DNA based vaccines are easy to manipulate, stable inexpensive and may be a better vaccination method for inducing anti tumor immunity.

Previously we have shown the ability of three class II restricted peptides from the non small cell lung cancer antigen IGFBP2 to partially protect mice vaccinated with an IGFBP2 expressing tumor cell line.

We created two DNA vaccines encoding the three IGFBP2 peptides one with defined flanking sequences between peptides previously demonstrated to augment vaccine efficacy of DNA vaccines encoding class I restricted peptides.

Here we compare the efficacy of the DNA based vaccines with the peptide based vaccine to protect mice from a challenge of IGFBP2 expressing tumor cells and evaluate the effect inclusion of the genes for the dendritic cell activating cytokines, GMCSF and IL-12, have on the induced anti tumor response.

Results

Background

Spacer Construct

Figure 1. Diagram of the DNA vaccines: p8, p251 and p291 refer to the three IGFBP2 peptides.

Vector Control

Vector Control

Figure 2: All three vaccines produce statistically significant improvement in tumor protection than their respective negative controls (p<0.05). Vaccination with the Spacer DNA vaccine induced an equivalent level of protection when compared to mice receiving the peptide based vaccine (p = 0.22). Surprisingly, the non spacer construct induced a superior level of anti tumor protection when compared to peptide or spacer construct vaccinated mice, p < 0.001 and p = 0.034, respectively.

Method

Tumor cells: The MMC cell line is derived from a spontaneous mammary cell carcinoma that developed in the neu transgenic mouse model. It produces subcutaneous tumors after injection of one million cells.

Mice: The neu transgenic mouse line is engineered to express rat HER2/neu on the MMTV promoter driving expression within the mammary tissue. These mice develop spontaneous mammary carcinomas beginning around four months of age.

Creation of DNA based vaccines: Due to the size of the vaccines, overlapping oligonucleotides encoding the sequence for each construct were purchased from Sigma. Use of the Herculase II Fusion PCR enzyme (Stratagene) created double stranded sequences that were digested with the XbaI and NotI and cloned into the corresponding sites in the vector pL3MCI. All three constructs were sequenced and found to produce mRNA of appropriate size when transfected into the MMC cell line.

Protection experiments: Cohorts of 4-5 mice are given three doses of vaccine separated by two weeks. Fourteen days following the last vaccine, mice are challenged with one million MMC cells. Mice are evaluated every three to four days for development of tumors which are measured with calipers. All DNA vaccinated mice received 50 µg of DNA emulsified in CFA/IFA while peptide vaccinated mice received 50µg of each peptide emulsified in CFA/IFA.

ELISPOT ASSAY: Ten to fourteen days after the final vaccination, two hundred thousand splenocytes from each mouse are incubated with ten µg of each peptide and incubated on ELISPOT plates coated with anti-mouse interferon gamma antibodies for 48 hours. Subsequent incubations with a second biotinylated anti interferon gamma antibody, streptavidin-alkaline phosphatase and then substrate produce countable spots. A mouse is considered to have a peptide specific response if the mean number of spots in wells incubated with peptide are statistically greater than wells that only received splenocytes and media.

ELISPOT data for mice vaccinated with the IGFBP2 vaccines produces peptide specific T cell responses only in peptide vaccinated mice raising the question of the mechanism of action with which the DNA vaccines are mediating their anti tumor effect.

Conclusions

DNA based vaccines induce superior levels of anti tumor protection when compared to the peptide based strategy.

Inclusion of defined spacer sequences detracts from the induced immunity.

Inclusion of the genes for the dendritic cell activating cytokines GM-CSF and IL-12 augment the potency of the DNA based vaccine.

Future Studies

Evaluate the effect inclusion of the genetic construct has on the non spacer IGFBP2 vaccine.

Funding Source

Cooperative Ovarian Cancer Group for Immunotherapy (COGI) and Clayton F. Steward Endowed Research Fund