

Pancreatic Cancer (PaCa)-Derived Soluble Mediators Induce Dendritic Cells (DC) to Acquire an Immunosuppressive Phenotype by Downregulating CTLA4

Elisa Gnatta¹, Paola Fogar¹, Ada Aita¹, Chiara Frasson², Sara Teolato¹, Monica Facco¹,
Dania Bozzato¹, Eliana Greco¹, Andrea Padoan¹, Filippo Navaglia¹, Stefania Moz¹,
Carlo Federico Zambon¹, Giampietro Semenzato¹, Giuseppe Basso², Mario Plebani¹, Daniela Basso¹

¹Department of Medicine and ²Laboratory of Pediatric Oncohematology,
Department of Woman and Child Health; University of Padua. Padua, Italy

Context An altered function of lymphocytes, DC and immature myeloid cells appears to be an hallmark of tumor-mediated immune suppression and the two inhibitory co-stimulatory receptors PDL-1 and CTLA4 might have a role in this context. **Objective** The aim of the present *in vitro* study was to assess whether PaCa cells cross-talk with normal mononuclear circulating cells (PBMC) causing them to acquire an immune-suppressive phenotype and to evaluate whether PDL1 and CTLA4 are involved. **Methods** PBMC from blood donors were cultured for 4 days in control (CTL) and in the PaCa cancer cell line Capan1 conditioned media (CM). Lymphocytes subsets (CD4+, CD8+, CD4+CD25+) and CD33+ immature myeloid cells subsets (CD14+/-; HLA-DR+/-) expressing or not PDL1 and/or CTLA4 were analyzed by flow cytometry. To assess immunosuppressive function, myeloid cells were FACS sorted and co-cultured with allogenic total T lymphocytes in 1:20 and 1:40 ratios. Total T lymphocytes proliferation was determined by ³H-thymidine uptake. **Results** Capan1 CM caused an expansion of CD4+CD25+ (P=0.01) and a reduction of

CD33+CD14-HLA-DR+ (P=0.03) cells. In this latter cellular subset, CM caused also an increase of PDL1 (P=0.046) and a decrease of CTLA4 (P=0.05) positive cells. FACS sorted CTL and CM CD33+CD14-HLA-DR+ cells did not significantly affect the proliferation of allogenic total T lymphocytes both at 1:20 (P=0.54) or at 1:40 ratios (P=0.81). The CD33+CD14-HLA-DR+ PDL-1+ cells did not significantly modify allogenic T cells proliferation with respect to PDL-cells (P=0.11), while those cells which were CTLA4 negative caused a significant inhibition of T cell proliferation in comparison of CTLA4 positive cells (P=0.008). **Conclusions** PaCa-derived soluble factors induce the expansion of the inhibitory lymphocyte subset CD4+CD25+ and a reduction of the immature CD33+CD14-DR+ dendritic cells. The tumor associated reduced expression of the inhibitory molecule CTLA4 in this cell population was demonstrated to characterize an immunosuppressive phenotype and this study suggests to take care in the use of anti-CTLA4 therapies.