Tumor Associated Antigens Harbor Readily Defined and Universally Immunogenic Regions Relevant for Cancer Immunotherapy

Dustin B. McCurry, MSIV[1,2], Latha B. Pathangey, MSc[2], Sandra J. Gendler, PhD[2], and Peter A. Cohen, MD[2]

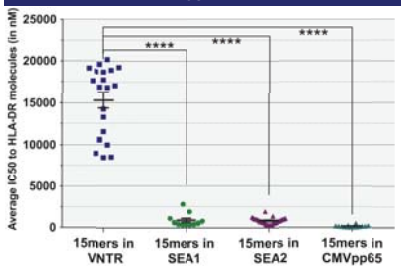
[1]University of Arizona College of Medicine-Phoenix, [2]Mayo Clinic Arizona

Abstract

Recent advances in cancer immunology, highlighted by immune checkpoint inhibitors, have demonstrated that immunotherapy is a viable option in the oncologist's armamentarium. Despite these advances, many patients are non-responders; and strategies to convert non-responders into responders by inducing anti-tumor immune responses are needed. We hypothesized that many tumor associated antigens (Ag) are readily susceptible to immune attack, but only in the context of identifying the tumor antigen epitopes that can reliably initiate a response, regardless of individual patient human leukocyte antigen (HLA)-haplotypes. Utilizing known HLA-serotype frequencies and the open source epitope prediction algorithm netMHCIIpan, we made epitope predictions relevant for over ninety percent of the population from the widely expressed oncoproteins MUC1, HER2/neu and CMV-pp65. Unexpectedly, predicted promiscuous HLA-binding epitopes clustered together in high priority regions: unique "hot spots" with high densities of non-HLA-serotype constrained epitopes. Synthetic long peptides spanning these regions were synthesized and utilized in an antigen specific T-cell culture system involving peripheral blood mononuclear cells (PBMC). Exposure of PBMC to these peptides was synchronized with aggressive surrogate activation of innate immunity by GM-CSF, LPS, and R848, followed by step 2 IL-7-modulated T-cell hyperexpansion. Added long peptides (>20aa) derived from the "hot spot" regions of MUC1, HER2/neu, and CMVpp65 reliably produced selective and sustained expansion of both CD4+ and CD8+ peptide-specific, interferon-γ (IFNγ)-producing T-cells. MUC1 peptide-specific T-cells preferentially recognized MUC1 expressing tumor cell lines, either in tumor cell killing assays or as tumor lysate when pulsed onto restimulatory PBMC. This mechanistically rational antigen selection sequence, effective even for unvaccinated donors, regardless of HLA-haplotype, enables rapid identification of tumor protein regions relevant for cancer immunology, including adoptive immunotherapy, cancer vaccines, and even identification of tumor neo-antigens unique to each patient.

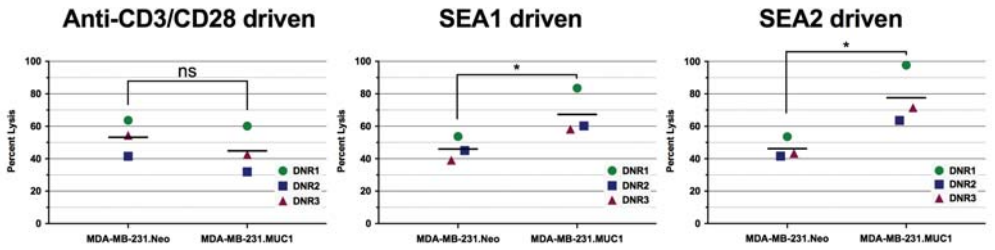
Results

Figure 4



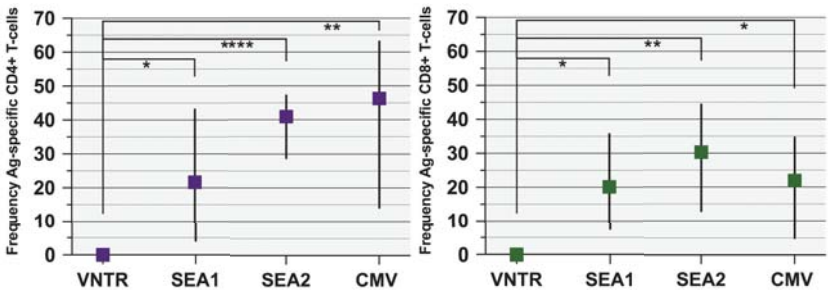
15mers Within "Hot Spot" Derived Peptides are Predicted to Bind to HLA-Serotypes More Strongly than 15mers Within Non-Hot Spot Derived Peptides: *Each data point represents the average predicted IC50 for each 15mer, calculated from the predicted affinities for every HLA-DRB1-type examined. Lower values indicate higher affinity (two-tailed p<0.0001 for each comparison, applying Mann-Whitney test).*

Figure 6



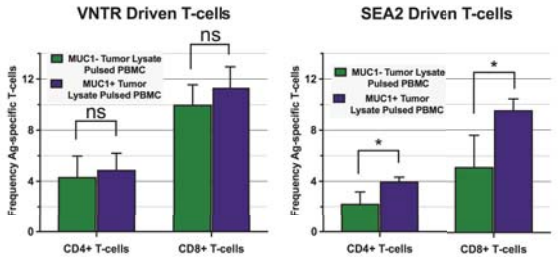
SEA1 and SEA2 Driven T-cells Preferentially Lyse MUC1 Expressing Tumor Targets: *HLA-A2.1+ PBMC from 3 healthy donors were either driven polyclonally with anti-CD3/CD28 or driven by long peptides derived from the sea urchin sperm protein, enterokinase and agrin (SEA) domain of MUC1 (SEA1 and SEA2). At the end of T-cell culture expansion, each of the T-cell groups was cultured for 8h at a 100:1 ratio with Cr51-labelled HLA- A2.1+ human breast cancer line MDA-MB-231, either transduced to express MUC1 (MDA-MB-231.MUC1) or Neo control (MDA-MB-231.Neo). % Lysis was calculated as ((Experimental Lysis – Spontaneous Cr51 release)/(Complete Lysis in Triton X–100 - Spontaneous Cr51 release)) x 100. % lysis of MDA-MB-231.Neo was statistically indistinguishable for all 3 donors whether cultures were polyclonally-, SEA1- or SEA2-driven. SEA1- and SEA2- driven T-cells from all 3 donors lysed MDA-MB-231.MUC1 targets significantly more than MDA-MB-231.Neo targets (two-tailed SEA1 p=0.039 and SEA2 0.038, applying Student's paired t-test).*

Figure 5



Long Peptides Derived from "Hot Spot" Regions Engender Tumor Antigen Specific CD4+ (Left) and CD8+ (Right) T-cells: *ICC assays were performed to determine the percentage of T-cells producing antigen-specific IFNγ in response to peptide pulsed restimulatory PBMC. Four successive donor responses are shown: vertical lines indicate the response range, boxes indicate the response average. "Hot spot" derived peptides (SEA1, SEA2, CMVpp65) enriched Ag-specific T-cells compared to a non-hot spot derived peptide (VNTR) (CD4+: p=0.040, p<0.0001, p=0.0059; CD8+: p=0.033, p=0.0048, p=0.023; all two-tailed unpaired t-tests).*

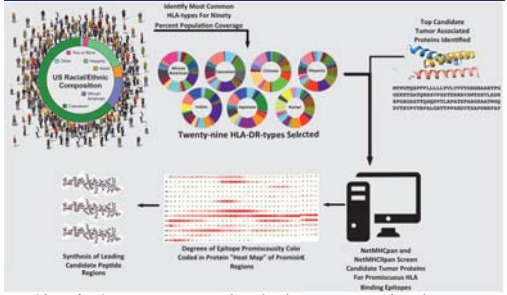
Figure 7



SEA2 Driven T-cells Preferentially Recognize PBMC Pulsed with MUC1+ Tumor Cell Lysate: *MUC1 non-expressing and MUC1 expressing tumor cell lines were lysed by repetitive freeze thaw cycles. ICC assays were performed to determine the percentage of T-cells producing IFNγ in response to tumor lysate pulsed restimulatory PBMC. SEA2 driven T-cells preferentially recognized MUC1+ tumor cell lysate pulsed PBMC (CD4+: p=0.022; CD8+: 0.025; two-tailed unpaired t-test).*

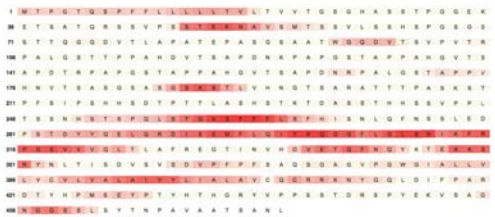
Methods

Figure 1



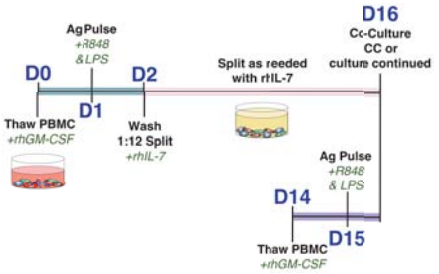
Peptide Selection Strategy: *Racial and ethnic groups within the US were assessed for HLA-DRB1 frequencies. In order to account for 90% of the population, 29 different HLA-DRB1 serotypes were chosen for prediction. The tumor antigens MUC1, HER2/neu, and CMVpp65 were examined. Candidate promiscuous HLA-DRB1 binding epitopes clustered together, and long peptides encapsulating these epitopes were sequenced for further study.*

Figure 2



MUC1 "Heat Map": *Each unique 15mer epitope within the tumor antigen MUC1 was assessed for predicted binding affinity to the 29 HLA-DRB1 serotypes under study utilizing the open source software NetMHCIIpan 3.1. If the predicted IC50 nM concentration was sufficiently low enough, the 15mer was considered a binder. Each 15mer was color-coded from white to red based upon the number of predicted binding HLA-DRB1 serotypes. High frequency binders were not uniformly distributed across the protein and clustered together, resulting in "hot spots." SEA1 and SEA2 sequences lie within the "hot regions" from the ~250-320 amino acid positions. The VNTR sequence lies within the ~130-155 amino acid positions.*

Figure 3



In vitro Ag-specific T-cell Culture: *PBMC were treated with GM-CSF, peptide Ag, and the TLR agonists, LPS and R848 during the initial culture. Cells were then washed and cultured in IL-7 containing media for two weeks. Ag-specificity was assessed on day 16 with intracellular cytokine (ICC) assays for IFNγ involving freshly thawed restimulatory PBMC, pulsed with the initial peptide Ag or irrelevant peptide Ag to determine the percentage of T-cells producing Ag-specific IFNγ.*

Conclusions

- Tumor associated antigens harbor universally immunogenic regions which are readily defined and are attractive targets for cancer immunotherapy
- Tumor associated proteins harbor clusters of high priority areas that can speed the discovery of immunologic regions and screen out low priority domains unlikely to be relevant to cancer immunotherapy (*Figures 1,2,4,5*)
- CD4+ and CD8+ T-cells are easily expanded and enriched for tumor-Ag-epitopes from high priority regions with the potential to recognize tumor derived-Ag in multiple contexts (*Figures 5,6,7*)
- These findings are applicable to cancer vaccine development, adoptive immunotherapy, and immunotherapeutic strategies targeting patient specific neo-Ag

Acknowledgements

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