706. DNA Vaccine Strategy Against Chronic B-Cell Lymphoma: Anti-Idiotypic CDR3 Vaccination

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B-cell lymphomas express tumor-specific immunoglobulin, the variable regions of which (Id determinants) are tumor-specific antigens and therefore are suitable targets for vaccine immunotherapy. Promising results have been obtained in clinical studies of Id vaccination using Id proteins or naked DNA Id vaccines. Several reports have indicated that the immunodominant epitopes of the clone-specific Ig lie within the hypervariable CDR3 regions. Our group has previously tested the possibility of using the short peptide encompassing the CDR3 of immunoglobulin heavy chain (V_H-CDR3) as a target for eliciting a tumor specific immune response via DNA-based vaccination. We demonstrated that DNA immunization of outbred mice with different patient-derived V_H-CDR3 peptides elicited antibodies able to recognize native antigens on individual patient's tumor cells. These findings prompted us to investigate the immune response and tumor protection following CDR3-based DNA vaccination in the 38C13 B lymphoma as tumor model.

In the present study, the nucleic acid sequence of the idiotypic IgM from the murine 38C13 B-cell lymphoma was analyzed and the region corresponding to the V_L-CDR3 sequence was chosen for the production of a synthetic mini-gene. By using a computer algorithm, one epitope within murine 38C13 B-cell lymphoma heavy-chain variable region was selected, "enhanced" and used for the production of a distinct mini-gene. The restricted V_H-CDR3 sequence was also fused to a pathogen-derived sequence, with the aim to enhance the immunogenicity of the corresponding DNA fusion vaccine. A high-level expression bicistronic plasmid DNA vaccine was designed to express both the tumor antigen and the mouse IL-2 sequences. To increase plasmid delivery and expression the new DNA constructs were improved by inclusion of a DNA nuclear targeting sequence (NTS) and were delivered by i*n vivo* electroporation and hyaluronidase pre-treatment. Therefore, we evaluated the humoral immune response and tumor protection recruited by CDR3-directed DNA vaccines. Here we show that vaccination of syngenic C3H/HeN mice with CDR3-based DNA vaccines protects vaccinated mice against a lethal tumor challenge and generates an immune response to the 38C13 tumor, inducing specific circulating antibodies.

707. Transduction and Expansion of T Lymphocytes Genetically Engineered To Target the CD19 Antigen for the Treatment of CLL Using Xcyte™ Dynabeads®

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In order to conduct a clinical trial of adoptive therapy with genetically modified T cells in patients with CLL, we are developing an ex vivo transduction and expansion protocol to generate biologically active tumor-reactive T cells. T cells are genetically modified to express the CD19-targeted 19-28z chimeric antigen receptor (CAR). The 19-28z CAR is derived from a single chain fragment length murine antibody specific to the human CD19 antigen expressed on CLL tumor cells, fused to the transmembrane and cytoplasmic domains of the human CD28 co-stimulatory receptor and the cytoplasmic domain of the CD3ζ chain of the human T cell receptor. We have previously shown that PHA-stimulated T cells subsequently expanded by co-culture on artificial antigen presenting cells (AAPCs) efficiently lyse CD19+ tumor cells in vitro and eradicate established CD19+ tumor cells in vivo in a SCID-Beige mouse tumor model (Brentjens et al. Nat. Med. 2003). However, as PHA is not suitable for clinical use, we are now investigating the feasibility of T cell transduction and expansion using Xcyte™ Dynabeads®. In four experiments, we find that transduction efficiencies of healthy donor-derived T cells initially activated with Xcyte Dynabeads range from 58% to 80% 19-28z+ T cells as measured by flow cytometry analysis. The degree of 19-28z+ T cell expansion ranges from 2 to 3 log over 14 days of culture. Significantly, the biologic activity of the T cells activated with Xcyte Dynabeads was comparable to that of T cells activated with PHA/AAPCs based on standard 51Cr release assays targeting CD19+ Raji tumor cells (70 to 90% killing at 25:1 E:T ratio). To determine whether 19-28z+ T cells activated with Xcyte Dynabeads have cytotoxic activity in vivo, SCID-Beige mice with disseminated Raji cell lymphoma were treated with a single intravenous injection of either 2 x 107 19-28z transduced T cells (n=10) or 2 x 107 control Pz1 T cells transduced with an irrelevant CAR (n=4). After treatment, mice were routinely checked for tumor progression as determined by hind-limb paralysis. Mice with hind-limb paralysis were sacrificed. As expected, control mice treated with Pz1 transduced T cells all developed hind-limb paralysis 4-5 weeks after tumor cell injection. In contrast, mice treated with 19-28z+ T cells activated with Xcyte Dynabeads had either delayed progression of disease (n=3) or remain disease free (n=7) at >60 days. Based on our earlier studies, this result suggest that 19-28z+ T cells activated with Xcyte Dynabeads are as potent as 19-28z+ T cells activated with PHA/AAPCs. In order to confirm this result, mice will be observed for a longer period of time. Additional experiments including direct comparisons with PHA/AAPCs activated T cells and expansion of T cells derived from patients with CLL will be presented. These preliminary results suggest that an Xcyte Dynabeads-based approach should be suitable to transduce and expand T cells for our planned clinical trial.

708. Combination of Allogeneic Hematopoietic Stem Cell Transplantation and Allogeneic MHC Gene Transfer Against Solid Cancers

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Introduction: Allogeneic hematopoietic stem cell transplantation (HSCT) often leads to a significant graft-versus-tumor (GVT) immune response mediated by donor T cells. Allogeneic HSCT is recently applied not only for hematological malignancies but also for solid cancers such as renal cancer. It is commonly believed that the target antigens of GVT in MHC-matched allogeneic HSCT are minor histocompatibility antigens and tumor-specific antigens, and the benefit of GVT activity is often offset by the occurrence of graft-versus-host disease (GVHD). In order to enhance the immune recognition of tumor antigens by donor T cells while preventing exacerbation of GVHD, we combined an allogeneic MHC gene transfer with allogeneic HSCT against solid cancers in this study.