Developing a CR281 Peptide Specific T Lymphocyte Immune Response To Target Pancreatic Cancer CCK2i4sv Receptor

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Developing a CR281 Peptide Specific T Lymphocyte Immune Response To Target Pancreatic Cancer CCK$_{2i4v}$ Receptor

Taylor Hook

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Abstract

Pancreatic cancer is a debilitating disease with a poor survival rate. There are no effective treatments for pancreatic cancer necessitating research to find new targets for treatment. The basis of this research is to determine whether a novel target located inside of pancreatic cancer cells can be utilized to control or eradicate pancreatic tumors by T cell-based immunity. Gastrin and Cholecystokinin (CCK) are hormones that regulate activity of the gastrointestinal tract. Each can signal via CCK-BR, which normally acts as an on/off switch for pancreatic cells to secrete enzymes and bicarbonate. An altered form of the CCK-B receptor, CCK_{2i4sv}, is expressed by pancreatic cancer cells. This unique form of CCK-B contains an extra loop of amino acids in an intracellular domain; which may serve as targets for immunotherapy. The goal of this research is to determine whether immunization with synthetic peptides that correspond to amino acid sequences found within the loop may induce loop-specific CD8+ and/or CD4+ T cell immune responses. A synthetic peptide corresponding to CCK_{2i4sv} intracellular loop amino acids 281-300 has been used to immunize mice in two trials, and peptide-specific cellular immunity was monitored by ELISPOT assays and Intracellular Cytokine Staining. Preliminary results confirm robust induction of T cells by the 281 peptide without a helper peptide, HBV. ICS results were inconclusive due to low cell numbers. Repeat ICS will be needed to determine whether the responding T cells consist of CD8+, CD4+, or a mixture of both T lymphocytes.
Introduction

Cancer affects more people today than ever before; nearly 14,500,000 people currently live with cancer in the United States alone. While cancer research has made astounding breakthroughs to limit its prevalence in the recent years, ongoing research is critical to rid this devastating disease. Specifically, pancreatic cancer is the fourth most common cancer in the United States, and has an incredibly poor survival rate (1). 95 percent of those diagnosed do not survive after 5 years due to the difficult and late diagnosis of pancreatic cancer. At the point of common detection for pancreatic cancer, it is too difficult to surgically remove cancerous cells, and nonsurgical techniques have struggled to produce significant results. Pancreatic cancer infiltrates the lymphatic system quickly and easily, and the cancerous cells are quite resistant to chemotherapy. Pancreatic cancer is aggressive, and part of the difficulty in treating it comes from the lack of knowledge about its metastasis. For many cancers, serum markers allow for early detection. Early detection of pancreatic cancer is extremely difficult due to the lack of serum markers, and the deficiency of other screening tools such as mammograms or antigen specific tests that some cancers employ. Usually by the time pancreatic cancer is diagnosed it has already spread to the surrounding organs. Korc (1) has named four mutations that are linked with pancreatic cancer in the hopes of furthering research, one of which provides pertinent background information for this research. Three are loss of function mutations that affect the P16/CDKN2A, TP53 and SMAD4/DPC4 genes, while one is an activating mutation that affects KRAS. Korc mentions the mutation of the Kras gene in conjunction with the presence of a CCK receptor, CCK-B, has been linked to pancreatic cancer cell proliferation. Currently, unique targets are being identified to discover potential effective treatments, specifically in the area of immunotherapy.
Gastrointestinal cells express G protein-coupled receptors for the cholecystokinin hormone of which there are two present in the pancreas, CCK-A and CCK-B (2). These receptors play a large role in secretions of the pancreas. Cholecystokinin and its binding is extremely important in normal recognition of hunger and satiety, and is released in response to signals from the pancreas. Gastrin, another hormone produced by gastrointestinal cells, binds with the CCK-B receptor to monitor acid contents in the stomach. These receptors have been under recent investigation for their potential usefulness in treating gastrointestinal cancers (3). The CCK-A receptor is not involved in the tumor growth of pancreatic cancer (4). CCK-B receptor downregulation has been associated with decreased cancer cell growth (5). Suppressing the ligand, gastrin, also appears to inhibit growth of pancreatic cancer in humans (6). In fact the severity of the activating mutation involved in signal transduction, KRAS, appears to be suppressed by reduction of gastrin binding to the receptor, leading to inhibition of growth of pancreatic cancer tumors.

A unique and unusual form of the CCK-B receptor contains an extra 69 amino acids that are encoded by the retained intron IV sequence. Failure to splice intron IV results in an extra intracellular loop of 69 amino acids. It is unusual for an in frame 69 amino acid intracellular loop to be retained in spicing. According to Ding, this receptor isoform is found in both colon and pancreatic cancer, and potentially plays a role in tumor growth (7). This CCK-B isoform, CCK_{2i4sv}, is absent in noncancerous tissue leading to the association of unspliced intron IV with the regulation and growth of pancreatic cancer (8). The fraction of people diagnosed with pancreatic cancer whose tumor contain this unique receptor is unknown, but knowing the CCK_{2i4sv}R is found only in cancerous cells necessitates further research. The CCK-B isoform also has a higher affinity for gastrin than the normal receptor. A more recent study conducted by
Smith has found a single nucleotide polymorphism (SNP) associated with intron IV found in the CCK$_{2i4sv}$ receptor (4). SNPs are deviations of DNA sequences where a single nucleotide, C, G, A or T, is changed. Single nucleotide polymorphisms are more commonly found in noncoding regions of genes, like an intron. The particular SNP identified is a switch from C to A at position 32 in the fourth intron. Cells carrying this SNP are 274 times more at risk of developing pancreatic cancer along with lower chances of survival. While the CCK$_{2i4sv}$ receptor and its corresponding SNP have serious implications on the prognosis of pancreatic cancer, they also present a novel target for treating this aggressive cancer.

As part of the study where the SNP was discovered, Smith developed antibodies specific for the CCK$_{2i4sv}$ receptor (4). The antibody is specific enough to stain cells containing pancreatic cancer, while not recognizing healthy tissue. The development of antibodies demonstrates the ability to target the receptor using immunology. However, an immunotherapeutic strategy relying on loop-specific antibodies should not be effective in targeting this unique receptor due to the intracellular location of the loop. Antibodies are too large to cross cellular membranes limiting their function to identifying extracellular antigens, proving the impossibility of utilizing antibodies to target the receptor. T lymphocytes are alternative immunological defenses that have potential to target to the intracellular loop in CCK$_{2i4sv}$R.

T and B lymphocytes are part of the acquired immunity line of defense the body can employ to prevent the growth of pathogens such as viruses or intracellular bacteria. Acquired immunity has the ability to remember specific pathogens once exposed. Acquired immunity is generated by vaccination, where an attenuated form of a pathogen is injected into the body, recognized by immune cells, which will remember the pathogen in the case of later exposure. Immune cells, such as B or T lymphocytes, involved in acquired immunity express receptors
(antibodies or T cell receptors, respectively) with the ability to specifically recognize antigens that are not of the host; recognition leads to destruction and elimination of the pathogen (antigen) through a variety of mechanisms.

As a population, B and T lymphocytes have the ability to recognize and remember unique structural facets of antigen proteins by virtue of the diverse variety of distinct antigen receptors expressed by the population of B and T cells available within a given host. Each developing B and T cell must form the genes that will encode its antigen receptor during development in the bone marrow (B lymphocytes) or thymus (T cells). Because the process is random, each developing B cell generates a pair of genes that will encode a unique antibody and each developing T cell generates a pair of genes that will encode a unique TCR. Therefore, the population as a whole represents a vast array of distinct receptors. Infection with a given pathogen only causes the activation (and proliferation) of those subsets of B and T lymphocytes whose receptors (antibodies or TCRs, respectively) recognize structural facets of the infecting pathogen. Activated B and T cells become active and proliferate to combat the infection, and some of the activated, pathogen-specific lymphocytes mature into long-lived, memory lymphocytes. Memory lymphocytes activate much faster and respond more aggressively than previously naïve lymphocytes that must be activated for the first time. Acquired immunity develops as pathogen specific B and T memory lymphocytes are activated during an initial infection and survive over years to be rapidly reactivated in a subsequent infection by the same pathogen.

T lymphocytes can be divided into two subsets, CD4+ and CD8+, which are distinguished by the coreceptor protein (CD4 or CD8) they express. CD4+ T cells, helper cells, recognize and secrete cytokines or engage in cell-cell interactions that enable humoral or cellular
immunity, including induction and development of CD8+ cells. CD8+ T cells, cytotoxic cells, recognize peptide fragments derived from foreign antigens and kill the host cell(s) presenting the antigen fragments. Killer T cells secrete cytotoxins to kill cells presenting foreign antigens. In this way, CD8+ T cells can eliminate host cells in which a virus has begun to multiply before the virus particles have become infectious and host cell ruptures releasing more infectious virus particles to spread the infection to neighboring cells.

Neither type of T cells recognizes free-floating antigen targets. Instead, pathogen peptide fragments must be presented on the surface of a host cell by major histocompatibility complex molecules. A T cell recognizes a foreign peptide when its receptor binds efficiently to the molecular surface formed by peptide and upper surface of the major histocompatibility complex, MHC, molecule, which are expressed on the surface of the host cell. The MHC is the reason for rejection after organ transplants because it is composed of polymorphic genes that differ among unrelated individuals and therefore control the compatibility of tissues. MHC molecules present peptides acquired within the cell onto the surface of the cell where T cells can detect them. The peptides may come from the degradation of host cell proteins or pathogen proteins. There are two classes of MHCs, class I and class II, and they present peptides to CD8+ or CD4+ T cells, respectively. MHC molecules are very broad in their peptide binding, but the T cell recognition is extremely specific. Cytotoxic T cells specifically bind and recognize MHC class I molecules, and these types of MHC molecules are present on all nucleated cells where as class II molecules are not. CD4+ T cells could be important in limiting pancreatic cancer, but do not have direct tumorcidal effects like CD8+ cells. MHC class II molecules are present on antigen presenting cells. Antigen presenting cells only encompass B-lymphocytes, macrophages, dendritic cells and
Langerhans cells. Because of the universality of MHC class I molecules, CD8+ T cells are the best choice for lymphocyte utilization in recognizing and directly destroying cancer cells.

Use of vaccination against amino acid sequences found within intron IV sequences in the cancer-associated CCK\textsubscript{2i4sv} form of the gastrin receptor to proliferate specific CD4+ or CD8+ T cells was the goal of this research. Mainly, this research dealt with inducing memory T cell proliferation to one specific amino acid sequences in intron IV of the CCK\textsubscript{2i4sv} receptor to understand if this type of a response will be successful in controlling the growth of pancreatic cancer. We hypothesize that immunizing mice with the specific peptide (CR281-300) corresponding to the CCK\textsubscript{2i4sv} receptor will cause a T cell immune response which will suppress tumor growth. Previous robust T cell reactivity was observed following immunizations containing CR281 peptide, compared to another corresponding CCK\textsubscript{2i4sv},R derived peptide, CR272. We hope to achieve a proinflammatory response, by having T helper lymphocytes identify foreign antigens and induce a cytokine response by CD8+ T cells. ELISPOT assays were used to measure cytokine production from activated T lymphocytes. Intracellular Cytokine Staining was employed to determine the subset of T lymphocytes being activated by immunizations. Similar research has been completed using CD8+ T cells to generate an anti-tumor response for cancers and diseases (9).
Peptide Emulsion Preparation

Two peptides (synthesized by Penn State Hershey Macromolecular Core Facility) were used for immunization: CR281-300 and HBV core antigen. CR281 peptide was prepared to 8.5 mM, while HBV was prepared to 42.5 mM. Four emulsions to a total volume of 600 µL were prepared using warmed 300 µL Freund’s Incomplete Adjuvant (InvivoGen): 240 µL CR281 and 60 µL HBV, 240 µL CR281 and 60 µL PBS, 60 µL HBV and 240 µL PBS, and 300 µL PBS.

Immunization of C57BL/6 Male Mice

The first trial entailed C57BL/6 male mice (10; Taconic) immunized following four immunization groups. Three mice received CR281 and HBV emulsion, three mice received CR281 emulsion, two mice received HBV emulsion, and 2 mice received the mock emulsion (PBS alone). The second trial entailed C57BL/6 male mice (5; Taconic) immunized following two immunization groups. Three mice received CR281 emulsion, and the remaining two mice were left naïve for control. 1 cc syringes (without rubber plunger) were attached to 27G½ needles (Becton Dickson Co). Mice were immunized via tail skin injection to both sides, using emulsion volume of 50 µL.

Harvest and Preparation of C57BL/6 Mice Splenocytes for ELISPOT and ICS

Mice were euthanized by cervical dislocation, and spleens were removed using sterile, aseptic technique and placed into 15 mL conical tubes containing 5 mL of RPMI + GlutaMAX-I (1x) (RPMI(0))(Life technologies). Spleens were placed on wire screens washed previously with 5mL RPMI(0). Spleens were penetrated with the plunger from a 3 mL syringe (Becton
Dickinson Co) and disrupted to release all splenocytes. Cells were suspended using an additional 5mL of RPMI(0) media and placed back in original 15 mL conical tubes. All cell suspensions were held on ice. Tubes were centrifuged at 4°C for 7 minutes at 1,000 rpm (Jouan CR412 table top refrigerated centrifuge; M4 swinging bucket rotor). Supernatant was aspirated and cells were resuspended rapidly in 7 mL prewarmed (37°C) Tris ammonium chloride (obtained from PennState Hershey). Tubes were incubated in a 37°C water bath for 5 minutes, and then 7 mL RPMI(0) was added to each tube and inverted. Tubes were centrifuged at 4°C for 7 minutes at 1,000 rpm, and supernatants were aspirated. Pellets were resuspended in 5.5 mL of RPMI(0) media and stood on ice for 10 minutes. Cell suspension (supernatant) was pipetted into a new 15 mL conical tube without unsettling cell debris. Cells were counted at a suitable dilution using a hematocytometer and Trypan Blue (obtained from PennState Hershey). Tubes were centrifuged at 4°C for 7 minutes at 1,000 rpm. Supernatant was aspirated, and cell pellets were resuspended in a suitable volume of HL-1 media (Lonza) to have a cell concentration of 1.0 x 10^7 cells/mL for ELISPOT and 2.0 x 10^7 cells/mL for ICS.

**ELISPOT**

One 96-well filtration plate (Multiscreen-IP sterile 0.45 µm hydrophobic) (Merck Millipore) was used for ELISPOT analysis of IFN-γ. Capture antibodies were prepared for IFN-γ (eBioscience) with dilution in Elispot coating buffer (powder from eBioscience, 1L dH2O) at 1/250. Capture Antibody was added to each plate with 100 µL of capture antibody per well. Plate was incubated at 4°C overnight. Routinely 24 hours later, coating antibody was aspirated from each well, and plate was washed twice with 200 µL of Elispot coating buffer. Plate was blocked using 1% (w/v) BSA (eBioscience) in PBS (1x eBioscience) for one hour at room temperature.
Blocking solution was aspirated from wells and each well was washed twice with 200 µL of Elispot coating buffer. Coating buffer was aspirated from wells. 40 µL of CR281, HBV core antigen, T529 (Trial 1), and mPyT 678 (Trial 1) peptides were added to plate with 60 µL HL-1 media according to control set-up. 100 µL of cell suspension was also added to each well for a total well volume of 200 µL. Plates were incubated at 37°C, 5% CO₂ for 24 hours. After incubation time, plate was removed from incubator. Assay diluent was prepared with sterile water at a 1/5 dilution using 5x ELISA/ELISPOT Diluent (eBioscience) for a total volume of 25 mL. Cells and medium were aspirated from 96-well plate, and washed six times with Elispot Wash Buffer (200 µL/well) (1x PBS with 0.05% Tween-20). 100 µL of detection antibody was added to each well, and incubated at room temperature for two hours on a plate rocker. Avidin-HRP (eBioscience) was diluted in 11 mL Assay Diluent at a dilution of 1/250. After incubation on plate rocker, plate was aspirated and washed six times with Elispot wash buffer (200 µL/well) allowing wells to soak for 1 minute in each wash. 100 µL of Avidin-HRP was added to each well and the plate was incubated at room temperature for 45 minutes on plate rocker. AEC substrate solution was prepared during this incubation by adding 400 µL of AEC stock solution (100 mg AEC in 10 mL DMF) to 12 mL acetate solution (0.1M, pH 5) and filtered through a 0.45 µm syringe-mounted filter (Millex-HA). AEC substrate solution was held on ice. After plate rocker incubation, Avidin-HRP was aspirated from wells and wells were washed four times with Elispot wash buffer (200 µL/well) and three times with PBS lacking Tween 20 (200 µL/well). Before AEC substrate solution was added to wells, 6 µL of H₂O₂ was added to the AEC substrate solution. 100 µL of AEC substrate solution was added to each well, and plate was covered with aluminum foil to protect from light. Spots were allowed to develop (protected from light using aluminum foil) on plate rocker (routinely between 10-15 minutes). After the reaction had run to
completion, the reaction was terminated by rinsing with distilled water (routinely >20 times). Plates were allowed to air dry at least 24 hours protected from light.

**ELISPOT Counting**

Spots on ELISPOT membranes were counted at Dr. Todd Schell’s laboratory at Penn State Hershey using the C.T.L. ELISPOT Plate Scanner. Pictures of all plates were taken using ImmunoCapture 6.3.5, and counted using ImmunoSpot 5.0 Academic Basic Count.

**Intracellular Cytokine Staining**

One 96-well tissue culture plate (Falcon U-Bottom Plate, 0.25 mL) (BD biosciences) was used for ICS IFN-γ analysis. 100 µL of cell suspension added to each well. 100 µL of HL-1 containing 10 mM of CR281 and 1 mg/ml of Brefeldin A (Sigma-Aldrich) for wells testing CR281 T cell reactivity. 100 µL of HL-1 containing 10 mM of HBV and 1 mg/ml of Brefeldin A (Sigma-Aldrich) for wells testing HBV T cell reactivity. Plate was incubated at 37°C and 5% CO₂ for 5 hours. Plate was centrifuged (3 minutes, 2,000 rpm in Jouan CR412). Supernatant was removed via one inversion. Cell pellet was washed twice with 200 mL of FACS buffer. FACS buffer prepared by 8 mL Fetal Bovine Serum (HyClone) and 5 mL of 10% NaN₃ added to 400 mL PBS, with final concentration 2% (v/v) FCS and 0.125% (w/v) NaN₃ in PBS. 100 µL FACS buffer containing 200 µL FC block (BD Pharmingen) added to each well, and cells were resuspended. Plate was incubated at room temperature for 10 minutes. 100 µL of FACS buffer added to each well, and centrifuged (3 minutes, 2,000 rpm). Supernatant was removed, and wells were washed with 200 µL FACS buffer. Cell pellet was resuspended in 50 µL FACS buffer containing 10 µL anti-mouse CD8-APC (TONBO Biosciences, 20-0081-U100), and 20 µL anti-
mouse CD4-FITC (TONBO Biosciences, 35-0041-U500). Plate was incubated for 15 minutes at room temperature protected from light. 150 µL of FACS buffer was added to each well and plate was centrifuged (3 minutes, 2,000 rpm). Plate was washed twice with 200 µL of FACS buffer added to each well. 150 µL Cytofix/CytoPerm (BD Biosciences) was added to each well and cell pellet was resuspended. Plate was incubated for 20 minutes at room temperature protected from light. 50 µL of PermWash (BD Biosciences, PW/10) (diluted to 1:10 in sterile glass distilled water) was added to each well, and then plate was washed twice with 200 µL PW/10 per well. Cell pellets were resuspended in 50 µL PW/10 containing 10 µL anti-mouse IFN gamma PE (TONBO Biosciences, 50-7311-U100). Plate was incubated for 15 minutes at room temperature protected from light. 150 µL of PW/10 was added to each well and plate was centrifuged (3 minutes, 2,000 rpm). Plate was washed twice with 200 µL of PW/10. Cell pellet was resuspended with 300 µL of 2% (w/v) paraformaldehyde and transferred to 1.2 mL microtubes (Marsh Bio Products) for flow cytometry analysis.

*Flow Cytometry*

Intracellular Cytokine Staining was analyzed by flow cytometry. Flow cytometry was performed at Penn State Hershey using BD Biosciences 16-color LSR Fortessa.
A CR281 Peptide Specific T Lymphocyte Immune Response to CCK$_{2i4sv}$R

**Results**

C57BL/6 mice were immunized with the CR281 peptide to attempt to induce memory T cell proliferation specific to the amino acid sequences. The amino acid sequences of the CR281 peptide is derived from the amino acid sequence in intron IV of the CCK$_{2i4sv}$ receptor. It is from a highly conserved sequence retained in intron IV (Figure 1). The CR281 peptide produced robust T cell reactivity previously. HBV core antigen was immunized as a helper peptide and a positive control for an immune response. The T529 peptide was used previously as an independent positive control and irrelevant ELISPOT analysis, but exhibited cross reactivity with CR281. T529 was tested for the cross reactivity again in the first trial using two different T529 peptides: one from Dr. Todd Schell’s lab at Penn State Hershey and one from Dr. Lawrence Mylin’s lab at Messiah College. Murine polyomavirus T antigen 676-688 was used as an irrelevant control to CR281 in Trial 1 for ELISPOT analysis. Both trials were completed with a series of immunizations. Mice were immunized according to consecutive week intervals: week 1, week 2, week 3. Harvest occurred 7 days following the third immunization for both trials. Splenocytes were collected from each mouse and used in ELISPOT assay for cytokine IFN-$\gamma$ for both trials, and also for Intracellular Cytokine Staining for trial 2.

Results from ELISPOT (Figure 2) for trial one show T cell induction and reactivity to CR281. T cell reactivity to CR281 alone was more robust than reactivity to CR281 with HBV as helper peptide by almost 4.5 times (Table 1). T cell reactivity to both T529 peptides was not notable above background.

Results from ELISPOT (Figure 3) for trial two show T cell induction and reactivity to CR281 without HBV as a helper. Again, T cell reactivity to CR281 was quite robust (Table 2). Originally 3 mice were immunized with CR281, but one mouse died following the first
immunization. Another mouse that was immunized with CR281 was a non-responder and was excluded from data collection. A naïve mouse was also excluded from data collection due to abnormal results.

Results from Intracellular Cytokine Staining (Figure 4) from trial two show little CD4+ or CD8+ T cell reactivity to CR281 peptide when compared to overall population of T lymphocytes from each mouse. ICS results were largely inconclusive in determining subset of T cells responding to CR281 peptide (Table 3).
Discussion

The results have indicated that peptide immunizations corresponding to the retained intracellular loop of CCK$_{2i4sv}$R are capable of inducing memory T cell proliferation and reactivity. The T cell reactivity to CR281 alone without HBV was significantly higher than with use of HBV as a helper peptide. This leads to thought of potential immune-competition between CR281 and HBV. CD8+ epitopes need a helper peptide with a known CD4+ epitope in order to produce strong T cell induction. The HBV peptide has a known strong CD4+ epitope within it. Since CR281 without HBV produced a robust amount of T cells, we believe that CR281 contains a CD4+ epitope rather than a CD8+ epitope. There may still be a CD8+ epitope within CR281, but we would have expected to see stronger T cell induction with HBV as a helper if that were the case. The CCK$_{2i4sv}$ receptor may still contain a CD8+ epitope within it, but our results indicate that it is not with CR281-300. Investigation into other amino acid sequences corresponding to the CCK$_{2i4sv}$ receptor may have potential CD8+ epitopes.

As stated, the cross reactivity with CR281 and T529 observed previously is not notable when compared to naïve, unimmunized, mice. We tested T529 from two different locations to be sure that there was not a mistake in the synthesis of the peptide in our previous study. We have concluded that the previous cross reactivity was due to contamination in our previous study, and not due to similar MHC binding between the peptides that could make it difficult for T cells to differentiate between the two peptides.

Intracellular cytokine staining was attempted in order to determine the exact subset of T cells reacting to the CR281 peptide, whether it be CD4+, CD8+, or a mixture of both. Our results were quite inconclusive. Trial two consisted of five mice originally, but ended with four mice after one died following the first immunization. Along with one mouse dying, a mouse
immunized with CR281 was a non-responder, and had very little T cell proliferation. Both of those factors combined, lead to very low cell numbers in total for the experiment. Intracellular Cytokine Staining has a much higher background than ELISPOT, being 1/10th the sensitivity of ELISPOT, explaining why we saw robust T cell reactivity with ELISPOT to CR281, but in ICS we saw essentially no response by either CD8+ or CD4+ T lymphocytes. Our results were inconclusive because we needed higher cell numbers due to the lack of sensitivity with ICS.

Further research is needed to determine which classes of T lymphocytes are responding to the peptide immunizations. Repeat Intracellular Cytokine Staining can be employed, but with higher cell numbers to have a percentage of CR281 specific T cells above background. In vitro culture should be employed to amplify the T lymphocytes reacting to CR281. There still could potentially be a CD8+, CD4+, or a mixed T cell response to CR281. If repeat Intracellular Cytokine Staining reveals a CD8+ response, future research could include evaluating the effect of T cell reactivity on a pancreatic cancer tumor in mice, with hopes of observing slowed tumor growth or T cell caused regression of tumor growth.

In conclusion, peptide immunizations corresponding to intron IV of the CCK$_{2i4sv}$ receptor induced robust T cell reactivity to the CR281 peptide without a helper peptide of HBV in C57BL/6 mice, leading to the belief that there is a CD4+ epitope present with CR281.
Acknowledgments

Thank you Dr. Harms and Dr. Mylin for patience, guidance, and support. Thank you PennState Macromolecular Core Facility at Hershey for making the peptides, Dr. Todd Schell’s lab at the Penn State College of Medicine for the use of the C.T.L. ELISPOT Plate Scanner, and the Flow Cytometry Core at Penn State Hershey for ICS analysis. Finally, thank you Messiah College for the equipment and funding.

References


Figure Legends

Figure 1. Amino acid sequence of the peptides used for immunization and as targets in ELISPOT assays. CR281-300 was derived from the retained fourth intron in CCK\textsubscript{2i4sv}R. T529-543 is found in SV40 T antigen, and was tested for previously observed cross reactivity with CR281. HBC128-140 is found in the Hepatitis B virus core antigen, and was used as a helper epitope and positive control when co-immunized with CR281. Murine Polyomavirus T antigen 676-688 (mPyT 676) was an irrelevant control for ELISPOT analysis.

Figure 2. Results of ELISPOT for immunization trial one. Graph displaying the reactivity of each immunization group to each of the peptides based on number of spots counted in each well. Error bars measuring standard deviation between each mouse in the immunization group.

Figure 3. Results of ELISPOT for immunization trial two. Graph displaying the reactivity of each immunization group to each of the peptides based on number of spots counted in each well.

Figure 4. Results of ICS for immunization trial two. Flow cytometry analysis of ICS from trial two testing reactivity to CR281 and HBV. First slide displays ICS results of the CR281 immunized mice, and second slide displays ICS results of the naïve mice. The boxed area in each flow chart displays T cells specific for peptide being tested. Two side-by-side columns display results for one mouse, with the first column testing for CD4\textsuperscript{+} T cells and second column for CD8\textsuperscript{+} T cells.
Table 1. Spots counted by C.T. L. ELISPOT Plate Scanner for trial one of peptide immunizations. Columns are immunization groups, and rows across are peptides being tested for reactivity. T529 (S) indicates T529 peptide obtained from Dr. Todd Schell’s lab, while T529 (M) indicates peptide obtained from Dr. Lawrence Mylin’s lab.

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<th>Naïve</th>
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<td>329.8</td>
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<tr>
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Table 2. Spots counted by C.T. L. ELISPOT Plate Scanner for trial two of peptide immunizations. Columns are immunization groups, and rows across are peptides being tested for reactivity.

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<tr>
<td>HBV</td>
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<td>66.7</td>
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Table 3. Number of CD4+ and CD8+ T cells specifically reactive to CR281 and HBV peptides from ICS. Mouse 1 and 2 were immunized with CR281, while Mouse 3 and 4 were unimmunized naïve mice.

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<th>CD8+ for CR281</th>
<th>CD4+ for HBV</th>
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<td></td>
<td>0.15</td>
<td>0.19</td>
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<td>0.12</td>
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A CR281 Peptide Specific T Lymphocyte Immune Response to CCK$_{24}$vR

Figure 1.

GEAELWRATGPAGVGGTEMK
CR281-300

NEYSVFKTLQARFVK
T529-543

TPPAYRPPNAPIL
HBc$_{128}$-$_{140}$

NEYLLPQTVWARF
mPyT 676-688
Figure 2.
Figure 3.
A CR281 Peptide Specific T Lymphocyte Immune Response to CCK$_{24\text{av}}$R

Figure 4.

281 Peptide Immunized

CD4+  |  CD8+  |  CD4+  |  CD8+  

281

281

HBV

HBV
A CR281 Peptide Specific T Lymphocyte Immune Response to CCK_{24-46}R

CD4+  CD4+  CD8+  CD8+

Naive

281

HBV

HBV