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Mini-review

Immune modulation by ER stress and inflammation in the tumor microenvironment

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ABSTRACT

It is now increasingly evident that the immune system represents a barrier to tumor emergence, growth, and recurrence. Although this idea was originally proposed almost 50 years ago as the "immune surveillance hypothesis", it is commonly recognized that, with few rare exceptions, tumor cells always prevail. Thus, one of the central unsolved paradoxes of tumor immunology is how a tumor escapes immune control, which is reflected in the lack of effective autochthonous or vaccine-induced anti-tumor T cell responses. In this review, we discuss the role of the endoplasmic reticulum (ER) stress response/unfolded protein response (UPR) in the immunomodulation of myeloid cells and T cells. Specifically, we will discuss how the tumor cell UPR polarizes myeloid cells in a cell-extrinsic manner, and how in turn, thus polarized myeloid cells negatively affect T cell activation and clonal expansion.

Introduction

Modern tumor immunology is predicated on the original Burnettian hypothesis of immune surveillance [1], which posits that the immune system is able to recognize tumor-associated antigens and acts as a cell-extrinsic regulator of tumor growth. Although over the years this hypothesis was regarded with skepticism, there have been numerous instances in which T cell responses against self tumor antigens have been detected in humans [2–6]. However, CD8 T cells generated by vaccination in melanoma patients are functionally heterogeneous and have a predominantly quiescent phenotype [7,8], reflecting perhaps a defective activation during priming. Consistent with this interpretation, studies in sporadic cancer initiated in mice through the rare spontaneous activation of a dormant oncogene showed that these tumors are in fact immunogenic and do not passively escape recognition by T cells but rather actively induce tolerance associated with the expansion of non-functional T cells [9]. Collectively, this suggests the anti-tumor T cell responses depend on a delicate balance between activation of the residual T cell repertoire specific for self tumor antigens and mechanisms controlling the state of activation and function of T cells against these antigens.

The anti-tumor adaptive immune response

Adaptive anti-tumor T cell responses are based on the recognition of antigens expressed on the surface of tumor cells in association with molecules of the major histocompatibility complex (MHC). However, there are several mechanisms by which self tumor antigens evade immune surveillance: tolerance/anergy [10–12], ignorance [13] and active immunosuppression through soluble mediators and metabolic derangement [14,15]. In addition, escape also occurs through immune suppression mediated by CD4 and CD8 regulatory T cells (Tregs) [16,17], a class of cells increased in patients with malignancies and in tumor tissues [18–21].

Studies in mice show that antigen specific tumor-infiltrating CD8 T lymphocytes display an activated phenotype but little cytotoxicity when transferred into tumor-bearing mice [22]. Sporadic tumors in mice are immunogenic but induce tolerance associated with the expansion of non-functional T cells [9]. T cells tolerant to self antigen return to a tolerant phenotype even after having resumed proliferation and function [23]. This shows that tumor-initiated *active* regulation of the adaptive T cell response plays an important role in the lack of effectiveness of anti-tumor immunity. The recent clinical success of immune checkpoint inhibitors (e.g. ipilimumab and

Abbreviations: APC, antigen presenting cells; ATF6, activating transcription factor 6; CHOP, CCAAT/-enhancer binding protein homologous protein; DC, dendritic cell; ECM, extracellular matrix; eIF2α, eukaryotic translation initiation factor 2 alpha; EMT, epithelial to mesenchymal transition; ER, endoplasmic reticulum; FOXP3, forkhead box 3; GRP78, glucose regulated protein 78; IDO, indoleamine 2,3-dioxygenase; IL-, interleukin; iNOS, inducible nitric oxide synthase; IRE1α, inositol requiring enzyme 1 alpha; LAG3, lymphocyte activation gene 3; MDSC, myeloid derived suppressor cell; MHC, major histocompatibility complex; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PD-L1, programmed cell death ligand 1; PERK, protein kinase-like endoplasmic reticulum kinase; PGE2, prostaglandin E2; SERCA, sarcoendoplasmic reticulum calcium transport ATPase; TAM, tumor associated macrophage; TCR, T-cell receptor; Tregs, regulatory T cells; TERS, transmissible endoplasmic reticulum stress factor; TIDC, tumor infiltrating dendritic cell; TNBC, triple negative breast cancer; TLR, toll like receptor; UPR, unfolded protein response.

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nivolumab) that release cytotoxic T cells from immunosuppressive signaling within the microenvironment of solid tumors of different histologic subtypes [24] further supports this contention.

Tumor-associated myeloid cells

Virtually all adult solid tumors (carcinomas most notably) contain infiltrates of diverse leukocyte subsets, mainly myeloid cells [25], which express the CD11b$^+$ surface marker [26,27]. These cells have been stratified into tumor-associated macrophages (TAM) (F4/80$^+$/Gr1$^+$), myeloid-derived suppressor cells (MDSC) (Gr-1$^+$) and tumor infiltrating myeloid dendritic cells (CD11c$^+$). Myeloid cells that infiltrate solid tumors are key players in the cell-extrinsic regulation of tumor growth and produce a variety of pro-tumorigenic factors (discussed further below) that effectively modify the tumor microenvironment and the immune cell landscape, ultimately leading to the inhibition of T cell responses *in vitro* and *in vivo* [28,29]. Early studies suggested that tumor-associated CD11b+/Gr1+ myeloid cells possess an anti-inflammatory and suppressive (M2) phenotype [30]. Similarly, tumor infiltrating dendritic cells (TIDCs) were described as having an immature phenotype characterized by low levels of MHC Class I and II, and co-stimulatory molecule (CD86/CD80) expression. Thus, it was suggested that these cells were responsible for defective T cell priming and anergy via a lack of antigen presentation function, which have been observed in the peripheral blood of cancer patients [31–34].

Recent evidence suggests that the tumorigenic phenotype of myeloid cells is concomitantly pro-inflammatory and actively immune suppressive [35]. For instance, in tumor-associated myeloid cells, the generation of reactive oxygen species is crucial for the inhibition of T cell responses either via arginase (Arg1), a classical M2 marker, or via iNOS, an inflammatory (M1) marker [29,36]. Furthermore, tumor-derived myeloid cells produce inflammatory cytokines that play key roles in tumor growth and in regulating anti-tumor immunity, including IL-6, IL-23, and TNF-α [14,37]. Moreover, TIDCs in melanoma, lung carcinoma, ovarian cancer, and breast cancer express high levels of MHC Class I/II, CD80, and CD86, yet they still inhibit anti-tumor CD8 T cell responses *in vitro* and *in vivo* due to a combination of inadequate antigen presentation, Arg1 production, or PD-L1 expression [38–42]. In a murine model of ovarian carcinoma, as well as in human ovarian tumor samples, "regulatory" TIDCs promote tumor outgrowth by suppressing T cell function within the tumor via PD-L1, Arg1, and IL-6 [41,43].

Importantly, large cohort studies in breast cancer patients have shown that the presence of CD68$^+$ macrophages correlates with poor prognostic features [44], increased angiogenesis [45] and decreased disease-free survival [46]. Likewise, increased numbers of CD68$^+$ macrophages in tumor stroma of patients with non-small-cell lung carcinoma (NSCLC) correlates with poor overall survival [47–49].

Tumorigenic cytokines in the tumor microenvironment

Inflammatory cytokines, often under the control of the transcription factor NF-κB, promote tumor cell survival, proliferation, and immune subversion. The predominant source of tumorigenic inflammatory mediators is tumor-infiltrating myeloid cells [37]. For example, inhibition of the NF-κB by ablation of IKKβ in liver macrophages results in loss of TNF-α and IL-6 production, which in turn, impairs tumor growth [50]. The deletion of IKKβ in macrophages leads to decreased production of PGE_2 and IL-6, resulting in reduced incidence of colitis-associated colorectal tumors [51]. CD11b$^+$ macrophages and dendritic cells of the lamina propria have been found to produce IL-6, which drives tumorigenesis in a mouse model of colitis-associated cancer [52]. Likewise, IL-6 and TNF-α produced by myeloid cells in response to tumor-derived versican drive lung cancer growth and progression in a TLR2-dependent manner [53]. To this one may add that progenitor cells and Kupffer cells in early dysplastic lesions in a model of carcinogen-driven liver carcinogenesis promote IL-6 production and progression to hepatocellular carcinoma (HCC) [54]. IL-23, produced predominantly by tumor associated macrophages [55], was found to block CD8 T cell infiltration into skin tumors and promote regulatory T cell differentiation in the melanoma microenvironment [56]. The neutralization of IL-23 with antibody combined with agonistic CD40 antibodies reduces primary fibrosarcoma and metastatic melanoma tumor burden [57]. Bacterial TLR ligands promote IL-23 production by adenoma-infiltrating myeloid cells, ultimately leading to secondary induction of other tumor-promoting cytokines (IL-17 and IL-6) and tumor promotion [37].

The TGFβ family of cytokines has different roles at different stages of tumorigenesis. The source of TGFβ can be tumor cells themselves, especially early in tumor growth; however, infiltrating myeloid cells are a major TGFβ source later during tumor progression (reviewed in Reference 58). Early during tumor growth, TGFβ restrains tumorigenesis by (a) repressing the cell cycle and inducing cell cycle inhibitors, (b) promoting cellular differentiation and senescence, (c) activating apoptosis, (d) suppressing autocrine and paracrine mitogenic signaling in neighboring stromal fibroblasts, and (e) inhibiting innate and adaptive immune cell function and tumorigenic cytokine production (reviewed in References 58 and 59). However, during tumor progression malignant cells inactivate downstream TGFβ signaling and co-opt tumorigenic functions of TGFβ signaling that include extracellular matrix (ECM) degradation via matrix metalloproteinase production [60], epithelial-to-mesenchymal transition (EMT) [61], and stimulation of angiogenesis [62]. TGFβ also promotes tumorigenic inflammatory and immunosuppressive effects in invading immune cells. For instance, TGFβ and IL-6 drive CD8 and CD4 T cell differentiation to Tc17 and Th17, which in turn facilitate tumor growth via promotion of angiogenesis and tumor cell proliferation [59]. On the other hand, TGFβ signaling polarizes tumor-associated myeloid cells to a suppressive phenotype, leading to the inhibition of T cell function *in vitro* and perhaps *in vivo* [59,63]. In addition, TGFβ signaling in CD8$^+$ T cells represses the expression of the *NKG2D* receptor, hence inhibiting their lytic activity [32,64].

The unfolded protein response (UPR) and its cell-intrinsic effects in tumor adaptation and progression

In mammalian cells, the ER stress response/UPR is mediated by three initiator/sensor ER transmembrane molecules: inositol-requiring enzyme 1 (IRE1α), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6), which, in the unstressed state, are maintained in an inactive state through association with 78 kDa glucose-regulated protein (GRP78) [65]. When a cell experiences ER stress, GRP78 disassociates from each of the three sensor molecules to preferentially bind un/misfolded proteins, allowing each sensor to activate downstream signaling cascades, which act to normalize protein folding and secretion. PERK phosphorylates eIF2α, resulting in the selective inhibition of translation, effectively reducing ER client protein load. IRE1α autophosphorylates, activating its endonuclease domain, resulting in the cleavage of *Xbp-1* to generate a shortened Xbp-1 isoform (Xbp-1s), which drives the production of various ER chaperones to restore ER homeostasis. IRE1 also activate JNK (c-JUN N-terminal kinase) through TRAF2-ASK1 signaling [66]. In addition, under prolonged ER stress or forced autophosphorylation, IRE1α's RNase domain can cause endonucleolytic decay of many ER-localized mRNAs through a phenomenon termed regulated IRE1α-dependent decay (RIDD) [67]. ATF6 translocates to the Golgi where it is cleaved into its functional form, and acts in parallel with XBP-1s to restore ER homeostasis [68]. If ER stress persists such that compensatory mechanisms fail, downstream signaling from PERK

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