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200

NEUTROPHIL-DERIVED MICROPARTICLES MODULATE MONOCYTE MIGRATION IN AN ENDOTHELIAL DEPENDENT MANNER

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Introduction Neutrophil depletion can delay atherogenesis and conversely increasing circulating neutrophils enhances plaque progression in mice. Lack of evidence for the presence of neutrophils in atherosclerotic plaques makes their role in disease progression less clear. Neutrophils may have a greater role in facilitating the movement of other cells into the vessel wall, possibly through the release of microparticles. The ability of microparticles to deliver cargo to target cells may be an explanation for neutrophil-specific markers being co-localised with endothelial cells in atherosclerotic plaques, despite the absence of neutrophils themselves.

Methods Neutrophils were incubated with PBS, fMLP (10–5 M), and acetylated LDL (AcLDL, 20 μg/ml) to stimulate microparticle formation. To remove residual fMLP, microparticle suspensions were dialysed. Microparticles were characterised and quantified using a standardised flow cytometry method. Their adhesion to human coronary artery endothelial cells (hCAEC) after incubation with anti-ICAM-1 or isotype control was quantified using flow cytometry. Internalisation was visualised using confocal microscopy. Microparticle binding to and activation of monocytes was also investigated using flow cytometry. Monocytes migration to CCL-2 in the presence of neutrophil-derived microparticles with and without hCAEC was also investigated.

Results Neutrophil-derived microparticles adhered to hCAEC in an ICAM-1 dependent manner and were internalised. Microparticles bound to monocytes regardless of stimuli, but their adhesion was lower than observed with hCAEC. Only microparticles from neutrophils stimulated with fMLP induced monocyte L-selectin shedding and significantly enhanced monocyte-hCAEC adhesion. Microparticles increased monocyte migration to CCL2 in an endothelial cell and CD18-dependent manner.

Conclusions Microparticles bind to ICAM-1 on the surface of hCAEC and are internalised. Monocyte migration is enhanced by neutrophil-derived microparticles only in the presence of endothelial cells. In conclusion, neutrophil-derived microparticles may play a role in activating hCAEC, potentially by their uptake, and thus amplify inflammatory responses at sites of atherosclerotic lesion development.

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