Presentation
Chronic Inflammation and Cancer

Speaker:
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Abstract:
Chronic Inflammation and Cancer. HMGB1, A Tumor Derived Damage Associated Molecular Pattern Molecule, is a Proximal Mediator
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Introduction: Tumor progression in adults is associated with increased necrosis and reactive inflammation. Aponecrotic cells release several endogenous danger signals (including the nuclear protein HMGBI), which recruit and activate inflammatory cells, modulating the activity of cytokines in mouse and man[1-5]. Necrosis, or Type III death, is distinguished largely morphologically from apoptotic [Type I] and autophagic [Type II] death, and has even been identified in protists. These distinctions have critical import not so much for the dying cell as for the nature of the subsequent host response.

Distinguishing these types of cell death has been less than straight forward. High-mobility group B 1 protein (HMGB1) is primarily a nuclear chromatin-binding protein released when cells die following necrotic cell death and also secreted by inflammatory cells, but sequestered in the cells during apoptotic, autophagic or platinum-induced death[6]. Histone H1, also a chromatin-binding protein, conversely is not released when cells die following necrosis such as the setting of ischemia/reperfusion injury[7-10]

Material and Methods: We developed a highly sensitive method to analyze necrotic cell death by concurrently detecting both intracellular HMGB1 and histone H1 using flow cytometry which should be widely applicable in distinguishing it from Type I and Type II death and superior to ELISA and Western blotting. HMGB1 [but not histone H1] is released following detergent lysis, but not released by UV irradiation induced apoptosis. Mass spectrometric studies show that HeLa derived material is less than the expected molecular mass in preliminary studies. We have evaluated human tumor cell lines, including lymphoma, leukemia, ovarian, melanoma and colon cancers by immunohistochemistry, western blot of nuclear and cytosolic fractions, and nude mouse xenografts. HMGBI is not only released by necrotic tumor cells but also actively secreted. *In vitro* and *in vivo*, HMGBI is over-expressed in tumors and unlike normal cells, it is primarily extranuclear, located within the cytoplasm. Reparative stromagenesis, angiogenesis, epithelial proliferation and altered host immune function by HMGB1 thus may paradoxically promote tumor growth[11]

Eosinophilic granulocytes are predominantly found in tissues that interface with the external environment including the skin, gastrointestinal, respiratory and urogenital tracts[12]These sites are at high risk of tissue damage and neoplastic transformation. Eosinophils are often found in cancer tissue and in the setting of responses to cancer following immunotherapy (IL-2, IL-4, GM-CSF, CTLA4-antibody). Eosinophils isolated from normal human leukopacks by negative selection[13] were 99% pure using H&E

staining. Granulocytes were isolated from normal human whole blood by density gradient centrifugation followed by ACK lysis of remaining red cells. Eosinophils were negatively separated using magnetic beads. Immature dendritic cells were generated from CD-14 positively separated monocytes which have been then treated 6 days with GM-CSF and IL-4. CpG ODN 2395 (CpG-C) as a PAMP-surrogate, adenovirus or influenza-virus were used to induce eosinophil based DC maturation. Transwells were used in order to assess cell-cell interaction between eosinophils and DCs. Eosinophil survival was assessed by flow cytometry, cells which did not stain with Sytox-orange were considered as viable.

Results: Melanoma cells were lysed by MART1 specific CTL and LAK/NK in short term assays with HMGB1 release. In the presence of CpG-C, adenovirus, and influenza-virus eosinophils induced DC maturation. Similar results were obtained when eosinophils were pretreated with CpG or pathogens for 4h and cocultured afterwards with DCs. Eosinophil-induced maturation of DCs directly correlated with the eosinophil:DC-ratio. Transwell studies showed that the direct cell-cell interaction between eosinophils and DCs enhances the maturation-inducing effect but was not obligatory. CpGs did not have any negative effect of eosinophil survival, thus we could exclude the possibility that DC maturation was caused by sensing eosinophil cell death.

Conclusions: HMGB1 is released from dying tumor cells when lysed by activated NK cells or specific T-cells. CpG activated eosinophils mature conventional DCs. The role of viral or bacterial products or potentially host derived DNA as eosinophil activators with consequent DC maturation should be considered in more detail in the inflammatory settings in which eosinophils have been observed. rHMGB1 enhanced eosinophil survival from 40 to 70% after 24h and caused upregulation of expression of the survival factor, CD40 on eosinophils. In addition, HMGB1 enhanced eosinophil-chemotaxis alone and synergistically with eotaxin. Furthermore, HMGB1 caused eosinophil activation and eosinophil degranulation of CD69 and promotion of MBP (major basic protein) and EPO [eosinophil peroxidase] release. Thus HMGB1 serves not only as a survival factor but also as a chemoattractant and activator for eosinophils[12]. The ability of HMGB1 to alter miRNA in DCs and other inflammatory cells is being evaluated.[13] The role of oxidation to eliminate DAMPs in the setting of chronic inflammatory conditions is also being assessed[14].

References.

1. Lotze MT, DeMarco RA. Dealing with Death: HMGB1 As a Novel Target for Cancer Therapy. Current Opinion in Investigational Drugs 2003 4(12):1405-1409.
2. Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. Nature Reviews Immunology 4:641-647, 2004.
3. Lotze MT. Inflammation, necrosis, and cancer. In *Cancer and Inflammation*; edited by Doug Morgan, Ulf Forssmann, and Marian Nakada; Birkhauser Publishing, Basel Switzerland; pp. 189-196, 2004.
4. Demarco RA, Fink MP, Lotze MT. Monocytes promote Natural Killer Cell Interferon Gamma Production in Response to the Endogenous Danger Signal HMGB1. Molecular Immunology 42(4), February 2005, 433-444.
5. Zeh HJ, Lotze MT. Addicted to Death: Invasive Cancer and the Immune Response to Unscheduled Cell Death; J Immunother. 2005 Jan-Feb;28(1):1-9.
6. Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. Nature Reviews Immunol. 2005 Apr;5(4):331-42.

7. Tsung A, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, Yang H, Li J, Tracey KJ, Geller DA, Billiar TR. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J Exp Med. 2005 Apr 4;201(7):1135-4.
8. Dong XD, Popovic P, Lotze MT, DeMarco RA, Watkins S, Ito N, Winikoff S, Bartlett DL, Zeh HJ. Sequestration of HMGB1 in the nucleus of dying tumor cells following treatment with the platinating agent oxaliplatin. J Immunotherapy, in press.
9. Tsung A, Hoffman RA, Izuishi K, Critchlow ND, Nakao A, Chan MH, Lotze MT, Geller DA, Billiar TR. Hepatic Ischemia/Reperfusion Injury Involves Functional TLR4 Signaling in Nonparenchymal Cells. J Immunol. 2005 Dec 1;175(11):7661-8.
10. Tsung A, Zheng N, Jeyabalan G, Izuishi K, Klune JR, Geller DA, Lotze MT, Lu L, Billiar TR. Increasing numbers of hepatic dendritic cells promote HMGB1-mediated ischemia-reperfusion injury. J Leukoc Biol. 2007 Jan;81(1):119-28.
11. Ito N, Demarco RA, Mailliard RB, Han J, Rabinowich H, Kalinski P, Stolz DB, Zeh HJ 3rd, Lotze MT. Cytolytic cells induce HMGB1 release from melanoma cell lines. J Leukoc Biol. 2007 Jan;81(1):75-83.
12. Lotfi R, Lee JJ, Lotze MT. Eosinophilic granulocytes and damage-associated molecular pattern molecules (DAMPs): role in the inflammatory response within tumors. J Immunother. 2007 Jan;30(1):16-28.
13. Lotze MT, Yu Y. Cancer genomics: the unknown unknowns. Curr Opin Investig Drugs. 2006 Jun;7(6):497-500.
14. Ellerman JE, Brown CK, de Vera M, Zeh HJ, Billiar T, Rubartelli A, Lotze MT. Masquerader: high mobility group box-1 and cancer. Clin Cancer Res. 2007 May 15;13(10):2836-48.
15. Lotze MT, Deisseroth A, Rubartelli A. FOCiS on damage-associated molecular pattern molecules. Clin Immunol. 2007 Apr 27; [Epub ahead of print]
16. Rubartelli A, Lotze MT. Inside, outside, upside down: DAMPs and Redox, submitted.