A modified skin test for diagnosis of subclinical enteric paratuberculosis in cattle

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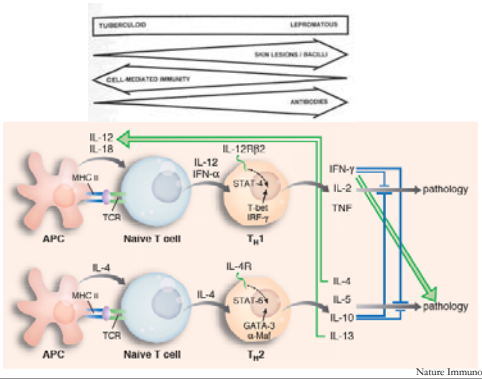
The problem...

- Diagnosis of subclinical *Map* infections
  - Difficult but vital to successful control
    - Current ante-mortem testing strategies
      - Fecal culture
      - Antibody tests, ELISA
      - CMI tests
    - Lengthy incubation period
    - Incompletely understood host immunologic responses
      - Early disease
      - Subclinical disease
      - Transition to clinical disease

Immunology of mycobacterial infections



Nature Immunology 4(6):503

Our goals

- To study the immunology of early and subclinical *Map* infection, we need a suitable model
  - Mice: immunology and pathology are distinct
  - Calves: recognized as appropriate model

Knowledge gaps


ScienceDirect

Review

Experimental challenge models for Johne's disease: A review and proposed international guidelines

- What are the significant gaps?
  - How does early tissue colonization relate to protection or shedding?
  - What is influence of host genetics?
  - How comparable are mouse challenge models to ruminant challenge model?
  - What is the ideal challenge model?
    - Oral infection, ligated ileal loop, cannulated ileum


Development of a Bovine Ileal Cannulation Model To Study the Immune Response and Mechanisms of Pathogenesis of Paratuberculosis

Early Phase Morphological Lesions and Transcriptional Responses of Bovine Ileum Infected with *Mycobacterium avium* subsp. *paratuberculosis*

Study Design

- How to model early/subclinical disease?
  - Modeling immune responses at an 'artificial' *Map* challenge site, the SQ model
  - Modeling enteric paratuberculosis


ScienceDirect

Live *Mycobacterium avium* subsp. *paratuberculosis* and a Killed-Bacterium Vaccine Induce Distinct Subcutaneous Granulomas, with Unique Cellular and Cytokine Profiles

ORIGINAL ARTICLE

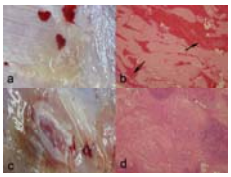
Gamma–delta T cell subsets are differentially associated with granuloma development and organization in a bovine model of mycobacterial disease

Limited phenotypic and functional maturation of bovine monocyte-derived dendritic cells following *Mycobacterium avium* subspecies *paratuberculosis* infection in vitro

Direct Inoculation of *Mycobacterium avium* Subspecies *Paratuberculosis* Into Ileocecal Peyer's Patches Results in Colonization of the Intestine in a Calf Model

Study Design

- SQ modeling: Matrigel
  - BD Matrigel is injected SQ, left in calf for 48 hours, then surgically removed via skin incision.
  - Liquid ➔ polymerization ➔ depolymerization



  - Cellular recruitment, phenotype, culture, cytokines

Study Design

- Enteric model:
  - Infection: right paralumbar laparatomy approach to distal ileum, sub-serosal Peyer's patch inoculation

- The current study:
  - Enteric infection, vaccination (Mycopar®); n=10
  - 30 days: SQ matrigel injections
  - matrigel alone vs. matrigel with 10^5 *Map*

Pathology, bacteriology

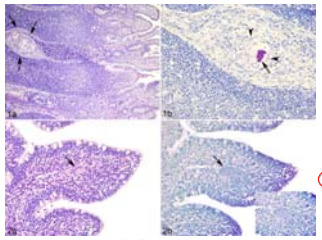


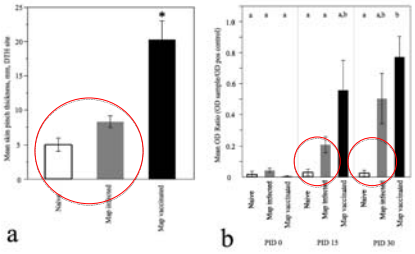
Table 2. Tissue and Fecal Culture Summary

Map Dose	Collection Day (PID)	Tissue Positive	Fecal Positive
Uninfected control	7	0/2	0/2
	30	0/2	0/2
	60	0/2	0/2
	90	0/2	0/2
Low dose	7	2/2	0/2
	30	1/4	0/4
	60	1/4	0/4
	90	2/4	0/4
High dose	7	4/4	0/4
	30	4/4	1/4
	60	4/4	4/4
	90	6/6	4/6

Sample positivity was defined by BACTEC liquid culture system with confirmation by *Map*-specific IS900 sequence polymerase chain reaction. High-dose group, 10^7 colony-forming units (CFU) and 10^8 CFU; low-dose group, 10^5 CFU and 10^6 CFU; PID, postinfection day.

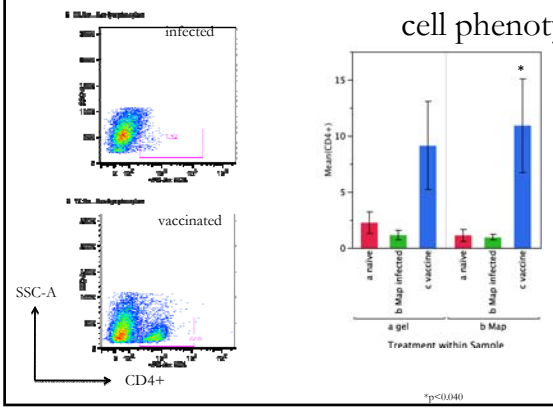
Plattner et al, *Veterinary Pathology* 48:584-92

Systemic immune response, WB IFNγ



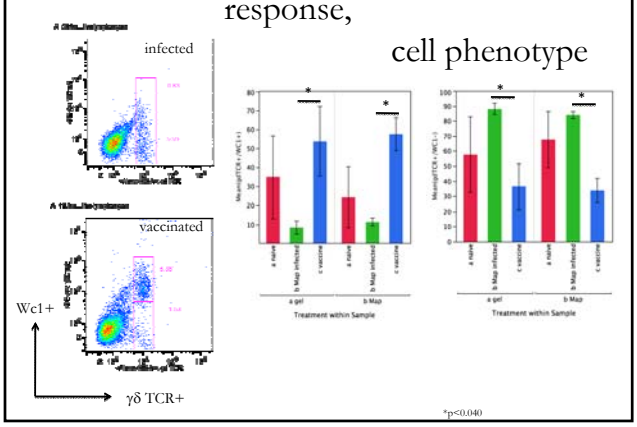
KEY*Cannot reliably distinguish enteric *Map* infection from others

Local immune response, cell phenotype



*p<0.040

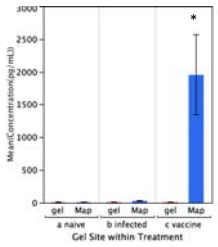
Local immune response, cell phenotype



*p<0.040

Local immune response, matrigel site IFNγ

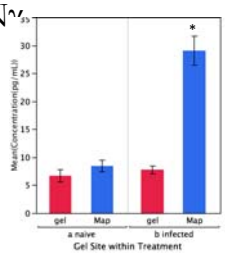
Luminex ELISA assay to detect secreted IFNγ at matrigel *Map* challenge sites



Matrigel assay distinguishes vaccinates (but so does the WB IFNγ assay)

Local immune response, matrigel site IFNγ

Though at a much smaller scale compared to IFNγ production in vaccinates….



We can detect differences in IFNγ secretion at matrigel *Map* sites of naïve calves vs. subclinical enteric *Map* infected animals

*p=0.0001

Summary, key findings

Naïve calves	Subclinical enteric *Map*	*Map* vaccinates
•Systemic response: -WB IFNγ negative -DTH negative	•Systemic response: -WB IFNγ weakly positive and not significant -DTH weakly positive, not significant	•Systemic response: -WB IFNγ strongly positive -DTH strong positive
•Local response: -no specific pattern -matrigel IFNγ negative	•Local response: -few CD4+ T cells -Wc1- γδ T cells -matrigel IFNγ weakly positive, and statistically significant	•Local response: -CD4+ T cells -Wc1+ γδ T cells -matrigel IFNγ strongly positive

Conclusions, Discussion

- Data suggests that matrigel/*Map* assay, as modified skin test, could be useful for:
  - Diagnosis of subclinical enteric *Map* infections
  - Differentiation of subclinical infections from *Map* vaccinates
  - Further investigation of the local, infection-site immune response to *Map* infections
- Use of live *Map* in the assay?
  - We substituted whole cell sonicate (WCS) for live *Map*
    - Results are similar, data not shown

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