Distribution of T Cell Cytokine Responses to Tumor-Associated Antigens in Healthy Adults and Breast Carcinoma Patients

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Abstract

The extent of pre-existing T cell immunity to tumor-associated antigens is largely unknown, either in healthy individuals or cancer patients. A database of CD4+ and CD8+ T cell responses to a variety of overlapping peptide mix antigens, including those corresponding to tumor-associated antigens (Her2/neu, MAGE-3 and CEA) and infectious disease-associated antigens (HIV, cytomegalovirus (CMV) and influenza A) was created. Data was collected for IFNγ, TNFα and IL-2 responses to each antigen, providing a distribution of responses in healthy individuals that was compared to a cohort of patients diagnosed with stage III breast carcinomas. Responses to Her2/neu in healthy individuals demonstrated a trend toward higher responses than those to MAGE-3 or CEA, suggesting either pre-existing or cross-reactive immunity in some individuals. Responses to CEA in healthy donors were not different from background or from responses to HIV gag. The latter was used as a negative control, since all donors were known to be HIV-negative. Differences between healthy donors and breast carcinoma patients will be analyzed. This database of cytokine responses to selected malignant disease and infectious disease associated antigens can provide a baseline for clinical trials of vaccines using these antigens. This work is partly supported by grant no. U-54 CA090818 and grant no. RO1-AI047062.

1 Method for lyophilized reagent, plate-based CFC assays

1. Isolate PBMCs from Cell Preparation Tubes
2. Add antibody to plate
3. Lyophilize the antigen plate
4. Add cells to plate

2 Responses of healthy donors to cancer and other antigens

CMV pp65 mix
Flu HA+M1 mix
Her2/neu mix
MAGE-3 mix
CEA mix
HIV mix (neg)

Heat maps were generated to demonstrate the percent cytokine response of 40 healthy donors.

Percent cytokine positive cells for Heat Maps

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3 Significance of differences in responses to various antigens

Kolmogorov-Smirnov tests were used to conduct pairwise comparisons of the response of cancer antigens to negative controls. ***p<0.0001

4 Cytokine responses of unvaccinated breast carcinoma patients versus healthy donors

Cytokine responses of two breast carcinoma patients were overlaid over box and whisker graphs generated for cytokine (TNFα, IL-2, IFNγ) responses of 40 healthy donors to Her2/neu, MAGE and CEA.

Conclusions

1. Healthy donors demonstrate correlation between CMV serology and CFC responses to CMV peptides.
2. In healthy donors, TNFα responses in CD4 and CD8 cells to Her2/neu and MAGE were low, but were significantly different (p<0.0001) from negative antigens (HIV).
3. IFNγ, TNFα, and IL-2 responses to CEA antigens were not significantly different from negative antigens in healthy donors.
4. We are gathering additional breast carcinoma patients to compare unvaccinated cancer patients with baseline levels of healthy donors.