

**The Development of model systems
to address important pathogenic
mechanisms of MAP associated
with the survival in ruminants**

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Before anything...

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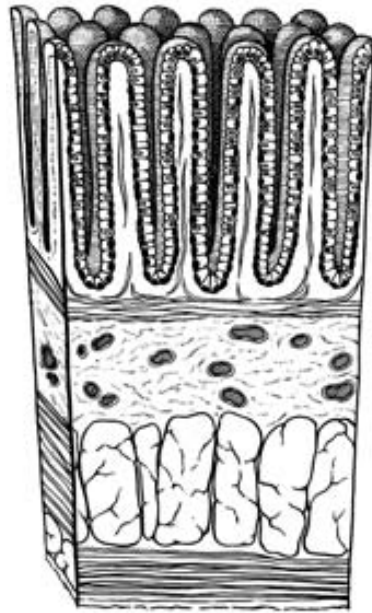
Jonathan Roussey

Johne's Disease

- Chronic inflammatory bowel disease in ruminants
- Asymptomatic for a 2-5 year incubation
- Causes severe diarrhea, leading to malnutrition/dehydration
- Economic loss (> \$250 million/year in US)
 - Reduced fertility
 - Reduced milk production
 - Expenses lost to replacing culled cattle

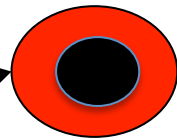


In vivo MAP infection

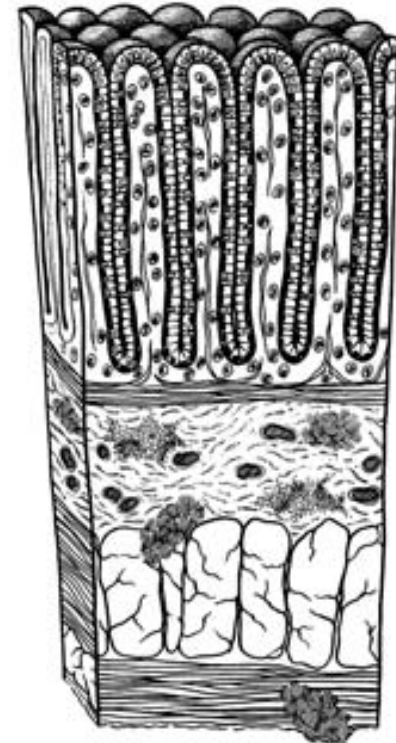


Normal colon

- Normal histology
- No granulomas



macrophages



Johne's Disease

- Severe lamina propria inflammation
- Granulomas
- Transmurial process

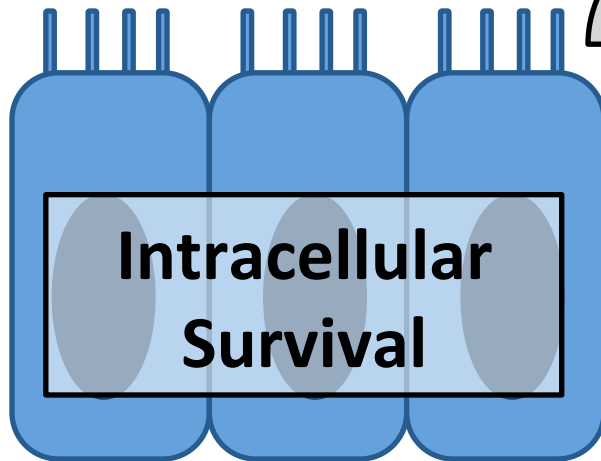
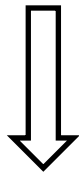
Lymph nodes

Mammary gland

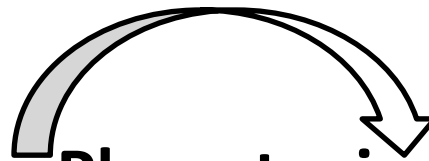
Liver ? Spleen ? Blood ?

How can one explain the difference in MAP behavior?

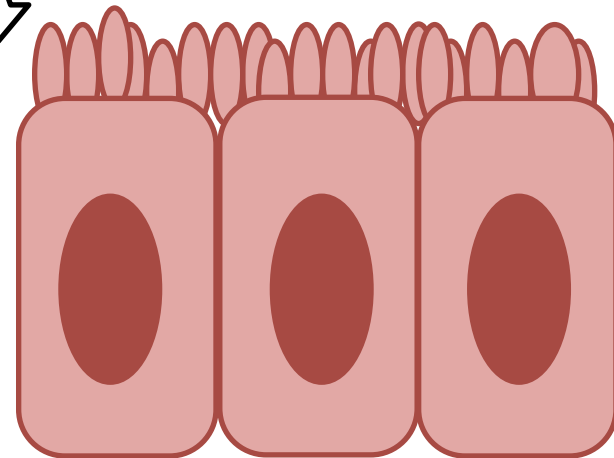
Methods of
Invasion



EARLY
Bind and Invade
No inflammatory response

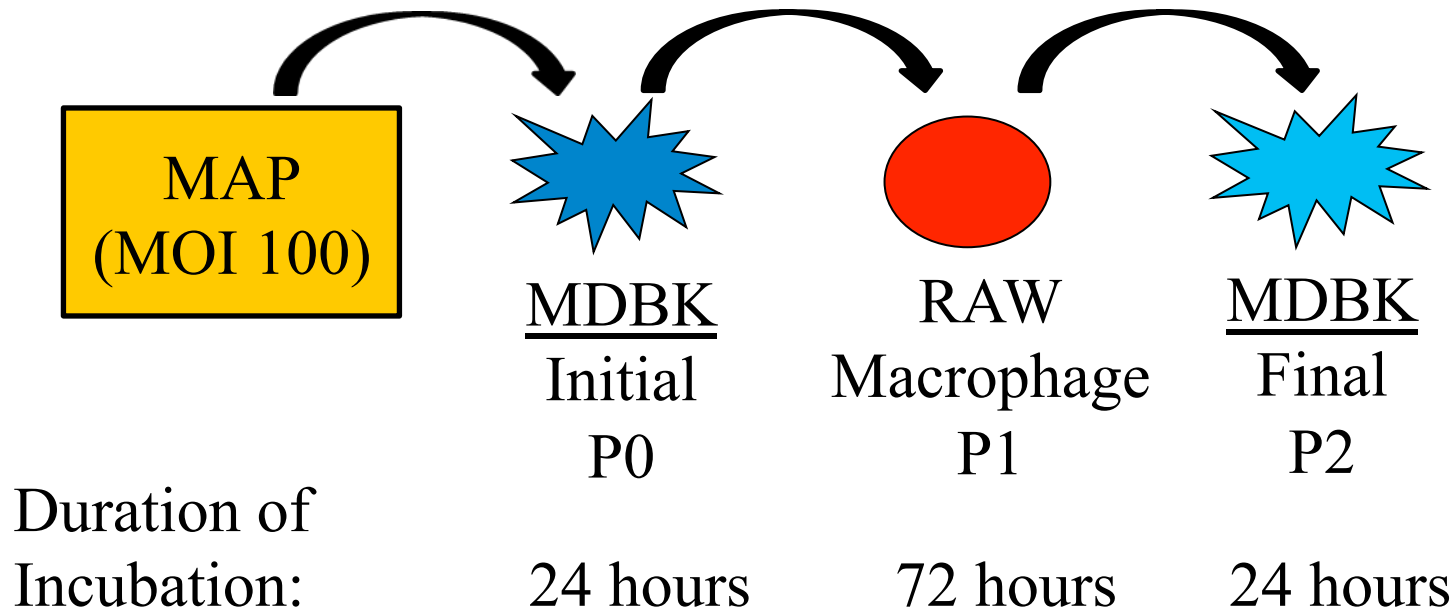


Phenotypic
Changes
?

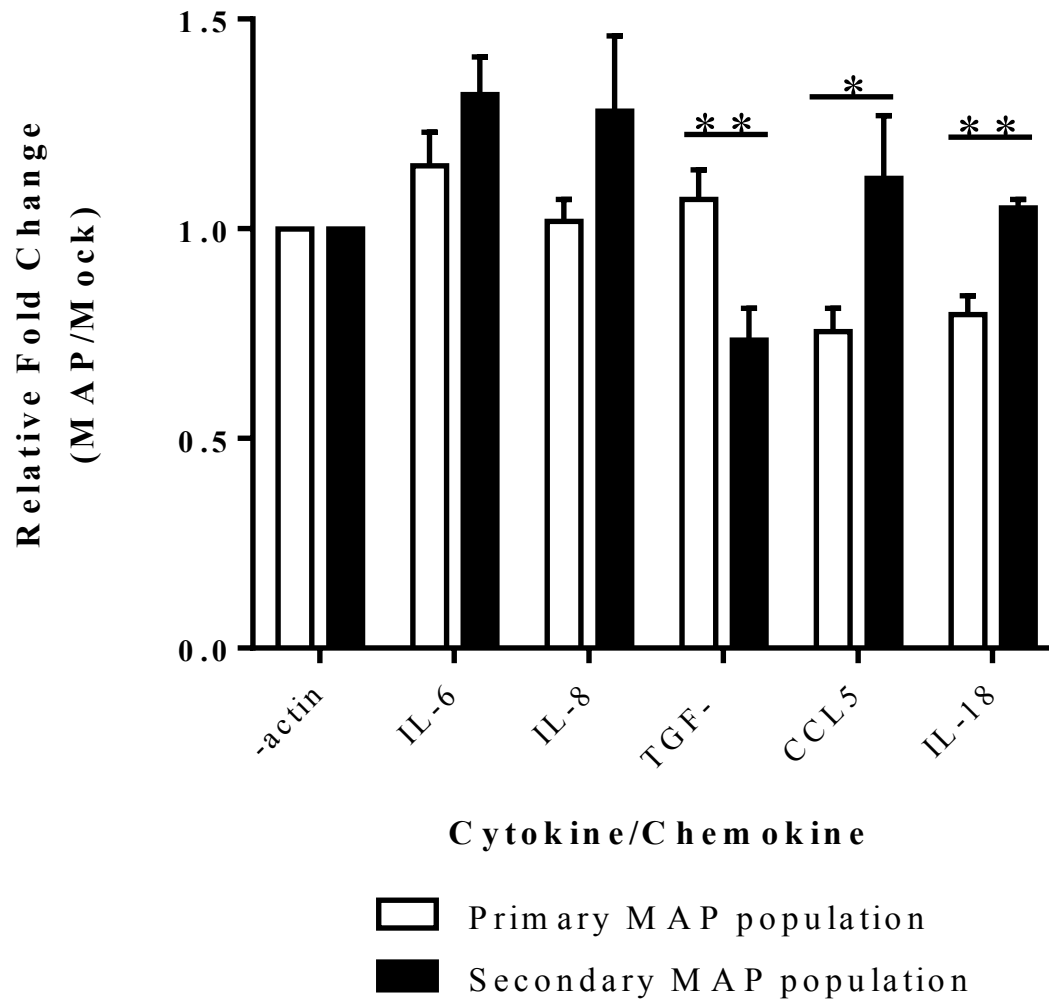


LATE
Severe inflammatory
response

In Vitro Cell Culture Passage Model for MAP Infection



Inflammatory response of MBK epithelial cells during *in vitro* cell culture passage model



protein

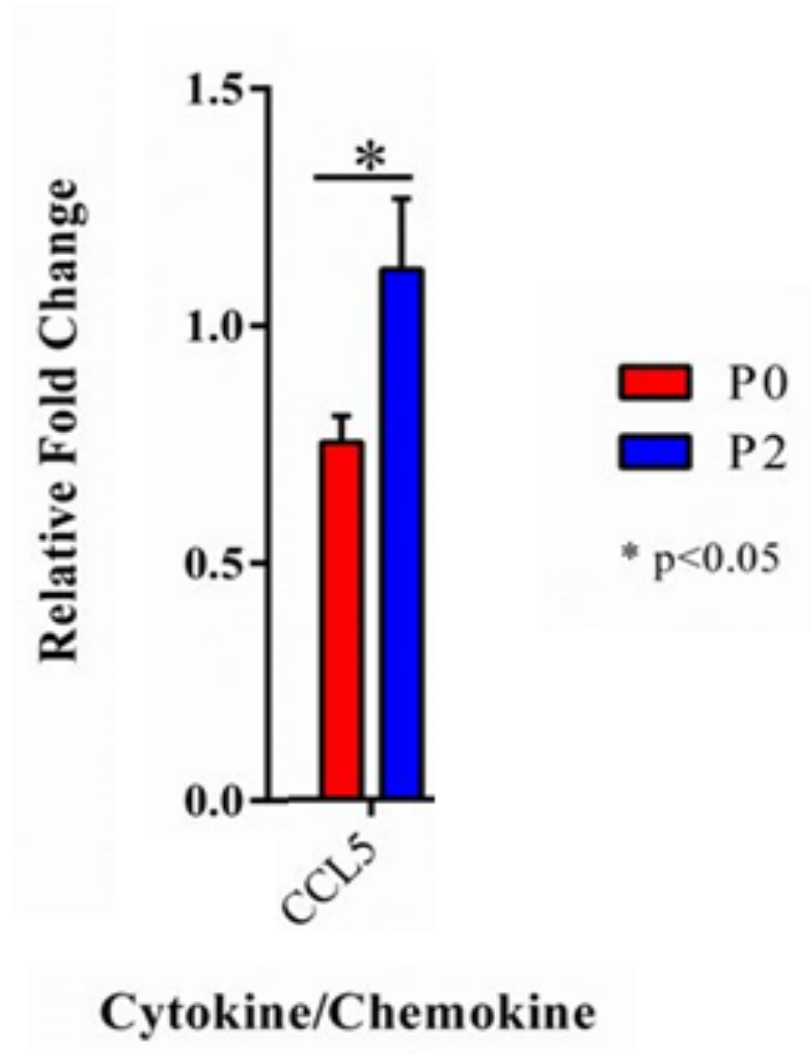


Table 1: Cytokine and chemokine express (mRNA) by epithelial cells infected in the second passage. Approximately 7-10% cells were infected.

cytokines/chemokines	first passage	second passage	p value
β actin	1.0	1.0	-
IL-6	1.1 ± 0.3	12.2 ± 0.4	-
IL-8	1.0 ± 0.4	13 ± 0.5	$p < 0.05$
TFG- β	1.1 ± 0.3	6.3 ± 0.4	$p < 0.05$
CCL-5	0.8 ± 0.4	11.7 ± 0.3	$p < 0.05$
IL-18	0.8 ± 0.2	10.8 ± 0.4	$p < 0.05$

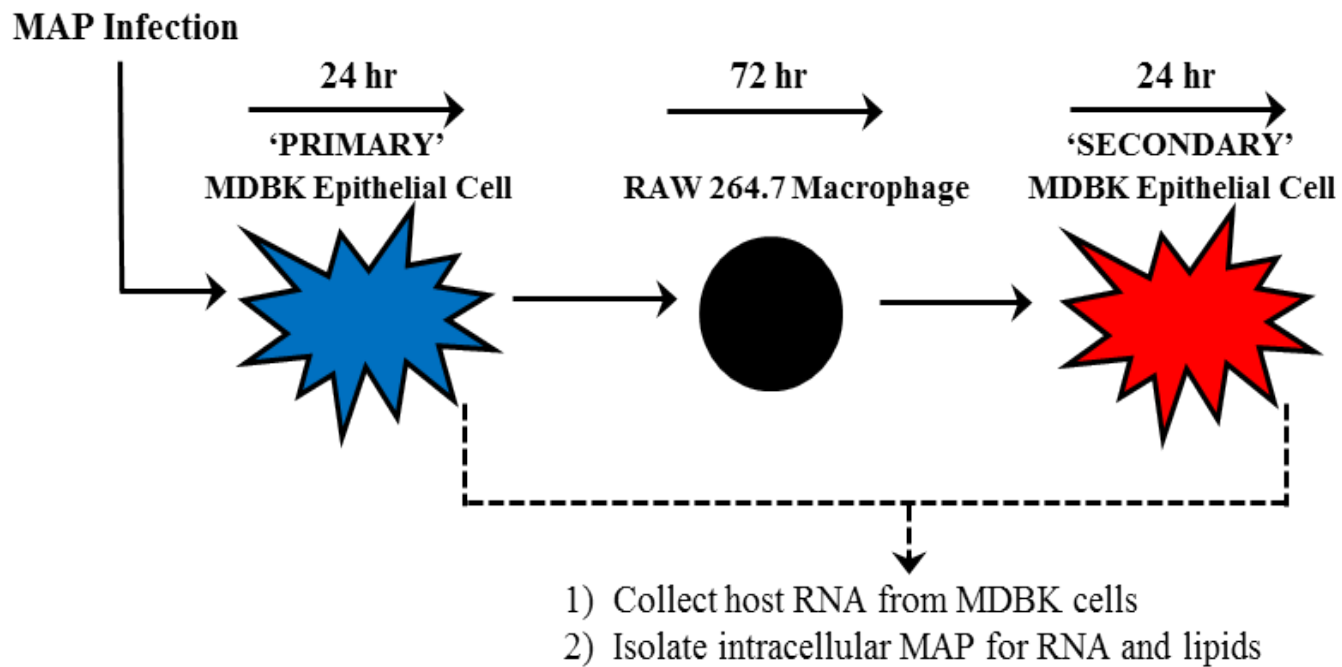
We then hypothesize that “**something has changed**” in the bacterium



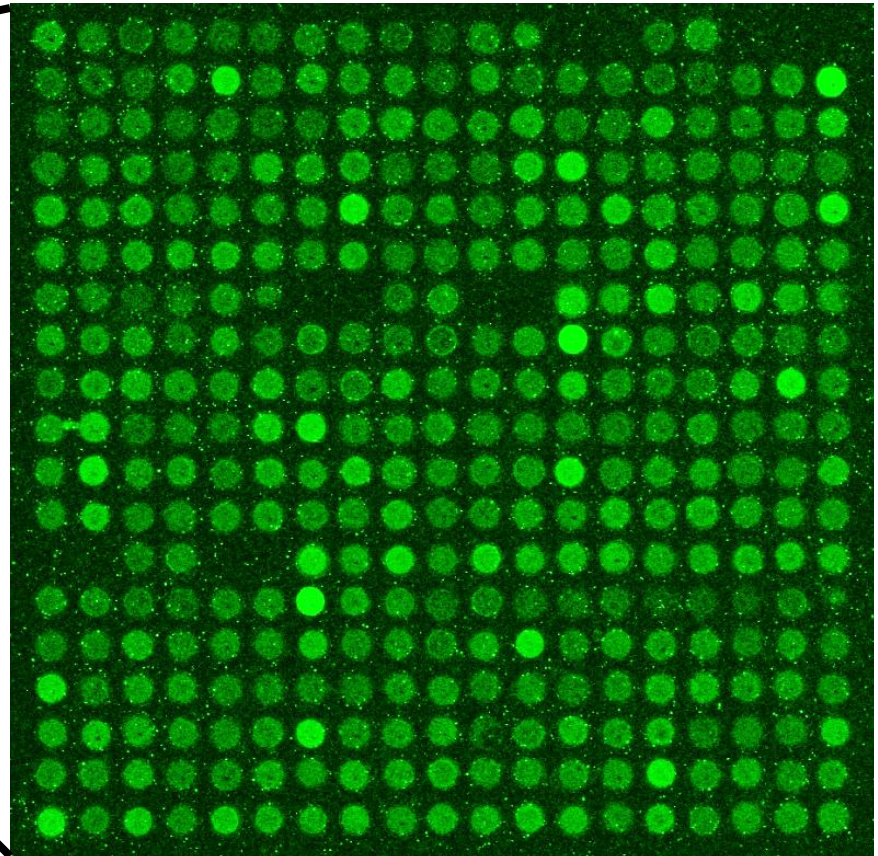
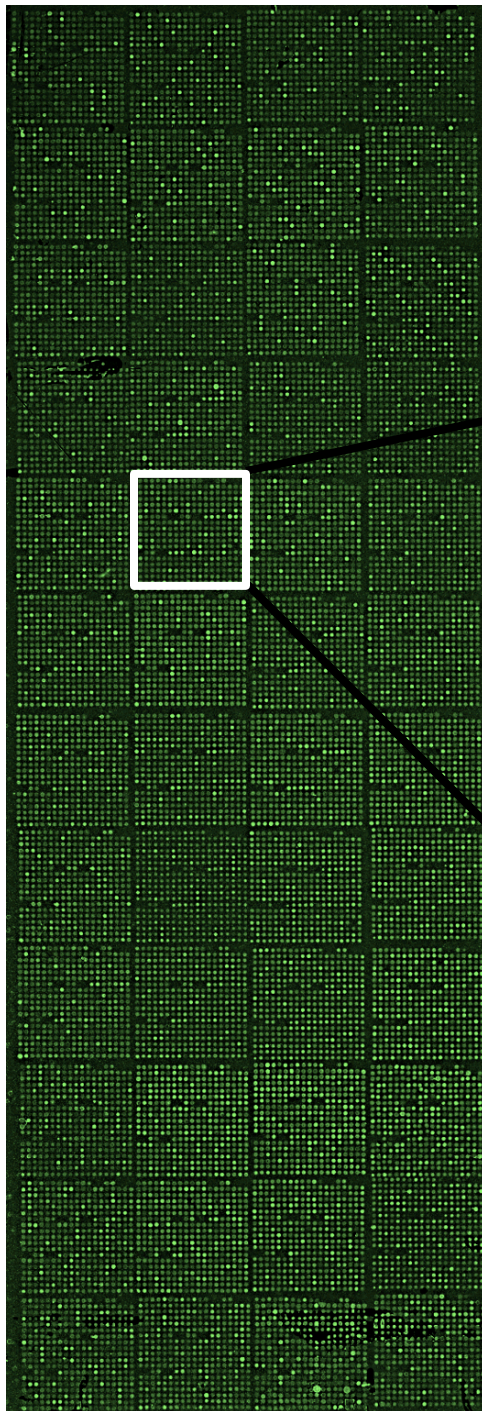
To investigate it we collaborate with John Bannantine and Torsten Eckstein

TRANSCRIPTOME and LIPIDOME

In vitro cell culture passage model



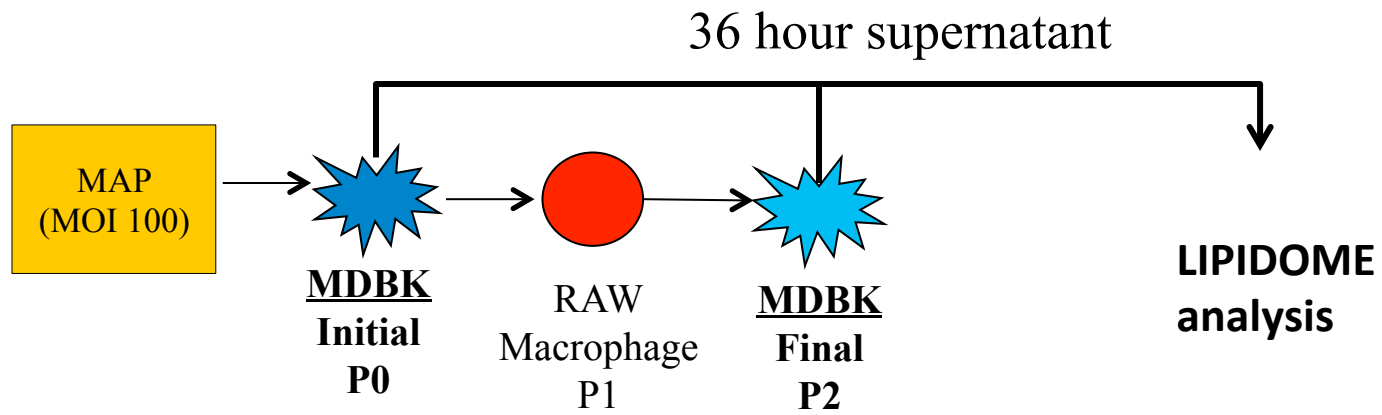
Oligo-Spotted
MAP K10Array Chip

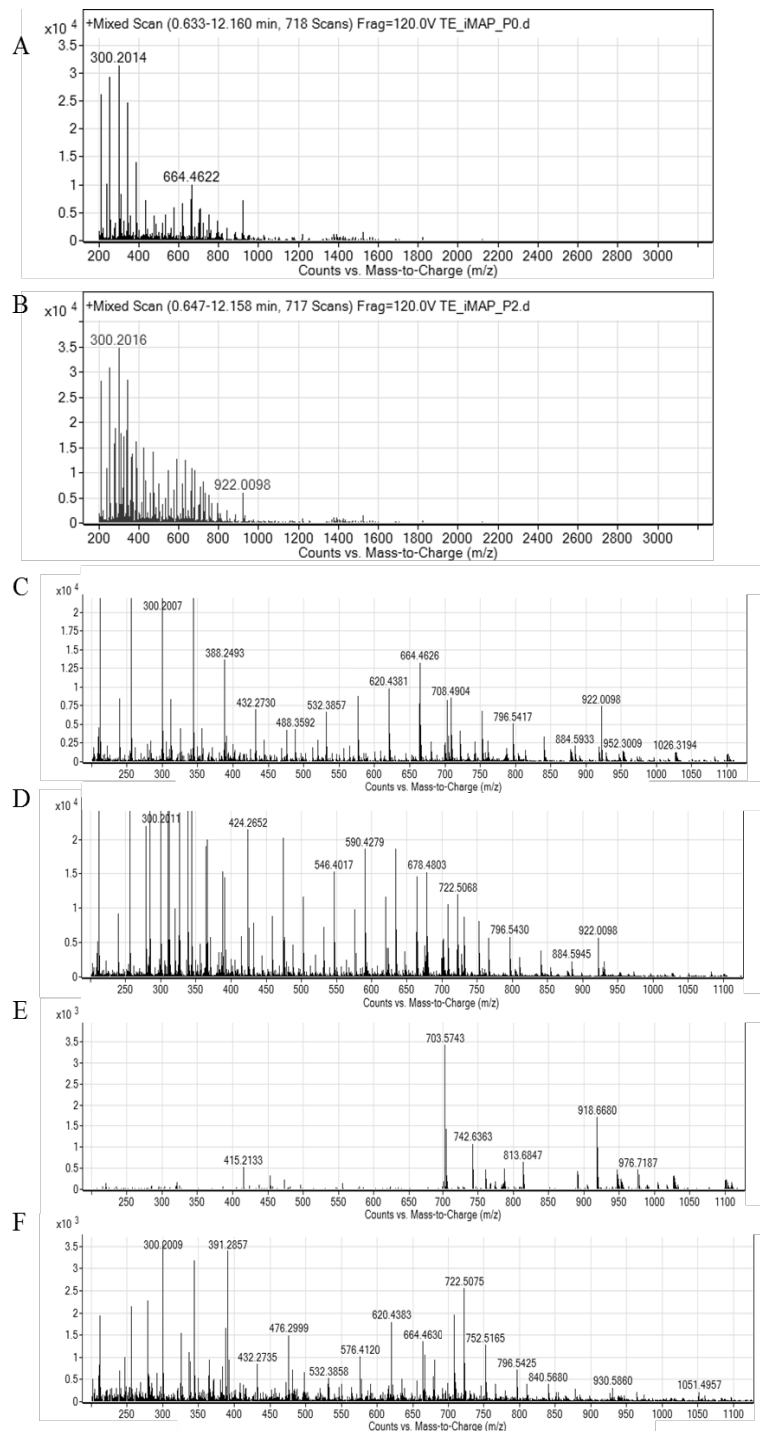


Microarray analysis of MAP phenotypes

Gene	Fold Change	Gene Description	Biological Process
MAP2974c	3.20	cyclopropane-fatty-acyl-phospholipid synthase 1	Lipid Biosynthesis
MAP3121	3.14	enoyl-CoA hydratase	Lipid Metabolism
MAP3433	2.99	hypothetical protein	Unknown
MAP3763c	2.98	conserved polyketide synthase associated protein 3	Lipid Metabolism
MAP1584c	2.92	ATP-dependent Lon protease	Stress Response Proteolysis
MAP2660	2.85	NAD-dependent epimerase dehydratase	Nucleic Acid Metabolism
MAP0385	2.82	restriction endonuclease family protein	DNA Binding
MAP0808	2.81	molybdenum cofactor biosynthesis protein	Metabolism
MAP3111c	2.81	regulator of sig8	Metabolism
MAP3516	2.77	hypothetical protein	Unknown
MAP1456	2.76	hypothetical esterase lipase	Metabolism
MAP1485c	2.72	acyl-CoA synthetase	Metabolism
MAA2452	2.72	hypothetical protein	Unknown
MAP1137c	2.71	aminoglycoside tetracycline-transport membrane protein	Membrane Transport
MAP0350	2.70	short chain dehydrogenase	Oxidoreductase
MAP2604c	2.68	mycocerosic acid synthase	Oxidoreductase
MAP2239	2.67	mmp14 protein	Unknown
MAP2751	2.67	21 kDa protein	Unknown

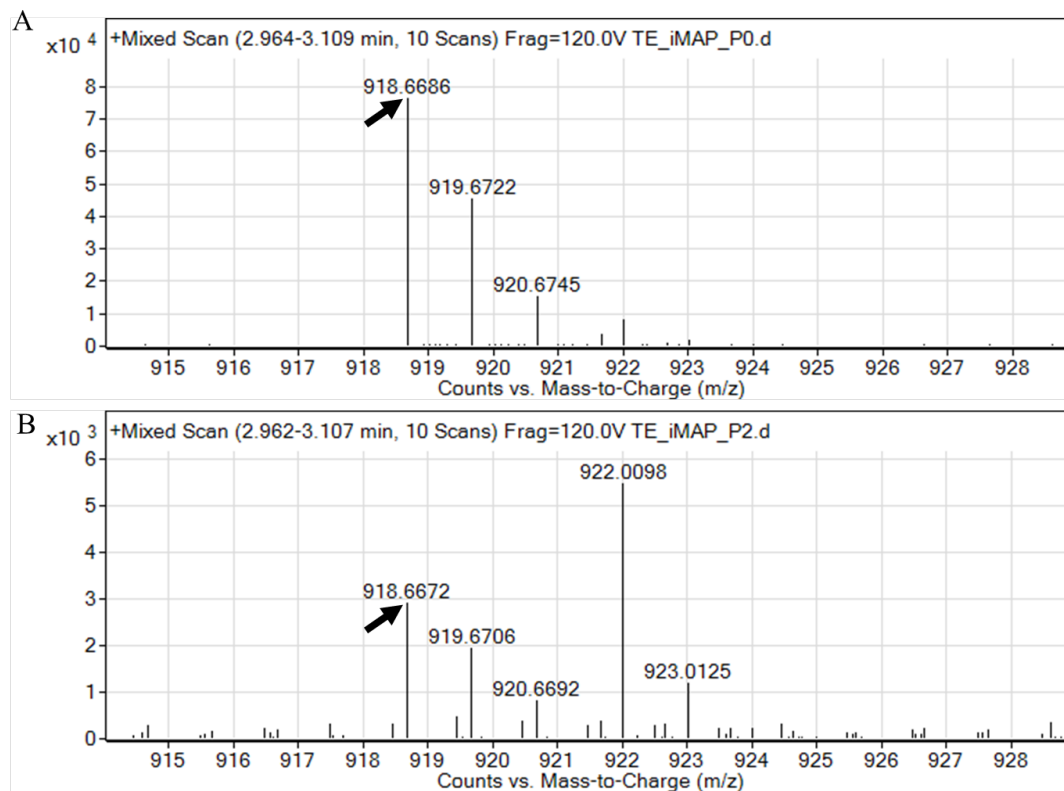
Because it was clear that one of the main changes (not the only one by all means) was in the lipids, we decide to investigate what were the differences in lipids (if any) **in MAP infecting the first intestinal cell and the MAP infecting the “second” intestinal cell.**





The complete spectra of 200 to 3000 m/z is shown (A and B) as well as a more detailed representation of the spectra from 200 to 1100 m/z (C and D). The lipidome of the primary population phenotype (A and C) and the secondary inflammatory phenotype (B and D) have distinct differences in both the presence and abundance of detected lipid ion fragments. Lipid ions unique to the primary population phenotype (E) and the secondary inflammatory phenotype (F)

Abundance of cell wall lipid para-LP-01 in MAP phenotypes

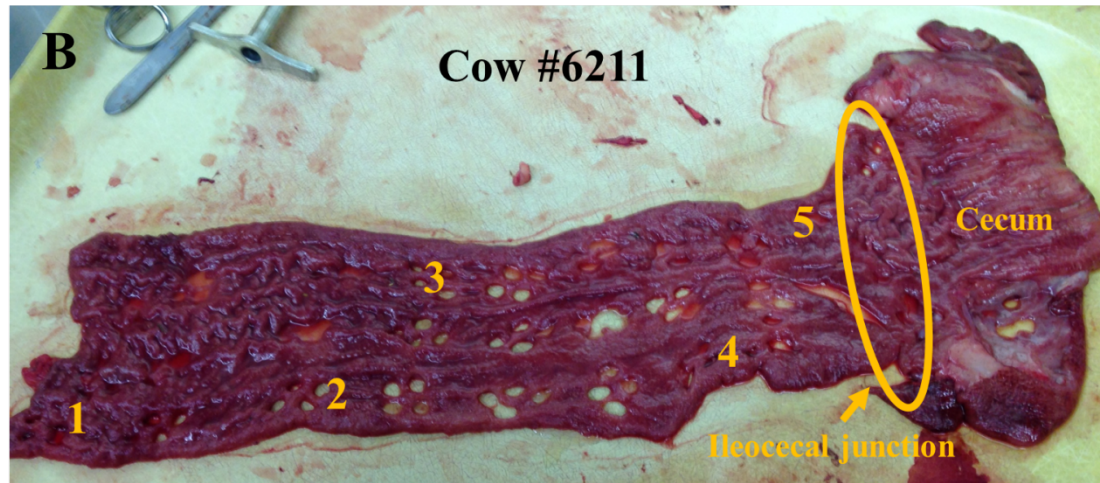
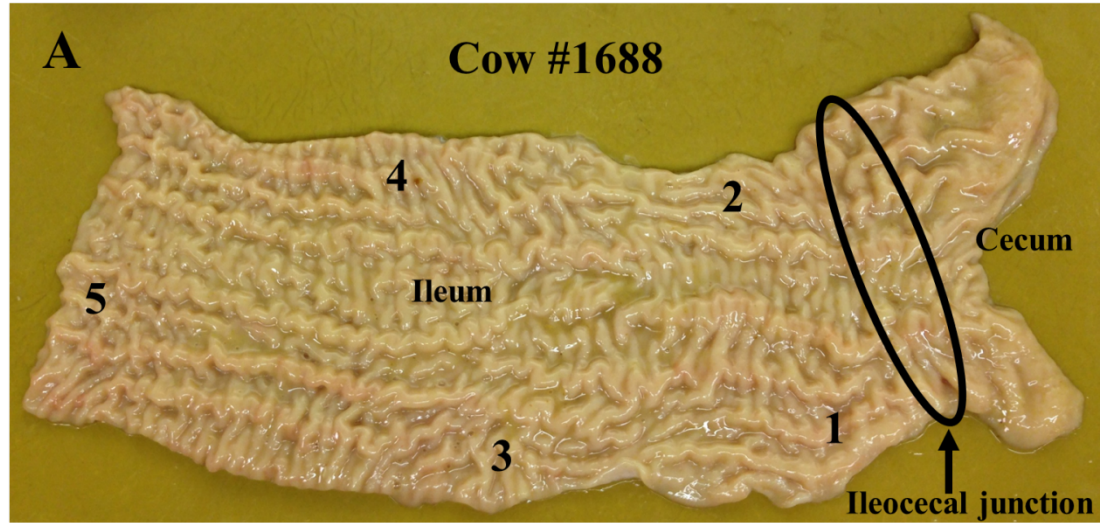


The “BIG” question

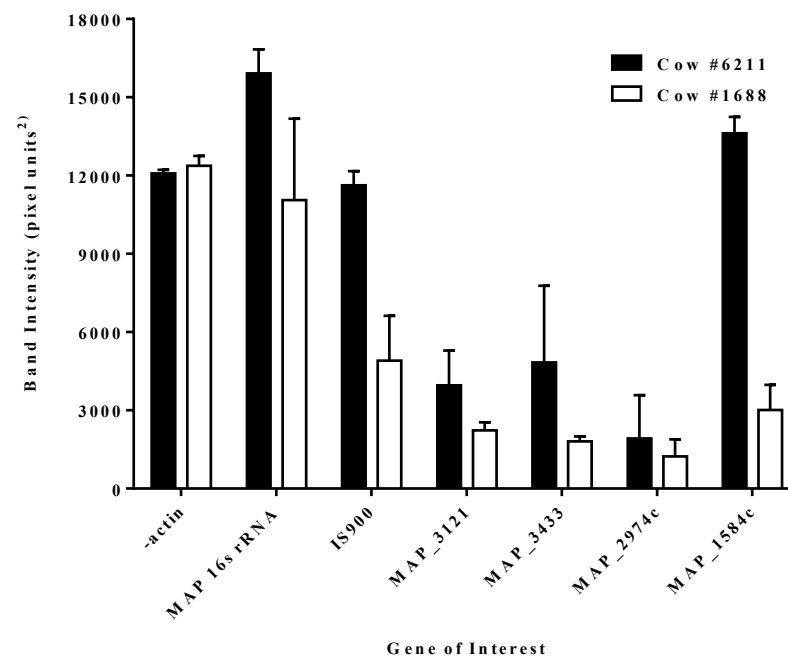
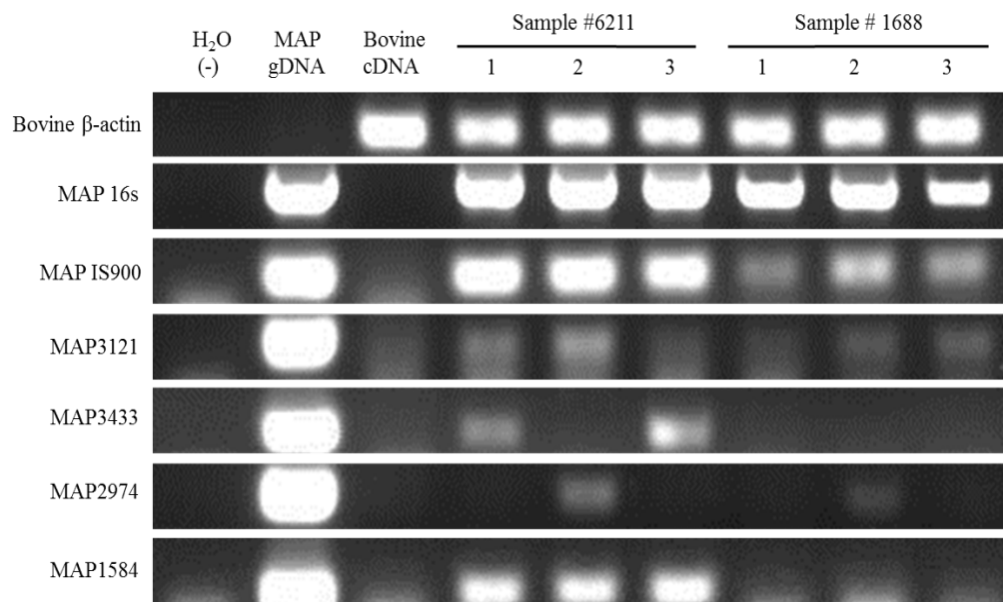
To answer it we collaborate with Paul Coussens
and Jonathan Roussey



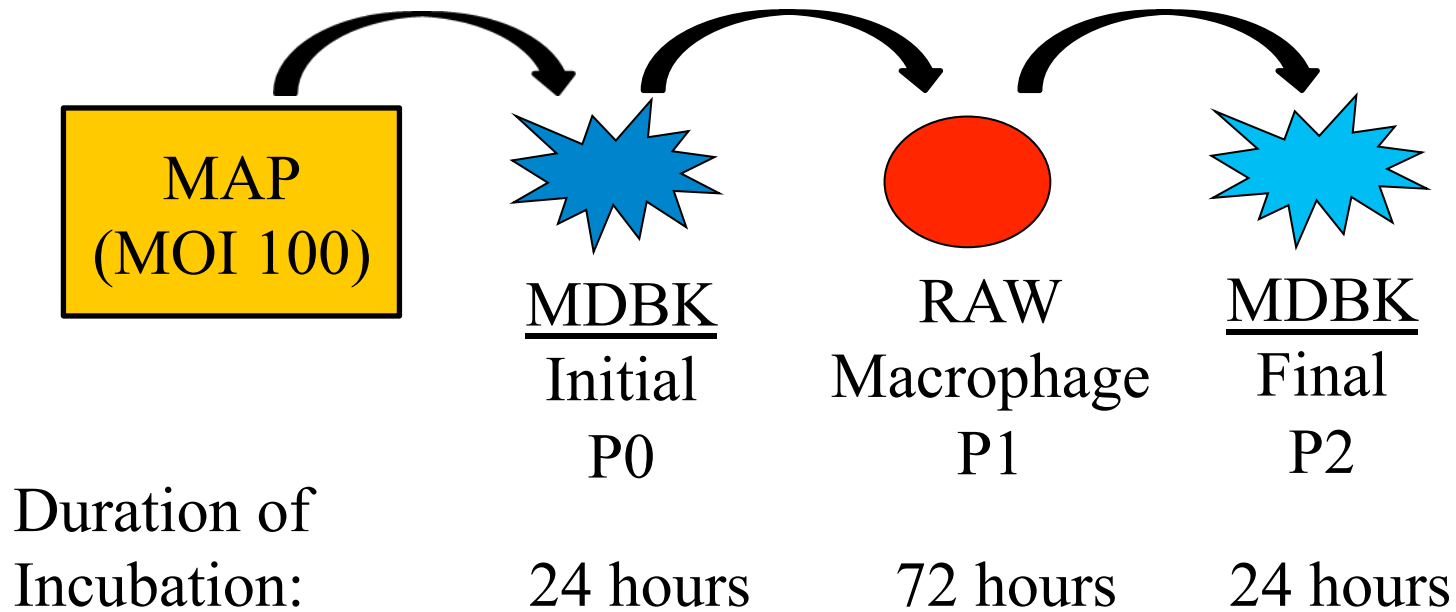
Tissue from cows with Johne’s Disease



MAP transcripts from infected bovine intestinal tissue



In Vitro Cell Culture Passage Model for MAP Infection



And now?

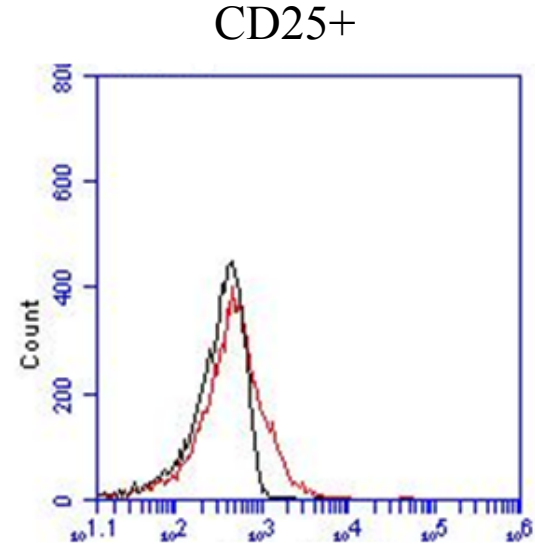
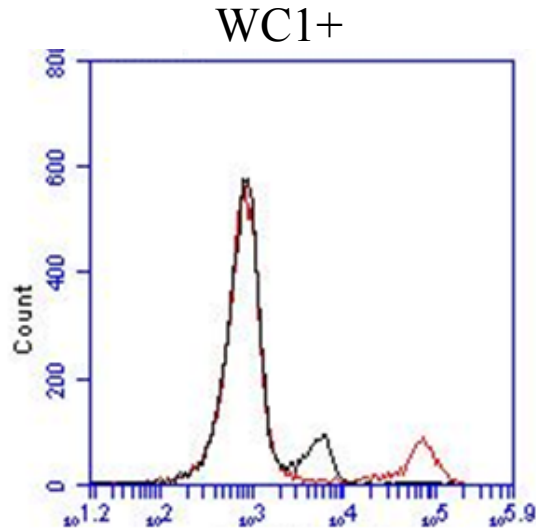
We are in the process of:

- a. Purifying lipids fractions
- b. Expressing and purifying proteins
- c. Studying the immune response of bovine intestinal cells

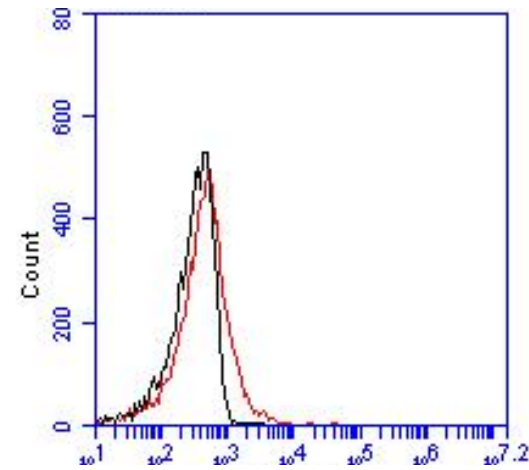
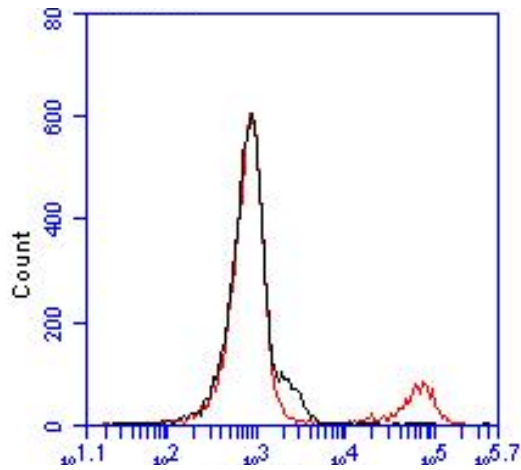
Supernatant from MAP-infected and Mock-infected epithelial cells

P0 Samples

Mock-infected



MAP-infected

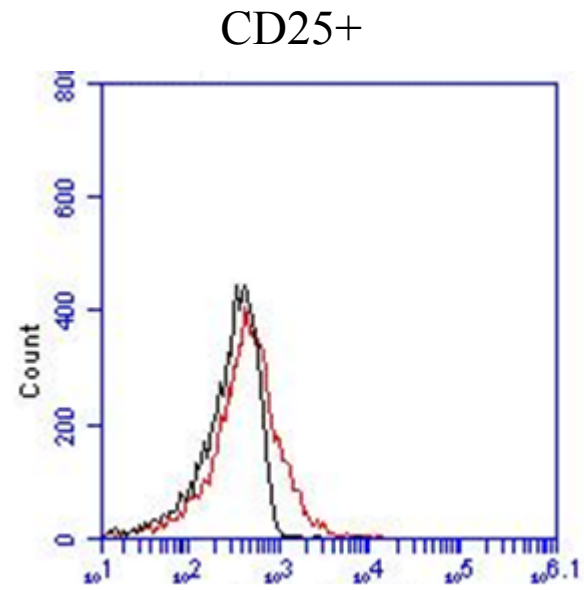
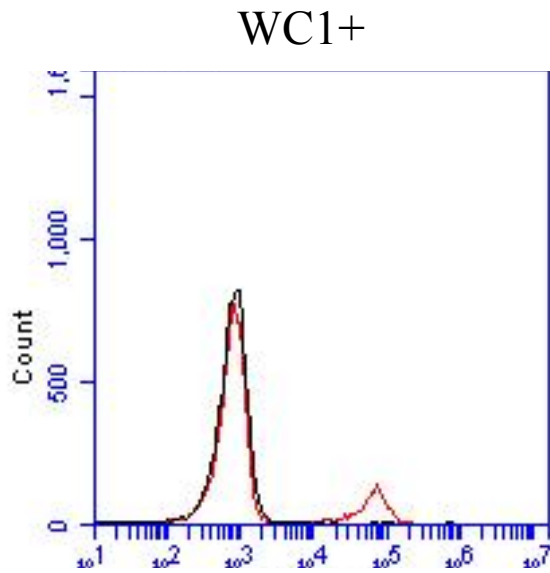


→ WC1
FITC

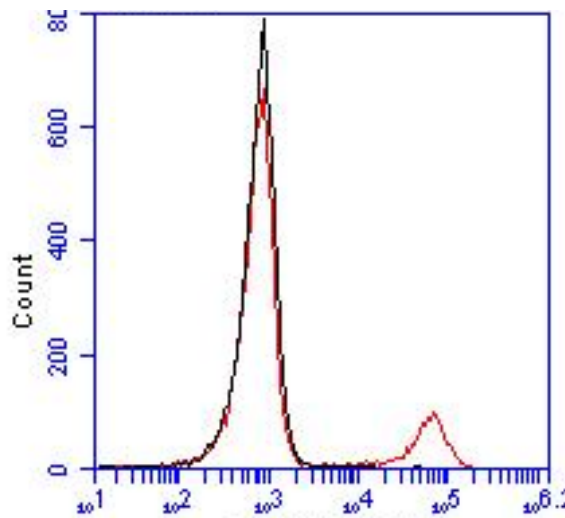
→ CD25
RPE

P2 Samples

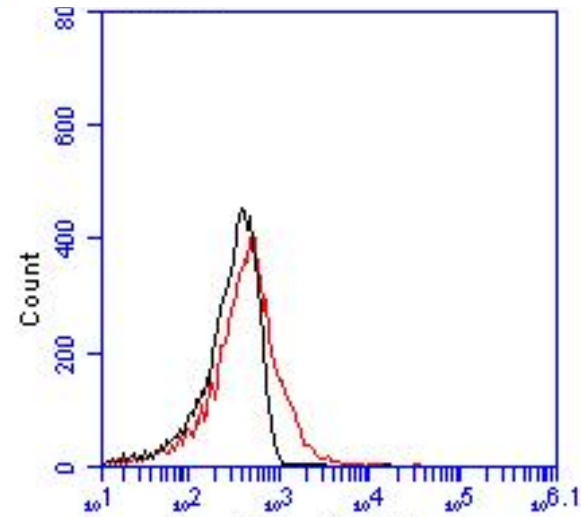
Mock-
infected



MAP-
infected



WC1
FITC



CD25
RPE

However...

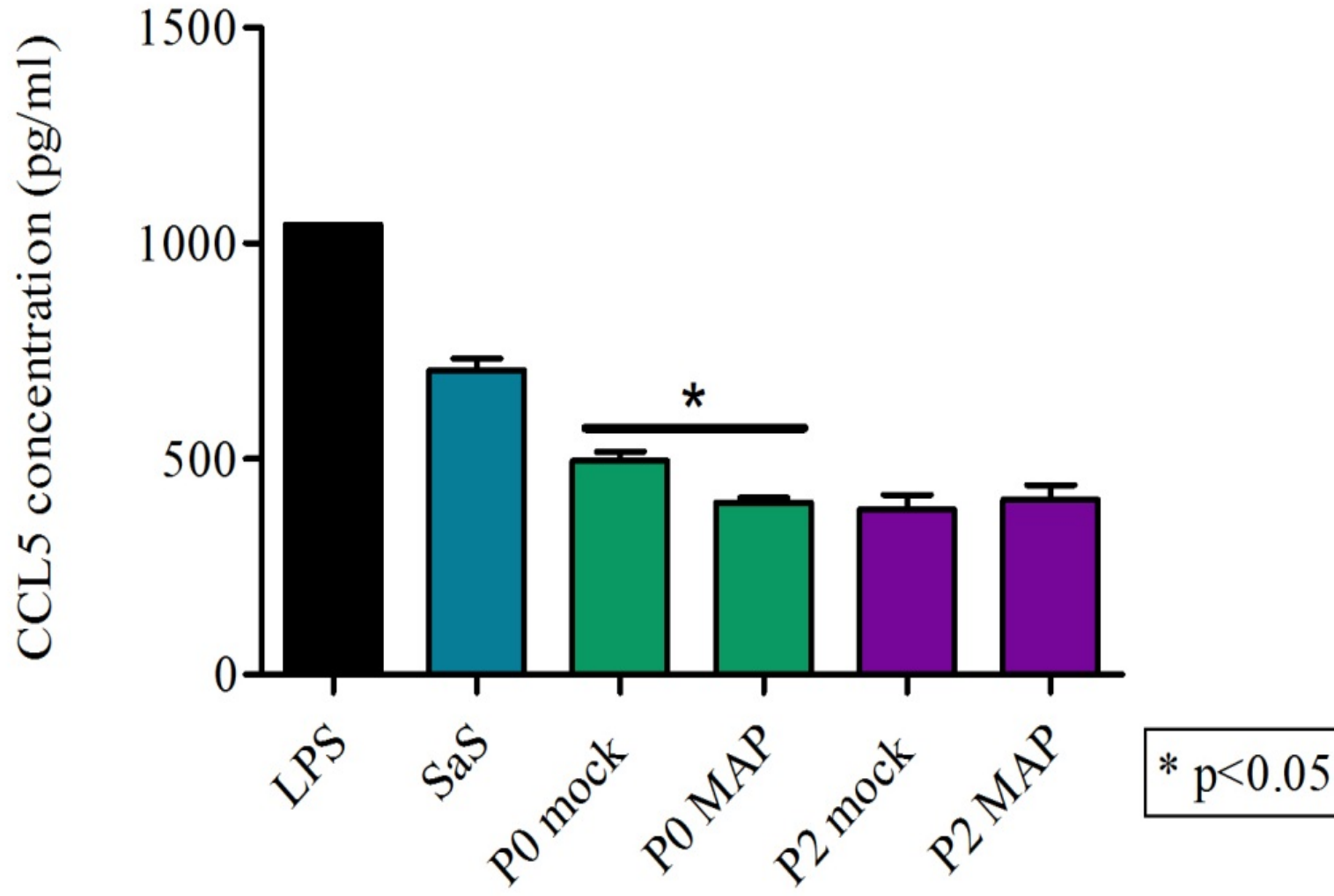
Intestinal intestinal mononuclear cells: 48 h incubation

	RNA (pixels)			Protein (ng)	
	<u>TNF</u>	<u>IL-6</u>	<u>IL-18</u>	<u>TNF</u>	<u>IL-6</u>
Control supernatant	260	184	145	12	20
Supernatant of MAP epithelial cells	2,374	1,023	2,669	387	276

Conclusions

1. That is a model system...
2. Can we obtain important information from it? A: Apparently yes.
3. Can the data be confirmed in diseased cows?
A: So far.
4. Can it be employed as method to obtain key knowledge to develop new strategies to deal with the disease? A: I think the answer is yes. Time will tell...

CCL5 protein production by MAP-infected and mock-infected epithelial cells



Quantify chemokine ligand 5 (CCL5) protein expression:

- Recruits T-cells and leukocytes to inflammatory sites
- Determine if protein levels match mRNA expression

Determine macrophage stimulation:

- Inflammatory cytokine (TNF- α)
- Produced in high amounts by macrophages

Activated gamma delta ($\gamma\delta$) T-cells:

- May serve a regulatory function *ex vivo*
- Abundant in bovine
- Present in mucosal tissues
- Activated by non-traditional MHC presentation