Review

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Checkpoint blockade for cancer therapy: revitalizing a suppressed immune system

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Immune checkpoint receptors are crucial molecules for fine-tuning immune responses. Checkpoint signaling dampens T cell activation to avoid autoimmunity and the destructive effects of an excessive inflammatory response. It is well established that tumors use several mechanisms to avoid elimination by the immune system, and one involves hijacking these checkpoint pathways. Checkpoint blockade therapy utilizes monoclonal antibodies to release the brakes from suppressed T cells, allowing them to be activated and recover their antitumor activity. This therapeutic approach has revolutionized cancer immunotherapy, and extraordinary increases in overall survival were noted, first with anti-CTLA-4 (cytotoxic T lymphocyte-associated protein 4) and subsequently with anti-PD-1 (programmed cell death receptor-1) in melanoma and other malignancies.

'Natural forces within us are the true healers of disease' (Hippocrates)

An immune response against tumor cells is clearly beneficial for the prognosis of most cancer patients. Spontaneous regression of tumors associated with detectable antitumor immune cells has been reported, albeit rarely [1,2]. Circulating tumor-reactive and tumor infiltrating lymphocytes (TILs) are considered a favorable prognostic marker in several malignancies including melanoma, hepatocellular carcinoma, and ovarian cancer [3–6]. The graft-versus-tumor effect generated by the ability of allogeneic lymphocytes to eradicate residual tumor cells is now believed to be responsible for much of the therapeutic effect of allogeneic stem cell transplantation [7]. Direct evidence for the potent antitumor effects of immune cells can be gathered from the observation that infusion of donor lymphocytes can produce remissions in patients with hematological malignancies who have relapsed after allogeneic stem cell transplantation [8]. Moreover, *in vitro* expansion and adoptive transfer of TILs has proved to be successful in the treatment of different types of cancers [9].

The idea of actively harnessing the immune system as an approach to cancer therapy is not new, but conviction about the clinical effectiveness of this therapeutic approach has been variable. Initial enthusiasm for therapeutic vaccines against cancers in the 1980s through mid-1990s gave way to increasing skepticism and dejection as clinical trial after clinical trial failed to demonstrate significant, consistent, and durable effects. The renaissance of cancer immunotherapy began with the publication of reports that ipilimumab, an antibody that blocked CTLA-4 (see Glossary) [10] produced clinical responses in 13% of patients [11]. Indisputable evidence for the anticancer activity of CTLA-4 blockade was demonstrated in a Phase III trial with advanced melanoma patients, where ipilimumab reduced the risk of death by 32% [12]. After CTLA-4, several other ligand–receptor signaling axes with additional immune-regulatory functions have been described and are presently in various stages of clinical testing (Figure 1) [13]. These signaling pathways,

Glossary

Antigen-presenting cell (APC): any type of cell that can process antigens and present them to T cells in conjunction with MHC and other costimulatory molecules, typically leading to the activation of T cells.
CD28: a costimulatory receptor expressed on the surface of T cells. It is responsible for the second signal of T cell activation after peptide recognition by the TCR.
Cytotoxic T lymphocyte-associated protein 4 (CTLA-4, CD152): a protein expressed mainly on the surface of T cells that transmits inhibitory signals when bound to its ligands, CD80 and CD86.
Immune checkpoints: a group of inhibitory pathways in the immune system whose function is maintaining self-tolerance and modulating the immune response to avoid tissue damage and autoimmunity.
Major histocompatibility complex (MHC): a set of molecules expressed on all nucleated cells and at high levels on APCs that are crucial for antigen-specific activation of T cells.
Myeloid-derived suppressor cells (MDSCs): a heterogeneous group of cells of myeloid origin that possess immune suppressive activity.
Programmed cell death 1 (PD-1, CD279): a surface protein, expressed on T cells and B cells. It transmits inhibitory signals when bound to its ligands PD-L1 and PD-L2.
Regulatory T cells (Tregs): a subset of $CD4^+$ T cells with immune-modulatory properties. In healthy individuals they maintain tolerance to self-antigens and abrogate autoimmune diseases.
T cell receptor (TCR): a receptor expressed on the surface of T cells. Each TCR specifically recognizes unique peptides displayed on MHC molecules, thus providing the T cell with the initial activation signal, signal 1.
Tumor-associated antigen: an antigenic protein produced by tumor cells that has the potential of triggering an immune response in the host.
Tumor infiltrating lymphocytes (TILs): lymphocytes found inside tumors or in the tumor stroma. Although they can also include B cells and natural killer (NK) cells, the term usually refers to $CD4^+$ and $CD8^+$ T cells.

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Keywords: cancer immunotherapy; PD-1; CTLA-4; checkpoint blockade; MDSCs.

1471-4914/
 http://dx.doi.org/10.1016/j.molmed.2015.05.005

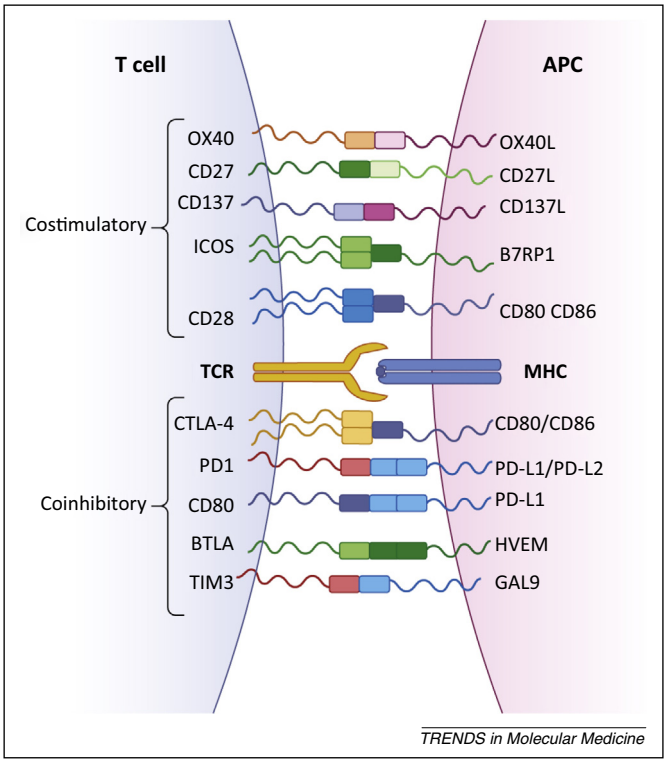

Figure 1. Costimulatory and coinhibitory receptors in the immune synapse. The fine-tuning of the immune response is coordinated by a plethora of coreceptors that are responsible for amplifying or dampening the initial immune response. Most of these receptors require the T cell receptor (TCR) to specifically recognize a peptide displayed by a MHC molecule on an antigen-presenting cell (APC), to deliver their costimulatory or coinhibitory signal. These interactions can take place either in secondary lymphoid sites where naïve T cells encounter antigen for the first time, or in the periphery where effector cells may be activated (or suppressed). Abbreviations: B7RP1, B7-related protein 1; BTLA, B and T lymphocyte attenuator; GAL9, galectin 9; HVEM, herpesvirus entry mediator; ICOS, inducible T cell costimulator; TIM3, T cell membrane protein 3.

collectively termed 'immune checkpoints', have proved to be an excellent target for cancer immune therapy. The focus of this review is on two pathways that are most advanced in terms of clinical development and use: CTLA-4 and PD-1 (Box 1).

Checkpoint blockade and antitumor immunity

To recognize the significance of immune checkpoint inhibitors, one needs to understand the elements of T cell mediated antitumor immunity. The classical model for T cell activation was first described in 1970 [14] as a two-step process that requires engagement of the T cell receptor (TCR) to an antigen presented by an antigen-presenting cell (APC), and a second costimulatory signal delivered by the engagement of CD28 to its ligands CD80 and CD86 [15,16]. This rudimentary model has been further elaborated by the discovery of additional costimulatory and coinhibitory pathways that work concurrently or in sequence to boost or dampen T cell activation (Figure 1). This interplay of the diverse and opposing signals cumulatively regulates the thin line between an effective and a destructive immune response.

Tumors commandeer this finely tuned immune homeostasis, actively suppressing T cell activation and effector function. Tumors create an environment hostile for T cell function, and drive T cells into adaptive tolerance after excessive antigen stimulation while simultaneously usurping the inhibitory pathways to avoid elimination by T cells.

Box 1. CTLA-4 versus PD-1: different time, different place

While both CTLA-4 and PD-1 are involved in setting the brakes on the immune system, there are substantial differences between them. CTLA-4 is displayed on the surface of naïve T cells upon activation in the lymph node. PD-1 is expressed on effector T cells after activation in the periphery. In the case of CTLA-4, the checkpoint function avoids systemic destructive immune responses. By contrast, PD-1 is involved in controlling possible tissue collateral damage after an inflammatory response. Both pathways can be hijacked by tumors to avoid antitumoral immune responses.

Cytotoxic T lymphocyte-associated protein 4 (CTLA-4)

The first immune checkpoint molecule to be described, CTLA-4 [10], is a type 1 transmembrane protein that is expressed on the surface of T cells upon activation, and a member of the immunoglobulin superfamily. It shares ligands with CD28, but binds to them with a ~10-fold higher affinity [17]. The binding of CTLA-4 to CD80 or CD86 results in downstream signaling that culminates in T cell inhibition, diminished cytokine production, and restricted proliferation.

CTLA-4 is present within intracellular vesicles in T cells that are not activated and appears on the cell surface following activation [18]. The role of CTLA-4 spans the entire continuum of T cell mediated immune activity. At the most essential level CTLA-4 competes with CD28 for binding of CD80 and CD86, thereby antagonizing the positive signaling axis for T cell activation [19]. Other mechanisms include blocking T cell receptor downstream signaling [20] and inhibition of maturation and antigen presentation by APCs [21]. Cell-extrinsic mechanisms by which CTLA-4 negatively influences immune responses include expansion of regulatory T cells, that produce immune-suppressive cytokines such as transforming growth factor β [22], as well as inducing indoleamine-pyrrole 2,3-dioxygenase (IDO) production in APCs following interaction with CTLA-4-expressing T cells [23].

Effect of CTLA-4 blockade on myeloid-derived suppressor cells

Although CTLA-4 expression was initially considered to be T cell specific, there are reports suggesting that ipilimumab may also act on additional cellular populations. Two independent studies have shown that ipilimumab treatment reduces the frequency of granulocytic (Gr) [24] and monocytic (Mo) myeloid-derived suppressor cells (MDSCs) in the circulation [25]. In the first report [24], the reduction in the frequency of GrMDSC was accompanied by a reduction in the frequency of $Arg1^+$ (arginase 1) myeloid cells, which suggests that the suppressive capability of these cells (via arginine starvation of T cells) was also diminished after treatment. In the second study [25], not only was a decrease in MoMDSC observed but the magnitude of this decrease was associated with progression-free survival (PFS). It is also important to highlight that response to