

## ANNUAL REPORT PROJECT NC-229

**PERIOD COVERED:** June 2008 to November 2009

**INSTITUTION OR STATION:** University of Maryland, College Park, MD

**A. NC-229 REPRESENTATIVE:**

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**Other PRINCIPLE LEADERS associated with the projects**

Zhu, Xiaoping, UMD

**B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:**

*Per station for ALL Accomplishment = Maximum 3,000 characters including spaces;  
Full NC229 report for ALL Accomplishment = Maximum 30,000 characters):*

This section focuses on intended activities, outputs, and short-term outcomes. The report should also reflect on the items that stakeholders want to know, or want to see. The accomplishments should cover only the current year of the project.

**Objective 1.** Elucidate the mechanisms of host-pathogen(s) interactions.

Indicate progress in the following areas.

**1. Research related to pathogenesis/persistence**

We continued studies on developing anti-PRRSV peptide-conjugated phosphorodiamidate morpholino oligomers (PPMOs). To examine PPMO-mediated inhibition of PRRSV replication in primary culture of porcine pulmonary alveolar macrophages (PAMs), we found that:

1. PRRSV infection of PAMs induced the activity of caspases-3/7, -8, and -9 significantly.
2. Compared with mock-infected control, the expression of CXCL10, IFN- $\beta$ , and CCL2 in PRRSV-infected PAMs were 2157-, 1740-, and 108-fold higher, respectively. The expression of double-stranded RNA-activated protein kinase R (PKR), interferon regulatory factor 1 (IRF-1), signal transducers and activators of transcription 1 (STAT-1), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), toll-like receptor 3 (TLR-3), and nuclear factor-kB p65 (NF-kB) in infected PAMs were 7.5-, 6.4-, 6.4-, 8.3-, 2.8-, and 1.7-fold higher, respectively.

**2. Research related to virus evolution**

**3. Research related to mechanisms of transmission**

**4. Research related to viral Immunity and cross-protection**

**5. Research related to epidemiology**

**Objective 2.** Understand the ecology and epidemiology of PRRSV and emerging viral diseases of swine.

Indicate progress in the following areas.

1. Research related to pathogenesis/persistence
2. Research related to virus evolution
3. Research related to mechanisms of transmission
4. Research related to viral Immunity and cross-protection
5. Research related to epidemiology

**Objective 3.** Develop effective and efficient approaches for detection, prevention and control of PRRSV and emerging viral diseases of swine.

Indicate progress in the following areas.

1. Research related to pathogenesis/persistence

We continued studies on developing anti-PRRSV PPMOs. PPMOs are single-stranded DNA analogs containing a modified backbone and cell-penetrating peptide. PPMOs are nuclease-resistant, water-soluble, enter cells readily, and exhibit highly specific binding to complementary RNA. We examined PPMO-mediated inhibition of PRRSV replication in PAMs. We found that:

1. PAMs uptake PPMO efficiently. The uptake efficiency of PAMs of 72-hour pre-incubation was higher than PAMs of 24-h pre-incubation.
2. Treatment of PAMs with PPMO 5UP2 resulted in protection from PRRSV-induced cell death for at least seven days, and produced no elevation in the activity of the caspase 3, 7, 8 and 9.
3. 5UP2 treatment of PRRSV-infected PAMs also prevented the vigorous induction of interferon- $\beta$  and chemokines observed in infected and mock-treated PAMs.
4. Administration of this PPMO to piglets that were experimentally infected with PRRSV resulted in lower viremia and less lung lesion than those without the PPMO treatment.

These results provide further rationale for the development of PPMO 5UP2 as an antiviral to control PRRSV infection.

2. Research related to virus evolution
3. Research related to mechanisms of transmission
4. Research related to viral Immunity and cross-protection
5. Research related to epidemiology

## **C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDERS:**

Our studies of PPMOs against PRRSV have demonstrated that PPMOs inhibited PRRSV replication and protected the cells from PRRSV-induced cell death. Administration of PPMO 5UP2 to piglets that were experimentally infected with a PRRSV strain resulted in lower viremia and less lung lesion. Specific antiviral PPMOs can complement other approaches for PRRS prevention and control, because there are highly conserved target sequences among PRRSV

strains. Application of the antiviral PPMOs will yield significant economic benefits to the swine industry, especially for breeding farms.

Impact statements (500 characters per statement)

This section focuses on actual or intended potential long-term outcomes and impacts, covering only the current year of the project. The report should also reflect on the items that stakeholders want to know, or want to see. List any grants, contracts, and/or other resources obtained by one or more project members as a result of the project's activities. Include the recipients, funding source, amount awarded and term if applicable.

#### **D. PRRS PUBLICATIONS ISSUED OR “IN PRESS”**

##### **1) Refereed publications**

1. Deendayal Patel, David A. Stein, and **Yan-Jin Zhang**: Morpholino Oligomer-Mediated Protection of Porcine Pulmonary Alveolar Macrophages from Arterivirus-Induced Cell Death. *Antiviral Therapy* 2009. In Press.
2. Xue Han, Sumin Fan, Deendayal Patel, and **Yan-Jin Zhang**: Enhanced Inhibitory Effect on PRRSV Replication by Combination of Two Morpholino oligomers. *Antiviral Research* 2009. 82:59-66.

Give full citation

##### **2) Abstracts or Proceedings**

Cite authors, year, title, meeting (use abbreviations, e.g., Proc., CRWAD, AASV, 2008 PRRS Symp., etc.) Do not give full dates.

##### **3) Book chapters or monographs**

Give full citation

#### **E. FUNDING SOURCES FOR PRRSV RESEARCH**

##### **1) Current: Institutional start up funding.**

##### **F. WORK PLANNED FOR NEXT YEAR:**

**We will continue studying the PRRS virus-cell interactions and exploring PPMOs for PRRSV control.**