (Figure 7B,E). Furthermore, in line with the results from previous experiments, activation of MDCs with poly IC (TLR3L) led to a reduced activation of the pp65-specific HLA-A2-restricted CTLs (Figure 7C). Addition of the irrelevant DHFR protein control had no effect on the IFN-γ production by the CTLs (Figure 7D).

To further confirm the cross-presentation of CMV-derived antigens, we performed 51Cr-release assays using the pp65-peptide-specific CTL line generated from an HLA-A2- and CMV-positive donor as above (Figure 7). As target cells, we used autologous
MDCs pulsed with the pp65-peptide or incubated with HLA-A2–negative HFFs that were either infected with CMV (CMV⁺) or left untreated (CMV⁻). As shown in Figure 8A, the CTL line recognized and efficiently lysed autologous MDCs pulsed with the cognate pp65-peptide as well as MDCs cocultured with CMV⁺ HFFs with increasing E/T ratios. There was no recognition of MDCs incubated with CMV⁻ HFFs, an irrelevant HIV peptide, or supernatant (SN) obtained from the CMV⁺ HFF cell cultures. The specific lysis could be blocked using a monoclonal antibody directed against HLA class I molecules indicating that the elicited T-cell responses were HLA class I restricted. Consequently, the antibody directed against HLA class II molecules did not inhibit the lysis of the MDCs presenting CMV-pp65 epitopes. In addition, we included MDCs that were incubated with HFFs transfected with pp65-IVT in these assays. These targets were recognized by the pp65-specific CTLs, whereas MDCs that were cocultured with HFFs transfected with an irrelevant EGFP-IVT were spared (Figure 8B).

In our next experiments, we sought to analyze if cross-presentation of pp65-derivable epitopes requires proteasome degradation. Cytosolic protein degradation is performed by the proteasome, a large multicatalytic protease complex. Lactacystin specifically inhibits the 20S and 26S proteasome activity by targeting the catalytic subunit.32-35 MDCs were preincubated with lactacystin, cocultured together with HLA-A2–negative CMV-infected HFFs, and used as targets in standard ¹¹⁹Cr-release assays. As demonstrated in Figure 8B, addition of lactacystin completely inhibited the presentation of pp65-derived peptides indicating the involvement of the proteasome in these processes.

The fungal product brefeldin A blocks the MHC class I processing pathway by specifically inhibiting the vesicular egress from the ER and the Golgi complex.36,37 In line with previous findings, incubation with brefeldin A almost completely abolished the lysis of MDCs incubated with CMV⁺ HFFs.

To further analyze whether the cross-presentation of CMV-derived peptides on HLA class I molecules was dependent on lysosomal proteases, MDCs that were coincubated with HFFs as above were treated with the lysosomotropic agent chloroquine that prevents acidification of the lysosomal compartment involved in the exogenous pathway of antigen presentation. As shown in Figure 8B, the addition of chloroquine had no effect on the cross-presentation of CMV-derived epitopes on HLA class I molecules. However, in line with previous findings, the addition of curdian or poly IC but not of R848 significantly reduced the specific lysis of target cells.

Discussion

Apoptotic cell death and the removal of cells dying by apoptosis is an essential process in maintaining normal tissue homeostasis. Several receptors and ligands have been reported to be important in the uptake of apoptotic cells. The present study demonstrates that hDectin-1, a member of the C-type lectin–like receptor family, binds to apoptotic cells, facilitates their uptake by DCs, and mediates cross-presentation of cell-derived antigens.

Pattern recognition receptors (PRRs) are part of the innate immune system that is important in specifically recognizing pathogens and mediates their uptake by phagocytic cells. The
most prominent member representing these receptors is the TLR family that acts mainly to sense the environment and submit danger signals to the cell. Further on, there are several other molecules involved in recognition and uptake of antigen, such as C-type lectins, including the soluble collectins lung surfactant protein A (SP-A) and SP-D or the mannose-binding lectin (MBL) that opsonizes pathogens and mediates uptake through its specific receptors. Several membrane-bound C-type lectins such as MMR, Dec205, or DC-SIGN are reported to directly bind and internalize their ligands, which leads to efficient antigen presentation in the case of DCs. In recent years, it became clear that these receptors function not only in pathogen recognition but are also involved in the binding or uptake of endogenous ligands. For example, SP-A, SP-D, and MBL are not only a component of the innate immune system but also facilitate the binding of phagocytes to apoptotic cells.

hDectin-1 has been shown to be the major β-glucan receptor that is expressed mainly on human DCs and macrophages. Until now, it has been known that hDectin-1 mediates the nonopsonic phagocytosis of yeast and that it synergizes with the TLR2 in increasing proinflammatory cytokine production and their release in response to these pathogens. Furthermore, binding of this receptor to T lymphocytes in a carbohydrate-independent manner and induction of proliferation in these cells using the recombinant protein or transfected cells suggests that there is/are further ligand(s) for hDectin-1 endogenously expressed on mammalian cells. These data and the observed binding behavior of the hDectin-1 ECD to cell lines cultured under different conditions prompted us to investigate if there is a selective increase in the expression of this potential ligand on these cells. In our experiments, we could show that the binding of the ECD of hDectin-1b increases upon induction of apoptosis in HEK-293 cells. Furthermore, we could observe in phagocytosis experiments that uptake of apoptotic cells by immature MDCs is dramatically reduced upon presentation of mammalian cells such as DC-SIGN, which binds to mannan contents, is described. Therefore, the ECD of hDectin-1b was used as a competitive inhibitor to directly measure the participation of this molecule in apoptotic cell clearing. Although it was revealed as not as efficient as zymosan or curdlan in inhibiting apoptotic cell uptake, a significant reduction of phagocytosis could be observed. The remaining phagocytic effect is probably due to other molecules involved in phagocytosis. Interestingly, in the case of MDCs that were matured with poly I:C but not with Pam3Cys or R848, a...
and CMV-positive donor.

transcription that functions as a direct response to viral infection
This molecule is known to be involved in IFN-stimulated gene
activation, but ligand binding by this receptor, likewise stimulation of
TLR3, could also induce activation of a MyD88-independent
function of NFκB.

Figure 8. 51Cr-release assays using a CTL line generated from an HLA-A2–
and CMV-positive donor. (A) 51Cr-release assays using a pp65-peptide–specific CTL
line generated from an HLA-A2– and CMV-positive donor. Autologous MDCs pulsed with
the pp65-peptide or incubated with HLA-A2–negative HFFs that were either infected with
CMV (CMV +) or left untreated (CMV −) served as target cells. MDCs incubated with an
irrelevant HIV peptide or supernatant (SN) from infected HFFs were applied as controls.
Inhibition of HLA class I or class II was performed by incubating MDCs prior to the assay
with anti (−)HLA class I or II antibodies. MDCs that were cocultured with HFFs transfected
with pp65-IVT as well as MDCs that were cocultured with HFFs transfected with an
irrelevant EGFP-IVT as a control were included. (B) Cross-presentation of pp65-derived
HLA-A*02–binding T-cell epitopes is sensitive to proteasome inhibitor lactacystin or the
blocker of the MHC class I processing pathway brefeldin A but not to the lysosomotropic
agent chloroquine. Data represent means (± SD) of quadruplicates. One representative
experiment is shown.

reduced phagocytic activity could be observed that was accompa-
nied with a decrease in hDectin-1 expression as demonstrated in
quantitative real-time PCR and Western blot experiments. This
might be due to the different signaling pathways engaged upon
activation of the specific TLRs.38 TLR2 and TLR7 signaling is
MyD88 dependent and leads to the activation and nuclear localiza-
tion of NFκB. This is also true for TLR4-mediated signal transduc-
tion, but ligand binding by this receptor, likewise stimulation of
TLR3, could also induce activation of a MyD88-independent
pathway that leads to activation of the transcription factor IRF-3.
This molecule is known to be involved in IFN-stimulated gene
transcription that functions as a direct response to viral infection
and is potently involved in inhibiting viral replication.42 However,
the impact of the function of IRF-3 in the light of hDectin-1
regulation remains to be clarified.

hDectin-1 also seems to be involved in the uptake of
CMV-infected HFFs leading to cross-presentation of CMV-
derived peptides on MHC class I molecules and activation of
CMV pp65–specific CD8+ T lymphocytes. This is mediated at
least in part in an hDectin-1–dependent manner as uptake and
presentation of CMV antigens could be inhibited through
addition of zymosan, curdlan, or hDectin-1 ECD to the cocul-
tures of MDCs and HFFs. In line with the phagocytosis
experiments using apoptotic HEK-293 cells, stimulation of
MDCs with the TLR3 ligand led to a reduced activation of
pp65-specific HLA-A2–restricted CTLs. Presentation of CMV-
specific peptides is likely to be mediated by cross-presentation
and not due to secondary infection of MDCs in the coculture
because the laboratory strain of CMV (AD169) used shows a
HFF-specific cell tropism and does not infect DCs.43,44 Further-
more, we used HLA-A2–negative HFFs in our experiments.
Incubation of MDCs with viral particle containing supernatant
obtained from infected HFFs had no effect on stimulation of
CMV-specific CTLs (data not shown). These results were
further confirmed using HFFs electroporated with the pp65-IVT
in the CTL assays.

The observed down-regulation of hDectin-1 upon activation
of MDCs with poly I:C (Figures 2 and 3) may reveal as a novel
escape mechanism from immune recognition of host invading
microorganism. These pathogens could activate TLR3 and
hence would not be recognized by the heterodimeric receptor
complex that is formed by TLR2 and hDectin-1.39 Furthermore,
our data demonstrate that the production and secretion of
β-glucan by pathogens may interfere with the uptake of cellular
antigens derived from infected cells and thus support their
spread and inhibit their recognition and elimination by the
immune system. This assumption is confirmed by recent studies
in which it was shown that several pathogenic microorgan-
isms45,46 and even tumor cells47 take advantage of TLRs to evade
immune recognition and destruction.

In summary, the data reported here show that hDectin-1 not only
functions as a pattern recognition receptor in innate immunity but is
also involved in the clearing of apoptotic cells and cross-
presentation of cellular antigens on MHC class I molecules to
specific CTLs. Furthermore, we demonstrate that TLR ligands
differentially affect this process.

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Authorship

Contribution: M.M.W. designed research, performed research, and
wrote the paper; S.A., D.W., and A.B. performed research; C.S.
contributed vital new reagents; F.G. and P.B. designed research and
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Authorship

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hDectin-1 is involved in uptake and cross-presentation of cellular antigens

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