RESEARCH ABSTRACT

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SWINE HEALTH

Title: Expanding the immune toolkit for assessing pig health and improving swine disease and vaccine studies NPB project **[NPB# 06-043 renewal of # 05-015**]

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Scientific Abstract:

Swine disease and vaccine research has been advanced by the development of sophisticated tools to measure physiologic parameters associated with immunity, pathology, and disease prevention. Our goal is to expand the immune toolkit for pigs, by developing and characterizing reagents that can be used to identify and quantify a major class of immune proteins, the antibodies or immunoglobulins (Igs) that swine produce in response to infection or vaccination. Scientists measure pathogen exposure and vaccine efficacy by quantitating Ig levels in serum. But not all Igs are equal. We know for porcine reproductive and respiratory syndrome virus (PRRSV) infections that there is a well-characterized antibody response as measured by the IDEXX ELISA. Neutralizing Igs are known to take longer to develop but are important in recovery from PRRSV infection. It is likely that these sets of Igs represent different IgG subclasses. Currently most investigators rely on polyclonal antisera that are tedious to prepare, lack immortality, are usually not class specific, and vary between batches. For this NPB project we characterized known, and attempted to develop new, monoclonal antibody (mAb) reagents that uniquely recognize the various swine IgG subclasses.

Our first objective was to “Identify all immunoglobulin-G (IgG) subclass genes; express Ig proteins for each swine IgG subclass gene.” We cloned genes from the previously known5 swine IgG genes. Research at Dr. Butler’s lab at the Univ. of IA actually resulted in the discovery of numerous other swine IgG genes, thus adding additional complexity to our goals. We worked with a collaborator, Dr. Serge Muyldermans, in Belgium to express the original 5 known swine IgG subclass proteins in vitro using his novel camelid-swine Ig expression system that efficiently produces single chain porcine-camelid chimeric IgGs. With this camelid system we have the means to express only constant regions of each specific swine IgG heavy chain protein and to use each porcine-camelid chimeric IgG to immunize mice and to produce and characterize mAb. The expressed proteins prepared in Belgium were shipped to the US where they were used to address objectives B “Characterize the reactivity of known anti-swine Ig monoclonal antibody (mAb) reagents with each Ig gene product” and C “Develop new mAbs that are specific for each of the expressed IgG subclass proteins.” For Obj. B previously developed hybridoma cell lines expressing mAb reactive with swine Igs were collected at BARC from labs worldwide, including anti-IgG hybridomas from the UK and anti-swine IgA and IgM hybridomas from Canada.

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project’s principal investigator. This report has not been peer-reviewed.

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Hybridoma supernatants containing mAb were prepared, purified at BARC and sent to Iowa for use in their tests of specific reactivity with the expressed swine IgG subclass. As expected none of the currently available anti-swine IgG mAbs was specific for just one IgG subclass. Thus Obj. C had to be pursued.

We first concentrated our efforts on what research had affirmed are the most highly expressed swine IgGs: IgG1, IgG3 and IgG5. We targeted a broadly reactive anti-IgG1, the major IgG in the blood of pigs; currently we have anti-allotypic mAb only. We targeted IgG3, the most important IgG for Complement fixation as well as the major IgG in the intestinal tissues of the newborn piglet. Finally we tried to make mAb to IgG5, the third most expressed IgG in the pig. For Obj. C we used the Hybridoma Facilities at Iowa State Univ. for hybridoma production. Mice were separately immunized with each swine IgG subclass protein, as the specific camelid-porcine IgG. All hybridoma fusion supernatant mAb produced were tested for their reactivity with swine Igs and the camelid-swine IgG subclass proteins. Several fusions were performed but unfortunately to date no mAb has been developed that reacted against just one swine IgG subclass protein. New fusion efforts have been put on hold waiting for new supplies of porcine-camelid chimeric IgG construct proteins to continue this immunization work. [It should be noted that as part of a separate funding initiative these immunizations and hybridoma fusions are continuing.]

The final objective was “Distribute mAbs and develop reference standard sera.” We worked with the USDA Animal and Plant Health Inspection Service (APHIS) National Veterinary Services Laboratories (NVSL) facilities in Ames IA to establish a resource for veterinary reagents; NVSL has made two anti-swine reagents, anti-IgM (M160) and anti- IgA (1459), available to researchers. It is hoped that this will be just the first step in NVSL making a broader panel of immune reagents available to veterinary researchers. The development of the USDA APHIS NVSL resource is a major accomplishment for this grant. Our overall goal has been to have a full panel of well-characterized mAb that react specifically with each swine Igs to expand our understanding of disease control mechanisms, immunity and pathology.