

ANNUAL REPORT PROJECT NC-229

PERIOD COVERED: June 2008 to November 2009

INSTITUTION OR STATION: University of Connecticut

A. NC-229 REPRESENTATIVE:

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Other PRINCIPLE LEADERS associated with the projects

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Progress (Risatti): Mapping Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Genetic Determinants of Macrophage Host Range and Immune Modulation. New knowledge has derived from this research. Since Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) genetic determinants affecting the response of the host primary target cell, the macrophage, to infection are yet to be defined. We have used recombinant viruses encompassing ORF 1A to identify PRRSV determinants associated with growth and modulation of pro- and anti-inflammatory cytokine expression in primary pulmonary alveolar macrophages cultures (PAMs). Three genomic chimeras encompassing ORF 1A of PRRSV live attenuated vaccine Prime Pac (LAV SP) in the genetic background of pathogenic strain NVSL 97-7895 (FL12v) were characterized *in vitro*. Unlike parental viruses, two of the recombinant viruses encompassing the area of the genome encoding for NSP2 to NSP8 showed reduced growth in PAM cultures. The effect of virus infections on gene activation was studied for 25 immunomodulatory cellular genes in PAMs at 24 and 48 hours post-infection (hpi). Steady state mRNA levels in PAMs infected with recombinant viruses were compared to levels observed in cells infected with parental virus FL12v. Recombinant viruses induced patterns of transcriptional activation differing from patterns induced by parental FL12v, suggesting a regulatory role of PRRSV ORF1A on PAM gene expression.

Progress (Garmendia): "Assessment of Virulence of PRRSV Isolates Based Both on their Sensitivity to IFN β and Ability to Induce Type I IFN Responses". The aims of the study are to determine the sensitivity to and induction of IFN β by the isolates, to identify mechanisms of evasion of host's innate immune responses by PRRSV and to determine correlations with virulence. We have phenotyped several PRRSV field isolates in their sensitivity to and ability to induce IFN β *in vitro*. A series of chimeric viruses derived from infectious clones of FL12 (virulent isolate) and a vaccine virus (attenuated), provided by Dr. Osorio (University of

Nebraska at Lincoln, Nebraska), are also being tested. Preliminary data shows significant differences in sensitivity to IFN β among different PRRSV isolates and between MARC-145 cells and porcine alveolar macrophages (PAM). The data on induction obtained thus far, shows that PRRSV isolates do induce IFN β in PAM but at variable levels. The sensitivity to and ability to induce IFN β *in vitro* will be the basis to formulate phenotypes for further testing. Selected isolates of different phenotypes will be utilized to identify segments in the type I IFN pathway that may be blocked by the virus. Efforts to test the effects of the virus on the signaling phase are under way. The pathogenicity of defined isolates will be investigated in swine.

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

Indicate progress in the following areas.

1. Research related to pathogenesis/persistence

Investigations on transcriptional activation in macrophages upon PRRSV infections have produced the following results (Risatti): Infection of primary porcine alveolar macrophages (PAMs) with virulent PRRSV FL12v resulted in transcriptional activation of 14 out of the 25 genes analyzed. A sustained transcriptional activation was observed for IL-1 α , IL-6, TNF- α , IFN- β , IRF-7, PKR and Mx1, consistent with activation of an antiviral state within PAMs upon infection with virulent FL12v. At 24 hour post-infection (hpi), which corresponds to the peak logarithmic phase of FL12v infectious virus assembly and release, transcription of IL-1 α , IL-10, IL-15, IRF-7, VCAM, and Mx-1 genes was significantly different in PAMs infected with cP5U.NSP12 or cPNSP3.8 relative to PAMs infected with FL12v. Live attenuated vaccine (LAV) SP infection induced a pattern of transcriptional activation at 24 hpi similar to that of FL12v. At 48 hpi, at the decline of virus yield in PAMs, the transcriptional activation profile differed from the profile observed at 24 hpi. IL-1 α , IL-1 β , IL-10, IL-15, TNF- α , MCP-2, IRF-7, and Mx1 mRNAs accumulation was significantly different in cells infected with the recombinant viruses or LAV SP relative to cells infected with FL12v.

Sensitivity to IFN β and Ability to Induce Type I IFN Responses by PRRSV (Garmendia): Preliminary data shows significant differences in sensitivity to IFN β among different PRRSV isolates and between MARC-145 cells and porcine alveolar macrophages (PAM). The data on induction obtained thus far, shows that PRRSV isolates do induce IFN β in PAM but at variable levels. The sensitivity to and ability to induce IFN β *in vitro* will be the basis to formulate phenotypes for further testing. Selected isolates of different phenotypes will be utilized to identify segments in the type I IFN pathway that may be blocked by the virus. Efforts to test the effects of the virus on the signaling phase are under way. The pathogenicity of defined isolates will be investigated in swine.

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
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:

Output for project: “Mapping Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Genetic Determinants of Macrophage Host Range and Immune Modulation”, PI: Guillermo Risatti. A poster was presented at the 27th Annual Meeting of the American Society for Virology (P7-11) held on July 12-16, 2008 at Cornell University, Ithaca, NY. Results of this research were presented by Ms Inga Gudmundsdottir (MS student) at the 2008 College of Agriculture and Natural Resources Graduate Student Council Research Forum of the University of Connecticut on March 2008. A poster was also presented during the 2008 International PRRS Symposium (P266) held on December 2008 in Chicago, IL. Ms. Inga Gudmundsdottir was mentored under this project and received a Master of Science from the University of Connecticut on July 2008 with a dissertation entitled “Mapping Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Genetic Determinants of Macrophage Host Range and Immune Modulation”.

Output for project: “Assessment of Virulence of PRRSV Isolates Based Both on their Sensitivity to IFN β and Ability to Induce Type I IFN Responses”. Active participation in the NC229 meeting held in Chicago, Illinois December 2008. Active participation at the International PRRSV Symposium held in Chicago, Illinois, December 2008. Mr. Christopher Overend a doctoral candidate in Pathobiology and Veterinary Science who is working in the project had a poster presentation. Mentoring Dr. Raj Maganti a post-doctoral fellow and participant in the study. Data obtained in this project are discussed with our collaborator Dr. Marvin Grubman who shares interests in the area of type I IFN as it relates with viral diseases of swine. Data are also presented and discussed internally at seminars in our department.

D. PRRS PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed publications

INFECTION OF PORCINE ALVEOLAR MACROPHAGES WITH RECOMBINANT CHIMERIC PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS: EFFECTS ON CELLULAR GENE TRANSCRIPTION AND VIRUS GROWTH. I. Gudmundsdottir and **G.R. Risatti**. 2009. **Virus Research** 145(1) 145-50.

He D., C.C. Overend, R.J. Maganti, J. Ambrogio, , M.J. Grubman and **A.E. Garmendia** 2009. Marked differences between MARC-145 cells and alveolar macrophages in IFN β -induced activation of antiviral state against PRRSV. Submitted to Vet Imm Immunopath

2) Abstracts or Proceedings

PATTERNS OF GENE EXPRESSION IN PORCINE ALVEOLAR MACROPHAGE CELLS UPON INFECTION WITH VIRULENT, ATTENUATED, AND RECOMBINANT CHIMERIC PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS. I. Gudmundsdottir¹, E. Tulman¹, D. L. Rock² M. V. Borca³, **G. R. Risatti**¹. ¹Dept. of Pathobiology and Veterinary Science, College of Agriculture and Natural Resources, University of Connecticut, Storrs, CT. ²Dept. Of Pathobiology, College of Veterinary Medicine, Urbana, IL. ³Plum Island Animal Disease Center, ARS, USDA, Greenport, NY. **2008 International PRRS Symposium, Dec. 5-6, 2008. Chicago, Illinois.**

MAPPING PRRSV GENETIC DETERMINANTS OF MACROPHAGE HOST RANGE AND IMMUNE MODULATION. Ingigerdur Gudmundsdottir (1), Edan Tulman (1), Daniel L. Rock (2), Manuel V. Borca (3), and **Guillermo R. Risatti (1)**. (1) Univ. of Connecticut, Storrs, CT 06269. (2) Univ. of Illinois, Urbana, IL 61802. (3) Plum Island Animal Disease Center, ARS, USDA, Greenport, NY 11944. **American Society for Virology**. 27th Annual Meeting 12-16 July, 2008. Cornell University, Ithaca, New York.

Overend C, R. Maganti, **A.E. Garmendia** 2008. COMPARISON OF N PROTEIN NUCLEOLAR LOCALIZATION AMONG PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS AN THEIR ABILITY TO INDUCE TYPE I IFN. 2008 International PRRS Symposium, December 5-6, Chicago, IL

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

F. WORK PLANNED FOR NEXT YEAR

Continue with investigations related with pathogenesis, virulence, and attenuation of PRRSV (Objective 1).