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Pattern recognition receptors (PRRs) influence the formation and spatial organization of podosomes in murine dendritic cells *in vitro*

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INTRODUCTION & AIM

Pattern recognition receptors (PRRs) expressed by immune cells play a crucial role in sensing pathogen-associated molecular patterns (PAMPs), thereby initiating innate immune responses. As professional antigen-presenting cells, dendritic cells (DCs) regulate the pericellular environment, capture antigens, and present them to T lymphocytes following their migration to secondary lymphoid organs. The migration of immature DCs is facilitated by specialized adhesion structures, such as podosomes.

Since podosome assembly and disassembly are highly associated with immunoregulatory and migratory functions of DCs, we evaluated the effects of various PRR agonists on podosome organization in mouse bone marrow-derived DCs (BMDCs).

METHOD

Primary cultures of BMDCs were generated using recombinant mouse granulocyte-macrophage colony-stimulating factor (rmGM-CSF) and subsequently stimulated for 24 hours with selected PRR ligands, including TLR agonists (Pam2CSK4, Pam3CSK4, Poly I:C, LPS, flagellin, CpG ODN) and NOD agonists (C12-iE-DAP, MDP). Podosome types were morphologically classified as single, clustered or rosette structures and quantified in individual cells following immunofluorescent staining for F-actin and vinculin.

RESULTS

Our data demonstrate that PRR stimulation promotes podosome dissolution and significantly alters podosome organization (Figure 1, Figure 2)

1. In unstimulated control BMDCs, more than 85% of cells exhibited podosomes, predominantly clustered (70%), followed by rosettes (10%) and single podosomes (6%).
2. The most pronounced podosome disassembly was induced by LPS, resulting in over 80% of cells losing podosomes and an increased proportion of cells displaying single podosomes (11%). Similar effects were observed following stimulation with Pam2CSK4 and Pam3CSK4.
3. Weaker podosome-disrupting effects were noted with flagellin, C12-iE-DAP, MDP, and Poly I:C, where podosome-negative cells accounted for 60%, 42%, 40%, and 36%, respectively.

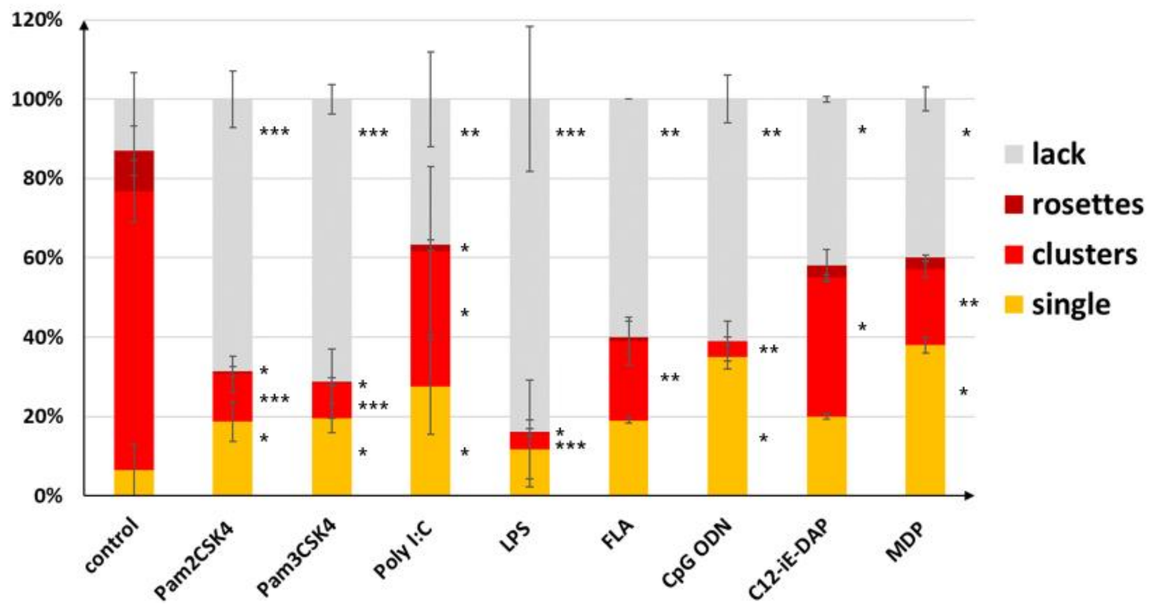

Figure 1. The mean percentage of cells with no podosomes or having single, clusters, or rosettes podosomes in unstimulated DCs and DCs stimulated with Pam2CSK4, Pam3CSK4, Poly I:C, LPS, flagellin, CpG ODN, and C12-iE-DAP and MDP 24 hours post stimulated. Asterisks [*] indicate statistically significant differences compared to control cells, as, determined using the Student's t-test: *p<0.05; **p<0.01; ***p<0.001.

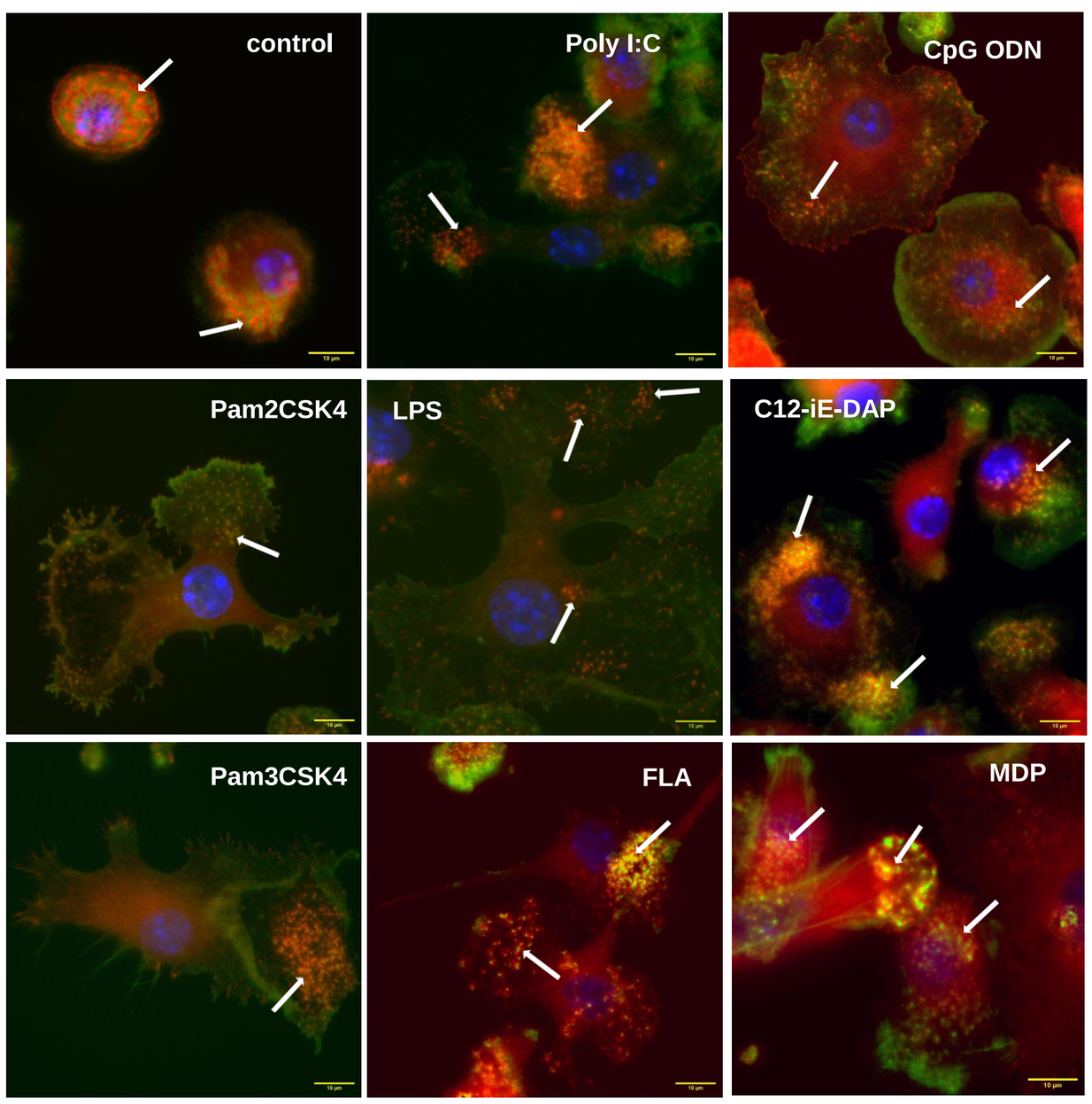

Figure 2. Podosomes in control DCs and DCs stimulated with Pam2CSK4, Pam3CSK4, Poly I:C, LPS, flagellin, CpG ODN, and C12-iE-DAP and MDP at 24 h. Vinculin (red fluorescence), actin (green fluorescence), and nuclear DNA (blue fluorescence). White arrows indicate podosomes. Scale bars: 10 μm.

REFERENCES

(1) Linder and Kopp (2005) Podosomes at a glance. Journal of Cell Science 118: 2079–2082. (2) Schachtner et al. (2013) Podosomes in adhesion, migration, mechanosensing and matrix remodeling. Cytoskeleton 70: 572–589

CONCLUSION

PRR stimulation influences podosome dynamics in BMDCs, suggesting a regulatory role in DC maturation and functional plasticity