

Editorial

Breaking the Immune Tolerance to Apoptotic Cancer Cells Ingested by Phagocytes

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Abstract

Apoptosis occurs routinely during tissue turnover/remodeling and in cancer treatment by irradiation and chemotherapy. Clearance of autologous apoptotic cells by professional phagocytes suppresses autoimmune responses in part through the release of key immunosuppressive cytokines such as TGF- β , IL-10, IL-23, prostaglandin E2, and inhibition of immunostimulatory cytokines such as IL-12 and TNF α . The dilemma is that, when apoptotic cancer cells are removed by phagocytes, this evolutionarily homeostatic mechanism causes immune tolerance, limiting anti-tumor immunity. It represents a major hurdle to cancer therapy. How to break this intrinsic tolerance is a fundamental problem that we are actively addressing. Using molecular, cellular, and genetic approaches, we are attempting to identify the novel signaling molecules that control the activation of this intrinsic self tolerance mechanism and demonstrate in animal models of cancer the feasibility and impact of blocking these novel pathways in the reversal of the immunosuppression and facilitation of tumor-specific immune responses without causing overt autoimmune damage.

Cell loss in malignant disease is a very significant component of tumor dynamics and apoptosis is a common process in high-grade malignancy, with high apoptosis indices generally reflecting poor prognosis and a likely indication of the rapidity of apoptotic cell (AC) clearance *in situ* [1]. Activation of apoptosis is a well-established approach to cancer therapy. However, constitutive or therapy-induced apoptosis of tumor cell populations generates an immunosuppressive environment that protects malignant tissue from potential host anti-tumor immune mechanisms. It has been postulated that apoptosis contributes to oncogenesis through recruitment and appropriate activation of tumor-associated macrophages that support tumor growth and evolution; direct and indirect trophic effects resulting in net increases in tumor cell numbers; and anti-inflammatory and tolerogenic properties that suppress innate and adaptive anti-tumor immune responses [2].

Rapid removal of ACs is considered central to the resolution of inflammation and in preventing autoimmune disease [3]. In health, more than 1×10^9 ACs are cleared from the human body each day in the immune system alone [4], underscoring the importance of tightly regulated mechanisms to prevent the activation of proinflammatory responses to self. ACs express various autoantigens, and their persistence is thought

to generate harmful autoimmunity through the activation of self-reactive lymphocytes [5,6]. Conversely, the efficient removal of ACs can generate an immunoregulatory milieu and promote the resolution of inflammation, a process that relies on their recognition and engulfment by professional phagocytes macrophages and dendritic cells [3,7,8]. Phagocytosis of ACs usually results in an anti-inflammatory state with the induction of immunosuppressive cytokines such as TGF- β , IL-10 and prostaglandin E2, and inhibition of proinflammatory cytokines such as IL-12 and TNF α . We first demonstrated how AC-derived signals inhibited IL-12 gene expression [9]. Cell-cell contact with ACs via phosphatidylserine (PS) was sufficient to induce profound inhibition of IL-12 production by activated macrophages. The inhibition did not involve autocrine or paracrine actions of IL-10 and TGF- β . We identified a novel zinc finger nuclear factor, named GC binding protein (GC-BP), which was induced following phagocytosis of ACs by macrophages. GC-BP, activated via tyrosine phosphorylation upon AC-induction, selectively inhibited IL-12 p35 gene transcription by binding to its promoter *in vitro* and *in vivo*, thus decreasing IL-12 production. Blocking GC-BP by RNA interference restored IL-12p35 gene transcription and IL-12 synthesis. GC-BP activity was regulated via tyrosine phosphorylation in response to ACs [9].

Subsequently, we reported that IL-10 production stimulated by ACs was regulated at the point of transcription in a manner dependent on p38 mitogen-activated protein kinase, partially on the scavenger receptor CD36, and required cell-cell contact but not phagocytosis [10]. Furthermore, we showed that AC-induced transcriptional activation of IL10 was mediated by pre-B cell leukemia transcription factor-1b (Pbx-1) and another Hox cofactor Pbx-regulating protein 1 (Prep-1). This study also revealed a novel role of the two developmentally critical factors in the regulation of homeostasis in the immune system.

Recent studies indicate that IL-23 is over-expressed by macrophages and DCs in human and mouse tumors and antagonistically regulates local inflammatory responses in the tumor microenvironment and infiltration of intraepithelial lymphocytes. IL-23 upregulates proinflammatory cytokines IL-17 and IL-22, matrix metalloproteinase MMP9 and increases angiogenesis. In addition, IL-23 reduces tumor infiltration of cytotoxic T lymphocytes (CTLs), in contrast to IL-12's promotion of CTL infiltration. Conversely, blocking IL-23 via genetic deletion or antibody-mediated neutralization causes increased CTL infiltration into the transformed tissues and protects against chemically induced carcinogenesis and transplanted tumors [11].

We recently observed that in the absence of microbial stimuli, human and mouse myeloid DCs and macrophages engulfing ACs produced substantial amounts of IL-23, IL-6, and TGF- β , the essential cytokines for the development of Th17 cells that have been strongly implicated in human tumor immunity [12]. Furthermore, we identified two novel transcription factors, LRRC16B and FLJ44967, as critically important and direct for the induction of IL-23 in phagocytes exposed to ACs.

Taken together, these data supports our hypothesis that phagocytosis of apoptotic cancer cells (ACCs) induces immunosuppressive cytokines and inhibits immunostimulatory cytokines in part through the induction of GC-BP, Pbx-1/Prep-1, LRRC16B and FLJ44967, (collectively abbreviated as **GPLF**), generating an immunosuppressive microenvironment favoring tumor progression. Targeting these molecules, instead of interfering with the phagocytosis itself, will have anti-tumor benefits without causing overt autoimmune damage (Figure 1).

Our research in this area is focused on three aspects: (1) investigating how the cancer-promoting cytokine IL-23 is induced during phagocytosis of ACCs through the two novel transcription factors we have identified, LRRC16B and FLJ44967; (2) assessing the effects of targeting the three major transcription regulators (GC-BP, Pbx-1/Prep-1, and LRRC16B/FLJ44967) induced in the phagocyte/ACC interaction that regulate the expression of IL-12, IL-10, and IL-23, respectively, in a mouse tumor model via immunization using ACC-pulsed dendritic cells; (3) deciphering the role of TGF- β expression and signaling in phagocytes in regulating immune tolerance to ACCs via genetic targeting of the TGF- β gene and its signaling pathway in the myeloid compartment.

This research carries the potential of uncovering novel inner workings that underlie some of the most profound networks of nature and evolution. It will conceptually move the field forward, and inspire the development of innovative strategies to overcome immune tolerance to self tumor antigens in cancer-therapeutic modality and vaccination.

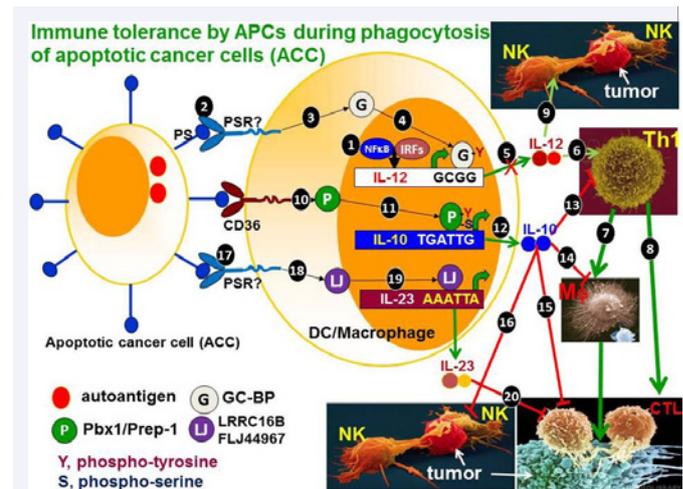


Figure 1 Model of apoptotic cancer cell-induced inhibition of IL-12 production, induction of IL-10 and IL-23 synthesis, and inhibition of cell-mediated immunity against cancer. IL-12 gene transcription is stimulated in professional antigen-presenting cells (dendritic cells and macrophages) by innate immune cues, such as TLR-mediated signaling, and by adaptive immune signals such as CD40L through activation of NF- κ B and interferon regulatory factors (IRFs) 1 and 8 that induce IL-12p35 gene transcription (#1). Apoptotic cancer cells (ACCs), principally via the "eat me" signal phosphatidylserine (PS) on their surface interacting with certain phagocytic receptors (PSR) on DCs (#2), induce GC-BP tyrosine phosphorylation (#3). Phosphorylated GC-BP translocates to the nucleus (#4) and blocks IL-12 production by binding to the proximal p35 promoter region at the "apoptotic cell response element" (ACRE) (#5). The lack of IL-12 results in the block of Th1 differentiation and activation from naive T (Th0) cells (#6), which limits cell-mediated immune responses against malignant tumors via two major cell types: macrophages (#7) and CTL (#8). Lack of IL-12 also directly dampens NK activation (#9). This process also induces the production of IL-10, a highly immunosuppressive cytokine, via the activation of homeoproteins Pbx-1 and Prep-1. The induction of IL-10 by ACCs involves the scavenger receptor CD36 on DCs (#10). The signaling triggered by the ACC-DC interaction induces serine and tyrosine phosphorylation of Pbx-1 and Prep-1, respectively (#11). The activated Pbx1/Prep1 complex binds to the IL-10 promoter at the "TGATTG" motif (#12), driving its transcription. IL-10 produced in this context can inhibit all four effector cell types critical for anti-tumor immunity: Th1, macrophages, CTL and NK (#13-16). The ACC-phagocyte interaction can induce IL-23 through a yet to be defined phagocytic receptor (#17), which activates LRRC16B/FLJ44967 (LJ) (#18). These two transcription factors bind to the proximal IL-23p40 promoter region at the "AAATTA" motif (#19) driving its transcription. IL-23 can inhibit CTL infiltration into tumors and upregulates IL-17 and IL-22, matrix metalloproteinase MMP9 and increases angiogenesis. (#20). Thus, blocking these transcriptional regulators during ACC/DC interaction may benefit anti-tumor immunity without interfering with the process of clearance of ACCs by phagocytes.

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