

Uptake of microparticles by B cells

with

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What are microparticles ?

Matthias Mack: Microparticles are small membrane vesicles that are spontaneously released from the plasma membrane of a variety of cell types. Shedding of microparticles can occur independently of apoptosis or cell death and seems to be part of a normal cellular function. Microparticles typically range in size from 0.1 to 2 µm and include cytoplasmic components and cell surface derived elements, such as membrane receptors. Microparticles are defined by their size and the presence of negatively charged phospholipids like phosphatidylserine in the outer membrane leaflet (1). In most cases stimulation

of cells increases the release of microparticles. The shedding of microparticles was first described from activated platelets and subsequently discovered in various haematopoietic and non-haematopoietic cells. Microparticles should be distinguished from exosomes that are not derived from the plasma membrane but have an endocytic origin (multivesicular endosomal bodies). Exosomes are smaller than microparticles, more homogeneous in size (30–90 nm) and enriched for certain membrane proteins like tetraspanins or MHC II molecules allowing them to stimulate CD4⁺ T cells (2,3).

Which cells can take up microparticles?

Matthias Mack: Transfer of microparticles has been described to endothelial cells, epithelial cells and leukocytes. The conditions that result in uptake of microparticles by different acceptor cells are not well defined. Uptake will depend on properties of the microparticles that are determined by their cells of origin and by modifications of microparticles after their release. In addition, the ability of the acceptor cell to interact with microparticles will also determine how efficiently microparticles are transferred.

Microparticles can carry soluble molecules as well as lipid membranes and transmembrane proteins from one cell to another. Thereby micro-

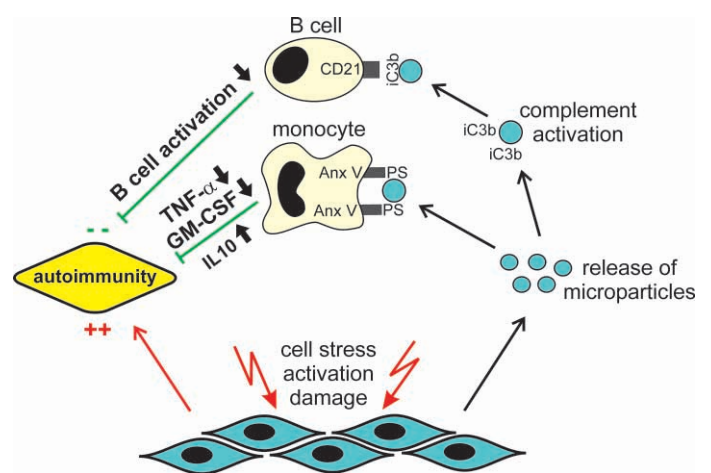
particles might function as vectors for intercellular communication independent of a direct cell-to-cell contact. We have shown previously that microparticles can transfer the transmembrane receptor CCR5 from one cell (e.g. CCR5⁺ CHO cell or leukocytes) to another cell (e.g. T cells, monocytes or endothelial cells). This transfer of CCR5 enables infection of previously CCR5 negative cells with m-tropic HIV-1 strains (4). To date it is unclear to which extent membrane fragments and transmembrane receptors are integrated in the plasma membrane of acceptor cells, to which extent microparticles are taken up by phagocytosis or simply adhere on the acceptor cells.

What mechanisms are involved in the uptake of microparticles by monocytes and B cells?

Matthias Mack: Incubation of microparticles derived from various epithelial cells with human PBMC results primarily in uptake of microparticles by monocytes. If human serum is present during this incubation microparticles are also efficiently taken up by B cells. The transfer of microparticles to monocytes is dependent on the presence of calcium and also requires an intact cytoskeleton, while B cells take up serum exposed microparticles independently of calcium or actin polymerization. Annexin V is also involved in the uptake of microparticles by monocytes, as antibodies against annexin V or FITC-/biotin modified annexin V interfere with the microparticle transfer to monocytes (5).

As microparticles are present in the peripheral blood and therefore exposed to plasma components, we analyzed how plasma or serum enables the uptake of microparticles by B cells. Heat inactivated serum completely lost the ability to mediate the

transfer of microparticles to B cells, pointing towards the involvement of complement components. Use of serum, depleted of distinct complement components, and re-addition of complement components demonstrated that the complement component C3 is essential for the transfer of microparticles to B cells. Microparticles are able to activate complement resulting in a deposition of iC3b on their surface. We further demonstrate that the complement receptor CR2 is crucially involved in the uptake of iC3b coated microparticles by B cells. Blockade of CR2 with a monoclonal antibody almost completely prevented the uptake of iC3b coated microparticles by B cells (5). In contrast, blockade of the complement receptor CR1 had no effect, indicating that either most of the C3 is converted on microparticles to iC3b or that the interaction of C3b and CR1 plays no role for uptake of microparticles by B cells.



Do you believe that microparticles can downregulate inflammation?

Matthias Mack: As microparticles are preferentially released during cell stress or activation we hypothesized that microparticles might be involved in downregulating inflammation during tissue injury, where an enhanced generation of microparticles might counterbalance endogenous and exogenous proinflammatory signals.

Transfer of microparticles to isolated monocytes clearly reduced the LPS induced release of pro-inflammatory cytokines, such as GM-CSF and TNF- α and increased the release of the anti-inflammatory cytokine IL-10. Induction of an anti-inflammatory phenotype in macrophages has extensively been analyzed with apoptotic cells (6). However, the inhibitory effect of microparticles on monocyte activation was even somewhat more pronounced than that of apoptotic cells. The influence

of microparticles on monocyte activation seems to be largely independent of the cellular source of microparticles, as microparticles derived from 3 different cell lines (Kato cells, CHO cells and human fibroblasts) were equally effective in altering the cytokine release from monocytes.

Transfer of iC3b opsonized microparticles to B cells markedly down-regulated the expression of MHC class II and CD86 by B cells under basal conditions. Moreover, the PMA-induced increase in B cell size and CD25 expression was significantly reduced after uptake of serum-treated microparticles. Again microparticles were at least as effective as apoptotic cells.

A summary of the proposed mechanisms of microparticle transfer and the biological effects of microparticles on monocytes and B cells is provided in the figure below.

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