Review Article

Effect of Pattern Recognition Receptor (PRR) Activation on Immune Suppression and Potential Implications for Cancer Treatment

Shanta Bantia¹*, and Nirmal Choradia²

¹Nitor Therapeutics, 689, Highland Lakes Cove, Birmingham, AL-35242, USA
²Palo Alto Veterans Affairs Hospital, 3801 Miranda Ave, Palo Alto, CA, 94304, USA

*Corresponding author: Shanta Bantia, Nitor Therapeutics, 689, Highland Lakes Cove, Birmingham, AL-35242, USA. Tel: +205-617-7655; E-mail: sbantia@nitortheraeutics.com

Received: August 16, 2019; Accepted: September 03, 2019; Published: September 10, 2019

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Abstract

Pattern Recognition Receptors (PRRs) are essential to the body’s innate immune response to both pathogens and malignant cells. PRR agonists activate the innate immune response and are currently being explored as immunotherapeutic treatment options in cancer patients. However, constitutive activation of PRR pathways can override the initial immune activation response and lead to immune suppression through various mechanisms including pyroptosis of immune cells, crosstalk and blunting of expression of other PRRs, and upregulation of genes encoding immunosuppressive proteins. This is the likely origin of tachyphylaxis noted in experimental dosing of PRR agonists. While there are numerous different PRRs, this paper focuses on Toll-like Receptors (TLRs), Stimulatory of Interferon Genes (STING), and RIG-I-Like Receptors (RLRs). These three PRRs utilize immune suppression pathways to curb damage due to inflammatory effects, and interestingly, they have a unique interplay to affect each other to help regulate immune activation. In this paper, we explore the pathways for immune suppression utilized by each of these PRRs, digging deeply into specific examples to elucidate the mechanisms of immune suppression, and discussing how these effects can be considered when modulating PRRs for the treatment of cancer.

Keywords: pattern recognition receptors, immune-suppression, immune-exhaustion

Introduction

The innate immune system employs germline-encoded PRRs for the initial detection and response to microbes and also internal byproducts of cellular damage. In response to microbe-specific molecular signatures known as pathogen-associated molecular patterns (PAMPs) and self-derived molecules derived from damaged cells, referred to as damage-associated molecular patterns (DAMPs), PRRs activate downstream signaling pathways that lead to the induction of innate immune responses [1]. These processes are essential for the clearance of the insult as they not only trigger immediate host defensive responses such as inflammation, but also prime and orchestrate antigen-specific adaptive immune responses. Immunotherapies that target adaptive immunity like checkpoint modulators have revolutionized the cancer treatment producing durable responses and improved overall survival albeit, in a small fraction of patients. Stimulation of innate immunity through PRR activation is another approach
that is being explored as a therapeutic intervention for cancer [2]. Many preclinical studies support the notion that combination immunotherapies eliciting convergent innate and adaptive immune responses may be capable of enhanced antitumor activity relative to therapeutic strategies focused on adaptive immunity [3]. Currently a number of clinical trials are ongoing, investigating the PRR agonists as a single agent and in combination with check point modulators as a treatment for cancer.

The role of PPRs in innate immune activation is well known; however, much less appreciated is the concept that the innate immune system also utilizes these same PRRs to trigger immune suppression if continuously activated for the purpose of protecting the host from tissue damage. This paper will forego discussion of the immune activation effects of PRRs since there is extensive literature available and instead, focus on elucidating how certain PRRs can lead to immune suppression due to constitutive activation. The phenomenon of T-cell immune-exhaustion/immune-suppression through persistent antigen exposure is well characterized in viral diseases [4]. Initial exposure to the viral antigen produces a robust immune response that can inhibit the viral replication as is noted with the primary immune response in patients newly infected with HIV [5]. However, chronic viral infections lead to constitutive activation of various PRRs which ultimately leads to pathway refractoriness and immune suppression through T-cell immune exhaustion and disease progression. Similar observations have also been noted with certain bacterial and fungal infection [6,7].

The mechanisms that PRRs use to limit immune activation include pyroptosis of activated immune cells, crosstalk between PRRs (Figure 1), and increased expression of enzymes and proteins related to immune suppression and exhaustion. A selection of these mechanisms will be explored in more detail in discussion of individual PRRs and their immunosuppressive effects. Approaches aimed at activating pattern recognition receptors (PRRs) in cancers are being tested as a treatment option, with the goal of stimulating innate immunity through production of pro-inflammatory cytokines, enhancing tumor neoantigen presentation, and increasing cytotoxic activity of tumor infiltrating lymphocytes [8-10]. Constitutive or hyper activation of these same PRRs leads to immune suppression, through multiple pathways.

**Figure 1.** Crosstalk between various PRRs leading to dynamic and opposing effects.

Mammals have several distinct classes of PRRs including toll like receptors (TLRs), RIG-I-like receptors (RLRs), Nod-like receptors (NLRs), AIM2-like receptors (ALRs), C-type lectin receptors (CLRs), and intracellular DNA sensors such as cGAS/STING. At the risk of neglecting the individual effects of each PRR on immune suppression, this paper will discuss a selection of PRRs and pathways that have recently been more aggressively studied for their use as immune activators for treating cancer. This includes TLRs, RLRs, and STING.
TLRs

There are 10 TLRs known, some of which are expressed on the cell membrane (TLR1, TLR2, TLR4, TLR5, and TLR10) and others are expressed intracellularly in the endosomes (TLR3, TLR7, TLR8 and TLR9) [11]. TLRs are expressed in innate immune cells such as dendritic cells (DCs) and macrophages as well as non-immune cells such as fibroblast cells and epithelial cells. Each TLR is activated by specific antigens ranging from bacterial lipopolysaccharides (LPS) to viral RNA to cellular DNA. Activation of innate immune response by TLRs is through NF-κB pathway.

Constitutive activation of TLRs leads to activation of various pathways that suppress the immune system and these include pyroptosis of immune cells, crosstalk with other PRRs, or modulating expression of various enzyme and cytokines. TLR ligands can induce pyroptosis, an inflammatory type of cell death caused by activation of NLRP3 multiprotein complex known as an inflammasome complex. The NLRP3 inflammasome comprises the sensor molecule NLRP3, the adaptor protein ASC, and pro-caspase-1. TLR agonists induce NF-κB-mediated NLRP3 and pro-IL-1β expression, thereby promoting NLRP3 inflammasome assembly and caspase-1-mediated IL-1β and IL-18 secretion and pyroptosis [12,13]. Another pathway for TLRs to induce immune suppression is through the production of a metabolite called itaconate which leads to increased transcription of anti-inflammatory proteins. Itaconate is made from cis-aconitate in the tricarboxylic acid cycle (TCA cycle) in macrophages activated by factors, notably by lipopolysaccharide (LPS) but also by other Toll-like receptor (TLR) ligands [14]. Itaconate is required for the activation of the anti-inflammatory transcription factor Nrf2 (also known as NFE2L2) which is normally kept inactive in the cytosol by its inhibitor protein KEAP1 (Kelch-like ECH-associated protein) [15]. Itaconate alkylates cysteine residues on the protein KEAP1, enabling release of Nrf2 which causes increase in anti-oxidant and anti-inflammatory factors like heme oxygenase I (HMOX1), and glutathione (GSH), attenuate NFκB-inflammatory response, and decrease reactive oxygen species (ROS), nitrite and inducible nitric oxide synthase (iNOS). Itaconate also exerts anti-inflammatory effects by modulating macrophage metabolism and decreasing STING expression and responsiveness to STING agonists [16]. Activation of TLRs by [poly(I:C)], rapidly reprograms murine APCs by simultaneously augmenting sensitivity of endosomal TLRs and inhibiting activation of RIG-I-like receptors (RLRs) [17]. Finally, activation of the innate immune response by TLR agonists is counteracted by the induction of immunosuppressive cytokines and enzymes including interleukin-10 (IL-10), transforming growth factor-β (TGF-β), indoleamine 2,3-dioxygenase (IDO), and induced nitric oxide synthase (iNOS) [18]. When a TLR is constitutively activated each of these mechanisms act as a negative regulator counteracting activation of the immune system which then leads to immune exhaustion.

A clinical example of constitutive activation of TLRs leading to immune exhaustion can be observed in purine nucleoside phosphorylase (PNP) deficient patients. Although the initial rationale for developing PNP inhibitors as immunosuppressive agent, for the treatment of autoimmune diseases and hematologic malignancies, was that the PNP deficient patients were presented with lymphopenia, it was recently discovered that PNP deficiency or PNP inhibitors activate the immune system through elevation of guanosine which in turn activates TLR2, TLR4 and TLR7 [19,20]. Activation of these TLRs via PNP inhibition or deficiency shows expected manifestations of immune activation such as the appearance and worsening of autoimmune disease, improved immune response to tetanus toxoid vaccine, and elicitation of graft-versus-host disease (GvHD) in post-hematopoietic stem cell transplant (HSCT) relapse leukemia patients treated with PNP inhibitor and in PNP deficient patients receiving blood transfusion [19,21]. However, chronic PNP deficiency either through inhibitor or genetic manifestation leads to hallmarks of immune exhaustion, most obviously characterized by lymphopenia but also noted by recurrent infections.
STING

STING is considered a critical hub in cytosolic DNA-sensing pathways for innate immune responses directed against numerous bacterial, viral, and parasitic pathogens. STING activates host defense via induction of type I interferons through a well-characterized pathway involving TANK Binding Kinase-1 (TBK1) and Interferon Regulatory Factor-3 (IRF3) which leads to the production of the NF-κB-driven cytokines. However, exploratory models of STING in autoimmune disease note that despite its immune activating effects, it still has an important role in immune suppression.

MRL.Fas<sup>lpr</sup> mice develop autoimmune disease resembling systemic lupus erythematosus (SLE). Since STING is known to induce a pro-inflammatory state, STING deficient MRL.Fas<sup>lpr</sup> mice would be expected to have unchanged or improved disease, but contrary to expectation, these mice developed more severe disease and accelerated mortality [22]. Lymphoid hypertrophy, autoantibody production, serum cytokine levels, and other indicators of immune activation are markedly increased in STING-deficient MRL.Fas<sup>lpr</sup>. This is considered to be due to STING causing immune suppression through negative regulators of TLR signaling, including A20 and suppressor of cytokine signaling 1 (SOCS1) and 3 (SOCS3). STING-deficient macrophages demonstrated markedly decreased expression of these negative regulators of TLR signaling and are hyperresponsive to TLR ligands. Conversely, stable overexpression of STING in macrophages resulted in elevated basal levels of A20, SOCS1, and SOCS3, further demonstrating STING-dependent regulation of these components [22]. The importance of STING's role in regulation of TLR-mediated inflammation is similarly shown in the 2,6,10,14-tetramethylpentadecane (TMPD)-mediated peritonitis model [22].

A slightly different example of STING-mediated immune exhaustion can be seen in mouse tumor models treated with STING agonists. STING ligands have been shown to potently induce antitumor activity in several cancers, including breast cancer, chronic lymphocytic leukemia, colon cancer, and squamous cell carcinoma. Recently, Sivick et al. demonstrated that STING agonists have differing anti-tumor effects at lower versus higher doses. In a mouse mammary tumor model, lower doses and/or infrequent dosing of STING agonist produced tumor specific CD8+ responses and provided better protection when re-challenged with the same tumor cells compared with mice treated with higher doses and more frequent dosing [23]. The higher doses led to direct tumor cell death via interferon-independent and caspase-3 dependent apoptotic pathways and produced limited to no immune response. This suggests a tachyphylactic response to dosing likely due to competition of immune activation and immune suppression. Similar to TLR, activation of STING is also known to induce pyroptosis through NALP3 activation [24] and release immunosuppressive factors like IDO and IL-10 [22,25]. Progression of disease in MRL.Fas<sup>lpr</sup> STING knock out mice and lower doses or infrequent dosing of STING agonist providing better protection in mouse tumor model are consistent with the concept that constitutive or frequent activation of STING limits immune activation and can result in immune suppression and exhaustion.

RLRs

RLRs, a family of related RNA helicases including RIG-I, MDA5, and LGP2 detect viral oligonucleotide patterns of certain RNA viruses. RLR activation induces immunogenic cell death (pyroptosis) of virally infected cells accompanied by increased inflammatory cytokine production, antigen presentation, and antigen directed immunity against virus antigens. This also extends to identification of and activation via endogenous RNA which has been noted in patients treated with radiation [26].

In addition to releasing type I interferon, activation of cytosolic RLRs results in selective suppression of transcription of the gene encoding the p40 subunit of interleukin 12 (IL12b; immune activating cytokine) that is effectively induced by the activation of TLRs. The activation of RLRs in mice attenuates TLR-induced responses of the
T-helper type 1 cell (TH1 cell) and interleukin 17–producing helper T-cell (TH17 cell) subset types [27]. RLR-mediated induction of type I interferon, while simultaneously suppressing il12b, may be viewed as a mechanism for protecting the host from full brunt of immune responses. Although activated by distinct nucleic acids, STING and RIG-I signaling are functionally interconnected and coordinate their expression levels through positive feedback mechanisms [28].

Recently, it has also been shown that in vitro activation of another PRR, AIM2 inflammasome in murine macrophages and dendritic cells, leads to reduced activation of the STING pathway, in part through promoting caspase-1-dependent cell death [29]. This crosstalk and interconnectedness of PRR-mediated signaling pathways highlights the importance of further understanding of mutually cooperative or antagonistic aspects of the PRR signaling pathways for deciphering complex outcomes as this information would be extremely important in the clinical development of PRR modulators in cancer.

Conclusion

Although the primary function of the PRRs is to contain the pathogen or the disease-causing stimuli and induce adaptive immunity for complete elimination of the insult, persistent activation of PRRs can result in pathways refractoriness and subsequent immune exhaustion. Within this paper, we have explored a select group of pathways to elucidate this immune exhaustion mechanism. Other PRRs likely have different mechanisms for abrogating their primary immune activation response which will range in effectiveness. Hence, it is important in the development of the PRR agonists as anti-cancer treatment to evaluate the pathways leading to tachyphylaxis and possible long-term immune suppression when considering medication dose and frequency of administration. This runs counter to the approach for traditional chemotherapy where high doses and continued treatment are typically pursued until disease progression. If these delayed mechanisms of immune suppression are not considered, otherwise effective treatment may fail to show benefit and the burgeoning field of cancer immunotherapy may miss out on important tools for treatment.

Conflict of Interests

The authors declare no potential conflict of interests.

Acknowledgement

The authors would like to acknowledge Dr. Steven M. Albelda of Penn Medicine/Abramson Cancer Center for his valuable comments.

References


