Immature Dendritic Cells Phagocytose Apoptotic Cells via $\alpha_v\beta_5$ and CD36, and Cross-present Antigens to Cytotoxic T Lymphocytes

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Summary

Dendritic cells, but not macrophages, efficiently phagocytose apoptotic cells and cross-present viral, tumor, and self-antigens to CD8$^+$ T cells. This in vitro pathway corresponds to the in vivo phenomena of cross-priming and cross-tolerance. Here, we demonstrate that phagocytosis of apoptotic cells is restricted to the immature stage of dendritic cell (DC) development, and that this process is accompanied by the expression of a unique profile of receptors, in particular the $\alpha_v\beta_5$ integrin and CD36. Upon maturation, these receptors and, in turn, the phagocytic capacity of DCs are downmodulated. Macrophages engulf apoptotic cells more efficiently than DCs, and although they express many receptors that mediate this uptake, they lack the $\alpha_v\beta_5$ integrin. Furthermore, in contrast to DCs, macrophages fail to cross-present antigenic material contained within the engulfed apoptotic cells. Thus, DCs use unique pathways for the phagocytosis, processing, and presentation of antigen derived from apoptotic cells on class I major histocompatibility complex. We suggest that the $\alpha_v\beta_5$ integrin plays a critical role in the trafficking of exogenous antigen by immature DCs in this cross-priming pathway.

Key words: dendritic cells • phagocytosis • CD36 • integrins • cross-presentation

Abbreviations used in this paper: Ann V, Annexin V; DC, dendritic cell; MCM, monocyte-conditioned medium; PI, propidium iodide; PS, phosphatidylserine.
Materials and Methods

Media. RPMI 1640 supplemented with 20 μg/ml of gentamicin (GIBCO BRL, Gaithersburg, MD), 10 mM 1-hepes (Mediatech, Herndon, VA), and either 1% human plasma, 5% pooled human serum (c-Six Diagnostics, M equn, WI), or 5% single donor human serum was used for DC preparation, cell isolation, and culture conditions (18, 24).

Preparation of cells. PBMCs, DCs, macrophages, and T cells were prepared as previously described (18, 19, 24). In brief, peripheral blood was obtained from normal donors in heparinized syringes and PBMCs were isolated by sedimentation over Ficoll-Hypaque (Amersham Pharmacia Biotech, Piscatway, NJ). T cell–enriched and T cell–depleted fractions were prepared by rosetting with neuraminidase-treated sheep red blood cells (24). Immature DCs were prepared from the T cell–depleted fraction by culturing cells in the presence of GM-CSF and IL-4 for 7 d.

1,000 U/ml of GM-CSF (Immunex Corp., Seattle, WA) and 500–1,000 U/ml of IL-4 (Schering-Plough Corp., Kenilworth, NJ) were added to the cultures on days 0, 2, and 4. To generate mature DCs, the cultures were transferred to fresh wells on day 7 and MCM was added for an additional 3–4 d (18, 19). At day 7, >95% of the cells were CD14–CD83–HLA-DR–DCs. On days 10–11, 80–100% of the cells were of the mature CD14+CD83+ HLA-DR+ phenotype. FACSort® (Becton Dickinson, San Jose, CA) was used to generate highly pure populations of immature and mature DCs, based on their CD83– and CD83+ phenotypes, respectively. Macrophages were isolated from T cell–depleted fractions by plastic adherence for 1 h. After 24 h, cells were removed from the plates and placed in Teflon beakers for 3–9 d. T cells were further purified from the T cell–enriched fraction by removing contaminating monocytes, NK cells, and B cells (24).

Antibodies. Antibodies to the following proteins were used: CD85b, CD14–PE, CD83–PE, HLA-DR–PE, HLA-DR–biotin (Becton Dickinson), IgG 2b (clone 6603001; Coulter Corp., Hialeah, FL), CD8 (CRL 8014; American Type Culture Collection, Rockville, MD), CD83 (clone HB15a; Coulter Corp.), HMC I (W6/32, ATCC clone H975), CD36 (clone FA6; obtained from the fifth international workshop on leucocyte differentiation antigens, α5, (clone CLB-706, Chemicon International, Inc., Temecula, CA; clone 69.65, Coulter Corp.), β1, (clone 658; Chemicon International, Inc.), β2, (clone SS21, Coulter Corp.; clone RUU-PL 7F12, Becton Dickinson), β3, (clone BS-5F2; Upstate Biotechnology, Inc., Lake Placid, NY), αβ, (clone 23C6; Pharmingen, San Diego, CA), αβ, (clone P1F6; Chemicon International, Inc.), CD71 (Dako Corp., Carpinteria, CA), mannose receptor (clone 3.2P1; a gift from A. Lanzavecchia, Basel Institute, Sweden), and influenza nucleoprotein (ATCC clone HB85).

Induction of Apoptotic Death. Monocytes were infected with influenza virus in serum-free RPMI. These cells undergo viral-induced apoptotic death within 6–8 h. Cell death was confirmed using the Early Apoptosis Detection Kit (Kajima Biomedical Co., Seattle, WA) [7]. As previously described, cells were stained with Annexin V–FITC (Ann V) and propidium iodide (PI). Early apoptosis is defined by Ann V+/PI– staining as determined by FACScan® (Becton Dickinson). To ensure that we were studying the uptake of early apoptotic cells, the kinetics of death were carefully worked out. After infection, monocytes were stained with Annexin V and propidium iodide and the kinetics of death were determined using flow cytometry. The majority of the monocyte population included trypan blue into the cytoplasm, an indicator of secondary necrosis (Albert, M.L., and N. Bhardwaj, unpublished data, references 25, 26). Hela cells were triggered to undergo apoptosis using a 60 UVB lamp (Derma Control Inc.), calibrated to provide 2 mJ/cm2/s. The kinetics of cell death in these cells has been defined previously (4).

Phagocytosis of A Poptotic Cells. Monocytes and Hela cells were dyed red using PKH26-GL (Sigma Biosciences, St. Louis, MO), and induced to undergo apoptosis by influenza infection and UVB irradiation, respectively. After 6–8 h, allowing time for the cells to undergo apoptosis, they were cocultured with phagocytes that were dyed green using PKH67-GL (Sigma Biosciences), at a ratio of 1:1. Macrophages were used 3–6 d after isolation from peripheral blood; immature DCs were used on days 6–7 of culture and mature DCs were used on days 10–11. Where direct comparison of cells was needed, cells were prepared from the same donor on different days. In blocking experiments, the immature DCs were preincubated in the presence of 50 μg/ml of various mAbs for 30 min before the establishment of cocultures. After 45–120 min, FACScan® analysis was performed and double positive cells were enumerated.

Phagocytosis of Latex Beads. Immature DCs were preincubated at 37°C with mAbs specific for α5, and α5β3. 105 cells were then cultured with 5 × 105 red fluorescent microspheres (diameter 1 μm, 2.5% solids, carboxylate-modified latex; Sigma Chemical Co.) for varying periods of time. Alternatively, the cells were maintained at 4°C. At the end of the assay, cells were separated from unengulfed beads by density gradient centrifugation and analyzed by FACScan® analysis (27).

Immunofluorescence. Cells were adhered to Alcian blue (Sigma Chemical Co.)–treated coverslips and fixed using 100% acetone. Cells were stained with antiinfluenza nucleoprotein antibody (HB85; American Type Culture Collection) and Texas red conjugated goat anti–mouse IgG (Jackson ImmunoResearch Labs., Inc., West Grove, PA). This was followed by staining with biotinylated anti–HLA-DR (Becton Dickinson) followed by FITC–conjugated streptavidin (Jackson ImmunoResearch). Cells were visualized using a Zeiss Axioplan2 microscope (Carl Zeiss, Inc., Thornwood, NY).

A say for cross–priming of A poptotic cells. Various APC populations were prepared from HLA-A2.1+ donors as described above. Mature DCs were further purified by labeling with the DC–restricted marker CD83, followed by cell sorting on the FACSort®. Immature DCs were CD14+ and sorted by FACSort® as a CD83− population. Mature macrophages were generated by culturing an adherent mononuclear cell fraction in a Teflon beaker for 9 d. These APC populations were cocultured with HLA-A2.1– influenza–infected monocytes. After 12 h, the APCs were loaded with Na2CrO4 and used as targets for influenza–specific CTLs in an standard 51Cr–release assay (7, 24). Specific lysis indicates that the APC had cross–presented antigenic material derived from the apoptotic cell, leading to the formation of specific peptide–MHC class I complexes on its surface. Specific lysis = (% killing of APCs cross–presenting influenza–infected monocytes) – (% killing of uninfected APCs); Background lysis ranged from 0 to 8%. Controls included influenza–infected and uninfected mature DCs, immature DCs, and macrophages. The HLA–A2.1– monocytes used as a source of apoptotic material were also tested as targets to demonstrate the absence of lysis when using a mismatched target. For the control targets, specific lysis = (% killing of influenza–infected APCs) – (% killing of uninfected APCs). Background lysis ranged from 0 to 5%. Maximal influenza–specific killing was determined using T2 cells (a TAP−/− HLA–A2.1+ class II–cell line) pulsed for 1 h with 1 μM of the immunodominant influenza matrix peptide, GILGFVFTL, as targets (28). Responses varied from 25 to 60% as a function of the individual’s prior exposure to influenza.
Reverse Transcriptase PCR. RNA was purified from highly purified sorted cell populations of immature and mature DCs as described above. Messenger RNA for β3, β5, and CD36 were identified using a one-step reverse transcriptase PCR reaction (Titan kit; Boehringer Mannheim, Indianapolis, IN). The forward primer 5'-TGAAGATGCGCCCTGCCC was used for both β3 and β5. The reverse primers 5'-CTTGGTCCTGGCCCTTTTCTT and 5'-CTTGGTAGTTGTGACCTTG were used for β3 and β5 to obtain 438- and 509-bp products, respectively (primer sequences were provided by S. Silletti, Scripps, San Diego, CA). The forward primer 5'-GGGAATTCATATGAAATCATAAAAGCAACAAACAT and the reverse primer 5'-CGGAATTCTACATTTCACTTCTCATTTTCTG for CD36 yielded a product of 392 bp (29). The reverse transcriptase reaction was carried out for 30 min at 56°C followed by 30 cycles of amplification. After 30 cycles of PCR the samples were visualized on an agarose gel.

Results

Immature DCs Efficiently Phagocytose Apoptotic Cells. Based on previous observations that immature DCs are the cells responsible for capturing antigen (11), we predicted that apoptotic cells would be engulfed best by immature DCs. To test this hypothesis, we established a phagocytosis assay that allowed us to visually detect the uptake of apoptotic cells, and compare the phagocytic capacity of immature DCs, mature DCs, and macrophages. In brief, immature DCs were prepared by culturing a T cell–depleted fraction from peripheral blood in the presence of IL-4 and GM-CSF. Mature DCs were generated with the addition of MCM and these cells expressed the cell surface DC-restricted maturation marker CD83 (18, 19, 30). Macrophages were prepared by culturing a plastic adherent cell population in Teflon beakers for 3–9 d. As a source of apoptotic cells, we used influenza-infected monocytes (7); virus infection induces apoptotic death in these cells within 6–10 h (7, 25, 26). Monocytes were first infected with influenza virus as

Figure 1. Immature but not mature DCs efficiently phagocytose apoptotic cells. Freshly isolated blood monocytes were infected with live influenza A, PR/8 (Spafas Inc., Storrs, CT), labeled with the PKH26-GL fluorescent cell linker compound (Sigma Biosciences), and incubated at 37°C for 6–8 h, allowing apoptosis to occur. Macrophages, immature DCs, and mature DCs were dyed with PKH67-GL and added to the culture wells containing the apoptotic monocytes at a ratio of 1:1. Cells were analyzed by FACScan® where double positive cells indicate uptake of the apoptotic cells by the various APCs (iii, vi, and ix). We used the various APCs alone to establish the proper settings (i, iv, and vii). Note that as the forward scatter of the APCs increased, the dying monocytes were excluded from the established region (ii, v, and viii). After 2 h, 80% of the macrophages, 50% of the immature DCs, and <10% of the mature DCs had engulfed the apoptotic monocytes (A). In an independent experiment, macrophages (squares), immature DCs (diamonds), and mature DCs (circles) were prepared, and cocultures with apoptotic monocytes were established as described above. FACScan® was performed at various time points. Percentage of phagocytosis was calculated based on the number of double positive cells (B).

Figure 2. Low temperature, Cytochalasin D, and EDTA block phagocytosis of apoptotic cells by immature DCs. Apoptotic monocytes and immature DCs were prepared as described above. Immature DCs were preincubated at 4°C (A) in the presence of varying concentrations of Cytochalasin D (B) or EDTA (C) for 30 min. Apoptotic monocytes were then added to the DC cultures at 4°C (A) or 37°C (B and C). FACScan® analysis was performed after 1–2 h. Data shown are representative of five independent experiments in which influenza-infected monocytes or UVB-irradiated HeLa cells were sources of apoptotic food for the immature DCs. Percentages of inhibition ± SD for these experiments were: 4°C, 85 ± 7%; 10 μM Cytochalasin D, 69 ± 3%; and 2 mM EDTA, 76 ± 14%.
previously described (24), then dyed red using PKH-26 (Sigma Biosciences). After 6-8 h, the various APCs were dyed green using the fluorescent cell linker compound PKH67-Gl (Sigma Biosciences) and cocultured with the apoptotic cells at a ratio of 1:1. After 2 h at 37°C, cocultures of cells were analyzed by FACScan® analysis, allowing for quantification of phagocytic uptake as double positive cells. 80% of the macrophages, 50% of the immature DCs, and <10% of the mature DCs engulfed the apoptotic monocytes after 2 h of coculture (Fig. 1 A). The smear of double positive cells (PKH67-labeled APCs that engulfed the PKH26-labeled apoptotic cells) indicates that both apoptotic bodies and whole apoptotic cells served as ‘food’ for the phagocytic cell (Fig. 1, iii, vi, and ix). Note that as the forward scatter of the APCs increased and the setting of the FACS® shifted, the dying monocytes were excluded from the established region (Fig. 1, ii, v, and viii). Maximal uptake by all APC populations was achieved within 2-4 h and partially depended upon the source of apoptotic cell used (Fig. 1 B and data not shown). Given this kinetic data, we believe that macrophages and DCs engage and internalize dying cells while still displaying features of early apoptotic cell death. This data also demonstrates that it is the immature DC that preferentially acquires apoptotic material compared with the mature DC. The source of apoptotic cells was not critical, since we obtained similar results with UVB-irradiated HeLa cells (see Fig. 7, and data not shown). Given that maximal uptake of apoptotic cells by immature DCs occurs between 2 and 4 h (Fig. 1 D), we believe that cross-presentation of apoptotic material underlies the DC underlying the apoptotic cell can be seen.

Although mature DCs were efficient targets when infected with influenza, they were unable to cross-present antigens, presumably because they had downregulated the ability to phagocytose the apoptotic monocytes. However, the immature DCs did cross-present antigens from apoptotic cells. Furthermore, if the immature DCs were cocultured with the apoptotic cells in the presence of MCM, a maturation stimulus, they were even better targets. This is possibly due to the upregulation of costimulator and adhesion molecules (13, 31), or to the increased stability of peptide–MHC I complexes. Given that maximal uptake of apoptotic cells by immature DCs occurs between 2 and 4 h (Fig. 1 D), we believe that cross-presentation of apoptotic material reflects the phagocytosis and processing of early apoptotic cells rather than secondary necrotic cells (see Materials and Methods). With respect to this issue, it is important to recognize that the influenza-infected monocytes require 24 h to undergo secondary necrosis (Albert, M.L., and N. Bhardwaj, unpublished data; references 25, 26).
Notably, macrophages that efficiently phagocytose apoptotic cells (Fig. 1 A) did not cross-present antigens to CTLs (Fig. 4 B). Presumably, the engulfed material is degraded, not cross-presented, on HMC 1. This profound difference between the DC and macrophage populations is supported by our previous findings that macrophages do not cross-present antigens from apoptotic cells during the induction phase of class I–restricted antigen-specific T cell response. In fact, when put into culture with DCs in a competition assay, they sequester the apoptotic material and abrogate the CTL response (7).

Immature DCs can be distinguished from macrophages by intracellular expression of CD83 and a unique profile of phagocytic receptors. We investigated the possibility that immature DCs might phagocytose apoptotic cells via pathways distinct from macrophages. To clearly distinguish these cells, we characterized them phenotypically. Immature DCs are distinguished by the absence of both CD14, a macrophage restricted marker, and CD83, a maturation marker for DCs (30). We have extended the use of CD83, finding that immature DCs can be distinguished from both macrophages and mature DCs by their intracellular expression of CD83. Macrophages do not express CD83 intracellularly, whereas mature DCs express CD83 both intracellularly and extracellularly (Fig. 5).

These APC populations were examined for surface expression of receptors involved in phagocytosing apoptotic material (Table 1). These include αvβ3 and CD36, which act as coreceptors for engulfment of apoptotic neutrophils and lymphocytes by macrophages (32, 33), and CD14, which has been implicated in the uptake of apoptotic cells by macrophages (34). While studying the immature DC populations, we identified a discrepancy in the expression of the αv and β3 integrin chains and investigated the possibility that αv was binding an alternate β chain. Using antibodies that recognize combined epitopes of the αvβ3 and the αvβ2 heterodimers, we noted the selective expression of αvβ5 on immature DCs (Fig. 6 A). As is true for most receptors involved in antigen uptake (11, 12), the expression of CD36, αvβ5, and mannose receptor on DCs is downregulated with maturation (Fig. 6 B, Table 1).

To evaluate whether this downregulation could be observed on the level of mRNA expression, we performed reverse transcriptase PCR using primers specific for β3, β5, and CD36 (Fig. 6 C). Immature DCs (lane 1) showed amplified DNA of the appropriate size for β3, β5, and CD36. In contrast, in mature DCs (lane 2), no β3 and much fewer CD36 sequences were seen, whereas β5 sequences were comparable to those in immature cells. These data, although not quantitative, are consistent with the levels of protein expression observed by FACS® and suggest that phagocytic receptor expression in DCs may be regulated at a transcriptional level as mRNA expression of CD36 and β3 is downregulated during maturation.

αvβ3 and CD36 mediate phagocytosis of apoptotic cells in immature DCs. To demonstrate a direct role for αvβ3 in the recognition of apoptotic cells by immature DCs, we performed the phagocytosis FACS® assay in the presence of...
antibodies specific for αvβ5 (Fig. 7 A). In addition to the blocking observed using the mAb to αvβ5, blocking was also detected when using mAbs to αvβ3, and CD36. Blocking was not observed when isotype-matched mAbs were specific for β1, β3, or the transferrin receptor C D71. Note that control antibodies chosen recognized surface receptors present on the immature DCs (Fig. 7 A, Table 1). mAbs were tested in doses ranging from 10 to 80 μg/ml (data not shown). Maximal inhibition of phagocytosis of apoptotic cells was seen with mAbs specific for CD36, αv, and β3 at 50 μg/ml. The inhibition of phagocytosis of apoptotic cells by DCs was specific. We were unable to block the uptake of red fluorescent latex beads, a control particle, by DCs in the presence of these mAbs (Fig. 7 B). By histogram analysis, DCs phagocytose 1–6 particles per cell. mAbs to αvβ3 or αv did not alter the profile of these histogram plots (data not shown).

Although some inhibition of phagocytosis was observed when using αvβ3 this may be due in part to transdominance and/or the effect on the pool of free αv (35). For example, anti-αvβ3 antibodies suppress the intracellular signaling of the αvβ3 integrin (36). Alternatively, αvβ3 and αvβ5 may be working cooperatively in the immature DCs. We therefore tested combinations of anti-αvβ3 and anti-αvβ5 but did not observe an increase in the inhibition of phagocytosis. The low receptor density of αvβ3 on DCs (average mean fluorescence intensity of 7 ± 2; Table 1) also makes it unlikely that this integrin heterodimer is involved in the engulfment of apoptotic cells by immature DCs.

Our data do not exclude a role for other receptors in the phagocytosis of apoptotic cells, e.g., the putative PS receptor or the lectin receptor (5). In fact, other receptors are probably involved, as blocking observed did not exceed 60% even when combinations of all relevant mAbs were tested (data not shown). CD14 is unlikely to be involved in the engulfment of apoptotic cells, e.g., the putative PS receptor or the lectin receptor (5). In fact, other receptors are probably involved, as blocking observed did not exceed 60% even when combinations of all relevant mAbs were tested (data not shown). CD14 is unlikely to be involved in the engulfment of apoptotic cells by DCs, as DCs do not express this receptor (Table 1). In macrophages, phagocytosis of apoptotic cells was inhibited by antibodies to αv, β3, αvβ3, and CD36 but not by antibodies to β1, β3, or αvβ3 (data not shown). This correlates with published data (6, 33).

**Discussion**

Cross-presentation of antigens to CTLs appears to have two critical features: (a) it is mediated by DCs, and (b) apoptotic cells are the preferred source of antigen (7). The requisite stage of DC development for the acquisition of apoptotic cells is the immature phase. In fact, immature DCs are four to five times more efficient than mature DCs in phagocytosis, a feature that also correlates with their ability to cross-present antigen. This exogenous pathway for class I
MHC loading is highly effective: relatively few apoptotic cells (ratio of 1:10 DCs) are needed to charge the DCs as efficiently as the live replicating virus; exposure of 3–12 h is sufficient for generating a peptide–MHC complex that is capable of activating CTLs; and it is relatively indiscriminate, as the cellular source can be allogeneic or xenogeneic cells (7). We believe our earlier studies with mature DCs are explained by the fact that our cell populations were asynchronous and that only by sorting these cells have the differences become apparent. Based on the findings presented here, we suggest that the peripheral tissue DC, exemplified by the immature DC, has an additional important role. It is responsible for phagocytosing cells within tissues that undergo apoptosis (e.g., secondary to viral infection; during normal cell turnover) and migrating to the draining lymph nodes where appropriate T cells are engaged. This pathway may be used for stimulating or tolerizing CTLs and can account for the in vivo observations of cross-priming of tumor and viral antigens (9, 37) and cross-tolerance of self-proteins (10, 38) in their requirement for a bone marrow–derived APC.

A sharp distinction was also demonstrated between immature DCs and macrophages in the handling of apoptotic material. Although macrophages are more efficient at phagocytosing apoptotic cells than immature DCs, they fail to induce virus-specific CTLs (7). In fact, they cannot even generate effective levels of peptide–MHC I complexes. In a short-term assay, influenza-specific CTLs could not kill macrophages cocultured with apoptotic cells. Therefore, macrophages degrade rather than cross-present the ingested apoptotic cells. Our findings probably do not conflict with the report of Bellone et al. (39), as their ‘macrophages’ were prepared from bone marrow–derived precursors by culturing the cells in GM-CSF for 7 d. This method is tradi-
tionally used to generate DCs from bone marrow (40). We believe that contaminating DCs account for the cross-presentation observed in their studies.

Additional evidence exists that macrophages process apoptotic cells differently from DCs and prevent an immune response. Two groups have demonstrated that phagocytosis of apoptotic cells suppresses a subsequent inflammatory response to LPS stimulation. The macrophage's cytokine profile is skewed toward the synthesis of IL-10, IL-13, and TGF-β, whereas the production of proinflammatory cytokines such as TNF-α, IL-1β, and IL-12 is downmodulated (41, 42). Therefore, the resolution of inflammation is dependent on at least two pathways for removal of apoptotic cells: via (a) macrophages, which subvert and suppress proinflammatory responses, and (b) DCs, which stimulate T cell responses that clear pathogens responsible for the induction of the apoptotic death.

The αvβ3 integrin receptor may be pivotal in the distinctive handling of apoptotic cells by immature DCs versus macrophages, in that its expression is restricted to the former. We suggest that the unique profile of receptors expressed by immature DCs affects trafficking of phagocytosed apoptotic cells, and consequently facilitates cross-presentation. We have previously shown that NH4Cl inhibits the ability of DCs to process antigen derived from apoptotic cells, suggesting that processing in an acidic vesicle (e.g., CIIVs or MIIICs) is required (7). Indeed, class I MHC may interact with processed antigens in such a compartment, as MHC I molecules have been described in association with invariant chain (43) and can recycle from the cell surface to class II vesicles (44). Additionally, there may be as yet undescribed routes whereby antigens within vesicles can enter the classical endogenous pathway as described recently for antigens derived from the endoplasmic reticulum (45).

In contrast to our studies, Rubartelli et al. (46) have shown that immature DCs express high levels of the αvβ3 integrin but lack CD36. Their observations are hard to reconcile with data that indicates that αvβ3 and CD36 are both required for the engulfment of apoptotic cells by macrophages (33, 47). Furthermore, although they demonstrated inhibition of phagocytosis by DCs with anti-αv antibodies, they did not present phagocytosis data relevant to β3 or the αvβ3 heterodimer. However, it is possible that a different process is being studied, as phagocytosis in their hands is dependent on late stage Ann V+PI+ apoptotic cells. Here, we discuss data relevant to the phagocytosis of early apoptotic cells with intact plasma membranes.

αvβ3 and αvβ5 have both been described as important in angiogenesis, cell adhesion, migration, and now in their ability to phagocytose apoptotic cells. αvβ3 is critical in the phagocytosis of apoptotic cells in macrophages, where it acts in a cooperative way with CD36 and thrombospondin, collectively forming a ‘molecular bridge’ (47). Recently, it was reported that αvβ3 but not αvβ5 is critical for the engulfment of rod outer segments by CD36+ retinal pigment epithelial cells (48, 49). This phagocytic system is also inhibited by anti-CD36 antibodies, suggesting that αvβ3, like αvβ3, might cooperate with CD36. Taken together with our observations, thrombospondin, or possibly other soluble factors, may serve to bridge CD36, αvβ3, and the apoptotic cell.

Although similarities in function exist, αvβ3 can be distinguished from αvβ5 in its use of various ligands (e.g., vascular endothelial growth factor [VEGF] vs. basic fibroblast growth factor [bFGF]), by the requirements for activation, and by the intracellular signaling pathways (e.g., indirect activation of protein kinase C) (50). Also significant is the fact that the cytoplasmic domains of the two β chains are the portions that show the most considerable diversity (51). Thus, it is possible that the distinct use of the αvβ3 versus αvβ5 integrin receptors might account for the specialized functions of DCs in the route by which apoptotic material is trafficked and presented. In other words, differential expression of αvβ3 may be responsible for the ability of DCs to cross-present antigenic material derived from apoptotic cells, whereas macrophages scavenge and degrade such material.
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