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Immunomodulatory effects of European Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) on monocyte derived macrophages and dendritic cells

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PRRSV – Introduction

- First described in North America, 1987
- Small enveloped RNA virus, *Arteriviridae* family
- Two genotypes: N American and European
- Respiratory problems (young pigs)
 - Coughing/laboured exhalation
 - Interstitial pneumonia
- Reproductive problems
 - Late-term abortion
 - Neonatal death
- \$560 million annual losses
 - Vaccines are not protecting against all strains
 - New vaccine development/new control strategies required
 - Need for improved understanding of PRRSV interaction with the host




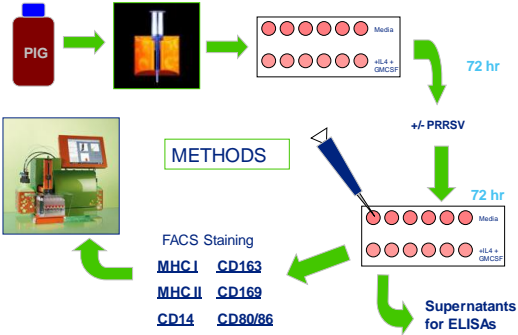
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Alveolar Macrophages and Dendritic Cells

- PRRSV infects alveolar macrophages (PAM) and dendritic cells (DC)
 - Both APC cell types play important roles in innate immunity
 - Do they contribute to ability of PRRSV to persist and propagate?
- Is the phenotype/function of macrophages or dendritic cells modulated by PRRSV?
 - Well described with US-lineage PRRSV but not yet EU-lineage PRRSV
 - Stimulation of IL-10 responsible?

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METHODS

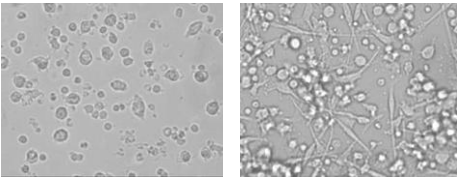
FACS Staining
MHC I **CD163**
MHC II **CD169**
CD14 **CD80/86**

Supernatants for ELISAs

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Differentiation of monocytes into macrophage and DC



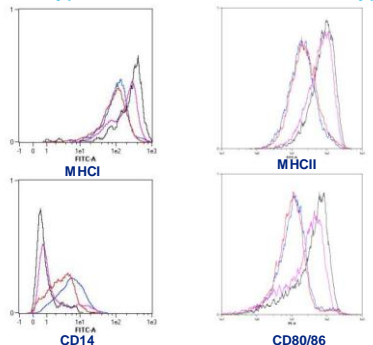
MoM0
+ RPMI media

MoDC
+ IL-4 & GM-CSF

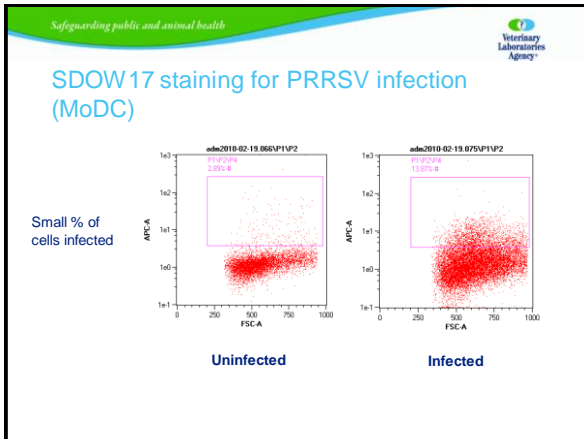
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Phenotypic differences between cell types



Immature MoM0
Mature MoM0
Immature MoDC
Mature MoDC

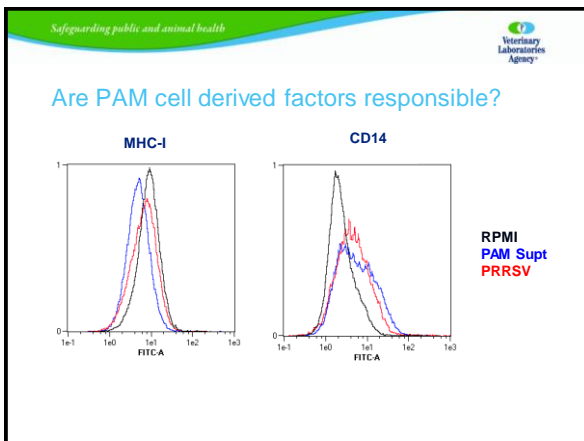
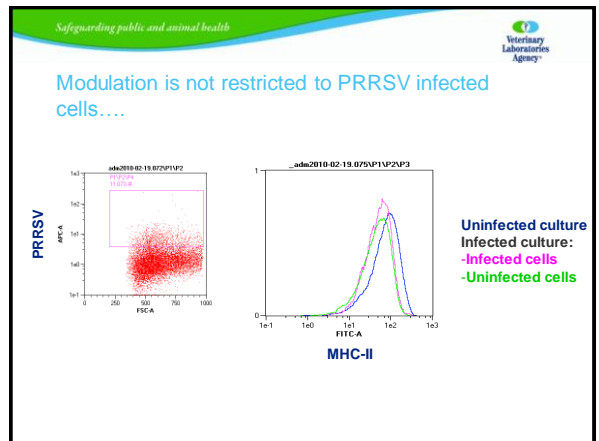
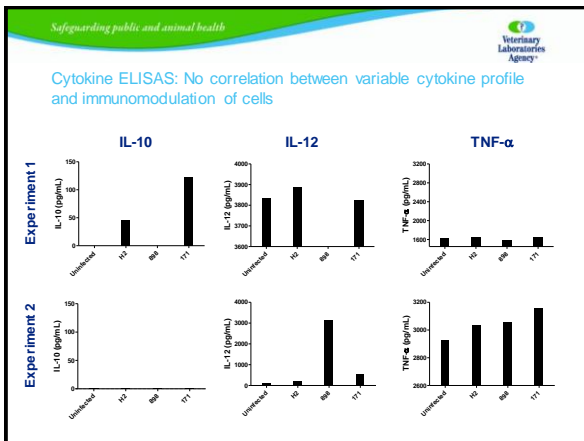


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Phenotypic changes induced by PRRSV

	Up-regulated	Down-regulated	No Change
MoMØ	CD14	MHC Class I CD163 CD80/86	CD169 MHC Class II
MoDC	CD14	MHC Class II CD80/86	CD169 CD163 MHC Class I



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PLoS PATHOGENS

Evasion by Stealth: Inefficient Immune Activation Underlies Poor T Cell Response and Severe Disease in SARS-CoV-Infected Mice

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Abstract

Severe Acute Respiratory Syndrome caused substantial morbidity and mortality during the 2002–2003 epidemic. Many of the features of the human disease are duplicated in BALB/c mice infected with a mouse-adapted version of the virus (MA15), which develop respiratory disease with high morbidity and mortality. Here, we show that severe disease is correlated with slow kinetics of virus clearance and delayed activation and transit of respiratory dendritic cells (DC) to the draining lymph nodes (DLN) with a consequent deficient virus-specific T cell response. All of these defects are corrected when mice are treated with liposomes containing clodronate, which deplete alveolar macrophages (AM). Inhibitory AMs are believed to prevent the development of immune responses to environmental antigens and allergic responses by interacting with lung dendritic cells and T cells. The inhibitory effects of AM can also be nullified if mice or AMs are pretreated with poly I:C, which directly activates AMs and DCs through toll-like receptors 3 (TLR3). Further, adoptive transfer of activated but not resting bone marrow-derived dendritic cells (BMDC) protect mice from lethal MA15 infection. These results may be relevant for SARS in humans, which is also characterized by prolonged virus persistence and delayed development of a SARS-CoV-specific immune response in individuals with severe disease.

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Conclusions...

- MoDC and MoMØ are susceptible to PRRSV infection
- Distinct phenotypic changes, consistent with immunosuppression, are induced by PAM culture supernatants regardless of PRRSV infection
- No apparent association with production of cytokines (IL-10, IL-12, TNF- α , TGF- β , IFN- γ , IFN- α)
- Is PRRSV taking advantage of the inherent immunosuppressive properties of PAMs to *indirectly* modulate DCs?

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What next...?

- Characterise cytokine profile of PAM supernatants +/- PRRSV
- Use different cells to grow PRRSV
 - MARC cell line
 - PK15 cell line (CD163 transfectant)
 - Both likely to have fewer immunomodulatory properties
 - Evaluate impact of M-CSF on differentiation of monocytes to MoMØ
- Purify Virus
 - Dialysis/filtration
- Microarray
 - Non-PAM derived PRRSV + MoMØ/MoDC
 - PRRSV + PAMs + DCs

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