Computational and Structural Biotechnology Journal 13 (2015) 265–272

COMPUTATIONAL AND STRUCTURAL BIOTECHNOLOGY JOURNAL

journal homepage: www.elsevier.com/locate/csbj

Mini Review

A2aR antagonists: Next generation checkpoint blockade for cancer immunotherapy

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ARTICLE INFO

Article history:
Received 21 November 2014
Received in revised form 26 March 2015
Accepted 31 March 2015
Available online 8 April 2015

Keywords:
A2a adenosine receptor
Immune checkpoint
Tumor
T cell
Immunotherapy
PD-1

ABSTRACT

The last several years have witnessed exciting progress in the development of immunotherapy for the treatment of cancer. This has been due in great part to the development of so-called checkpoint blockade. That is, antibodies that block inhibitory receptors such as CTLA-4 and PD-1 and thus unleash antigen-specific immune responses against tumors. It is clear that tumors evade the immune response by usurping pathways that play a role in negatively regulating normal immune responses. In this regard, adenosine in the immune microenvironment leading to the activation of the A2a receptor has been shown to represent one such negative feedback loop. Indeed, the tumor microenvironment has relatively high concentrations of adenosine. To this end, blocking A2a receptor activation has the potential to markedly enhance anti-tumor immunity in mouse models. This review will present data demonstrating the ability of A2a receptor blockade to enhance tumor vaccines, checkpoint blockade and adoptive T cell therapy. Also, as several recent studies have demonstrated that under certain conditions A2a receptor blockade can enhance tumor progression, we will also explore the complexities of adenosine signaling in the immune response. Despite important nuances to the A2a receptor pathway that require further elucidation, studies to date strongly support the development of A2a receptor antagonists (some of which have already been tested in phase III clinical trials for Parkinson Disease) as novel modalities in the immunotherapy armamentarium.

Contents

Abbreviations: A2aR, adenosine A2a receptor; APC, antigen presenting cell; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DLBCL, diffuse large B-cell lymphoma; Hif1-alpha, hypoxia inducible factor-1 alpha; LAG-3, lymphocyte-activation gene 3; NSCLC, non-small cell lung cancer; ORR, overall response rate; OS, overall survival; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; TFS, tumor free survival; TIM-3, T-cell immunoglobulin domain and mucin domain 3; T_{reg}, regulatory T cell.

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http://dx.doi.org/10.1016/j.csbj.2015.03.008

1. Introduction

The immune system has evolved an array of regulatory mechanisms to protect against tissue damage from autoimmunity or during active response to pathogen. Both central mechanisms (negative selection in the thymus) and peripheral mechanisms (e.g., deletion, anergy, and regulatory T cells (T_{regs})) contribute to establishing self-tolerance. Nonetheless, inherent in active immune responses against pathogens are inhibitory and negative feedback pathways which prevent collateral damage. Included in these protective mechanisms are a broad array of inhibitory receptors that are upregulated on lymphocytes during an active immune response. These inhibitory receptors and their related signaling networks, known as "immune checkpoint pathways," provide a negative feedback mechanism that is crucial for immunoregulation and protection of tissues from an overexuberant inflammatory response.

While the negative feedback loops created by checkpoint pathways are critical in modulating excessive inflammation, they are also subject to dysregulation in the presence of cancer and provide tumors with a means of immune evasion. Recently, clinical trials have confirmed that blockade of immune checkpoint pathways mediated by the CTLA-4 and PD-1 receptors can unleash an endogenous immune attack, leading to significant responses and long-term remissions in multiple solid tumor types [1–3]. In fact, antibody-mediated blockade of CTLA-4 and PD-1, alone or in combination, have led to unprecedented responses in refractory, metastatic melanoma, as well as in renal cell carcinoma and non-small cell lung cancer. The success of checkpoint blockade in these trials has been a major step forward in the development of immunotherapy for the treatment of cancer, confirming the clinical importance of tumor immune evasion through usurping fundamental pathways of immune regulation. With the success of CTLA-4 and PD-1 inhibition in clinical trials, significant effort has focused on uncovering other targetable checkpoint pathways active in the tumor microenvironment. In this regard, adenosine signaling through the A2a receptor has been found to function as one such promising negative feedback loop [4–7]. As we shall discuss, while the effects of A2a receptor inhibition in antitumor therapy can behave as a double-edged sword (depending on the degree and, likely, the duration of signaling blockade), preclinical studies have confirmed that blockade of A2a receptor activation has the ability to markedly enhance anti-tumor immunity. As such, A2a receptor blockade represents the potential next generation of immune checkpoint inhibition in cancer immunotherapy.

2. CTLA-4 and PD-1 and the arrival of cancer immunotherapy

Immune checkpoint pathways such as those mediated by CTLA-4 and PD-1 receptors are critical aspects of normal physiologic function. The hallmark of these pathways is the generation of a negative feedback loop that preserves self-tolerance and prevents excessive tissue damage in the setting of immune response. The pathways regulated by CTLA-4 and PD-1 receptors have somewhat distinct modes of action on the immune response [5,8]. CTLA-4 is upregulated during initial activation of effector T cells and is thought to counteract the activity of the co-stimulatory receptor CD28 by two mechanisms. By out-competing the lower affinity CD28 for engagement of shared, cognate ligands B7.1 and B7.2 on antigen presenting cells (APCs), as well as by providing a direct inhibitory signal, the CTLA-4 receptor dampens the effector T cell activation sequence [5,9–11]. CTLA-4 is also strongly expressed on regulatory T cells and enhances immunosuppression through enhancing T_{reg} activity and proliferation [12]. Like CTLA-4, PD-1 is induced upon effector T cell activation and is also highly expressed on T_{regs} [13–15]. Cognate ligands for PD-1 include PD-L1 and PD-L2. These are constitutively expressed on APCs and are induced in peripheral tissues during inflammatory responses or on the surface of tumor cells [13,16,17]. Among other inflammatory cytokines, interferon-gamma secreted during an immune response is a potent inducer of PD-L1 expression [18–20]. In contrast to CTLA-4, PD-1 is expressed on a broader range of immune cells (e.g., B lymphocytes and monocytes) [5,20–22]. And while PD-1 signaling is initiated during T cell activation, its primary effects of inducing CD8 + T cell anergy and, conversely, regulatory T cell activity and proliferation appear to be more pronounced during effector function in the peripheral tissues [5]. The critical roles of PD-1 and CTLA-4 in immune modulation were demonstrated in early studies that showed severe autoimmune pathologies in PD-1 and CTLA-4 knockout strains [22–24].

Although crucial in moderating inflammatory responses and preventing autoimmunity, checkpoint pathways can provide an immune evasion mechanism for tumors, allowing unchecked growth and progression. Preclinical studies have shown that PD-1 is expressed on a broad range of tumor infiltrating lymphocytes and is especially prominent on infiltrating T_{regs} and CD8 effector cells [25,26]. The PD-1 ligands, PDL1 and PDL2, are upregulated on a variety of tumor cells, and are also expressed by myeloid cells in the tumor microenvironment [27]. In studies by Dong et al., tumors expressing high levels of PD-L1 were found to promote apoptosis of tumor antigen-specific T cells in vitro as well as in mouse tumor models [17]. Early studies of antibody-mediated CTLA-4 blockade in a variety of transplantable tumor models (e.g., colon carcinoma, fibrosarcoma, ovarian cancer, and prostate cancer) demonstrated significant tumor response. An especially important finding in these studies was that once mice had experienced a response to CTLA-4 blockade, they were resistant to tumor rechallenge. These results demonstrated that, in addition to promoting regression of primary tumors, checkpoint inhibition facilitates the generation of an immunologic memory response that is associated with durable tumor remission [28,29].

These preclinical findings were validated in clinical trials of several immune checkpoint inhibitor antibodies [20]. In two large initial phase III trials, the anti-CTLA-4 monoclonal antibody ipilimumab significantly prolonged survival and produced durable responses in patients with advanced melanoma [1,30]. CTLA-4 blockade has also been shown to be active in patients with renal cell carcinoma and in patients with NSCLC [2,3]. Clinical studies of anti-PD-1 mAbs have also shown improvement in overall survival with durable responses in a variety of heavily pre-treated tumor types, including melanoma, NSCLC, and renal cell carcinoma [31]. Anti-PD1 mAbs have shown activity in hematologic malignancies as well, demonstrating a 66% ORR when combined with rituximab for follicular lymphoma [32], and a 51% ORR in patients with diffuse large B-cell lymphoma (DLBCL) who have progressed after autologous stem cell transplant [33]. Blockade of the PD-1 ligand PD-L1 has also shown activity in melanoma, renal cell cancer, and NSCLC, with overall response rates of 10–17% [34]. Importantly, a recent phase I trial demonstrated that the combination of PD-1 and CTLA-4 blockade produces greater than additive response rates in melanoma patients, with an ORR of 42% [35].

3. Adenosine-A2aR signaling: the emergence of a novel immune checkpoint pathway

While the clinical importance of immune checkpoints mediated by CTLA-4 and PD-1 has become clear, there are a number of other pathways active in the immune microenvironment that also appear to be important contributors to tumor immune evasion. While several of these pathways—like PD-1 and CTLA-4—are triggered by membrane-bound ligands (most notably LAG-3 and TIM-3 pathways), there are also soluble ligands found in the immune microenvironment that can function as triggers for checkpoint pathways [5]. Such soluble checkpoint ligands include tumor metabolites and cytokines such as IL-10 and TGF-beta [36]. Studies over the last two decades have also identified extracellular adenosine as a critical element in immune regulation [37–40].