PALADINS: PROTECTIVE APPROACHES LEVERAGING AD-APTIVE AND IN-NATE SYSTEMS

Therapeutic Efficacy Of A Potent Anti-veev Antibody Is Contingent On Humoral And Cell-mediated Cytotoxic Immunity

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The intrinsic value of specific, safe, and potent antibodies is a focal point of protein engineering that has led to a wave of exciting developments in highly effective, novel therapies for infectious disease, cancer, and chronic ailments. However, apart from the importance of antigenic specificity of classical antibodies, it is increasingly evident that the crystallizable fragment (Fc) of antibodies can be modified to differentially activate or suppress specific branches of the body's immune response. We demonstrate the importance of intact humoral and cell-mediated cytotoxic immunity in the context of Venezuelan Equine Encephalitis Virus (VEEV) infection. Using a number of in vitro and in vivo assays, we determined that the therapeutic value of the potently neutralizing anti-VEEV antibody, F5 (Hunt et al., 2010), is reduced with Fc mutations L234A and L235A (LALA). Of particular note, we describe a novel method of assessing Fc-mediated immune effector cytotoxicity in real time over the course of a VEEV infection in vitro. We describe an adaptable Real-Time Complement Dependent Cytotoxicity (RT-CDC) assay to inform the presence of productive binding between WT or LALA Fc and Complement with VEEV infected cells. We also detail a Real-Time Antibody Dependent Cell-mediated Cytotoxicity (RT-ADCC) assay, which identifies productive Fc binding to Fcγ receptors on immune effector cells in the context of VEEV infection. Our system not only informs VEEV-Ab functional specificity and infection kinetics, but also illustrates the in vitro kinetics of WT versus LALA Fc-mediated activation of the Complement system and FcγR-cell-mediated cytotoxicity. Finally, we bring our WT and LALA mutant therapeutics in vivo with prophylactic (24 hours pre-exposure) and therapeutic (48 hours post-exposure) studies. Our findings illustrate that diminishing engagement with the Complement system and Fcγ receptors is detrimental to host survival post exposure to VEEV but has little effect on prophylactic utility.

Discovering an optimal balance between immune effector functions, anti-microbial factors and their regulatory structures via Fc engagement highlights an opportunity to improve therapeutic outcomes for different pathogen-specific disease contexts that can affect the warfighter. Furthermore, identifying critical system components of Fc-mediated immunity by fine-tuning engagement with beneficial immune effectors and minimizing activity of those that cause excessive tissue damage, can mitigate development of harmful sequelae, and greatly improve disease outcomes, allowing us to better treat and protect the nation and world.

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