Christopher Alvarez-Breckenridge
PhD Candidate

“The Role of Natural Killer Cells in the Context of Oncolytic Simplex Viral Therapy for Glioblastoma”

May 4th, 2011
234 Meiling Hall
2:30-3:30 PM
VITA

1982 ............................................. Born – Columbus, OH

2005 ............................................. BS, BA The Ohio State University

COMMITTEE MEMBERS

Dr. E. Antonio Chiocca MD, PhD, Advisor

Dr. Michael Caligiuri MD

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AWARDS AND HONORS

Funding

1. NIH CTSA TL1 Mentored Clinical Research Training Program Award through OSU Center for Clinical and Translational Science (TL1RR025753)—2010-2011
2. American Medical Association Foundation Seed Grant—2010-2011
3. Sigma Xi, Grants-in-Aid of Research Award Winner, 2010

Awards

1. 6th International Meeting on Replicating Oncolytic Virus Therapeutics Travel Award, 2011
2. American Association for Cancer Research Minority Scholar in Cancer Research Award, 2010
3. Ohio State University Comprehensive Cancer Center Research Day—Poster Session Winner, 2010
4. Ohio State University Neuroscience Research Day—Poster Session Winner, 2009
5. American Physician Scientist Association Travel Award—ASCI/AAP Joint Meeting, 2009 & 2010

FUTURE PLANS

Christopher Alvarez-Breckenridge will return to medical school to complete his MD/PhD.
ABSTRACT

It is controversial as to whether the host immune response hinders or improves the efficacy of oncolytic Herpes Simplex viral (oHSV) therapy of glioblastoma (GBM). Natural killer cells (NK) limit viral infections, and previous work suggests they may similarly attenuate virotherapy. Using both xenograft and syngeneic intracranial GBM tumor models, we used flow cytometry to evaluate the temporal pattern and phenotypic characteristics of NK cells present in the periphery and recruited to the site of oHSV infection. Within hours after infection and continuing through 72 hours following oHSV inoculation, NK cells were rapidly recruited to tumor bearing hemispheres. Moreover, these NK cells exhibited an activated phenotype, including enhanced CD69, CD62L, CD27, NKG2D, and Ly49D staining compared to vehicle treated mice. However, neither the number nor phenotype of peripheral NK cells was altered following oHSV infection.

This robust NK response was confirmed to be detrimental to OV efficacy through the enhanced survival of NK depleted mice inoculated with oHSV compared to oHSV treated mice possessing NK cells. Interestingly, oHSV treated mice exhibited robust macrophage recruitment and activation at the site of infection. This was accompanied by the induction of macrophage/microglial associated inflammatory gene and protein expression, including CXCL9, CXCL10, CXCL11, iNOS, and TNF-α. However, when mice were depleted of their NK cells or IFN-γ knockout mice were used, their expression was abrogated.

In vitro, human NK cells preferentially lysed oHSV-infected GBM in a cell contact, perforin, and DNAM-1 dependent manner. Fusion proteins were used to detect currently unknown ligands for the NK natural cytotoxicity receptors (NCR) and decipher the critical NK activating ligands that mediate this response. Following oHSV infection of a panel of GBM stem cells and cell lines, we detected robust up-regulation of ligands for NKp46 and
NKp30. GFP expression was used to discriminate oHSV infected GBM cells and preferential NKp30/NKp46 ligand expression was found in the GFP+ population of cells. Additionally, blocking antibodies against either NKp30 or NKp46 abrogated NK mediated clearance of oHSV infected GBM, while antibodies against NKp44 did not inhibit killing.

We have previously shown that immunomodulation with cyclophosphamide (CPA) and valproic acid (VPA) enhances oHSV efficacy. CPA administered prior to virus inoculation abrogated the oHSV induced NK and macrophage recruitment into the tumor at all time points tested compared to oHSV alone. Similarly, VPA treatment resulted in a decline in NK and macrophage recruitment at 6 and 24 hours post-oHSV; however, a robust increase at 72 hours-post-oHSV was seen, resembling the response seen with oHSV alone. VPA was also found to have a profound immunosuppressive effect on human NK cells in vitro. NK cytotoxicity was abrogated following exposure to VPA through down-modulation of cytotoxic gene expression and granzyme B protein levels. In addition, IFN-γ was suppressed in a Stat5/T-bet dependent manner.

Collectively, these findings demonstrate that oHSV therapy for GBM is limited in part by a robust NK cell response mediated by specific NCRs, uncovering novel potential targets to enhance cancer virotherapy. Moreover, pharmacological co-therapies, such as CPA and VPA with oHSV, alter the host immune response to the virus albeit in differing ways. Future work will be needed to further define the nature of the innate immune response, how it coordinates downstream anti-tumor immunity, and how pharmacological agents can be optimized to modulate the host response to oHSV.

**RECENT ABSTRACTS AND PRESENTATION**

