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Critical Review

Role of the immunosuppressive microenvironment in immunotherapy

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Abstract Immunotherapy is reshaping cancer treatment paradigms; however, response rates to immune therapies are low and depend on the host's pre-existing antitumor immunity. The tumor microenvironment is comprised of malignant cells, stroma, and extracellular molecules and can hinder immune control of tumors. Herein, we review how anti-tumor immune responses are formed and how tumors avoid immune destruction. We also outline potential therapeutic targets in the immunosuppressive tumor microenvironment to promote immune control of tumors.

Introduction

Avoidance of immune control of tumor growth and spread is a hallmark of cancer.[1] Most human tumors express antigens, which are identified by the host immune system.[2] Clinical success with adoptive cell transfer demonstrates the potential for immune-mediated destruction of tumors.[3] Clinically evident tumors do not typically regress in the absence of immune therapy. Thus, in these patients, tumors have escaped immune control.

Immune therapy is based on the stimulation of the immune system to regain control of tumor growth, and its clinical practice predates modern chemotherapy and radiation. Immune therapy has evolved from Coley's toxin to systemic cytokines such as interleukin (IL) 2, to the more recently Food and Drug Administration—approved immune checkpoint inhibitors (ICIs) anti-CTLA-4 and anti-PD-1/PD-L1.[4-7]

Clinical successes with ICIs have led to new indications for immune therapy, with many active clinical trials underway looking to expand the role of immune therapy in modern cancer care.[8] Despite the potential for dramatic responses, initial response rates with ICIs are limited and depend on the host's pre-existing immunity to the cancer.[9-11] Methods to increase immune therapy

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response rates are under investigation and include identifying novel immune therapy targets and combinations with established therapies.

Tumors are composed of cancer cells and stromal features such as vasculature, fibroblasts, and infiltrating immune cells, which collectively form the tumor microenvironment (TME). The TME is highly variable between tumors and is vital for tumor growth, spread, and escape from immune-mediated destruction.[12,13] After clinical successes with ICI, preclinical models targeting other TME-mediated immunosuppressive pathways have identified novel targets.[14,15]

Combining immune therapy with conventional treatment modalities can improve response rates. Targeted ionizing radiation is a logical choice for combination with immune therapy because of its ability to cause focused cancer-cell death and release cancer-antigen and immune-activating molecules to prime T cell responses.[16-18] Preclinical models have demonstrated T cell priming after radiation[19] and increased homing of effector T cells into radiated tumors that display increased sensitivity to immune destruction.[20,21] Thus, tumor control after radiation therapy depends on T cell responses in the host.[19] However, radiation also induces changes in the TME, that inhibit immune control of tumors.[22] Understanding and targeting these radiation-induced changes will lead to rational radioimmune therapy treatments and are under active investigation.

In this review, the role of the TME in controlling antitumor immune responses is described. The basics of priming antitumor T cell responses and the mechanisms of T cell–mediated cell killing are discussed, and the impact of individual components of the TME on both processes will be examined.

Immune system and cancer

The immune system can detect and eliminate cancers before they manifest clinically. Mice that have been genetically engineered to have defective immune systems exhibit increased incidences of carcinogen-induced and spontaneous tumors.[23] Clinically, increased incidence of a variety of cancers is observed in immunosuppressed patients.[24] However, most patients with clinically apparent cancers are immune competent and harbor tumors that have escaped immune control. Regaining immune control of tumor growth by promoting robust, adaptive immune responses can lead to tumor regression and is the primary goal of immune therapy.

Antitumor adaptive immune responses are predicated on the processing of tumor antigens by antigen-presenting cells, which, when subjected to appropriate activation signals, migrate to secondary lymphoid tissue where they prime naïve T cells. Antigen-presenting cells in the tumor include macrophages, B cells, and dendritic cells (DCs). Of these, DCs express both major histocompatibility complexes (MHC) I and II for activation of cluster of differentiation (CD) 8 and CD4 T cells, respectively, and can also produce costimulatory molecules that overcome the activation threshold of naïve T cells. DCs uniquely possess the ability to access phagocytosed antigen in the cytosol for presentation on MHC I, a requisite to cross-present tumor antigen to prime cytotoxic CD8 T cells. Increased DC infiltration correlates with favorable outcomes.[25] Of note, there are multiple subsets of DCs with different impacts on tumor immunology.[26,27] Of these, $CD8^{+}CD103^{+}Batf3^{+}$ DCs are critical to priming CD8 T-cell responses in preclinical studies.[28-30]

Naïve T cells recognize antigen in complex with major histocompatibility via their T cell receptor. In the presence of a second, costimulatory signal such as B7 ligands binding to CD28, the naïve T cell is stimulated to proliferate and differentiate into effector T cells. Subsequently, effector CD8 T cells can recirculate back into the tumor, where they induce caspase-dependent apoptosis in cancer cells expressing the recognized antigen on MHC I. Durable immune control of tumors may depend on continuous priming of robust, adaptive immune responses to tumors that evolve under selective immune pressure.[31]

Tumor microenvironment is a barrier to effector immune cells

Clinically evident tumors have, by default, escaped host immune control despite evidence for tumor-reactive T cells.[32,33] Administration of ICIs may regain immune control of tumor growth by boosting the host's own pre-existing tumor immunity. In addition to expression of immune checkpoint–related molecules, mechanisms by which the TME may suppress efficacy of effector T cells are listed in Table 1 and displayed in Figure 1. Dysregulated cancer cell growth can lead to tumor intrinsic immunosuppressive features, such as regions of hypoxia and elevated levels of lactate that can inhibit effector T cell function.[34] However, the majority of immune suppression is due to the presence of normal immune regulatory cells and molecules within the tumor that inhibit T-cell priming or suppress cytotoxic T cell function.[35,36] Of these, immune checkpoint–related molecules have garnered the most attention, with proven clinical success of ICIs. Accordingly, expression of immune checkpoint–related molecules by both tumor and stromal cells has been reviewed extensively elsewhere.[37]

A highly variable component of the TME with significant impact on the immune control of tumors is tumor-infiltrating lymphocytes (TILs). TILs provide evidence for an antitumor immune response but do not always correlate with favorable prognosis.[38] Advances in analytical tools have identified diverse subsets of infiltrating immune cells with either directly cytotoxic,

Table 1 Mechanisms of immunosuppression in the tumor microenvironment

Mediator	Mechanism of immunosuppression	References
Cell surface proteins		
Programmed death-ligand 1	Induce T-cell tolerance/anergy after ligation with programmed cell death protein 1 on T cells	37
CTLA-4	Inhibit activation of naïve T cells	37
	Enhance regulatory T cell function	74
↓Major histocompatibility complex I	Avoid detection by effector CD8 T cells	75
↓FAS	Avoid FAS ligand–mediated cell killing	
↓TRAIL	Avoid TRAIL-mediated cell killing	
CD39/CD73	Convert extracellular immunostimulatory adenosine triphosphate to immunosuppressive adenosine	76
Secreted cytokines		
Transforming growth factor beta	Inhibit T cell priming and infiltration	77
	Suppress effector cell cytotoxicity	47
Vascular endothelial growth factor	Inhibit dendritic cell maturation	78
	Enhance programmed cell death protein 1/programmed death-ligand 1/2 expression	79
	Enhance interleukin-10 secretion	
Interleukin-10	Inhibit major histocompatibility complex II expression on antigen presenting cells	80
	Suppress M1 cytokine secretion	81
	Suppress iNOS (inducible Nitric Oxide Synthase)	82
	Induce T cell anergy	83
Metabolic pathways		
Indoleamine-2,3 dioxygenase	Convert tryptophan to kynurenine	55
	Inhibit T cell proliferation	84
Adenosine	Inhibit T cell proliferation and activation	85, 86
Hypoxia	Inhibit effector T cell function	87
	Promote prostaglandin E2 synthesis	88
Lactate	Inhibit effector T cell function	89
Arginase	Degrades L-arginine needed for cytotoxic iNOS production	90
Prostaglandin E2	Inhibit effector T cell function	91, 92
	Suppress M1 cytokine secretion	93
	Recruit myeloid-derived suppressor cells	94

Abbreviations: CD = cluster of differentiation; TRAIL = tumor necrosis factor-related apoptosis-inducing ligand.

immune-promoting or immunosuppressive functions. Thus, infiltrating lymphocytes may lead to an immune supporting or suppressing TME depending on which cell subsets dominate.

Of the immunosuppressive infiltrates, regulatory T cells (Tregs) are identified as $CD4^+CD25^+FOXP3^+$ and play an important role in normal physiology by moderating immune destruction and preventing autoimmune disease.[39] Tregs are commonly found in solid tumors and promote immunosuppression by several mechanisms including secretion of immunosuppressive cytokines, competing for activating cyokines with effector cells, and after direct cellular contact with infiltrating effector cells.[40,41] However, elevated levels of Treg infiltrates in tumors can accompany elevated levels of effector T cell infiltrates and thus are not absolute indicators of an overall immunosuppressive TME.[42] Recent data using advanced image analysis techniques demonstrated that the proximity of effector CD8 T cells to Tregs correlated with worse prognosis in patients with oral cancer,[43] suggesting that spatial distribution of subsets within the tumor, rather than mere presence or prevalence, may determine their immunosuppressive effect.

Tregs contribute to the level of transforming growth factor beta (TGFβ) in the tumor, which is a mediator of immunosuppression and subverts both adaptive immune priming and effector responses. TGFβ can disrupt T cell activation by limiting the mobility and longevity of DCs and separately may preferentially promote activation of Tregs.[44,45] TGFβ promotes alternatively polarized macrophages (discussed in the next section), which may compete with DCs for tumor antigen and further inhibit T cell priming.[46] TGFβ also inhibits the cytotoxicity of CD8 T cells.[47] Thus, inhibiting TGFβ has the potential to disrupt the immunosuppressive TME on multiple fronts, and preclinical studies have shown improved tumor responses to radiation when combined with TGFβ inhibition.[48,49]

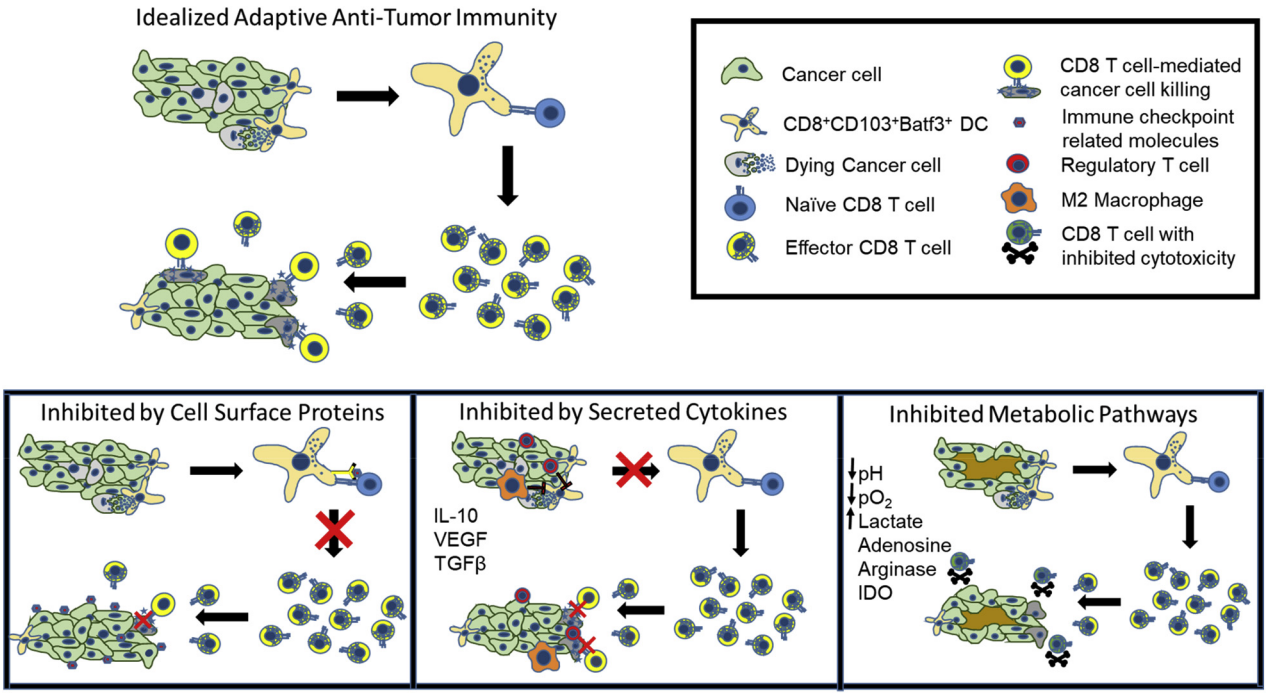

Figure. 1 Adaptive antitumor immunity and mechanisms of inhibition in the tumor microenvironment.

The most prevalent antigen-presenting cells within the TME are macrophages. Macrophages can be differentiated into opposing phenotypes, M1 and M2 (Fig 2). M1 phenotypes can be induced by Toll-like receptor stimulation in the presence of interferon gamma and express proinflammatory cytokines, such as IL-12. M2 phenotypes are induced by IL-4 and IL-13 produced by $CD4^+$ T helper 2 cells,[50] and are associated with the production of arginase I and anti-inflammatory IL-10.[51] Arginase converts arginine to ornithine and urea, making arginine unavailable for nitric oxide–mediated cell killing. Arginase also downregulates the ζ chain of T cell receptor, which inhibits T cell activation.[52] Preclinical models demonstrate a role for arginase expression by macrophages in limiting T cell control of irradiated tumors.[53]

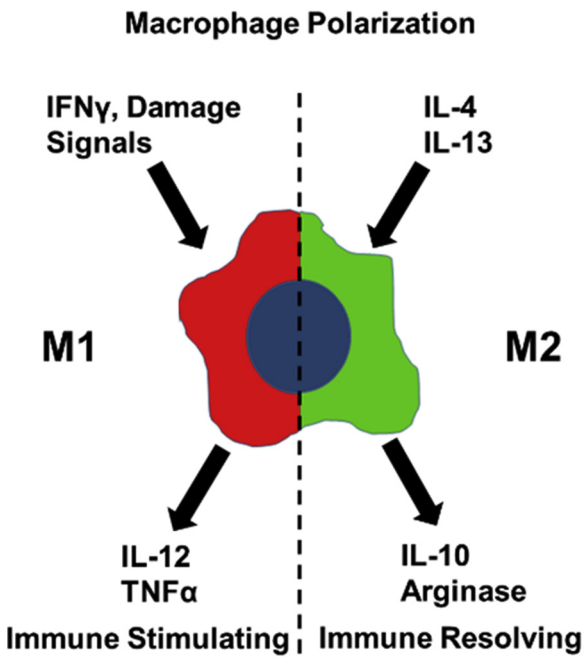

Figure. 2 Polarization states of macrophages.

A range of other metabolic features of tumors limit immune activation, including the enzyme indoleamine 2,3-dioxygenase (IDO). IDO plays a role in immune tolerance to apoptotic cells in normal physiology[54] and is readily induced in the tumor. IDO converts tryptophan to kynurenine, which suppresses effector T cell activation and promotes the generation of Tregs and infiltration of myeloid-derived suppressor cells.[55-57] In preclinical models, inhibiting IDO improves the efficacy of conventional cancer treatments and the immune control of tumors.[58] Moreover, $IDO^{-/-}$ mice showed increased responses to ICIs in a melanoma model, suggesting better immune therapy outcomes for ICIs in combination with IDO inhibitors.[59]

Although macrophages can promote or suppress adaptive immunity depending on phenotype, the presence of macrophages in human tumors is associated with poor prognosis.[60] In preclinical studies, macrophages are the most abundant infiltrating cell in the tumor stroma after tumor irradiation and exhibit M2 properties that suppress subsequent immune responses.[53,61,62] Depleting macrophages with anti–colony stimulating factor 1 antibodies or chemokine ligand 2 blockade slowed growth and improved responses to immune therapy and ionizing radiation in a number of tumor models.[63,64] Apart from

depletion, several strategies have been demonstrated to repolarize macrophages to an M1 phenotype, which restores adaptive immune control of tumors.[65] The prevalence of macrophages in tumors and the potential for repolarizing to M1 phenotypes makes them an attractive target for establishing an immune-promoting TME.

Another critical component of tumors and a prerequisite for antigen presentation is the presence of dead cancer cells. In tumors, cell death occurs from dysregulated growth and cytotoxic therapies. Identification and phagocytosis of apoptotic cells is a tightly regulated process and critical for normal physiology and cell turnover. Phagocytosis of apoptotic tumor cells could comprise a source for processing and presenting cancer antigens on MHC II. However, mounting evidence indicates that phagocytosis of apoptotic cell leads to immune-tolerizing rather than immune-activating antigen presentation.[66]

Blocking phagocytic pathways can improve tumor control after therapeutic radiation.[61,67] Invoking non-apoptotic cell death with release of inflammatory mediators may be key to promoting synergy between ionizing radiation and immune therapy.[68-70] Thus, in this scenario, the goal of radiation is not only to induce cancer cell killing, but to cause immunogenic cell death while controlling how the TME handles the dead cells to promote an adaptive immune response.[71]

Clinical trials combining radiation with immune therapy targeting the TME

Targeting the immunosuppressive TME can promote adaptive immune priming and improve the efficacy of effector cell killing and cytotoxic therapies. Radiation is a logical choice to synergize with immune therapies because it may induce immunogenic cell death intratumorally to prime an adaptive immune response and increase the homing of effector cells into tumors. Clinical success with ICIs has demonstrated the potential for targeting the TME to promote immune control of tumors. Hundreds of active phase 2 and 3 clinical trials are evaluating novel immune therapies and concurrent immune therapy combinations targeting the immunosuppressive TME. A comprehensive listing of active trials is beyond the scope of this review, and readers are directed to serial publications aimed at reviewing such trials for further edification.[72,73] The results from these trials will guide future studies of novel targeted immune therapies and immune therapy combinations.

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