Zhen Li
Dr. med.

Tagging of apoptotic cells by the β-chemokine CCL5/RANTES stimulates phagocytosis by macrophages

Geboren am 17.06.1974 in Hubei, V. R. China
Reifeprüfung am 07.07.1997
Studiengang der Fachrichtung Medizin vom WS 1992 bis SS 1997
Physikum und Klinisches Studium und Praktisches Jahr
an der Fourth Military Med. Universität in Shanxi
Diplomarbeit von 1997-2000 an der Fourth Military Med. Universität , Shanxi

Promotionsfach: Pathologie
Doktorvater: Prof. Dr. med. Hermann-Josef Gröne

The release of RANTES from CD8+ lymphocytes has been shown to be dependent on TCR triggering by MHC class I/peptide complex engagement [Wagner L et al, 1998]. Stimulation of $CD8^+$ lymphocytes by incubation with target cells induced a reorientation and distribution of RANTES onto the contact area with the target cells [Dr. Nößner E, Institute of Molecular Immunology, Munich, unpublished results]. We therefore hypothesized that RANTES may take some additional novel function, namely, that RANTES secreted by $CD8^+$ lymphocytes in the context of target cell lysis could promote phagocytic removal of target cells by tissue macrophages.

To test the effect of RANTES on phagocytosis, apoptotic colon 26 cells were labeled with DiI. After coincubation of the peritoneal macrophages with the labeled apoptotic bodies the number of DiI positive macrophages was determined as an index of phagocytosis. Pretreatment with either human or murine RANTES resulted in a significant increase in the uptake of the apoptotic bodies. Analogous to the results seen with colon 26 cells, the uptake of apoptotic CHO cells was significantly increased following treatment with human RANTES.

The RANTES protein readily forms large aggregates. The role of RANTES aggregation on the enhanced phagocytosis was then investigated using RANTES and nonaggregating variants, enabling comparison of aggregated, tetrameric, and dimeric RANTES forms. Apoptotic RANTES-GPI expressing CHO cells showed an approximately nine fold increase in phagocytosis as compared to control CHO cells. Modification of RANTES to a tetramer resulted in a 1.3 fold decrease in uptake relative to RANTES-GPI. Finally, mutation of RANTES to a dimeric protein resulted in a three fold reduced effect on uptake of the apoptotic bodies.

The addition of a Met group to the amino terminus of RANTES led to a complete abrogation of the increased uptake. To evaluate the potential role of CCR1 or CCR5 on this process, the receptors were blocked by either BX 471 a small molecule antagonist of CCR1, or a blocking antibody to murine CCR5. Complete inhibition of the enhanced uptake was seen at 50 μM BX 471. In contrast, the CCR5 blocking antibody did not significantly affect uptake of the CHO-RANTES-GPI apoptotic cells.

Our data demonstrating RANTES-stimulated phagocytosis of apoptotic cells *in vitro* suggested a role for this process in promoting the resolution of inflammation *in vivo*. Consistent with this hypothesis were the data that demonstrated that RANTES stimulated phagocytosis of murine apoptotic cells in the murine BALB/C model of thioglycollate-induced peritonitis, an established model of inflammation, while use of recombinant Met-RANTES did not lead to an increased phagocytosis of apoptotic bodies.

Furthermore the effect of other proinflammatory chemokines on apoptotic body uptake was tested. The effect of murine MIP-1α, MIP-1β and MCP-1 were compared to RANTES in the macrophage phagocytosis assay. The results show that only pretreatment with RANTES lead to a significant increase in macrophage phagocytosis.

Taken together, these results suggested that the phagocytosis of cellular corpses by macrophages is greatly enhanced by RANTES painted on the surface of the apoptotic cells. Aggregation of RANTES is required and the enhanced phagocytosis is mediated through CCR1. The phenomenon appears unique to RANTES as other chemokines did not affect phagocytosis. The efficacy of RANTES in modulation of Mϕ phagocytosis in models of inflammation has led to the proposal that this compound might have therapeutic potential in specific stages of inflammation.