# Inflammatory networks and immune surveillance of pancreatic carcinoma

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## Abstract

Cancer-associated inflammation plays an important role in restraining anti-tumor immunity, particularly in pancreatic ductal adenocarcinoma (PDA) for which a massive infiltration of immunosuppressive leukocytes into the tumor stroma is an early and consistent event in oncogenesis. This pathophysiology is in contrast to many other solid tumors for which infiltration of effector T cells is often prominent, associated with improved clinical outcomes, and mechanistically contributes to tumor immunoediting that ultimately can mediate immune escape. In PDA, increasing evidence suggests that the *ras* oncogene drives an inflammatory program that establishes immune privilege in the tumor microenvironment. Indeed, PDA cells might remain intrinsically sensitive to T cell killing because they have never been exposed to T cell selective pressure *in vivo*. In support of this hypothesis, recent studies demonstrate that derailing immune suppressive pathways in the PDA microenvironment, such as tumor derived GM-CSF, facilitates T-cell mediated tumor rejection. These findings carry major implications for the development of novel, combination immunotherapies for pancreatic cancer.

## Introduction

Leukocytes can be a major part of the tumor microenvironment, with evidence supporting both tumor-suppressing and tumor-promoting roles [1]. The host-protective and the tumor-sculpting actions of the immune system on a developing tumor are embodied in the prevailing model of cancer immunosurveillance, recently understood to occur in three phases – elimination, equilibrium, and escape [2,3]. In the elimination phase, the immune system acts as a powerful extrinsic tumor suppressor, and recent elegant studies underscore the large extent to which this process is T-cell dependent. [4–6]. In cases where the elimination phase fails to rid the host completely of transformed cells, a dynamic equilibrium ensues between residual malignant cells and the host immune system [7]. This phase is marked by genetic instability in the cancer cells, immune pressure, Darwinian selection, and in some model systems the outgrowth of tumor cells with reduced immunogenicity [2,3]. The selection of tumor cells with reduced immunogenicity ("immunoediting") renders them more capable of surviving in an immunocompetent host. In the escape phase, immunoedited tumor cells can grow unrestrained by immune pressure and manifest as invasive tumors. In other model systems, active immune suppression mediated

by the tumor enforces tolerance in tumor-specific T cells as a dominant mechanism of immune escape [8]. In so far as immunoediting has been documented in patients with cancer [9,10], a major implication of the "triple E" theory is that clinically apparent tumors in humans have escaped adaptive immunity and may therefore be inherently resistant to immune pressure. This may help explain why most therapeutic cancer vaccines and other immunotherapies have been ineffective, or only effective in a small fraction of patients, even in diseases such as melanoma which are considered to be immunogenic.

It is increasingly appreciated that tumor-associated leukocytes can promote tumor development [11–13]. Neoplastic cells are often capable of driving inflammatory pathways that recruit leukocytes to the tumor microenvironment. While the mechanisms underlying tumor-induced inflammatory responses remain incompletely understood, innate immune cells of the myeloid lineage such as tumor-associated macrophages (TAMs) and immature myeloid cells, are integrally involved [14]. These cell populations can promote tumor growth through the production of cytokines, chemokines, proteases, and growth factors; foster angiogenesis and tissue remodeling; and mediate local and systemic immune suppression [12].

## Immunobiology of pancreatic cancer

Determining the balance between tumor-promoting innate immune responses and tumor-suppressing adaptive immune responses is critical both to understanding the pathophysiology of cancer and to developing new immunotherapies. A number of laboratories have investigated immunosurveillance and inflammation in the context of pancreatic ductal adenocarcinoma (PDA) [15–22]. PDA is a devastating tumor, highly refractory to standard therapies and almost universally lethal [23], owing in large part to a striking desmoplastic reaction and fibrosis in the stromal compartment [24]. PDA is one of only two cancers among 21 identified in the 2012 AACR Cancer Progress Report for which the death rate in the United States has actually risen since 1990 (the other is liver/intrahepatic bile duct cancer). Indeed, a recent report from the Pancreatic Cancer Action Network estimates that by 2020; PDA will become the second leading cause of cancer-related death in the US (behind lung cancer) [25]. For patients with metastatic PDA, only one new drug has been approved by the FDA in the last decade, and despite recent advances with combination chemotherapy [26,27], PDA remains a significant medical problem that requires an innovative, aggressive, and novel approach.

To understand the immune reaction to PDA, we and others have evaluated genetically defined mouse models of PDA [15–20,28]. In one model, mutations in *Kras* and *p53* alleles are targeted to the pancreas via a Cre-lox approach [29]. These "KPC" animals develop PDA with reproducible kinetics and with features that closely resemble the human disease (histologically and molecularly), including progression from preinvasive pancreatic intraepithelial neoplasia (PanIN) to invasive and metastatic PDA (Figure 1). Furthermore, the dense desmoplasia and leukocytic infiltration classically observed in the tumor stroma of patients with PDA is reproduced with high fidelity in the KPC murine model [16,29,30]. Immunosuppressive cells, including TAMs, Gr-1^{+} CD11b^{+} myeloid cells, and regulatory T cells (Treg), are prominent at even the earliest stages of neoplasia and persist through invasive cancer [16,17]. Effector T cells are scarce in preinvasive and invasive lesions, and most T cells show a naïve phenotype without evidence of activation. In some model systems, tumor specific (non-Treg) T cells have been noted, but these are typically dysfunctional [28,31]. These findings are consistent with the immune infiltrate observed in human PDA which involves a marked macrophage and myeloid cell infiltration associated with few effector T cells (unless there is underlying chronic pancreatitis) [21,32,33]. Identifiable T cells are skewed toward a Th2 phenotype [32]. Autoantibody production to

pancreatic tumor cells is weak relative to antibody responses seen in other tumors [34]. Thus, suppressive cells of the immune system appear early during pancreatic tumorigenesis – preceding and outweighing antitumor cellular immunity. The absence of effector T cell surveillance in PDA may indicate that immunoediting is not a major outcome of immune surveillance in this cancer [16]. Rather, we have hypothesized that T cell evasion in PDA manifests in in four stages (the "four I" hypothesis): induction of cancer by an oncogene (i.e. mutant *ras*), inflammation linked to downstream pathways of the oncogene, immune suppression imposed early and persistently by the inflammatory reaction, and ultimately, immune privilege [16]. Potential mechanisms driving this pathophysiology range from immunological ignorance to defects in immune effector function, both of which might contribute to the failure of immune surveillance in pancreatic cancer. This scenario of tumor-induced immune privilege, if true, creates an apparent paradox as well as an opportunity for intervention: PDA cells intrinsically might be sensitive to T cell killing because they have never been exposed to T cell selective pressure *in vivo* (Figure 2).

## Impact of Gr-1⁺ CD11b⁺ cells on T cell responses in PDA

To understand the potential potency of T cell responses against PDA, immune reactions have been monitored in tumor-bearing KPC mice in which key microenvironmental regulators of immune suppression have been abrogated genetically or pharmacologically [18,19,30,35]. Targets evaluated include TAMs, Tregs, B cells, and mesenchymal cells, including cells expressing fibroblast activation protein. In recent work, we [18] and others [19] have also tested the immunological consequence of blocking the *in vivo* recruitment of Gr-1$^+$ CD11b$^+$ immature myeloid cells. These cells infiltrate both PanIN and PDA lesions in KPC mice, as well as metastatic foci. Cells of this phenotype have been well-recognized in multiple other tumor models (with an equivalent myeloid cell also being appreciated in cancer patients, including those with PDA) [36–38]. In mice, these immature myeloid cells, often termed myeloid-derived suppressor cells (MDSCs), are a heterogeneous population of cells that co-express CD11b and the myeloid-cell lineage differentiation antigen Gr-1, and represent precursors to macrophages, dendritic cells, and granulocytes [36–38]. Numerous studies have reported the expansion of MDSCs in a variety of tumor models and have demonstrated the ability of these cells to impair T cell responses *in vitro* in a cell contact-dependent manner [39–41].

In the KPC and other mouse models of PDA, Gr-1$^+$ CD11b$^+$ cells potently suppress both antigen-specific and polyclonal T cell responses *in vitro* (consistent with the nomenclature MDSCs) [15,17,18]. *In vivo*, there is an interesting mutual exclusion observed between Gr-1$^+$ CD11b$^+$ cells and CD8$^+$ T cells infiltrating PDA tumors [17]. Among the many cytokines and chemokines produced by PDA tumors, GM-CSF appears both necessary and sufficient for the generation of functional MDSCs from c-kit$^+$ Gr-1$^{neg}$ CD11b$^{neg}$ precursors in tumor-bearing mice [18]. *In vivo*, abrogation of GM-CSF in KPC PDA tumor cell lines with either neutralizing antibodies or shRNA inhibits the recruitment of Gr-1$^+$ CD11b$^+$ cells to the tumor microenvironment and blocks tumor development. Experimental depletion of CD8$^+$ T cells fully rescues tumor growth, even though Gr-1$^+$ CD11b$^+$ cell infiltration is not completely restored [18]. Likewise, GM-CSF knockdown PDA cells grow progressively and establish tumors in *Rag2* deficient or *perforin*-deficient mice (Bayne and Vonderheide, unpublished). CD8$^+$ T cell-dependent rejection *in vivo* also occurs for pancreatic epithelial lines harboring a *ras* mutation and injected orthotopically, provided GM-CSF is abrogated [19]. In patients, based on immunohistochemistry, more than 90% of both primary PDA and PanIN samples prominently express GM-CSF, suggesting that GM-CSF-mediated recruitment of immature myeloid cells is an early event in the clinical disease [18,19]. Interestingly, expression of GM-CSF has been linked to activity of mutant *ras* [19,42]. Thus, suppressive cells of the host immune system appear early during pancreatic tumorigenesis

and effectively shield developing tumors from immune pressure, thereby preserving the underlying susceptibility of PDA cells to T cell attack (Figure 2). Elaboration of a productive anti-tumor T cell response, therefore, requires uncoupling of the *ras*-driven pathway of immunosuppressive myeloid inflammation.

Interestingly, modulation of the PDA tumor microenvironment can have other consequences on immune surveillance that are independent of T cells. Although CD40 agonistic antibodies have been shown to drive anti-tumor T cell responses *in vivo* (a notion being aggressively tested in patients) [43,44], we found that in tumor-bearing KPC mice, agonistic CD40 mAb re-educates macrophages in the tumor microenvironment, transforming immunosuppressive macrophages into tumoricidal macrophages that facilitate the depletion of tumor stroma [30]. Similar tumor regressions are observed equally in T cell replete and T cell depleted mice. In the clinic, gemcitabine and the agonistic CD40 mAb CP-870,893 produces objective responses in 24% of patients with metastatic PDA [30]. Thus, productive immune surveillance of PDA is not necessarily T cell dependent. CD40-activation of macrophages appears to be a major mechanism of tumor rejection with implications beyond PDA immunobiology [30,45,46].

## The good and bad of GM-CSF

Further experiments are underway to understand the function of tumor-derived GM-CSF as a potential mechanism of immune suppression in PDA, i.e. that GM-CSF drives an early accumulation of MDSCs even around PanIN lesions and thereby blocks tumor-specific recognition by T cells. Immunosuppressive effects of tumor-derived GM-CSF have been well appreciated in other model systems [47,48], but it should be further emphasized that GM-CSF is neither the only tumor-derived cytokine implicated in MDSC biology [49–54] nor necessary in all tumor models in which MDSC accumulate [55]. Ironically, GM-CSF is also well-described as a key immunological activator (rather than suppressor) when tumor cells are transduced to express GM-CSF, irradiated and injected subcutaneously as therapy in tumor-bearing mice or humans. Moreover, the potency of such vaccines (often called "G-VAX") has been clearly demonstrated in patients with PDA [56]. The determinants driving immune suppression in the PDA microenvironment on one hand vs. immune activation via G-VAX therapy on the other remain to be fully understood, but are likely linked to differences in GM-CSF dose and location, as well as the immunogenicity of the tumor antigen released. In any event, it is hypothesized that G-VAX vaccines in patients with PDA will require concomitant therapeutic down-regulation of the relevant immunosuppressive factors in the tumor microenvironment to achieve full potency and ultimate clinical success.

## Conclusions

PDA is a highly inflammatory and lethal tumor for which infiltration of immune suppressive myeloid and other cells is an early event. In genetically engineered mouse models, myeloid inflammation occurs concomitantly with neoplastic induction, and T cell infiltration is scarce throughout the natural history of pancreatic cancer. These results raise the hypothesis that the consequence of immune surveillance in pancreatic cancer is more akin to immune privilege than immunoediting, although further studies are needed to understand mechanism. Pancreatic tumor cells therefore might be paradoxically sensitive to T cell attack, and an emerging body of experimental evidence suggests this is the case. Clinically, it will be important in pancreatic cancer to test novel cancer immunotherapies in combination with agents that inhibit or alter oncogene-driven immunosuppressive mechanisms in the tumor microenvironment.

## Acknowledgments

## References

* of special interest

** of outstanding interest

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## Highlights for Vonderheide and Bayne submission

- Immune suppressive leukocytes are prominent in pancreatic cancer

- T cell infiltration is rare throughout disease progression

- Pancreatic tumors may therefore remain intrinsically sensitive to T cell killing

- Blockade of tumor GM-CSF blocks myeloid inflammation and mediates T cell immunity

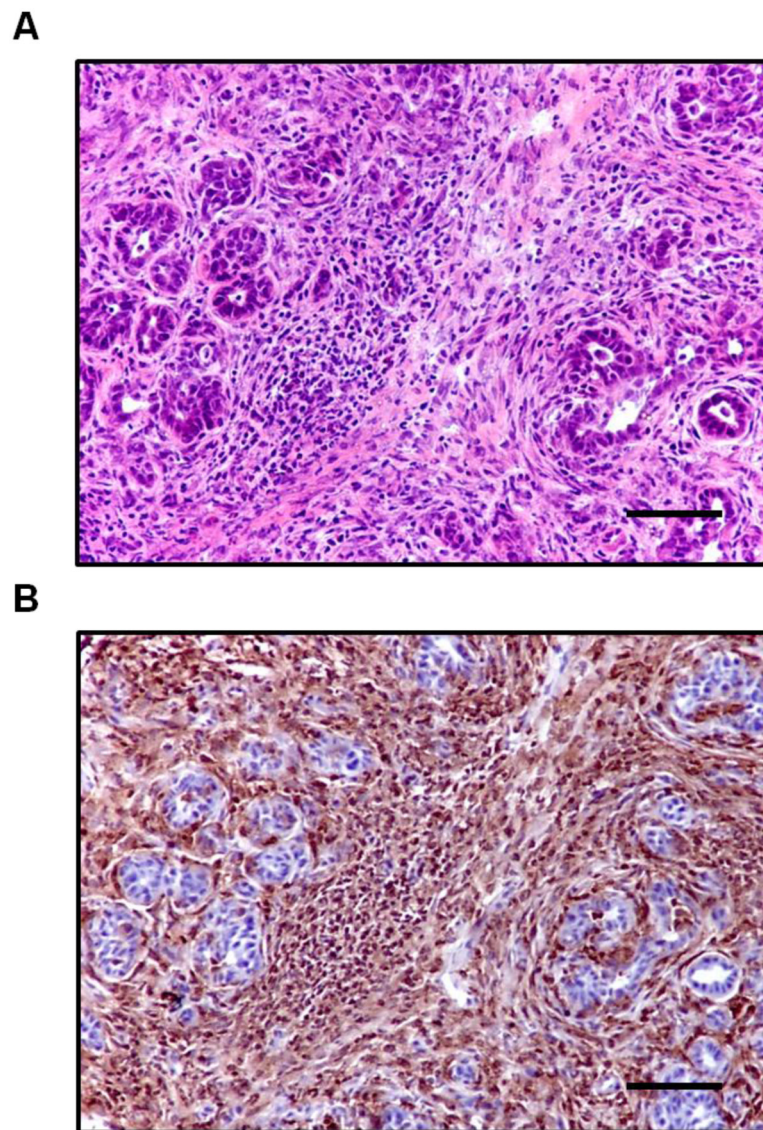- Combination immunotherapies in pancreatic cancer are warranted

**A**



**B**

**Figure 1.**
The PDA microenvironment is characterized by a dense desmoplastic reaction and prominent infiltration of leukocytes. (A) H&E stain and (B) immunohistochemistry for CD45 of a representative primary PDA lesion from a tumor-bearing KPC mouse. Scale bars, 100 μM.
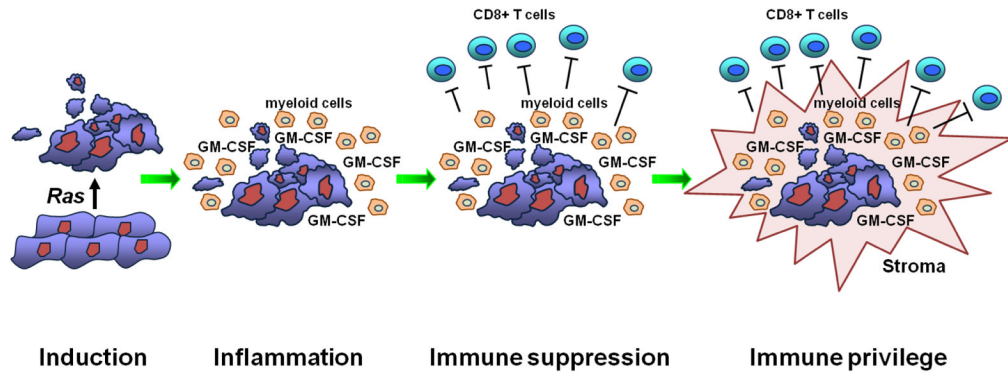
**Figure 2.**
In pancreatic ductal adenocarcinoma, the *ras* oncogene induces an inflammatory program that establishes immune suppression and ultimately immune privilege in the tumor microenvironment. One critical mediator is tumor-derived GM-CSF which drives the accumulation of immature myeloid cells that then directly suppress infiltrating T cells.