

**Role of Natural Killer Cells in FMDV in cattle-characterization and identification of modulation strategies**

DHS Priority Areas Addressed	<input checked="" type="checkbox"/> Prevention <input type="checkbox"/> Detection <input checked="" type="checkbox"/> Response <input checked="" type="checkbox"/> Recovery <input type="checkbox"/> Education/Risk Communication			
Proposal Section Addressed	Sections 5.1.2 and 5.3.2			
Investigators	UTMB: D. Mark Estes, Janice Endsley PIADC: Bill Golde, Luis Rodriguez, Marvin Grubman TAMU: L. Garry Adams			
<b>Objectives</b>	<b>Deliverables</b>	<b>Progress Toward Deliverables</b>	<b>Percent Complete</b>	
Determine the potential for NK cell mediated lysis of FMDV infected cells, and effect of cytotoxicity on reduction of infectious virus.	In vitro NK assay development for FMDV infected targets	This assay has been developed and implemented at PIADC	100%	
	Analysis of pathways for modulation of NK-mediated viral killing as an early intervention point in the disease	This aspect of the project is under development. Antibodies have been received for one of the markers and is under testing.	15%	
	Analysis of gene expression in FMDV infected and uninfected targets in the presence or absence of NK cells	This aspect of the project is ongoing. The assay has been developed for replicons but not for live virus	35%	
Determine if natural killer cells with enhanced cytotoxic/effector potential increase in peripheral blood and traffic to sites of infection following FMDV infection.	Evaluation of in vivo NK cell response in the early response to infection (d0-d4) with FMDV in vaccinated and nonvaccinated subjects	These studies are ongoing but near completion.	90%	
	<b>Evaluation of in vivo NK cell response in the early response (d0-d4) to infection with FMDV in conjunction with anti-viral intervention (type I IFN) with and without vaccination</b>	These studies are planned and dependant on scheduling by DHS	10%	

**Highlight for Research Briefs**

Published abstract from the American Association of Immunologists Meeting in Miami in the Journal of Immunology from the results of in vivo studies of NK modulation by virus (*The Journal of Immunology*, 2007, 178: 46.19.)

**Interpretive Summary**

NK cell populations in swine and cattle are not fully delineated and this is an aspect of investigation we are conducting. Differential stimulation is possible (lytic function versus IFN-gamma production or both) and this is part of the continuation of this project along with a separate project for a delivery platform. We have developed a high throughput assay for cytotoxicity on the FACS which is in use at PIADC as trials are ongoing. We have trained their scientists for this purpose. The replicon assay is still under development and we will have a new postdoc coming on board this month who will take over the development of this assay.

**Results and Interpretations**

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals. Immunological knowledge to assess more rapid acting

vaccines against FMDV is presently limited. We examined the reactivity of swine and cattle NK cells following infection for their capability to express intracellular perforin, to kill a human tumor cell line target in vitro, and to secrete IFN gamma. The cytotoxicity of NK cells from non-infected animals against the K562 cells is low with baseline levels at 5–15% in swine and 15–20% in cattle. Stimulation with rhIL-2 or rhIL-12 plus rhIL-15, increased the lytic activity against K562 cells. Infection with FMDV inhibited swine NK cell lytic activity but did not significantly increase IFN gamma secretion during the acute infection. Perforin expression increased but this did not correlate with the killing capability of the swine NK cells. Infection of cattle with FMDV initially activated the NK cells to increase target cell lysis. NK cell IFN gamma secretion and perforin expression were slightly elevated upon infection and coincided with the lytic activity in cattle. These results are a further indication of immune evasion by FMDV by inhibiting or limiting NK cell function. The potential to manipulate the innate immune response to block this evasion is discussed in the context of designing rapid acting vaccines for foot-and-mouth disease.

### **Technology Transition**

Once the candidate activating receptors are validated, we will begin the development of monoclonal antibodies. Polyclonal antibodies for one of the receptors are in hand and are undergoing testing and validation. We will be entering discussions with Merial regarding the application of this technology to our antibody modulation platform.

### **Status of Funding**

Continuing