

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



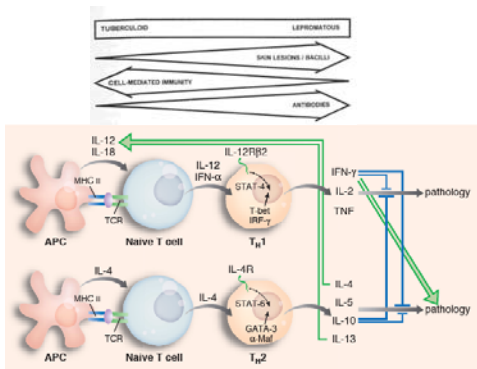
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The problem...



- 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

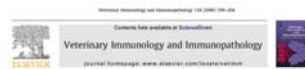
Immunology of mycobacterial infections



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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



The calf model of immunity for development of a vaccine against tuberculosis

Janice J. Enbeler¹, W. Ray Waters¹, Mitchell V. Palmer¹, Brian J. Niswender¹, Tyler C. Thacker¹, William E. Jacobs Jr.¹, Michelle H. Larson¹, Alison Hogg¹, Elizabeth Shell¹, Martin McKinlay¹, Charles H. Castro-Alamancos¹, Tracey Coffey¹, Chris J. Howard², Bernabé Vilgoral-Ramos², D. Mark Estes^{1*}

Review Article
Tuberculosis Immunity: Opportunities from Studies with Cattle

W. Ray Waters¹, Mitchell V. Palmer¹, Tyler C. Thacker¹, William E. Jacobs Jr.¹, Bernard Groszmann¹, Paul Casanova¹, Klaus G. Meade¹, Bruce C. Hogg¹ and D. Mark Estes^{1*}

Knowledge gaps

- 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



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

Martín E. Haim ¹*, Judith R. Subtil ², Raymond W. Swearingen ³, Frank Griffin ⁴, Adel M. Taha ⁵, Denise Bakker ⁶, Grant Benedictus ⁷, William C. Davis ⁸, Geoffrey W. de Lisle ⁹, Lisa A. Gardner ¹⁰, Ramon A. Juste ¹¹, Virek Kapur ¹², Ad Kiers ¹³, Jim McNair ¹⁴, Greg Pinner ¹⁵, Robert H. Whitlock ¹⁶

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

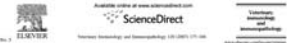
Andrew B. Allen ¹, Ryan York-Park ², George M. Barnum ³, Jay R. Leffler ⁴, Mary Jo Henderson ⁵, and William C. Davis ⁶

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

S. Kawai, J. A. Smith, J. P. F. Probst, M. S. Linton, C. M. Brown, T. Ohta, A. C. MacFadyen, J. L. Ross

Study Design

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



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

Lijing Lai ^{1,2}, Braden L. Plummer ¹, and Jean M. Humeau ^{1,3}

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

Lijing Lai, Jean M. Humeau

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

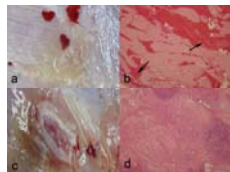
Bradley L. Plummer, Robert T. Shick, and Jean M. Humeau

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

B. L. Plummer, Y. W. Chang, J. A. Roth, R. Pinner, K. McRae, J. E. Ross, and J. M. Humeau

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 laparotomy 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 10⁵ *Map*

Pathology, bacteriology

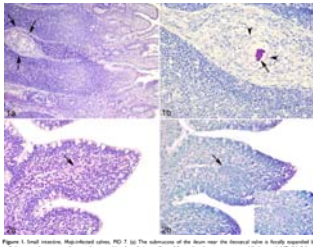


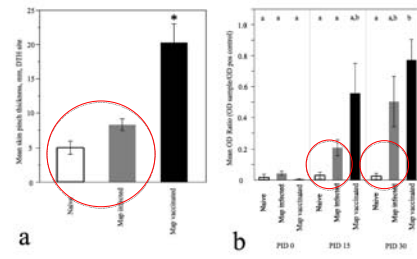
Table 2. Tissue and Fecal Culture Summary

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
Low dose	7	0/2	0/2
	30	1/4	0/4
	60	1/4	0/4
High dose	7	4/4	0/4
	30	4/4	1/4
	60	4/4	4/4
	90	6/6	4/8

Sample positivity was defined by BACTEC liquid culture system with confirmation by Map-specific 6700 sequence polymerase chain reaction. High-dose group, 10^7 colony-forming units (CFU) and 10^7 CFU low-dose group, 10^7 CFU and 10^7 CFU, postinfection day.

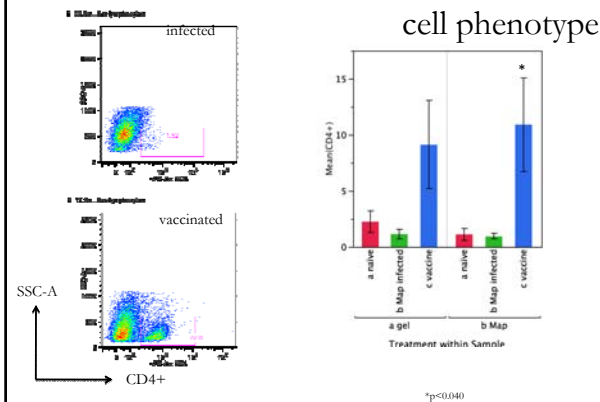
Figure 1. Tissue sections. Magnified views (PID 7). In the subacute of the lesion near the ileocolic site a body composed of well-differentiated aggregates of macrophages, mononuclear giant cells, and lesser lymphocytes are recognizable (arrow). In a large cluster of similar bodies in the center of a granule (arrow), smaller aggregates of bacteria are present within macrophage cytoplasm. In Figure 2, small response, magnified views (PID 30). In epithelial mucosal crypts and cryptiform macrophage cytoplasm, granules within the vacuole (arrows) (A). In (B) numerous mononuclear and multinuclear giant cells (arrows) and nuclei are present (B).

Systemic immune response, WB IFN γ



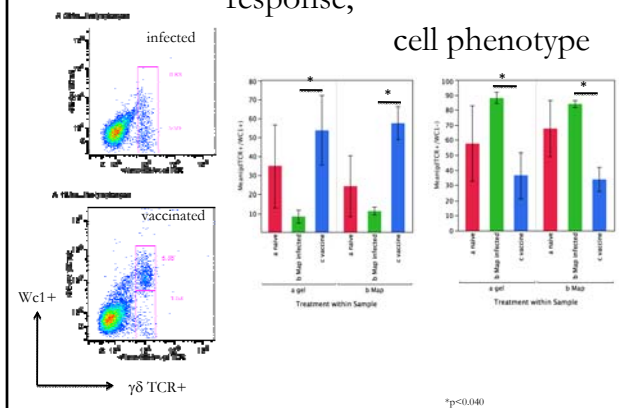
KEY*Cannot reliably distinguish enteric *Map* infection from others

Local immune response, cell phenotype



*p<0.040

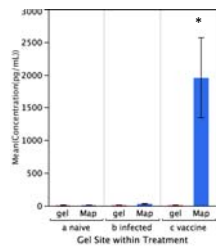
Local immune response, cell phenotype



*p<0.040

Local immune response, matrigel site IFN γ

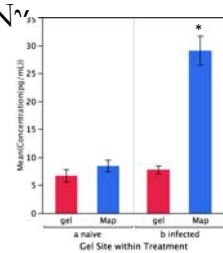
Luminex ELISA assay to detect secreted IFN γ at matrigel *Map* challenge sites



Matrigel assay distinguishes vaccinates (but so does the WB IFN γ assay)

Local immune response, matrigel site IFN γ

Though at a much smaller scale compared to IFN γ production in vaccinates....



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

*p=0.0001

Summary, key findings

Naïve calves	Subclinical enteric <i>Map</i>	<i>Map</i> vaccinates
<ul style="list-style-type: none"> Systemic response: <ul style="list-style-type: none"> -WB IFNγ negative -DTH negative Local response: <ul style="list-style-type: none"> -no specific pattern -matrigel IFNγ negative 	<ul style="list-style-type: none"> Systemic response: <ul style="list-style-type: none"> -WB IFNγ weakly positive and not significant -DTH weakly positive, not significant Local response: <ul style="list-style-type: none"> -few CD4+ T cells -Wc1- $\gamma\delta$ T cells -matrigel IFNγ weakly positive, and statistically significant 	<ul style="list-style-type: none"> Systemic response: <ul style="list-style-type: none"> -WB IFNγ strongly positive -DTH strong positive Local response: <ul style="list-style-type: none"> -CD4+ T cells -Wc1+ $\gamma\delta$ 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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