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IRX-2 increases the T cell-specific immune response to protein/peptide vaccines

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ABSTRACT

Therapeutic cancer vaccines are attractive due to the prospect of specificity and their lack of toxicity; however, their clinical development has been hampered by several biologic and clinical challenges. One of the most important biologic challenges is the relative lack of effective cellular immune adjuvants. Effective physiologic immune responses are characterized by the local generation of a complex cytokine environment that activates and regulates multiple immune cell types. IRX-2 is a primary cell-derived biologic with physiological levels of multiple active cytokine components, produced under pharmaceutical standards. The hypothesis that IRX-2 amplifies the T cell response to defined antigens was assessed in mice by measuring the T cell-specific peptide response to a dominant mouse peptide (NFT) derived from human prostate-specific membrane antigen (PSMA). IRX-2 enhances the T cell response to NFT when antigens were delivered either via irradiated cells expressing human PSMA, NFT peptide in Incomplete Freund's adjuvant (IFA) or NFT peptide conjugated to KLH. The T cell-specific activity was measured in spleen or lymph nodes cells by IFN-γ ELISpot and/or IFN-γ secretion over 6 days or in vivo by peptide-specific delayed-type hypersensitivity reaction (DTH). Further more, a single administration of IRX-2 with the antigen was not active as compared to 4 or 9 additional administrations which were sufficient to enhance the T cell response to antigens. The influence of IRX-2 on the B cell response to ovalbumin when it was used as a carrier protein was measured by ELISA. IRX-2 was compared to a commercially available combination adjuvant (MPL + TDM in squalene/Tween 80) which based on the literature is a potent adjuvant in murine systems. In the T cell assay IRX-2 was superior to the commercially available combination adjuvant and while IRX-2 also increased antibody titer, it was not as potent as the combination adjuvant. Mice immunized with IRX-2 and antigen also exhibited delayed tumor progression following challenge with PSMA-expressing tumor cells. These studies demonstrate that IRX-2 is an immunomodulator with adjuvant activity which preferentially enhances the T cell-specific responses to tumor associated antigens. Based on these studies, IRX-2 is a candidate for evaluation as a T cell adjuvant in a variety of preclinical vaccine delivery systems as well as in human clinical trials with cancer vaccine candidates.

1. Introduction

Development of effective therapeutic vaccines for cancer patients remains an ongoing challenge. Since immune-based therapies should be highly tumor specific and offer potential clinical efficacy with minimal side effects, this has led to a number of clinical trials evaluating a variety of therapeutic cancer vaccine regimens. Despite the multitude of studies with various antigens, adjuvants and vaccine constructs, the clinical experience to date with cancer vaccines has been disappointing [1–8]. A number of reasons for the modest results have been proposed, many with an underlying theme of immune dysfunction that necessitates immune enhancement as a component of the vaccine therapy.

The critical events for T cell-mediated anti-cancer immune response include antigen presentation to T cells primarily in the lymph nodes draining the source of the antigen (i.e. tumor or immunization site), followed by T cell activation in the lymph nodes and migration of cytotoxic cells (CD8) and helper cells (CD4) to the peripheral sites. The uptake of antigen by dendritic cells, tissue macrophages and other antigen presenting cells (APCs) and presentation of processed peptides in major histocompatability (MHC) class I and class II to the T cells in the lymph node are the first steps required for an effective immune response. Activation of the APC to enhance expression of MHC, costimulatory and adhesion molecules as well as various cytokines and chemokines are

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also required for a robust immune response. Although cytokines play a critical role in effective immune activation, relatively few studies have utilized combinations of cytokines as vaccine adjuvants. The early vaccine studies were with "classic adjuvants" such as Complete and Incomplete Freund's adjuvants and their derivatives either alone or in combination (MPL, TDM, MDP, bacterial DNA sequences, poly I:C, squalene, mineral oil, etc.). The results were interpreted as an increase in immune response due to the activation of the "inflammatory" cytokine network which would drive the immune response. With the observation that many components of Freund's adjuvant activated TLR receptors on antigen presenting cells, studies evolved to evaluate modified TLR based adjuvants as endogenous stimulators of cytokine production as a result of activation of antigen presenting cells. More recently, the use of viral constructs to deliver antigens is believed to stimulate cytokine production via innate immune mechanism since many TLR's are activated by viral products. These approaches to generate an effective local cytokine environment to initiate effective immune activation may be limited in the setting of cancer due to immune dysfunction and suppression. Despite the preclinical studies demonstrating that these newer adjuvant/delivery system approaches should be successful, clinical success remains elusive.

IRX-2 is a primary cell-derived biologic which has enhanced immune response and induced tumor rejection in preclinical studies [8–11]. The IRX-2 used in these studies is prepared under cGMP standards and has a demonstrated consistent profile of multiple cytokines [8,10,11] (unpublished data). The cytokines in IRX-2 are predicted to preferentially enhance the cell-mediated components of the immune response [8]. In multiple preclinical studies, IRX-2 has been shown to activate dendritic cells as well as T cells and, as such, is predicted to be a useful immune modulator with adjuvant activity [8–11]. This enhancement of the immune response probably occurs due to the presence of immune active cytokines such as IL-1β, IL-2, TNF-α, and IFN-γ. In early phase 1 and phase 2 clinical studies, IRX-2 (or IRX-2 like materials) administered perilymphatically so as to target the tumor draining lymph nodes of patients with head and neck cancer, was well tolerated and showed evidence of immune response [9,10,12,13].

The experiments reported in this paper were undertaken to confirm the hypothesis that IRX-2 will enhance T cell immune activation following vaccine administration. For the proof of principle preclinical studies presented here, the immune response directed towards a Balb/c mouse dominant T cell peptide (designated NFT) from prostate-specific membrane antigen (PSMA) was evaluated [14]. PSMA is a 750 amino acid surface protein expressed primarily in prostate epithelium and up-regulated 10-fold in prostate cancer [15–18]. There is an 80% homology between mouse and human PSMA so some epitopes of the protein are potentially self-tolerant in mice [19].

In the following study, IRX-2 was shown to amplify the T cell response to various prototypic cancer vaccine constructs. The activity of IRX-2 required 4–9 additional administrations following immunization with IRX-2 plus antigen, consistent with the shaping of the immune response by the cytokines over a period of time. IRX-2 enhanced the NFT peptide-specific immune response of mice immunized with irradiated 3T3 cells expressing PSMA (cell-based vaccine, P3T3) as well as to a peptide-carrier conjugate vaccine and to the peptide delivered with IFA. T cell specificity of lymphocytes harvested from immunized mice was assessed using both ELISpot assay and secretion of IFN-γ over 6 days. The T cell-specific in vivo activity was confirmed by measuring the peptide-specific DTH response. The T cell response using IRX-2 as the adjuvant was superior when compared to a commercially available combination adjuvant (MPL + TDM in squalene/Tween). The B cell response to ovalbumin when used as a carrier protein was also measured and IRX-2 enhanced the response compared to no adjuvant control, although the titer was less than for the combination adjuvant. Since these preclinical studies demonstrate that IRX-2 is an enhancer of T cell-specific responses, it is a candidate for evaluation as a T cell adjuvant in preclinical models as well as human clinical trials using a variety of cancer vaccine candidates.

2. Materials and methods

2.1. Reagents and cells

The NFT peptide used in these studies was synthesized by BioSynthesis Inc. (Lewisville, TX). The NFT peptide sequence was **NFTQIPHLAGTEQNF** which is from the human protein. The mouse PSMA has a similar sequence (**NFT**RT**PHLAGT**QN**NF**) but the non-identity as indicated by the non-bold amino acids, means that these studies are not specifically designed to demonstrate breaking of tolerance. The negative control immunization peptide for these studies was a class II influenza epitope for C57bl/6 mice (NGSLQCRICI). Ovalbumin, Keyhole Limpet Hemocyanin (KLH) cyclophosphamide, incomplete Freund's adjuvant (IFA), and the combination adjuvant (MPL + TDM in squalene/Tween 80, also called Ribi Adjuvant System or Sigma Adjuvant System Catalogue Number S6322) were purchased from Sigma (St Louis, MO). The combination adjuvant consisted of monophosphoryl lipid A (MPL; 0.5 mg) and synthetic trehalose dimycolate (TDM; 0.5 mg), and 44 μl squalene and 200 μl of Tween-80 in a final volume of 1 ml of PBS (i.e. oil in water). Freund's incomplete adjuvant consists of paraffin oil combined with mannide monooleate (0.85 ml paraffin oil and 0.15 ml mannide monooleate). The adjuvant was selected because it is commercially available and is similar to Freund's complete adjuvant but without the toxicity.

The PSMA transfected cells (P-3T3 and P-RENCA) used in these studies were previously described [20]. The expression vector used for PSMA constructs was pEF-BOS. The Xbal cloning site was converted to a BAMHI site giving pEF-BOSb. The human PSMA cDNA (GenBank accession M99487) was synthesized and cloned form LNCaP RNA by RT-PCR. The 2,200 bp, PCR-amplified PSMA cDNA was restricted with BamHI site modified vector pEF-BOSb and cloned in *Escherichia coli* DH5-α. The NIH3T3 cells were co-transfected with pEF-BOS/PSMA using Lipofectamine. G418 was used for selection of neo-resistant clones. The RENCA cells were also transfected with pEF-BOS/PSMA giving P-RENCA cells.

The PSMA-3T3 (P-3T3) and PSMA-RENCA (P-RENCA) cells were cultured with 10% FBS supplemented MEM [20]. The expression of the insert was confirmed by demonstrating geneticin resistance and expression of PSMA by immunohistochemistry and flow cytometry (data not shown and [21]). P-3T3 cells were irradiated with 800 Gy using a Cesium source and frozen at −20° C until use as a cell-based vaccine.

IRX-2 is a primary cell-derived biologic consisting of multiple active cytokines [8–11]. It is produced in a cGMP process that consists of stimulating human peripheral blood mononuclear cells from normal healthy blood donors with phytohemaglutinin (PHA). The PHA is removed prior to collecting the supernatant containing the cytokines which are then subjected to ion-exchange, and nano-filtration. Stringent QC testing that includes both bioassay and ELISA determination of cytokine levels assures the consistency of the components in IRX-2. Safety testing for sterility, DNA, mycoplasma, and endotoxin, and testing for HIV, CMV and EBV are also part of the process. IRX-2 dosing is based on the IL-2 content. Several lots of IRX-2 were used over the course of these studies. The average levels of the potential immune enhancing cytokines in the lots of IRX-2 used in these studies were IL-2 (6.3 ng/ml), IL-1 (0.8 ng/ml), IFN-γ (2.4 ng/ml), and TNF-α (4.0 ng/ml).