

Michael Lotze Title and Abstract

“The Grateful Dead: How Death Drives Regeneration”

Background: Eosinophilic granulocytes, at increased numbers, are found within tumor tissues and the blood of cancer patients. Little is known, however, about their role in this setting and which factors promote their recruitment and trafficking to sites of tumor and necrosis. HMGB1 (high mobility group protein B1) is a member of the damage associated molecular pattern molecules (DAMPs) released by necrotic tumor cells but also actively secreted by activated monocytes.

Objectives/Methods: We demonstrate, in addition to TLR-4, eosinophils and macrophages also express another HMGB1 receptor, RAGE. We used human PBMC and isolated eosinophils to evaluate the response to tumor lysates and HMGB1. Human granulocytes were purified from whole blood by density gradient centrifugation using Ficoll-Paque™ Plus (Amersham, Biosciences) followed by lysis of red cells using ammonium chloride solution (155 mM NH₄Cl, 10mM KHCO₃, and 0.1 mM EDTA). Human eosinophilic granulocytes were negatively selected out of the granulocyte population using MACS-separation (Miltenyi Biotec Inc.) following the manufacturer's instructions. The purity was assessed by H&E staining and was at least 95%. Cells were kept in RPMI 1640 (Cellgro; Mediatech Inc., Herndon, USA) supplemented with 10% FBS (Gibco-Invitrogen, USA) and containing 100U/ml Penicillin-G, 0.25µg/ml Amphotericin B, 100µg/ml Streptomycin (Cellgro; Mediatech Inc., Herndon, USA) in an humidified atmosphere at 37°C with 5% CO₂. For experiments assessing peroxidase release phenol-red free IMDM (HyClone, Utah, USA) without serum was used. All media were supplemented with 10µg/ml polymyxin B (Sigma, USA) in order to block any effects of contaminating endotoxin.

Generation of necrotic colorectal tumor cells: Two individual colorectal tumor cell lines, HCT-116 and CaCO₂ cells, were resuspended in PBS at a concentration of 1x10⁷ cells/ml and lysed by 3 cycles of freeze-thawing (-80 to 37°C) followed by sonicating and repeated passage through a 28G needle. The viability following treatment was assessed using trypan blue exclusion and was always less than 0.1%. For colorimetric measurement of EPO release, lysates were spun down hard (16,300x g) and the soluble supernatant was used.

Results: Subsequently we demonstrated that both natural and recombinant HMGB1 serve as survival factors and chemoattractants for granulocytes and in particular for eosinophils. HMGB1 promoted eosinophil degranulation with release of eosinophil peroxidase and major basic protein. We show that peroxide abolishes the effect of necrotic tissue on eosinophils in terms of further release of peroxidase as well as major basic protein, a factor promoting opsonization of cellular debris. We describe a new role for eosinophils in “sensing necrosis” and facilitating oxidation and removal of cellular

debris. Necrotic cells modulate immune responses. Similarly, incubation of peripheral blood mononuclear cells with either HMGB1 or tumor lysate induced expression of novel miRNAs, which are rapidly upregulated. We have identified several putative miRs involved in HMGB1-induced signaling and/or differentiation including hsa-mir-155, hsa-mir-545 and let-7g. These miRs are especially interesting to us, because of their computationally calculated potential targets (which can be found on <http://www.microrna.org/mammalian/index.html>). Hsa-mir-155 has computationally predicted targets of Spi-1 (or PU.1) and TLR4, as well as MAP4K5 (a Mitogen-activated protein kinase) while hsa-545 has computationally predicted target of IRF-8. Both Spi-1 and IRF-8 are myeloid-specific transcription factors involved in monocyte activation and differentiation. The MAP Kinase may be involved in early HMGB1 signalling, which in turn may be regulated by miR-155. Interestingly, let-7g is one of the upregulated microRNAs. This microRNA has putative targets of IRF-5 (an intermediate in TLR signaling) and Spi-1/PU.1.

Conclusions: Our results suggest a plausible role for eosinophils and macrophages infiltrating tumor tissue. In addition, these findings have implications for the immunotherapy of patients with cancer and suggests a linkage between the evolution of tumor necrosis, the host response, and carcinogenesis.