A subset of mature neutrophils contains the strongest PMN-MDSC activity in blood and tissue of patients with head and neck cancer

Yu Si, Kirsten Bruderek, Simon Merz, Philip Jansen, Matthias Gunzer, Joachim Klode, Anthony Squire, Sven Brandau, Sven Brandau*. University Hospital Essen, Essen, Germany

Background A high neutrophil-to-lymphocyte ratio in the circulation and high frequencies of tumor-associated neutrophils (TAN) in malignant tissue are associated with poor outcome and tumor progression in patients with cancer. It is hypothesized that immunosuppressive neutrophil activity (aka PMN-MDSC activity) contributes to this effect. In addition, this MDSC activity represents a major resistance mechanism in different types of immunotherapy. The exact cellular identity of human PMN-MDSC is still under debate. Improved immunomonitoring and functional characterization of MDSC is needed in order to exploit these cells as novel biomarkers and targets for combination immunotherapy.

Methods In this study, we sought to identify the neutrophil subset that contained the highest T cell suppressive activity. To this end, we purified different subsets of circulating neutrophils by FACS and performed multi-parameter immunofluorescence together with digital pathology on 2-D and 3-D tumor tissue samples.

Results We found that a population of circulating, mature, arginase 1+ neutrophils that co-purified with mononuclear cells in density gradients, most potently suppressed T cell function in multiple in vitro assays. These PMN-MDSC were also superior to mononuclear MDSC in T cell suppression. Using a novel technology of tissue whole mount labelling, clearing and imaging we derived 3-D spatial maps of neutrophil – T cell interaction in human tumors. We found that T cells, which were conjugated to arginase 1+, myeloperoxidase + TAN, had significantly reduced expression of proliferation and cytotoxicity markers. In patients, frequent conjugation of T cells to those PMN-MDSC was associated with poor prognosis. In contrast to circulating PMN-MDSC, tissue PMN-MDSC expressed high amounts of LOX-1 (oxidized low density lipoprotein receptor 1) and a high intratumoral frequency of LOX-1+ PMN-MDSC was associated with poor survival.

Conclusions We identified and characterized PMN-MDSC activity in human cancer patients. Our findings will facilitate and improve MDSC immunomonitoring and MDSC targeting in combination therapies.

TIM-4+ resident macrophages impair anti-tumor immunity in the serous body cavities by sequestering viable and cytotoxic CD8+ T cells expressing high levels of phosphatidylserine

Andrew Chow*, 1Sara Schad, 1Michael Green, 1Matthew Hellmann, 1Nicholas Ceglia, 1Viola Allaj, 1Gulia Zago, 1Nisang Shah, 1Sai Sharma, 1Marissa Mattar, 1Joseph Chan, 1Hira Rizvi, 1Hong Zhong, 1Callian Liu, 1Yonina Bykov, 1Dmitry Zamarin, 1Hongyu Shi, 1Sadna Buschu, 1Corrin Wohlhieter, 1Fatima Uddin, 1Aditi Gupta, 1Inna Khodos, 1Jessica Waninger, 1Angel Qin, 1Vinod Balachandran, 1Weiping Zou, 1Sohrab Shah, 1Andrew McPherson, 1Katherine Panagia, 1Jason Lewis, 1Justin Perry, 1Elisa de Sanchina, 1Triparna Sen, 1John Poirier, 1Jedid Wolcho, 1Charles Rudin, 1Taha Merghou, 1Memorial Sloan Kettering Cancer Center, New York, NY, USA; 1University of Michigan, Ann Arbor, MI, USA; 1NYU Langone Medical Center, New York, NY, USA

Background Malignant pleural effusions and peritoneal carcinomatosis are associated with poor outcomes in patients with cancer.1-3 Macrophages in these serous body cavities express the phosphatidylserine receptor Tim-4.4-8 Prior reports demonstrated that Tim-4 abrogation is associated with improved anti-tumor activity.9-11 Whether macrophages expressing Tim-4 contribute to immunosuppression in the serous body cavities has not been previously investigated.

Methods We retrospectively annotated sites of metastases in 500 patients with lung cancer and assessed for clinical outcomes. Utilizing a combination of flow cytometry, immunohistochemistry, and antibody biodistribution assays, we surveyed for Tim-4 expression across various tissues and cell types. We performed flow cytometry on 35 consecutive pleural and peritoneal effusions from patients with lung cancer. We utilized murine models of peritoneal carcinomatosis to determine whether Tim-4 abrogation could enhance the anti-tumor efficacy of anti-PD-1 therapy. We characterized CD8+ T cells with high levels of phosphatidylserine (PShigh) with flow cytometry, cytotoxicity assays, and paired single cell RNA and TCR sequencing. Confocal microscopy was utilized to visualize interactions between Tim-4+ macrophages and PShigh CD8+ T cells.

Results Metastatic disease involvement of the pleural or peritoneal cavity was associated with reduced response rate and progression-free and overall survival. We demonstrate that Tim-4 is highly expressed on pleural and peritoneal macrophages and other select resident macrophages, but not on monocytes, tumor-associated macrophages, or tumor cells in mice and humans. High levels of Tim-4 on macrophages from fluid biospecimens is associated with reduced levels CD39+ CD8+ T cells, which comprise the tumor-reactive portion of CD8+ T lymphocytes. In order to further elucidate the mechanism of Tim-4+ macrophage-mediated immunosuppression, we established a murine model of peritoneal carcinomatosis with MC38 and CT26 colon carcinoma. Genetic or pharmacologic abrogation of Tim-4 improved the efficacy of anti-PD-1 therapy and was associated with enhanced CD39+ CD8+ T cell numbers. In parallel, we observed in mice and humans that CD8+ T cell activation results in PS upregulation despite not undergoing cell death.

Ethics Approval Use of patient material was approved by the Ethics committee of the Medical Faculty of the University of Duisburg-Essen

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0523

524 TIM-4+ RESIDENT MACROPHAGES IMPAIR ANTI-TUMOR IMMUNITY IN THE SEROUS BODY CAVITIES BY SEQUESTERING VIALBE AND CYTOTOXIC CD8+ T CELLS EXPRESSING HIGH LEVELS OF PHOSPHATIDYLSERINE