ROLE OF TISSUE TRANSGLUTAMINASE IN PHAGOCYTOSIS OF APOPTOTIC CELLS BY MACROPHAGES

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The clearance of apoptotic cells by macrophages plays a crucial role in tissue repair, suppressing inflammation and regulating immune responses. Transglutaminase 2 (TG2) is a protein crosslinking enzyme with diverse biological functions. Among many others it acts as an integrin β_3 co-receptor. We have previously shown that in $TG2^{-/-}$ mice the *in vivo* clearance of apoptotic cells is defective leading to development of SLE like autoimmunity. This was partially related to a defect in TGF-β activation, as TGFβ released by macrophages digesting apoptotic cells promotes phagocytosis of apoptotic cells and inhibits inflammatory responses.

In the present work the role of TG2 was studied in details in the engulfment of apoptotic cells by macrophages. Here we report that TG2 promotes phagocytosis of apoptotic cells by acting on the macrophage cell surface in guanine nucleotide bound form. Besides being a binding partner for integrin β_3, a receptor known to mediate the uptake of apoptotic cells via activating Rac1, we also show that TG2 binds milk fat globule EGF-factor 8 (MFG-E8), a protein known to bridge integrin β_3 to phosphatidylserine on apoptotic cells. We report that in wild-type macrophages one or two engulfing portals are formed during phagocytosis of apoptotic cells that are characterized by accumulation of integrin β_3 and Rac1. In the absence of TG2, although the levels of integrin β_3 are enhanced, integrin β_3 and consequently Rac1 can not be concentrated and activated at one pole of the macrophage. The defect in the $\alpha_v\beta_3$ integrin signaling leads to an abnormal actin cytoskelatal organization and the efficient engulfing gate is not formed. Together, our data indicate that TG2 is a new protein member of the phagocytic cup, which together with MFG-E8 is required for proper apoptotic cell recognition and integrin β_3 signaling.

In the present study we also describe a subline of $TG2^{-/-}$ mice, in which a compensatory increase in integrin β_3 expression, which resulted alone in a high receptor concentration around the apoptotic cells without the requirement for accumulation, the elevated integrin β_3 levels were sufficient to overcome the defect caused by the loss of TG2 in the initiation phase of integrin β_3 signaling, but a significant accumulation of Rac1 around the apoptotic cells did not occur, and the phagocytosis of apoptotic cells was more severely

affected. The lack of Rac1 accumulation was partially related to a defect of PI-3-kinase activation. Our data provide a proof for the concept that the function of TG2 is to stabilize accumulated integrin β_3 concentration in the phagocytic cup.

Keywords: phagocytosis, tissue transglutaminase, integrin
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