[PS1.5]

Antibacterial Effect of Subcellular Vesicles Derived of Neutrophilic Granulocytes

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Introduction

Subcellular vesicles (SCV) were shown to be involved in blood coagulation, antigen presentation or transfer of important cell constituents such as plasma membrane receptors or RNA. We investigated the biological functions of SCV derived of neutrophilic granulocytes (PMN).

Methods

PMN were prepared from the blood of healthy volunteers. After incubation with physiological or pharmacological stimuli cell-free material was separated by centrifugation. Isolated SCV were investigated by light and fluorescent microscopy, flow cytometry, Western bloting, proteomics and a bacterial killing assay. The amount of produced SCV was quantitated on the basis of the protein content.

Results

Production of SCV was observed upon stimulation with chemoattractants or phorbol ester, however these SCV had no significant effect on bacterial growth. Incubation of PMN with opsonized *S. aureus* produced a significantly higher amount of SCV that was able to impair bacterial growth when coincubated with fresh *S. aureus*.

Formation of antibacterial SCV depended on opsonization of the bacteria with full serum and on intact cytoskeleton of the PMN. It was inhibited both by azide and di-phenyl-iodonium whereas it was not affected by DNAse treatment or lack of glucose. PMN recovered after SCV production retained their morphological integrity, viability and physiological functions such as superoxide production and killing of fresh bacteria. Moreover, PMN were capable of repeated production of antibacterial SCV.

Antibacterial SCV are heterogenously sized (d= 200-1000nm) vesicles surrounded by phospholipid membrane. Proteomic analysis showed that they contain several granule proteins and cytoskeletal proteins, but they are devoid of subunits of the NADPH oxidase. The antibacterial effect of SCV did not require opsonization of the bacteria, it was saturable and it was inhibited by destruction of the membrane or the cytoskeletal structure.

Conclusion

PMN-derived SCV may represent a new extracellular mechanism that limits bacterial growth.

Keywords: neutrophilic granulocytes, subcellular vesicles, bacterial growth, complement activation