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The S100A8/A9 complex reduces CTLA4 expression by immature myeloid cells

Implications for pancreatic cancer-driven immunosuppression

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An expansion of different myeloid derived suppressive cell (MDSC) subsets can be detected in the blood and secondary lymphoid organs of early and advanced pancreatic ductal adenocarcinoma (PDAC) patients. Double negative ($CD14^-$ $HLA\text{-}DR^-$) MDSCs are frequently induced by PDACs. In addition, by releasing S100A8 and S100A9, advanced PDAC lesions cause an expansion of highly immunosuppressive $CD33^+CD14^+HLA\text{-}DR^-$ monocytic MDSCs expressing low levels of cytotoxic T lymphocyte antigen 4 (CTLA4) on the cell surface.

The study of pancreatic ductal adenocarcinoma (PDAC), the fourth leading cause of cancer-related deaths in the U.S., is invariably associated with a question: What makes the mortality rate of this tumor almost equal to its incidence rate?[1] A median survival time of 20–22 months in patients affected by early PDAC declines to 9–10 months in patients bearing advanced lesions.[2] This scenario mainly depends on the clinical and biological features of PDAC, which causes symptoms that vary depending on the location of the tumor within the pancreatic gland and on tumor stage. Although the surgical removal of early PDAC lesions can be curative, such tumors are often asymptomatic or poorly symptomatic and hence often fail to be diagnosed. Conversely, advanced forms of PDAC quickly spread to other organs and are refractory to chemotherapy.

From a biological viewpoint, PDAC evolves from a premalignant state to a fully invasive tumor by accumulating genetic and epigenetic mutations that mainly involve the *KRAS* oncogene and the oncosuppressor genes *CDKN2A*, *TP53* and *SMAD4*. Although the rapid progression of PDAC stems from cancer cell-intrinsic genetic alterations, it is also the result of complex interactions between malignant and stromal cells.[3] Indeed, cancer cells and stromal inflammatory cells may create a microenvironment that supports rather than counteracts PDAC growth and dissemination to distant organs. In particular, this is achieved by the activation of immunosuppressive networks coupled to the inhibition of immune effector cells in the peripheral blood, secondary lymphoid organs and in the tumor stroma and/or via alterations in the expression of immunomodulatory molecules expressed on the surface of immune cells. As recently demonstrated by us,[4] PDAC is associated with reduced levels of circulating antitumor $CD8^+$ T cells as well as with reduced and increased numbers of dendritic cells (DCs) and myeloid derived immune suppressive cells (MDSCs), respectively, in the blood and spleen. These findings support the notion that the deregulation of both lymphoid and myeloid cells is a hallmark of PDAC. Human MDSCs comprise a set of immature myeloid cells (IMCs) that share the ability to exert immunosuppressive functions in spite of their phenotypic heterogeneity. Conversely, mouse MDSCs are relatively homogenous and always express CD11b and Gr1.[5] We found that different MDSC subsets accumulate in the blood and spleen of PDAC patients, according to different disease stage: (1) circulating $CD33^+CD14^-HLA\text{-}DR^-$ cells were increased in PDAC patients as well as subjects affected by borderline neoplasms (i.e., premalignant PDAC lesions with an uncertain malignant potential), suggesting that these MDSCs constitute a hallmark of early PDAC; (2) circulating and splenic $CD33^+CD14^+HLA\text{-}DR^-$ cells were increased in PDAC patients exhibiting vascular invasion, suggesting that this subset of monocytic MDSCs is a hallmark of advanced PDAC.

Based on the premise that the function of immune effector cells is controlled by a balance between positive and negative regulatory signals, we focused on the B7/CD28 receptor family and studied the expression and functional consequences of cytotoxic T lymphocyte antigen 4 (CTLA4) and programmed death 1 ligand 1 (PDL1) by IMCs in the context of

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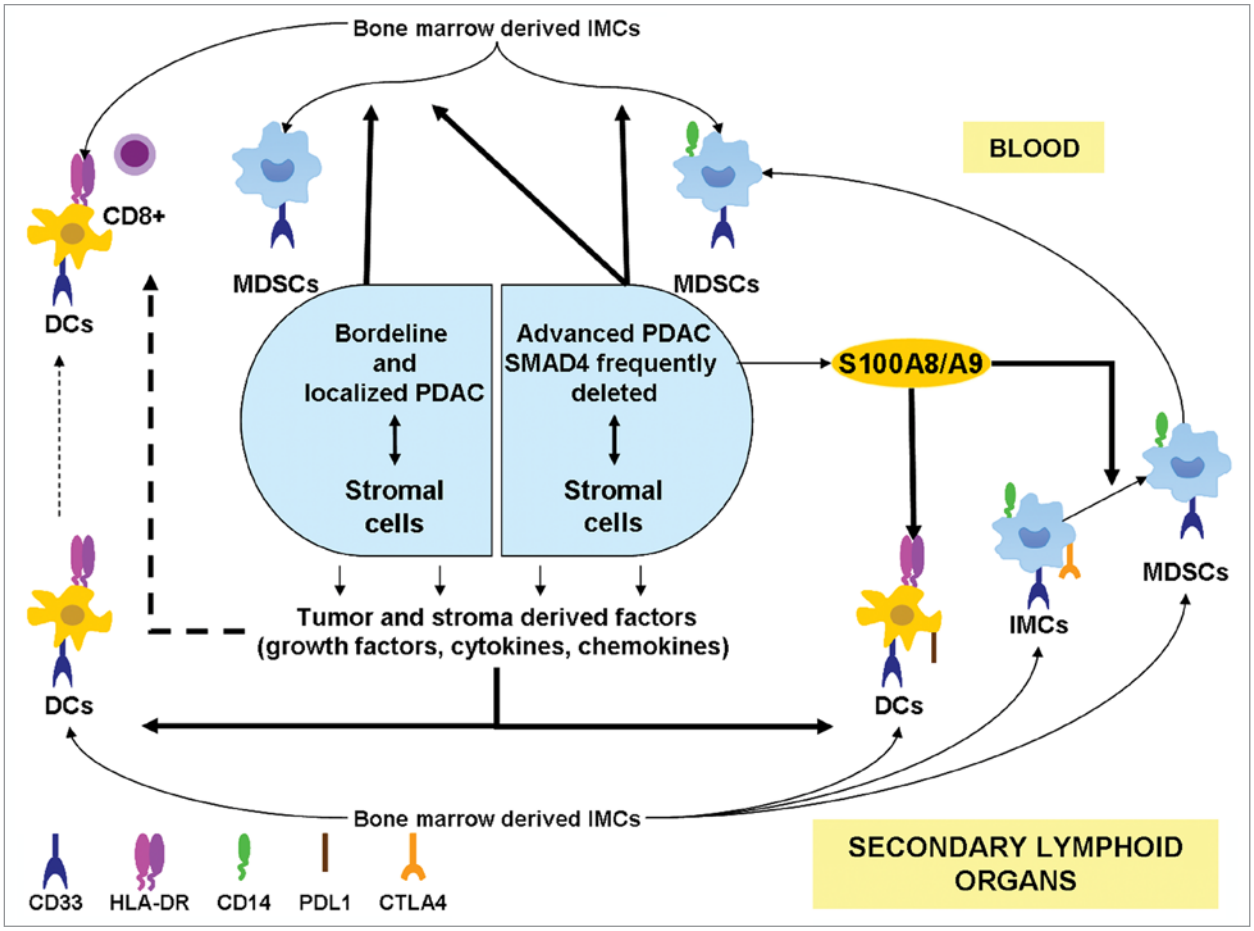

Figure 1. Crosstalk between malignant cells and the immune system during pancreatic cancer progression. Early and advanced pancreatic ductal adenocarcinomas (PDACs) exert partially overlapping effects on immature myeloid cells (IMCs) in the blood and secondary lymphoid organs. PDAC is often associated with a reduction in antitumor $CD8^+$ T cells and dendritic cells (DCs). An increase in $CD33^+CD14^-HLA\text{-}DR^-$ myeloid-derived suppressor cells (MDSCs) is induced not only by well-differentiated PDACs but also by borderline tumors (premalignant PDACs). Conversely, the expansion of $CD33^+CD14^+HLA\text{-}DR^-$ monocytic MDSCs is stimulated by poorly differentiated PDAC cells, occurs more frequently in the context of advanced PDAC and probably depends on the release of the S100A8/A9 complex by SMAD4-deficient cells. The S100A8/A9 complex downregulates cytotoxic T lymphocyte antigen 4 (CTLA4) expression by IMCs, resulting in the establishment of a potent immunosuppressive cell population.

PDAC.[6] CTLA4 is believed to participate in the immunosuppressive activity of regulatory T cells (Tregs). PDL1 is expressed by cancer cells as well as by cellular components of the tumor microenvironment and its expression by malignant cells appears to be of prognostic significance in PDAC patients. Furthermore, the blockade of both these molecules with specific antibodies has recently been demonstrated to constitute a promising immunotherapeutic strategy.[7]

In PDAC patients, splenic DCs expressed elevated levels of PDL1 while CTLA4 expression by splenic $CD33^+CD14^+HLA\text{-}DR^-$ monocytic MDSCs was reduced.[4] From a functional perspective, increased PDL1 expression was not associated with immunosuppression, whereas IMCs lacking CTLA4 exerted bona fide immunosuppressive functions. Immune cell alterations detected in PDAC patients may be the consequence of a direct or indirect crosstalk between cancer cells and the immune system, and this may occur within neoplastic lesions as well as at a systemic level. Soluble mediators directly secreted by tumor cells and/or indirectly produced by stromal inflammatory cells surely represent key players in this crosstalk. To get further insights into this issue, we studied the effects of culture media conditioned by PDAC cell lines exhibiting different metastatic potential on immune cells. In agreement with clinical data, well-differentiated and poorly metastatic PDAC BxPC3 cells induced the expansion of $CD33^+CD14^-HLA\text{-}DR^-$ MDSCs while downregulating the expression of CTLA4 on their surface. Conversely, poorly differentiated and highly metastatic Capan1 cells not only promoted the expansion of $CD33^+CD14^+HLA\text{-}DR^-$ monocytic MDSCs, but also caused a reduction in CTLA4 and an increase in PDL1 expression levels in all IMCs considered.

A variety of cancer cell- and immune cell-derived soluble proteins may be responsible for the effects of PDAC-conditioned media on immune cells. We focused on the S100A8/A9 complex because it has been (1) involved in the establishment of a favorable environment for metastases, (2) implicated in the recruitment of MDSCs and the stimulation of their immunosuppressive functions

and (3) shown to be intimately link with the deletion of *SMAD4*.[5,8] In fact, when *SMAD4* is deleted, PDAC cells acquire the ability to express high levels of the S100A8/A9 complex. S100A8/A9 caused an expansion of $CD33^{+}CD14^{+}HLA\text{-}DR^{-}$ monocytic MDSCs while reducing the levels of CTLA4 on their surface. As it failed to modulate $CD33^{+}CD14^{-}HLA\text{-}DR^{-}$ MDSCs, these findings suggest that the S100A8/A9 complex constitutes one of the immunosuppressive soluble mediators released by advanced PDACs. This complex probably operates by binding to the advanced glycosylation end product-specific receptor (AGER, best known as RAGE),[9] which has previously been shown to promote the accumulation of MDSCs in murine models of PDAC.[10] Thus, we suggest that the accumulation of genetic alterations by PDAC cells is paralleled by a progressive imbalance between regulatory and effector immune cells, ultimately resulting in the establishment of immunosuppressive networks that allow for rapid tumor progression (**Fig. 1**).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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