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TLR3 Agonists and Proinflammatory Antitumor Activities

Sherven Sharma^{1,2,3}, Li Zhu^{1,3}, Michael Davoodi^{1,3}, Marni Harris White³, Jay M. Lee^{1,2}, Maie St. John^{1,2}, Ravi Salgia⁴, and Steven Dubinett^{1,2,3}

¹Department of Medicine, UCLA Lung Cancer Research Program, Los Angeles, CA, USA

²Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

³Molecular Gene Medicine Laboratory, Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, CA, USA

⁴University of Chicago, Department of Medicine, USA

Abstract

Although tumor growth leads to inflammatory responses, the immune system develops tolerance to cancer. One way to break host tolerance to tumors is to activate key immune effector activities. Toward this end, various adjuvants are under investigation in an effort to harness the immune system to overcome tolerance to tumor associated self-antigens. There is enthusiasm for the use of specific ligands for toll-like 3 receptors (TLR3) that play a key role in the innate immune system. TLR3 agonists serve as immune adjuvants because they potently induce innate immune responses by activating dendritic cell (DC) maturation and inflammatory cytokine secretion. These activities facilitate the bridge between the innate and adaptive immune systems promoting the expansion of cytotoxic T lymphocytes (CTL) that destroy cancer cells. TLR3 agonists either alone or in combination with tumor antigens have shown success in terms of enhancing immune responses and eliciting antitumor activity in preclinical models. However, TLR3 agonists can also impact regulatory cells that dampen immune responses. Thus, immune strategies that utilize TLR3 agonists should consider the relative induction of suppressive as well as beneficial anti tumor immune activities. Herein, we summarize the TLR3 agonists that will hopefully come to clinical fruition.

Keywords

TLR3 Agonists; Immune therapy; Immune Suppression; Cancer

1. Basis for TLR3 Agonists in Cancer Therapy

The review (“Targeting TLR3 with no RIG-I/MDA5 activation is effective in immunotherapy for cancer”) discusses the basis for the use of TLR3 agonists for cancer immunotherapy. TLR3 is the specific intracellular recognition system found in innate DC and responds to RNA virus infection [1]. TLR3 located within the endoplasmic reticulum (ER) rapidly recruit to endosomallysosomal compartments, where they detect viral nucleic acids (dsRNA, ssRNA and ss DNA containing unmethylated CpG motifs) [2]. TLR3 is also expressed on the surface of fibroblasts, epithelial cells and tumor cells. In preclinical studies, chemically synthesized dsRNAs (polyI:C or polyA:U) induced Type 1 interferon production

by blood mononuclear cells and enhanced natural killer activities [3, 4]. In a mouse prostate tumor model, TLR3-mediated tumor suppression was demonstrated to be Type 1 IFN dependent [5]. In other studies, TLR3 directly triggered apoptosis of human prostate, breast, ovarian and head and neck cancer cells [6–10]. The ability of TLR3 agonists to potently activate DC maturation and cytokine secretion facilitate the bridge between the innate and adaptive immune systems leading to the expansion of cytotoxic T lymphocytes (CTL) that destroy cancer cells. For example, in a metastatic murine lung cancer model [11], a single administration of polyI:C reduced tumor outgrowth that was associated with an higher influx of mature DC promoting a cytotoxic immune environment. The anti-tumor effects of systemic polyI:C administration could be replicated by an adoptive transfer of bone marrow DC stimulated with polyI:C into tumor bearing mice [11]. In a preclinical tumor model, in the adjuvant setting, [12]TLR3 agonist complexed with cationic liposome augmented the immunogenicity of tumor antigens by impacting DC maturation and Type I IFN production. Overall, the immune potentiating activities of TLR3 agonists that support its use in cancer therapy include: (i) increased APC activities of DC that prime and activate naïve T cells, (ii) IFN secretion by DC that activate NK and $\gamma\delta$ T cells, (iii) direct apoptosis of tumor cells that express TLR3, (iv) cytokine production for maintenance of CTL and (v) low toxicity as well as non immunogenicity associated with several TLR3 agonists. For therapeutic use, three synthetic dsRNAs TLR3 agonists have been developed from polyriboinosinic polyribocytidylic acid (polyI:C) that include ampligen (poly(I:C12U), hiltonol (polyI:CLC) and polyadenylic polyuridylic acid (poly A:U). Poly I:C was introduced clinically for its ability to stimulate Type I interferon, its effect on myeloid DC and memory T Cells which led to evaluation of its therapeutic role in leukemia and HIV. In several cancers [13–15], poly I:C and poly I:CLC evaluated as single agents showed no clinical activity. On the contrary, poly I:C induced severe toxic side effects in some patients, including shock, renal failure, coagulopathies, and hypersensitivity reactions [13]. Modification of the poly I:C structure by the introduction of unpaired uracil and guanine bases resulted in a unique dsRNA, poly (I:C12U, ampligen) that has been shown to be safe in humans [16]. Poly I:C, hiltonol and poly A:U are ligands for TLR3 and the cytosolic sensor RIG1/MDA-5 whereas ampligen is a ligand for TLR3 only. It has been suggested that some of the known toxicity of these agonists may be linked to the combined signaling from MDA-5 and TLR3.

TLR3 Agonists as Adjuvants in Therapeutic Cancer Vaccines

TLR3 agonists' ability to induce DC maturation, Type 1 cytokine secretion and encouraging preclinical data has encouraged evaluations of their activity as adjuvants in therapeutic cancer vaccines. Therapeutic cancer vaccines hold promise; however, numerous challenges still preclude efficacy. These include: i) correct identification of optimal antigens and immune adjuvants; ii) quantification of the appropriate immune response to be generated; iii) elicitation of long term antitumor memory; and iv) tumor induced immune suppression and immune evasion. For an ideal cancer vaccine strategy, the requirements for i) immune cell activation, homing and accumulation in the tumors; ii) disruption of the regulatory mechanisms that limit immune responses; and iii) the ability to direct a coordinated and effective attack against tumors engaging multiple components of the immune system should evolve in parallel. Based on the immune potentiating activities of TLR3 agonists, they may prove to be an integral component of tumor antigen-based vaccines. The TLR3 agonists hiltonol and ampligen are being evaluated in clinical trials for cancer in combination with tumor antigen vaccines. For example, the trial, NCT01079741, Phase I/Phase II open label study of the TLR3 agonist hiltonol as an adjuvant for NY-ESO-1 protein vaccination with or without Montanide ISA-51 VG in patients with high risk melanoma in complete clinical remission, is a clinical trial that is actively recruiting (<http://clinicaltrials.gov>). A study of hiltonol administered with tumor lysate pulsed DC vaccines is ongoing in patients with malignant glioma trial NCT01204684. Efficacy data for the glioma trial has not yet been

reported. Another randomized phase I/II trial NCT01355393 will evaluate the side effects and best dose of ampliten when given together with the HER-2/neu peptide vaccine and GM-CSF to determine efficacy in patients with stage II–IV human epidermal growth factor receptor 2 (HER2)–positive breast cancer. The results of these trials will provide important information regarding the impact of TLR3 adjuvants in the therapeutic vaccination setting for different tumor types. Based on the preclinical data thus far, there is much anticipation that TLR3 adjuvants may augment vaccine efficacy in the clinical trials.

2. Expert Opinion

Although TLR3 agonists have reached the clinic, there remain essential questions regarding TLR3 agonists related to: formulation, dosage, route of administration, impact on tumor infiltrates and whether to combine with tumor antigens or antigen presenting cell loaded tumor antigens for improved antitumor efficacy. The immune infiltrates in the tumor microenvironment modulate tumor growth and invasion into the surrounding tissue. The tumor programs the cellular infiltrates to sustain tumor growth, progression and hypo responsiveness of the tumor. At present, the extent to which tumor infiltrates can be reprogrammed by TLR3 agonists into antitumor effectors is not yet fully understood and can be an area of future investigation. Such evaluations will provide insights on additional interventions for effective tumor control. In a preclinical murine lung cancer model, it has been demonstrated that TLR3 agonists can circumvent immune suppression induced by tumor infiltrating myeloid cells by converting them to tumoricidal effectors that control tumor progression [17]. Further work needs to be conducted to determine if the same is true in other tumor models. T regulatory cells (T reg) in tumor bearing hosts dampen immune responses to tumor antigens; however, the effect of TLR3 agonists on T reg activity is not fully understood and requires further investigation. Efforts on the identification of TLR3 agonist induced immune suppressive pathways may prove useful in attenuating such activities to improve antitumor activities. Further work is required to determine whether TLR3 agonists in combination with cytotoxic, targeted therapies or radiation therapy will improve the overall antitumor efficacy. Based on differences in the signaling by the various TLR3 agonists, it is important that the TLR3 agonists be compared side by side and that agonists developed for cancer clinical trials are made available for preclinical evaluations. This will aid in evaluating key issues of immune activation and suppressor activities as well as optimizing combinatorial approaches to realize the full potential of TLR3 agonists in cancer therapy.

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