Analysis of CD4+ T cell Neotigens in the Panc02 Tumor Model

Jocelyn Mathew1,2, Neeta Zaidi MD, Elizabeth M. Jaffee MD, Todd D Armstrong Ph.D.3
1Centennial High School, 2The Johns Hopkins University School of Medicine, Sidney Kimmel Comprehensive Cancer Center

Objective

To demonstrate the extent to which neotigens in the Panc02 tumor model are immunogenic to CD4+ T cells, in order to create a potent and effective cancer vaccine.

Abstract

Pancreatic cancer (PDA) is one of the deadliest cancers, with estimated 50,000 deaths per year.1 My lab developed a cancer-specific vaccine in the Panc02 tumor model by defining the genetic mutations and analyzing the resulting mutant peptides (neotigens) for their ability to be presented and activated CD4+ (killer) T lymphocytes. Peptides 218 and 230 of the vaccine showed positive responses only when presented by spleen cells, not T2+/Db- cells, which led to the hypothesis that these neotigens activated CD4+ T helper (Th2) cells which respond to MHC class II, and is permitted by the immune system function, and play an important role in tumor immunity.5 I performed ELISPOT analysis using T2+ cells (MHC class II-specific) and isolated CD4+ T helper cells from mice vaccinated with peptides 218 and 230 to confirm immunotherapy to demonstrate that these peptides activate CD4+ T cells. My results demonstrated a statistically significant increase in the number of peptide 218 and 230-specific CD4+ T cells in vaccinated mice. Future studies will analyze peptides not currently used in the vaccine system for their immunogenicity.

Introduction

In order to improve the outlook of pancreatic cancer, the Jaffe lab used RNA sequencing technology to identify mutations which code for tumor-specific peptides in the Panc02 mouse tumor model. These mutations are termed “neotigens,” and when given to tumor-bearing mice with an experimental adjuvant (STING-Vac), are seen to induce a significant anti-tumor response. (Figure 1, and Zaidi et al.1)

Figure 1: A) Peptide 218 and 230 induce a response when analyzed in whole spleenocytes not by CD8+ cells or APCs expressing MHC Class I/II. This neotigens vaccine is used to sensitize the tumor to checkpoint modulators, molecules which boost immune response to tumors. PD L 1 is a checkpoint protein which appears on the surface of T-cells, preventing them from attacking cells which express the proliferation indicator PDL 1. Antibodies which block the interaction between PD L 1 and PDL 1 increase T-cell recognition of tumor cells. (Figure 2) 

Figure 2: A. CD4 vs CD3 T cell recognition (5). B. Checkpoint complexes on T cells that activate (CD44)/ or dampen (PD-1)/ T cell activity (6).

Previous research has been conducted using antigen presenting cells (APCs) that express MHC class I alone, allowing only the CD8+ T cell response to be explored. However, CD4+ T cells play an integral role in tumor immunity, inducing dendritic cell activation, providing signals to CD8+ T cells, producing chemokines to enhance CD8+ T cell tumor infiltration, secreting cytokines such as IFN γ which increase apoptosis and increasing MHC I expression on tumors, allowing for better recognition effector CD8+ cells (5). Epitopes which bind to MHC class II and activate CD4+ cells may, therefore, increase the efficacy of a neotigens vaccine. Testing of the peptides 218 and 320 revealed IFN γ was only produced when whole spleenocytes were used (Figure 2A) but not APCs that express only MHC I and CD8+ were isolated, suggesting CD4+ T cells were isolated, suggesting CD4+ T cells were required for the CD8+ T cell response (Figure 3).

Hypothesis

Vaccination with peptides 218 and 230 leads to the activation of CD4+ T-cells. Therefore, ELISPOT analysis with isolated CD4+ T cells from vaccinated mice will give a positive readout when previous analyses with whole CD4+ T cells were negative.

Methods

ELISPOT Protocols

Day 1: Coat Plate with anti-IFN antibody

Day 2: Incubate CD4+ T-cells using Mouse EasySpot Kit (StemCell) per manufacturer’s instructions, and incubated for purity (Figure 4) plate 72 h APC with peptide. Mix APC and CD4+ T cells together, include overnight.

Day 3: Wash. Add detection antibody, SAP, and develop signal. Develop plate and let dry.

Day 4: Read plate (Figure 5)

Results

Comparison of experimental versus control groups was done using an ANOVA F-test as there were too few samples for a t-test to be accurate. The ANOVA F-test assesses whether any treatment group is different from the others (Figure 6). However, two vaccinations significantly increased the number of neotigens specific CD4+ T cells and increased the number of positive CD4+ T cell responses using ELISPOT assays. The vaccines were evaluated for CD4+ T cells using ELISPOT, using ELISPOT analyses of mice boosted 7 days after the last injection. 3x10^4 CD4+ T cells were analyzed. The average and standard deviation of duplicate wells is shown. (Figure 7).

Conclusion

1. The ELISPOT results demonstrate that CD4+ T cells are the T cells activated by vaccination with peptides 218 and 230 whether individually or as part of the complete neotigens vaccine.

2. The vaccination data indicate that one vaccination may lead to variable effectiveness, however, two vaccinations significantly increase the number of neotigens specific CD4+ T cells.

3. Checkpoint inhibition can increase the number of 218 and 230 specific CD4+ T cells in tumor bearing mice, however, this analysis will need to be repeated as the tumors were late-stage which may have affected the results.

4. The sample size was small (n=5), therefore, the experiments will need to be repeated with larger groups to confirm significance.

Relevant Applications to Biotechnology

Biotechnology capitalizes on discoveries about the body to create helpful, potentially life-saving products. This study utilizes previous knowledge about immune system functions and next generation sequencing to identify genetic mutations and create a cancer vaccine that effectively induces an immune response. Future research to confirm the role of CD4+ T cells and determine whether more mutations epitopes from Panc02 induce effective CD4+ T cell responses.

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References


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