pH-dependent recognition of apoptotic and necrotic cells by the human dendritic cell receptor DEC205

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Dendritic cells play important roles in regulating innate and adaptive immune responses. DEC205 (CD205) is one of the major endocytotic receptors on dendritic cells and has been widely used for vaccine generation against viruses and tumors. However, little is known about its structure and functional mechanism. Here we determine the structure of the human DEC205 ectodomain by cryoelectron microscopy. The structure shows that the 12 extracellular domains form a compact double ring-shaped conformation at acidic pH and become extended at basic pH. Biochemical data indicate that the pH-dependent conformational change of DEC205 is correlated with ligand binding and release. DEC205 only binds to apoptotic and necrotic cells at acidic pH, whereas live cells cannot be recognized by DEC205 at either acidic or basic conditions. These results suggest that DEC205 is an immune receptor that recognizes apoptotic and necrotic cells specifically through a pH-dependent mechanism.

DEC205 | pH dependence | apoptosis | cryoEM | mannose receptor family

In living organisms such as humans, billions of cells are turned over through apoptosis or killed by pathological infections or inflammation every day. Therefore, clearance of dead cells is critical for maintaining tissue homeostasis and preventing autoimmunity and inflammation (1–3). Dead cells are usually removed by the host immune system through phagocytosis by phagocytes. Antigen-presenting cells (APCs) such as dendritic cells and macrophages are professional phagocytes that can engulf target cells or fragments by recognizing specific ligands through their cell surface receptors (4), and after antigen uptake, processing, and presentation, they can lead to either immune activation or tolerance (5–8).

DEC205 (CD205 or Ly75, MW 205 kDa) is an endocytotic receptor with wide tissue distribution and is highly expressed on dendritic cells and thymic epithelial cells (8, 9). It has been shown that DEC205 is involved in antigen uptake and can induce either tolerance or immunity in the absence or presence of inflammatory stimulus (10). It has also been suggested that DEC205 may bind apoptotic and necrotic cells (11) and oligonucleotides (12); however, neither the structure nor the functional mechanism of DEC205 has been identified.

In contrast, the role of DEC205 in generating protective immunity has been studied extensively. It is probably the most widely used receptor target in dendritic cell-based immune therapies. The high efficiency of antigen delivery and presentation makes DEC205 an ideal vehicle for vaccine generation against various antigens such as tumors and viruses (13, 14), mainly through DEC205-specific antibodies fused with a fragment or intact protein of the target antigen (15–17). This strategy has been shown to be successful in generate protective immune responses and reveals good potentials in clinical applications (18).

DEC205 belongs to the mannose receptor family (19). To date, five proteins have been classified as the mannose receptor family members, including the mannose receptor itself (20), DEC205 (9), Endo180 (21), PLA2R (22), and FcRY (23). These receptors share similar structural features, but their physiological functions are made diverse by recognizing different ligands. The ectodomain of mannose receptor family members begins with a cysteine-rich domain (CysR), followed by a fibronectin type II domain (FNII) and eight (10 for DEC205) C-type lectin-like domains (CTLDs) (Fig. 1*A*). Probably because of the potential internal flexibility, no high-resolution structures have been determined for this family. Currently known structural information of this family comes from electron microscopy. A negatively stained image reconstruction shows that the mannose receptor has a compact conformation (24). The cryoelectron microscopy (cryoEM) reconstruction of FcRY indicates that its ectodomain adopts a double-ringed conformation at acidic pH (25). However, because of limited resolution, the detailed domain arrangements of these molecules, especially the interactions among these domains, are unclear.

Here we determined the 3D structure of human DEC205 ectodomain by cryoEM single-particle reconstruction, identified its pH-dependent conformational change, and also investigated the mechanism of the conformational change and its correlation with ligand binding and release. These results indicate that DEC205 is an immune receptor that recognizes apoptotic and necrotic cells specifically through a pH-dependent mechanism.

Results

pH-Dependent Conformational Change of DEC205. The human DEC205 ectodomain (residues 1–1,668) was expressed in HEK293 cells. The purified protein was analyzed by size-exclusion chromatography (SEC) at both acidic (pH 6) and basic (pH 8) conditions. The DEC205 ectodomain eluted earlier at pH 8 than at pH 6 (Fig. 1*B*), suggesting a more extended conformation at pH 8. Consistent

Significance

Dendritic cells are critical in regulating immune responses. DEC205 (CD205) is an endocytotic receptor on dendritic cells with antigen presentation function and has been widely used in immune therapies. Here, we report that DEC205 is an immune receptor that recognizes apoptotic and necrotic cells specifically through a pH-dependent mechanism. The ectodomain of DEC205 forms a double-ringed conformation at acidic pH and becomes extended at basic pH. DEC205 only recognizes apoptotic and necrotic cells at acidic conditions with its N-terminal small ring and has no binding activities to healthy cells at either acidic or basic conditions, thus representing a novel pathway for immune clearance of dead cells and a potential mechanism for tumor scavenging.

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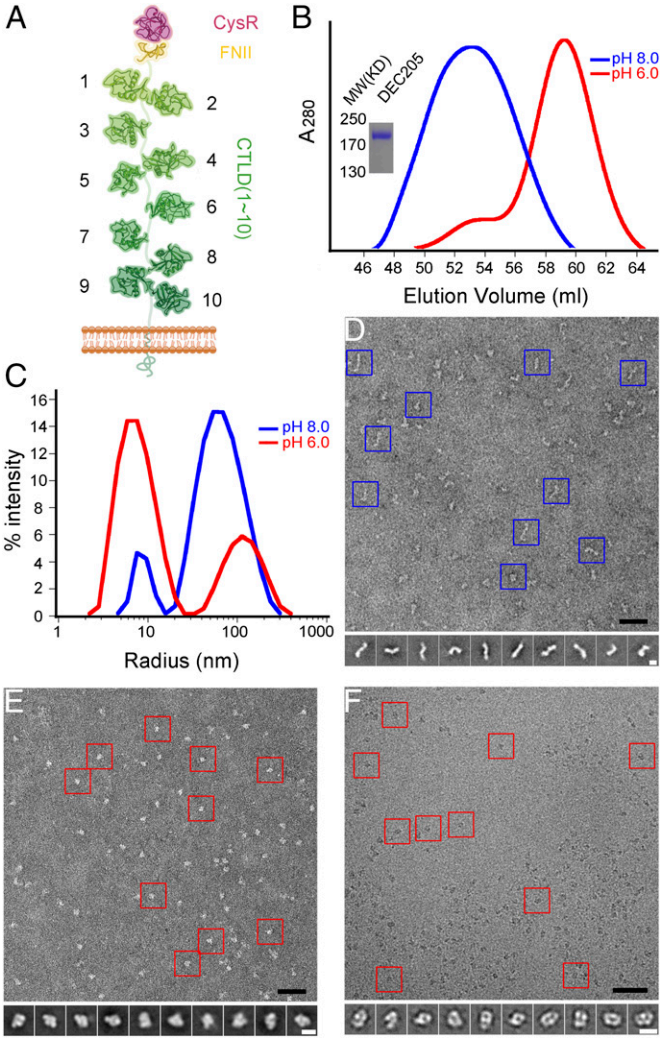

Fig. 1. The pH-dependent conformational change of DEC205. (*A*) A schematic representation of DEC205 domain arrangement. (*B*) SEC profiles of DEC205 at pH 6 and pH 8. (*C*) Dynamic light scattering analysis of DEC205 at pH 6 and pH 8. (*D*) A negatively stained EM micrograph (*Top*) and the representative reference-free 2D classes (*Bottom*) of DEC205 at basic pH. (*E*) A negatively stained EM micrograph (*Top*) and the representative reference-free 2D classes (*Bottom*) of DEC205 at acidic pH. (*F*) A cryoEM micrograph (*Top*) and the representative reference-free 2D classes (*Bottom*) of DEC205 at acidic pH. (Scale bars, 50 nm for micrographs and 10 nm for the reference-free 2D classes.)

with this interpretation, dynamic light scattering showed an increase in the hydrodynamic radius at basic pH compared with acidic pH (Fig. 1*C*).

To visualize the conformational change of DEC205 directly, the DEC205 ectodomain was negatively stained at both acidic (pH 6.0) and basic (pH 8.0) conditions and was imaged by electron microscopy. The images obtained at acidic pH showed that DEC205 had a homogeneous conformation with a globular shape (Fig. 1*E*), whereas the sample stained at basic pH showed mostly linear and extended particles with heterogeneous conformations (Fig. 1*D*). These results confirmed that DEC205 underwent a conformational change between acidic and basic conditions.

CryoEM Reconstruction of DEC205. To investigate the 3D structure of DEC205 at higher resolution, we collected images from the unstained frozen hydrated samples of human DEC205 ectodomain by cryoEM at pH 6 (Fig. 1*F*) and pH 8. 3D reconstruction of DEC205 at pH 8 was not successful, probably because of the flexibility and heterogeneity of the open conformation at basic conditions, as observed in the negatively stained images (Fig. 1*D*). The compact conformation at pH 6 was reconstructed and refined to 14.6 Å resolution, the highest resolution achieved for a mannose receptor family member to date (Fig. 2). The overall structure of DEC205 can be roughly divided into a head and a tail region. The head adopts a double-ringed conformation. The small ring contains CysR, FNII, and CTLD1~CTLD3, with CysR interacting with CTLD3. The larger ring is formed by FNII and CTLD1~CTLD6, with FNII interacting with CTLD6 (Fig. 2 and Fig. 3*A*). The tail of DEC205 includes domains from CTLD7 to CTLD10. A similar compact double ring-shaped conformation at acidic pH has been found for FcRY (25), suggesting the conformation might be conserved within the mannose receptor family.

The cryoEM reconstruction also allows the individual domain of DEC205 to be recognized (Fig. 2*B*). For example, the pseudo threefold structure of CysR (26) and the adjacent saddle-like shape consistent with the structure of FNII domain (27) are visualized in the reconstruction (Fig. 2 *B* and *C*). Crystal structures of CTLDs suggest these domains include a large loop region of about 20 residues (28), which may introduce internal flexibility

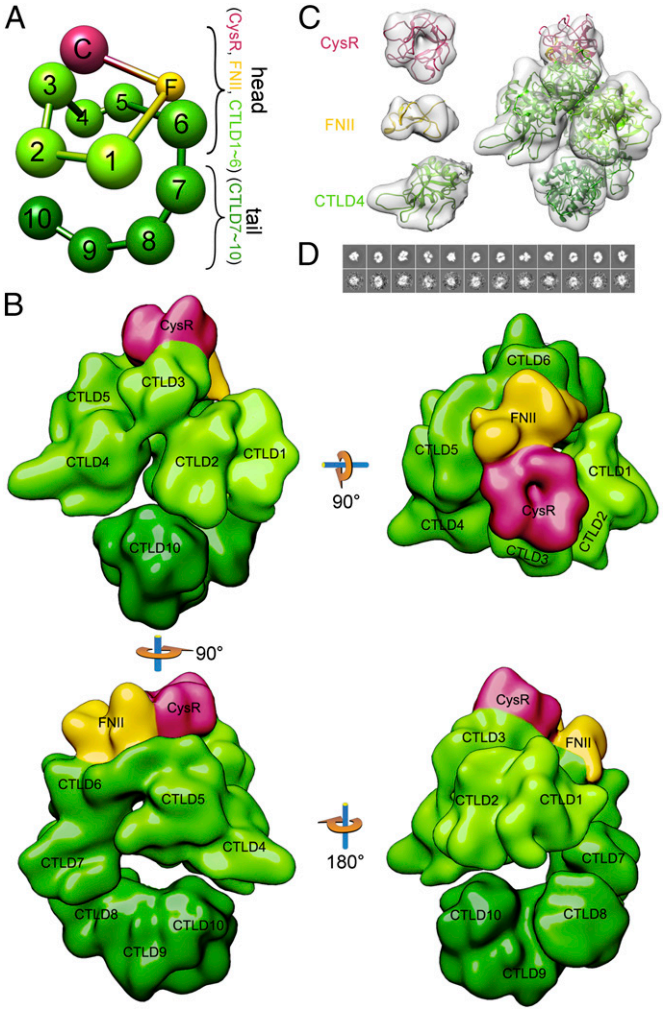

Fig. 2. 3D reconstruction of DEC205 by cryoEM. (*A*) A ball-and-stick model of DEC205 domain arrangement from cryoEM. (*B*) Views of the cryoEM reconstruction of DEC205. (*C*) CryoEM densities (gray) of the individual domains (CysR, FNII, and CTLD4) (*Left*) and DEC205 (*Right*) fitted with the corresponding homology models. (*D*) Back projections of DEC205 reconstruction (*Top*) and the corresponding reference-based 2D classes (*Bottom*).

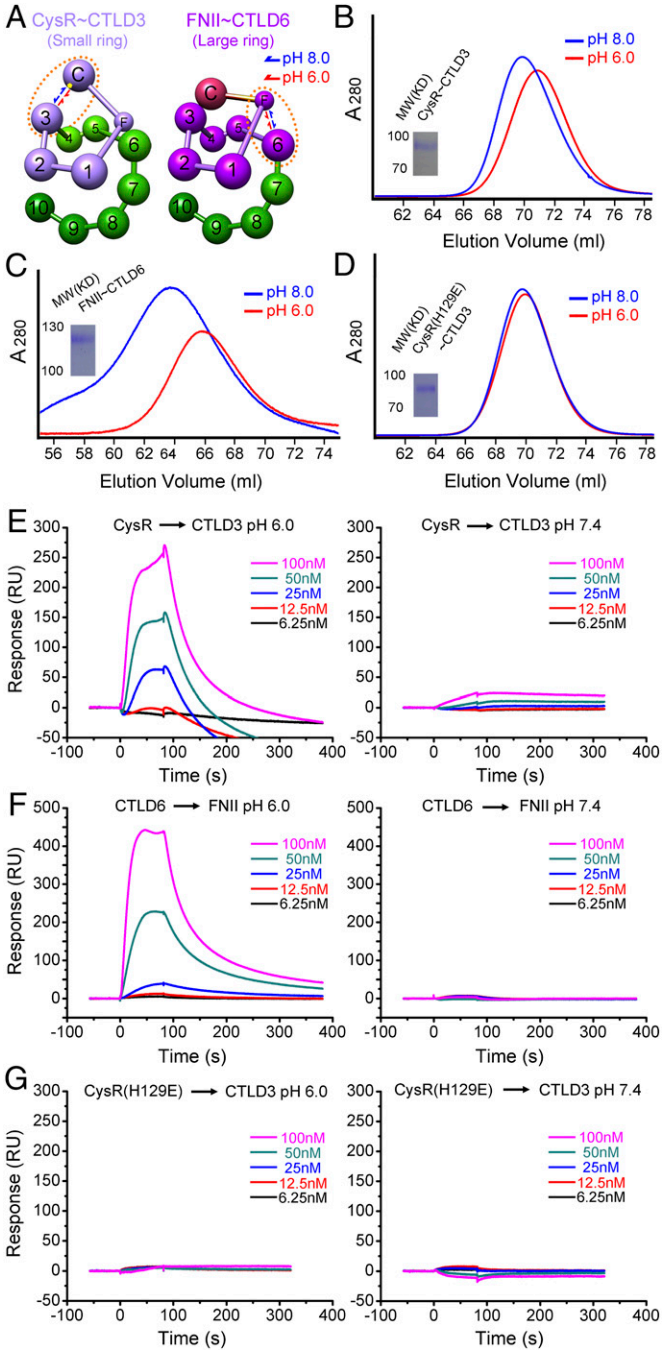

Fig. 3. The mechanism of DEC205 conformational change. (*A*) The conformational change of the small ring (CysR~CTLD3, light purple, *Left*) and the large ring (FNII~CTLD6, dark purple, *Right*) of DEC205 between acidic and basic pH. The approximate position of H129 is shown as a yellow dot. The domains involved in the intramolecular interactions are circulated by the brown dotted lines. (*B*) SEC profiles of the small ring at pH 6 and pH 8. (*C*) SEC profiles of the large ring at pH 6 and pH 8. (*D*) SEC profiles of the small ring H129E mutant at pH 6 and pH 8. (*E*) Sensorgrams for the interactions of CysR with CTLD3 at acidic (*Left*) and basic (*Right*) conditions. (*F*) Sensorgrams for the interactions of CTLD6 with FNII at acidic (*Left*) and basic (*Right*) conditions. (*G*) Sensorgrams for the interactions of CysR(H129E) with CTLD3 at acidic (*Left*) and basic (*Right*) conditions.

and reduce the resolution of reconstruction. However, the boundary of each CTLD is recognizable in the reconstruction and can be fit with CTLD models (Fig. 2*C*). Notably, the adjacent CTLDs of DEC205 can form groups, such as CTLD1~CTLD2, CTLD4~CTLD5, and CTLD8~CTLD10. The CTLDs within the same group associate with each other tightly, consistent with the results from protease digestions of the mannose receptor (29). In contrast, CTLD3, CTLD6, and CTLD7 are loosely associated with neighboring domains (Figs. 1*A* and 2*B*). The tight or loose association among neighboring CTLDs may contribute to the formation of the overall conformation and the transition between the open and the closed states.

Mutagenesis Studies of the DEC205 Conformational Change. To investigate the pH-dependent conformational change of DEC205, a series of mutants was constructed to probe the interactions among the extracellular domains. A truncation mutant containing the domains that form the small ring, including CysR, FNII, and CTLD1~CTLD3, was constructed and expressed. The purified protein of this mutant exhibited a pH-dependent elution shift by SEC at pH 6 and pH 8 (Fig. 3 *A* and *B*), suggesting the small ring is involved in the pH-dependent conformational change. Another mutant comprising the domains forming the large ring, including FNII and CTLD1~CTLD6, was also expressed and purified, and a similar pH-dependent elution shift was observed (Fig. 3 *A* and *C*). These results suggest that both rings are open at basic pH, driving DEC205 to adopt a linear and extended structure as observed by SEC and negatively stained EM.

The pH-dependent properties of proteins are commonly associated with histidine residues that undergo charge transitions between acidic and basic environments. We therefore investigated the potential locations of histidine residues in the ectodomain of DEC205 based on the cryoEM reconstruction. For forming the small ring, CysR and CTLD3 need to interact with each other, and histidine 129 of CysR locates at the potential interface between CysR and CTLD3 in the fitting model (Fig. 3*A*). Indeed, the SEC elution profiles at pH 6 and pH 8 of the purified H129E (Fig. 3*D*) and H129A mutants were almost identical, suggesting the pH-dependent conformational change was abolished for these mutants. These results also validate the cryoEM model. Identification of the histidines that regulate the conformational change of the large ring was not successful because of the low expression level of the large ring mutants.

To further validate the intramolecular interactions of DEC205 that are critical for forming the double ring-shaped conformation at acidic pH, four single domains, including CysR, CTLD3, FNII, and CTLD6, were expressed individually fused to an IgG Fc region, and the interactions of CysR with CTLD3 and FNII with CTLD6 were monitored by surface plasmon resonance (Fig. 3 *E* and *F*). The results showed that both interactions, CysR with CLTD3 and FNII with CTLD6, were pH-dependent, thus validating the structural model from cryoEM. We also tested the interaction between the CysR (H129E) and CTLD3, and no detectable interaction was found at either acidic or basic pH (Fig. 3*G*), confirming the importance of this residue in the DEC205 conformational change.

pH-Dependent Recognition of Apoptotic and Necrotic Cells by DEC205. We further explored functional correlation of the DEC205 conformational change by a series of cell assays. Binding of the GFP-tagged DEC205 to Jurkat and HEK293 cells were assayed at different pH conditions by flow cytometry and confocal microscopy. The flow cytometry data showed that DEC205 had no binding to healthy Jurkat cells at either acidic or basic pH (Fig. 4*A*). However, when these cells were treated with actinomycin D (ActD) to induce apoptosis and necrosis, DEC205 exhibited binding activities under acidic, but not basic, conditions (Fig. 4*A*). Similar binding characteristics were also observed for DEC205-GFP interactions with HEK293 cells (Fig. 4*B*), suggesting common ligands were expressed on both cell types. In addition, DEC205-GFP also bound frozen-thawed HEK293 cells at acidic pH (Fig. 4*C*), indicating that the ligand of DEC205 was likely to be a naturally expressed cellular

component before apoptosis. To further investigate binding differences between apoptotic and necrotic cells, both viable and ActD-treated Jurkat cells were stained by DEC205-GFP at acidic pH (Fig. 4*D*). DEC205 showed only weak binding to the preapoptotic Jurkat cells, but strong binding to the apoptotic and the early necrotic cells, and the strongest binding to the late necrotic cells. These results suggested that the cellular ligands of DEC205 were gradually exposed as the apoptotic process continued until the stage of necrosis.

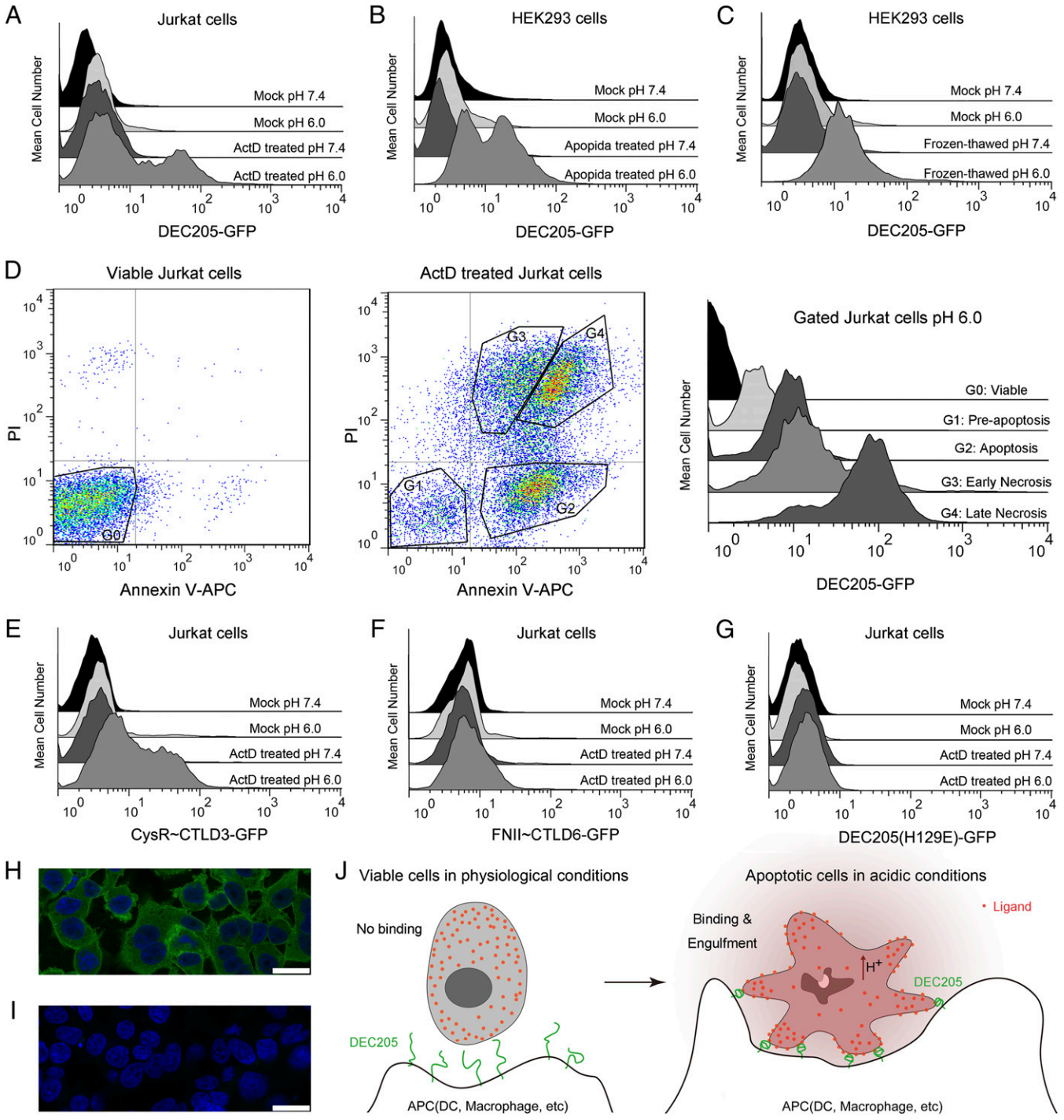

Fig. 4. The pH-dependent recognition of apoptotic and necrotic cells by DEC205. (*A*) DEC205-GFP binds to the ActD-treated Jurkat cells at acidic pH. (*B*) DEC205-GFP binds to the Apopida-treated HEK293 cells at acidic pH. (*C*) DEC205-GFP binds to the frozen-thawed HEK293 cells at acidic pH. (*D*) Triple staining of the viable Jurkat cells (*Left*) and the ActD-treated Jurkat cells (*Middle*) by Annexin V-APC, PI, and DEC205-GFP at acidic pH. The binding of DEC205 to the gated subsets of the viable and the ActD-treated Jurkat cells are shown on the right. (*E*) CysR~CTLD3-GFP (the small ring) binds to the ActD-treated Jurkat cells at acidic conditions. (*F*) FNII~CTLD6-GFP (the large ring) does not bind to the ActD-treated Jurkat cells at either acidic or basic conditions. (*G*) DEC205 (H129E)-GFP does not bind to the ActD-treated Jurkat cells at either acidic or basic conditions. (*H*) The fixed and permeabilized HEK293 cells stained by DEC205-GFP and DAPI at pH 6.0 by confocal microscopy. (*I*) The fixed and permeabilized HEK293 cells stained by DEC205-GFP and DAPI at pH 7.4 by confocal microscopy. (Scale bars, 25 μm.) (*J*) A cartoon representation of the pH-dependent recognition of apoptotic cells by DEC205.

The truncation mutants of DEC205, including the small ring fragment (CysR to CTLD3) and the large ring fragment (FNII to CTLD6) (Fig. 3), were also used for cell staining. The results showed that the small ring bound only to the apoptotic and necrotic cells at acidic pH and had no binding to healthy cells (Fig. 4*E*), similar to the results obtained from the intact DEC205 ectodomain (Fig. 4*A*). In contrast, the large ring fragment showed no binding to both ActD-treated and untreated cells at either acidic or basic pH (Fig. 4*F*). Furthermore, the H129E mutant of DEC205 was also tested for staining and showed no binding activity to both treated and untreated cells at either acidic or basic conditions (Fig. 4*G*). These data together demonstrated that the small ring of DEC205 was required and sufficient for recognizing apoptotic and necrotic cells, and the binding and release of ligand were correlated with the pH-dependent conformational change. To visualize cell staining by DEC205, fixed and permeabilized HEK293 cells were stained with DEC205-GFP and imaged by confocal microscopy at both pH 6 and pH 8. The images showed that DEC205 stained most of the cellular regions except nucleus at pH 6 (Fig. 4*H*), but at pH 8, DEC205-GFP showed only background level of cell staining (Fig. 4*I*), suggesting DEC205 only recognized cellular components at acidic conditions.

Discussion

Similar to other mannose receptor family members, DEC205 has a relatively large ectodomain that contains 12 domains; however, instead of having a flexible conformation, the cryoEM structure of DEC205 shows a rather compact double ring-shaped conformation at acidic pH. Similar conformation has been found for FcRY (25), another member of mannose receptor family, and it may also be true for the mannose receptor, according to the negatively stained EM results (24). It is noteworthy that this unique conformational feature has not been found outside this family, suggesting a common ancestor shared by the family members during evolution.

The high-resolution structural determination of the mannose receptor family members is not successful up to date, which could be because of the internal flexibility of the molecule; for example, the CTLD domains usually have large flexible loops, which would reduce the reconstruction resolution. Nevertheless, the 14.6-Å-resolution structure of DEC205 obtained by cryoEM is able to differentiate the individual domains of DEC205 clearly (Fig. 2), thus locating the interacting interfaces among these domains. The head of DEC205 contains two ring-shaped structures. The small ring is formed by CysR, FNII, and CTLD1~CTLD3, whereas the large ring is formed by FNII and CTLD1~CTLD6. The tail starts from CTLD7 to CTLD10. Consistent with the protease digestion results of the mannose receptor (29), the EM reconstruction shows that there are three tightly associated groups of CTLDs in DEC205, including CTLD1~CTLD2, CTLD4~CTLD5, and CTLD8~CTLD10, which may act as a scaffold for maintaining the double-ringed conformation (Fig. 1*A*). The domains that are loosely associated with neighboring domains such as CTLD3 and CTLD6 may have more flexibility in locating their binding partners. Indeed, the small ring is formed by the interaction between CTLD3 and CysR, and the large ring is formed by CTLD6 interacting with FNII. Similar domain arrangements and intramolecular interactions have been found in the EM structures of other mannose receptor family members (24, 25).

The pH-dependent conformational change has been observed for FcRY (23) and mannose receptor (24), suggesting pH dependence might be a conserved feature for the family. However, the mechanism and the residues involved in the conformational changes have not been identified in both cases. The mutagenesis studies of DEC205 show that both the small ring and the large ring undergo conformational changes as pH changes, resulting in a linear conformation at basic or physiological pH (~7.4). The cell binding results indicate that only the small ring is involved in ligand binding at its closed state; therefore, the opening and closing of the large ring may act as a facilitating factor for the conformational change of the small ring. It is not surprising that a histidine (His129) is identified as an important residue for the conformational change of the small ring and ligand binding. However, whether histidine residues are directly involved in ligand recognition remains unclear.

It seems unexpected that DEC205 is activated for apoptotic cell recognition at acidic pH, rather than at physiological pH. In fact, the intracellular acidification has been found to be common for apoptotic cells and occurs as an early event of apoptosis (30–32). As apoptosis proceeds, the extracellular environment around the apoptotic cells may also be acidified; for example, through hydrogen exchangers (33) or when cell membrane starts leaking. The acidification can then induce the conformational change of DEC205 and make it ready for ligand binding (Fig. 4*J*). The requirements for both acidification and ligand exposure increase the selectivity of DEC205 for target recognition. Unfortunately, the natural ligand or ligands of DEC205 still remain unknown. Nevertheless, the finding of the pH-dependent binding characteristic of DEC205 would help ligand identification in the future.

The extracellular acidification is usually associated with inflammation and tumorigenesis and treated as a "danger signal" by immune system (34, 35). For dendritic cells, extracellular acidification can affect their maturation and differentiation, especially antigen uptake and presentation (36). It has been shown that the antigen uptake of dendritic cells at pH 6.5 is almost 10-fold higher than at pH 7.3 (37), suggesting more receptors are activated at acidic pH, which is well consistent with the finding of DEC205.

To date, a number of receptors of phagocytes such as CD14 (38), CD36 (39), and integrin (40) are identified to be involved in removing apoptotic cells. The phagocyte receptors bind to dead cells through a variety of cell surface markers (3). For example, PtdSerR recognizes phosphatidylserine on the membrane of apoptotic cells (41). LRP of engulfing cells recognizes calreticulin on apoptotic cell surface for clearance (42). Clec9A recognizes actin filaments of damaged cells (43–45). However, none of these receptors has been shown to have pH-dependent activities. Therefore, the pH-dependent recognition of apoptotic and necrotic cells by DEC205 represents a novel mechanism for the immune system to regulate cell clearance and immune responses. Considering the high expression level of DEC205 on dendritic cells, DEC205 might be involved in routine screening over cells, detecting apoptosis at early stages by sensing pH changes and ligand exposure and triggering phagocytosis. Alternatively, DEC205 may also be able to access the internalized dead cells or fragments at phagosome and be activated under the acidic environment for antigen binding and presentation.

Unlike normal cells, tumor cells have relatively higher intracellular pH and lower extracellular pH, which could facilitate their proliferation, apoptosis evasion, and extracellular matrix remodeling for invasion (46). Therefore, the pH-dependent activities of DEC205 may give it advantages to target tumors specifically for immune recognition and clearance. Because DEC205 has been used for tumor vaccine generation (17, 18), a combination of the pH-dependent feature of DEC205 with its high antigen presentation efficiency may provide more potential to the immune therapies against tumors.

Materials and Methods

HEK293F cells were cultured with HyClone SFM4 HEK293 medium (HyClone Laboratories, Inc.) supplemented with penicillin and streptomycin. Five hours before transfection, the cells were collected by centrifugation and cultured in suspension with FreeStyle 293 expression medium (Gibco, Inc.) at 1×10^6 cells/mL for transfection.

Further experimental details can be found in *SI Materials and Methods*.

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