P-039

Possible mechanisms how *Mycobacterium avium subsp. paratuberculosis* may evade host immune responses in naturally-infected cows

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Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP), the causative agent of Johne's disease, is able to dampen or distort immune responses at the mucosal sites and co-exist with a massive infiltration of immune cells in the gastro-intestinal tract. The mechanism how MAP subverts protective immune responses in gut of cattle remains unsolved. Mucosal immune responses are tightly controlled by a network of cells and mediators which ensures that immune responses do not lead to uncontrolled inflammatory responses with associated mucosal tissue damage. It is hypothesised that MAP subverts the immune system by activating regulatory networks (T regulatory cells and indoleamine dioxygenase) to ensure its survival. In addition, there may be inefficient recognition of MAP by pattern recognition receptors such as Toll-like receptors (TLR) which are also important in stimulation of phagocytosis and development of cellular immunity.

The focus of the current study was to investigate gut immune responses in cows naturally-infected with MAP (late stage of infection) and calves experimentally-infected (early stage of infection, see P-047). For this study a range of techniques were used including cytokine responses at the protein level, expression of immuno-regulatory genes.

Materials and Methods

Animals. 38 cull cows (Friesian, Friesian-Jersey X and Jersey breeds) from lower north island of New Zealand.

Samples. Bacteriology and histopathology: mesenteric lymph node (MLN), ileo-caecal lymph node, distal ileum and the ileo-caecal junction.

Cell culture. PBMCs and MLN cells for MAP antigen stimulation.

Measurement. MAP culture, histopatholgical assessment , MAP-specific antibodies, released cytokines and gene expression by RT-PCR.

Histopatholgyscores for lesions in the distal ileum, ileo-caecal valve and mesenteric and ileo-caecal lymph nodes of cull cows naturally infected with *Mycobacterium avium* subsp. *paratuberculosis*.

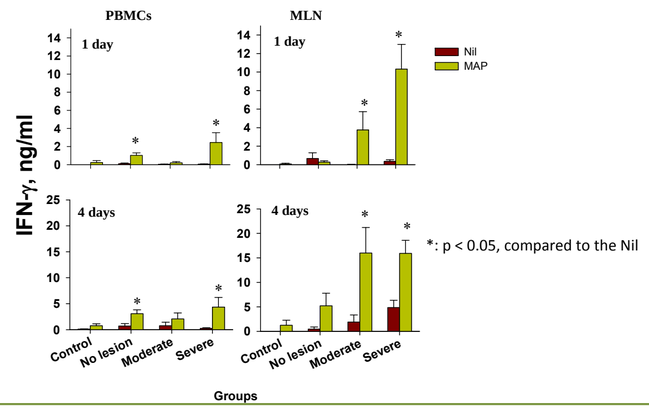
Parameters	Tissue	Scores	Description of histopathological changes
Villi	DI, ICV	0	No abnormality
		1	Mild blunting of villi
		2	Moderate blunting and fusion of villi
		3	Marked blunting and fusion of villi
Cellular infiltration	DI, ICV	0	No cellular infiltration
		1	Occasional scattered cellular infiltration
		2	Moderate cellular infiltration
		3	Severe and extensive cellular infiltration
Granuloma	DI, ICV, MLN, ICLN	0	No lesion
		1	Small focal granulomas or scattered giant cells
		2	Multifocal granulomas in some areas of tissue
		3	Diffused granulomas in large areas of tissue
AFB* per macrophage	DI, ICV, MLN, ICLN	0	No AFB
		1	<10
		2	10 to 50
		3	>50

Results

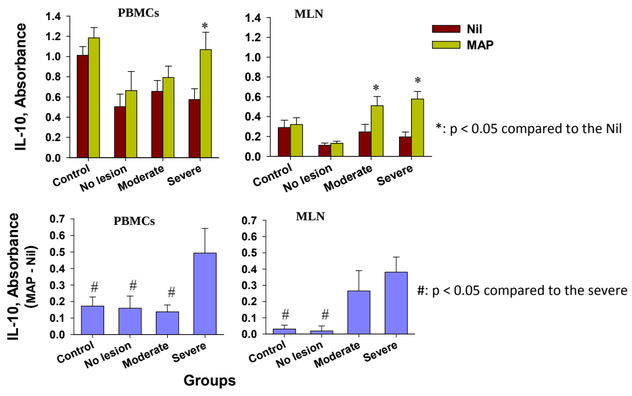
Classification of Johne's disease in cull cows based on culture of *Mycobacterium avium* subsp. *paratuberculosis* from gut tissue, followed by histopathological scores of sections from the distal ileum, ileo-caecal valve, and mesenteric and ileo-caecal lymph nodes.

Classification	No. of animals	MAP culture	MAP serology	Histopathology scores
Control	8	-	-	≤ 8
Non-lesioned/ MAP-infected	8	+	-	≤8
Moderately-lesioned	9	+	+	9 to 27
Severely-lesioned	13	+	+	≥ 28

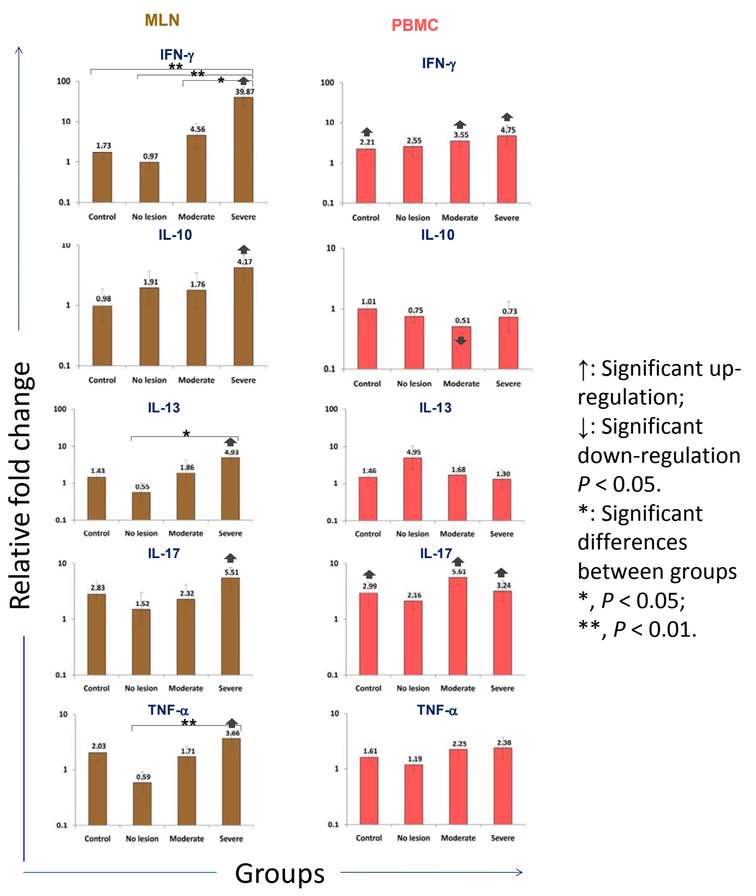
IFN-γ released from cultures of MLN cells and PBMCs from control (n = 8) and non-lesioned/MAP-infected (n = 8), moderately-lesioned (n = 9) and severely-lesioned groups (n = 13). Cultures were stimulated with MAP sonicate for 1 day or 4 days and compared to non-stimulated cultures. Data are presented as mean concentration (ng/ml) ± SE.



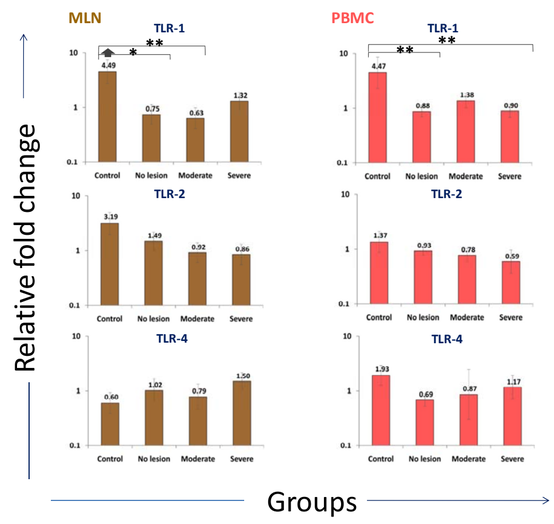
IL-10 released from cultures of MLN cells and PBMCs from control (n = 8) and non-lesioned/MAP-infected (n = 8), moderately-lesioned (n = 9) and severely-lesioned groups (n = 13). Cultures were stimulated with MAP sonicate for 4 days and compared to non-stimulated cultures. Data are presented as mean absorbance (OD 450 nm) ± SE.



Cytokine mRNA expression in cultures of MLN cells and PBMCs from control (n = 8), non-lesioned/MAP-infected (n = 8), moderately-lesioned (n = 9) and severely-lesioned groups (n = 13). Cells were stimulated with MAP sonicate for 20 hours. The levels of mRNA were normalised to reference gene. Non-stimulated cells were used as the calibrators to generate fold change values using the ΔΔCt method. The results were presented as mean relative fold change ± SE



TLR1, TLR2 and TLR4 mRNA expression following MAP sonicate stimulation for in MLN mononuclear cells and PBMCs from control, non-lesion, moderate lesion and severe lesion cows. The levels of mRNA were normalised to the reference gene relative to paired non-stimulated values and the results were presented as mean relative fold change ± SE.



Summary

- A classification of MAP-infected cull cows into three groups based on a histopathological score of Johne's disease lesions in gut tissues revealed major differences in their serological and cytokine profiles.
- Positive serological responses for MAP were restricted to those animals with lesions associated with Johne's disease.
- The severely-lesioned group had a significant up-regulation of mRNA expression for a diverse range of cytokine (IFN-γ, IL-10, IL-13, IL-17A and TNF-α) in MAP-stimulated MLN cell cultures, which was contrary to the paradigm of a distinct shift from Th1 to Th2 cytokines as the disease progressed.
- The MAP-infected cull cows had a significantly lower TLR1 mRNA expression and a trend for lower TLR2 expression in MAP-stimulated PBMC and MLN cell cultures compared to that for the controls.

Conclusions

Possible mechanisms how MAP may evade effective host immune responses include:

- Induction of a dysregulated immune response as seen in the severely-lesioned group.
- Failure to recognise MAP as foreign as shown by an impairment of up-regulation of TLR1 and TLR2 mRNA expression in MAP-stimulated MLN cell and PBMC cultures compared to controls.

References

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Subharat, S. et al. (2011) Vet Immunol Immunopathol http://dx.doi.org/10.1016/j.vetimm.2011.10.008

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