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Reaching for far-flung antigen: How solid-core podosomes of dendritic cells transform into protrusive structures

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Abbreviations: APC, antigen-presenting cell; DC, dendritic cell; ECM, extracellular matrix; MHC, major histocompatibility complex; MMP14 (MT1-MMP), matrix metalloproteinase-14; PRR, pathogen recognition receptor.

We recently identified a novel role for podosomes in antigen sampling. Podosomes are dynamic cellular structures that consist of point-like concentrations of actin surrounded by integrins and adaptor proteins such as vinculin and talin. Podosomes establish cellular contact with the extracellular matrix (ECM) and facilitate cell migration via ECM degradation. In our recent paper, we studied podosomes of human dendritic cells (DCs), major antigen presenting cells (APC) that take-up, process, and present foreign antigen to naive T-cells. We employed gelatin-impregnated porous polycarbonate filters to demonstrate that the mechanosensitive podosomes of DCs selectively localize to regions of low-physical resistance such as the filter pores. After degradation of the gelatin, podosomes increasingly protrude into the lumen of these pores. These protrusive podosome-derived structures contain several endocytic and early endosomal markers such as clathrin, Rab5, and VAMP3, and, surprisingly, also contain C-type lectins, a type of pathogen recognition receptors (PRRs). Finally, we performed functional uptake experiments to demonstrate that these PRRs facilitate uptake of antigen from the opposite side of the filter. Our data provide mechanistic insight in how dendritic cells sample for antigen across epithelial barriers for instance from the lumen of the lung and gut.

Dendritic cells (DCs) are antigen-presenting cells (APCs) ubiquitously present in all parts of the human body and constantly sample for antigens via PRRs on their surface.1-3 DCs constitute the link between the innate and adaptive immune systems, because they are the only cells that can induce a primary immune response in naive T-lymphocytes.4 In order to perform this function, DCs have to migrate between their sites of origin (bone marrow), sites of sampling activity and lymph nodes where T-cells are activated by the antigens presented by major histocompatibility complex (MHC) molecules on the surface of the DCs. DCs are thus a very motile cell type, that travel inside the body not only passively within the blood stream, but can also “crawl” between cells thereby reaching almost any part of the body within a relatively short time. For this crawling, DCs need to adhere to the ECM and this adhesion is facilitated by podosomes.5-9

Podosomes are cellular structures that consist of dense actin cores surrounded by adaptor proteins such as vinculin, talin, and paxillin that connect the actin cytoskeleton to the membrane, regulatory proteins WASP, and Arp2/3 as well as integrins that allow cellular adhesion to the ECM.10-13 Podosomes are also points of local degradation of ECM, which is achieved by concentrated release of proteases such as MMP14 (also known as MT1-MMP).7,9,14-16 Podosomes are well-known to facilitate cell migration through endothelium, epithelium, and connective tissues.8,17,18 A more recently discovered fact is that podosomes are mechanosensitive and can sense the local stiffness of the substrate.19,20

In an elegant study, Gawden-Bone and coworkers demonstrated that when grown on porous polycarbonate filters, podosomes of dendritic cells can evolve into protrusive structures.16 Although these protrusive structures morphologically resemble invadopodia of cancer cells, they are more dynamic with shorter lifetimes and lower protrusion depths and, in contrast to invadopodia,2,21 they still depend on the protein WASP for their formation. Very similar to invadopodia, these protrusive podosome-derived structures turned out to contain stretches of tubulin which likely mediate trafficking of metalloproteinases to the protrusive...
tips for degradation of ECM. Indeed, in our recent paper we showed the presence of the metalloproteinase MMP14 at the tips of protrusive podosome-like structures of human monocyte-derived DCs by immunofluorescence. We recently confirmed this finding by overexpression of MMP14 tagged with the fluorescent protein mCherry (Fig. 1A). Here, we co-expressed the F-actin binding reporter protein LifeAct tag to GFP to visualize the protrusion of the actin cores into the filter pores.

Not only is MMP14 released at the tips of protrusive podosome-like structures, but endocytosis also occurs at these spots and Gawden-Bone et al. already demonstrated uptake of degraded gelatin. In agreement with this notion that when podosomes become invasive, membrane trafficking is increased at these sites, we showed the presence of several endocytic and endosomal markers at these protrusive structures. We first established the presence of clathrin at protrusive podosome-like structures by immunofluorescence and recently confirmed this by overexpression of GFP-tagged clathrin (Fig. 1B). Several overexpressed GFP-tagged versions of other markers for endocytic activity also located to these protrusive podosome-evolved structures: Rab5, Rab8, and VAMP3 (Fig. 1C–E). Localization was specific for these proteins, as free GFP and GFP-tagged VAMP7 did not or only little protrude into the filter pores. Rab5 and Rab8 are members of the Rab family of small GTPases, and are well-known regulators of intracellular membrane trafficking of early and recycling endosomes. VAMP3 is a soluble NSF attachment protein receptor (SNARE) that catalyzes membrane fusion of early endosomes and is also involved in the extension of pseudopods in phagocytosis. Although Rab5, Rab8, and VAMP3 clearly localized in vesicles in the cytosol, our resolution was insufficient to discern a vesicular localization of these proteins in protrusive podosome-derived structures. Nevertheless, our results show that protrusions are sites of membrane trafficking and corroborate with the study of Gawden-Bone et al.

Since Gawden-Bone et al. demonstrated that endocytosis occurs at protrusive podosome-like structures and since the main function of DCs is antigen presentation, it seemed logical to suggest that these protrusive structures might...
be involved in endocytosis of antigens. Indeed, protrusive podosome-evolved structures contain various PRRs that can recognize foreign antigens and our immunofluorescence data showed the localization of several members from the C-type lectin family: DC-SIGN (CD209; Fig. 2A), DCIR (CLEC4A), dectin-1 (CLEC7A), and the mannose receptor (CD206). Further investigation by transmission electron microscopy and immunogold labeling confirmed that DC-SIGN and CD206 were present on the protrusive structures (Fig. 2B–D for CD206).

We then established that the PRRs residing at the protrusive podosome-like structures indeed were capable to sample for foreign antigen. We designed a functional assay where DCs were cultured on gelatin-impregnated filters and where fluorescently labeled mock antigen specific for either DC-SIGN or CD206 was present at the opposite side of the filter. With this assay, the ability of dendritic cells to endocytose antigens exclusively via protrusive podosome-like structures could be tested. Control experiments were performed: 1) without mock antigen, 2) with filters containing too narrow pores for podosome protrusion (<1 μm), 3) competitive blockage of PRRs with mannose, 4) with the endocytic inhibitor Pitstop II, and 5) with the WASP inhibitor wiskostatin. These functional uptake experiments demonstrated the exclusive role of protrusive podosome-evolved structures for uptake of antigen through the filter pores. We were also able to show that antigen which was taken up via protrusive podosome-like structures could be actively processed in the cell and loaded onto MHC class II to finally be presented to T-cells.

Our study directly demonstrates a novel role for podosomes in antigen sampling (Fig. 3). Podosomes sense for spots of low physical resistance of the substrate where they exert mechanical forces and actively degrade the ECM. This process results in remodeling of the ECM and podosomes become increasingly invasive and start protruding in the extracellular environment. At some point, when the pores in the
extracellular environment reach beyond a threshold size of about 3 μm, these protrusive podosomes-derived structures facilitate migration of dendritic cells through ECM and across endothelial barriers. It is increasingly well established that podosomes thereby help DCs to rapidly migrate for instance to sites of infection in the body. In our study, we showed that protrusive podosome-derived structures are sampling sites that actively probe for antigen which would otherwise not be accessible because of its deeper localization within the substrate or across epithelial/endothelial membranes. In these cases, PRRs locating to the protrusive tips of podosomes-derived structures can trigger receptor-mediated uptake and this propels subsequent antigen degradation, MHC class II presentation and finally T-cell activation. Our findings provide a mechanistic understanding for the well-known capability of DCs to probe for antigens across epithelial barriers, for instance in the lumen of the gut, lungs and small intestine. Thereby, our data constitute a novel way of how DCs sample for antigen in the human body and this aids our understanding of the immune system.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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