Dis-regulation of immune responses during early *M. paratuberculosis* infection of calves

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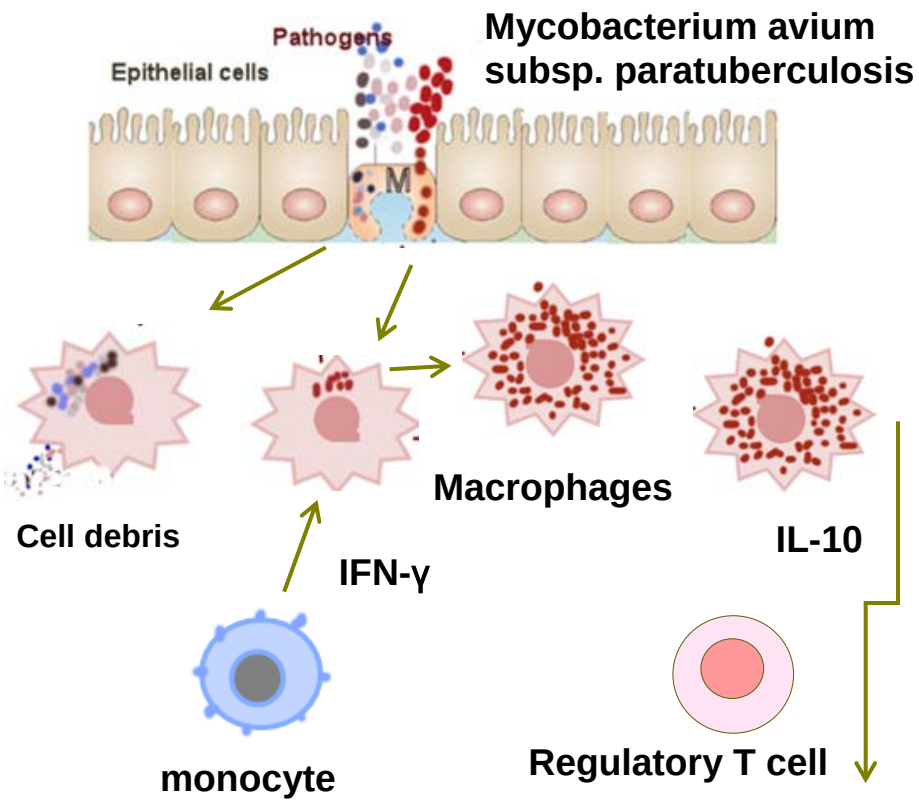
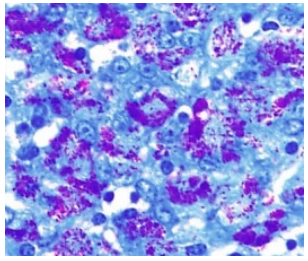
Introduction

Host / pathogen interaction → disease



Granuloma



Possible mechanisms how MAP subverts the gut immune system

- Immunoregulatory pathways
- Toll-like receptors (TLRs) which recognize foreign pathogens

Aims

- Investigate blood and gut immune response for early stage MAP infection
- Establish reproducible challenge model in calve

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Methods

Challenge calves = 20, Control calves = 11

- Experimentally challenge calves 5 - 8 wks old
  - MAP 10^9 bacilli/oral inoculum (pure culture)
  - Challenge 3 times at 1 week intervals
- Collect faeces and blood at 2-3 months intervals (culture, IFN-γ)
- 7 months kill (10 challenged and 5 controls) & 15 months kill (10 challenged and 6 controls)
  - Measure blood & gut immune response

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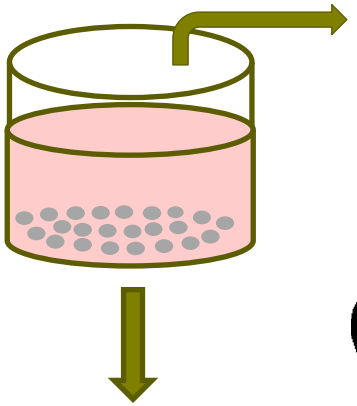
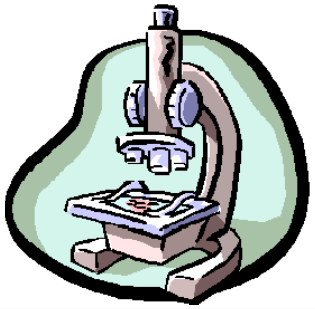
MLN and PBMC cells were cultured with MAP antigens



Supernatant (4 days)
ELISA
- Cytokines release (IFN-γ and IL-10)



Cells (1 day)
mRNA expression by qRT-PCR
- Cytokines (IFN-γ, IL-10, IL-13, IL-17A and TNF-α)
- TLR1, 2 and 4

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Culture of MAP from faeces of calves (2-15 months PC)

	Non-challenged											MAP-challenged																			
2 months		-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	-	+	+
4 months	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-		+	+	-	+	+
7 months	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	+	-	-	-	+	+	+	-	+	-
9 months						-	-	-	-	-	-											-	-	-	-	-	-	+	-	+	-
11 months						-	-	-	-	-	-											-	-	-	-	-	+	+	-	+	-
13 months						-	-	-	-	-	-											+	-	-	-	-	-	-	-	+	-
15 months						-	-	-	-	-	-											+						+		+	

+ faecal culture +ve for MAP

No sample

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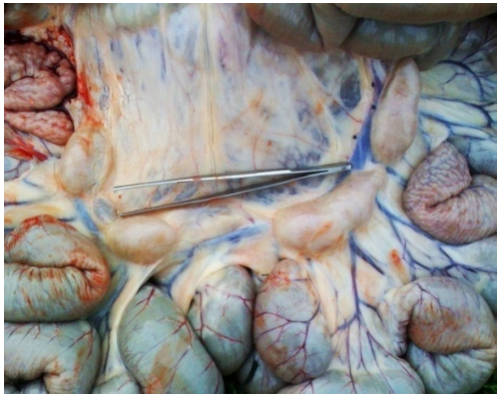
Culture of MAP from tissues of calves 7 and 15 months post-challenge

Group	Months post-challenge	Mesenteric LN	Ileo-caecal LN	Distal Ileum	Ileo-caecal region
Control	7 (n=5)	0/5	0/5	0/5	0/5
	15 (n=6)	0/6	0/6	0/6	0/6
MAP-challenged	7 (n=10)	10/10	10/10	8/10	8/10
	15 (n=10)	6/10	4/10	3/10	3/10



Mild histopathological lesions observed (7 months > 15 months)

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IFN-γ released from MAP-stimulated whole blood culture

Group	Months post-challenge						
	3	6	7	9	11	13	15
Control	0/10	0/11	0/11	0/6	0/6	0/6	0/6
MAP-challenged	7/20	**19/20**	**18/20**	7/10	8/10	9/10	7/10

No antibody response (JD-ELISA) at any time points

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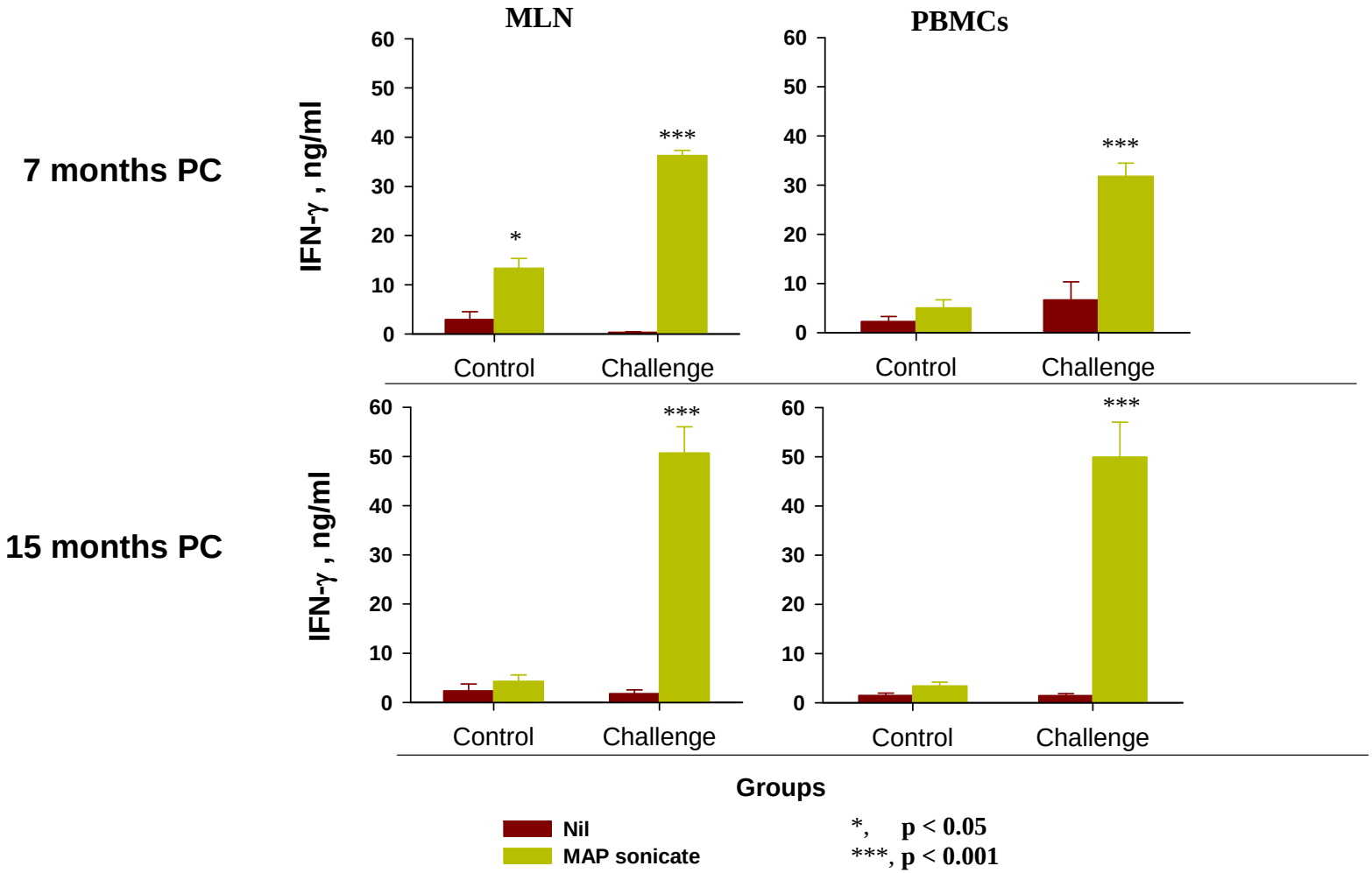
IFN-γ released from MAP-stimulated MLN and PBMC cultures



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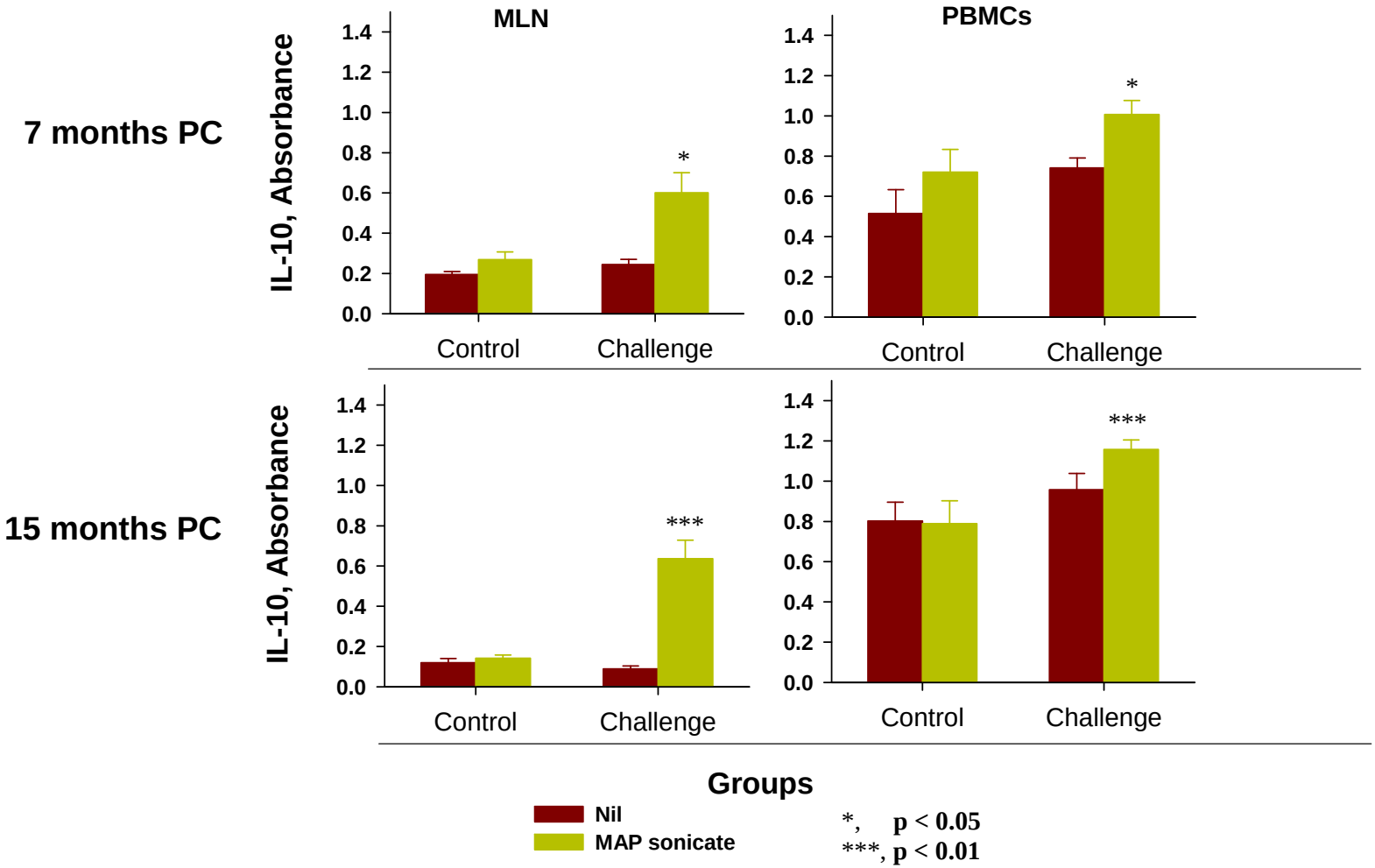
IL-10 released from MAP-stimulated MLN and PBMC cultures



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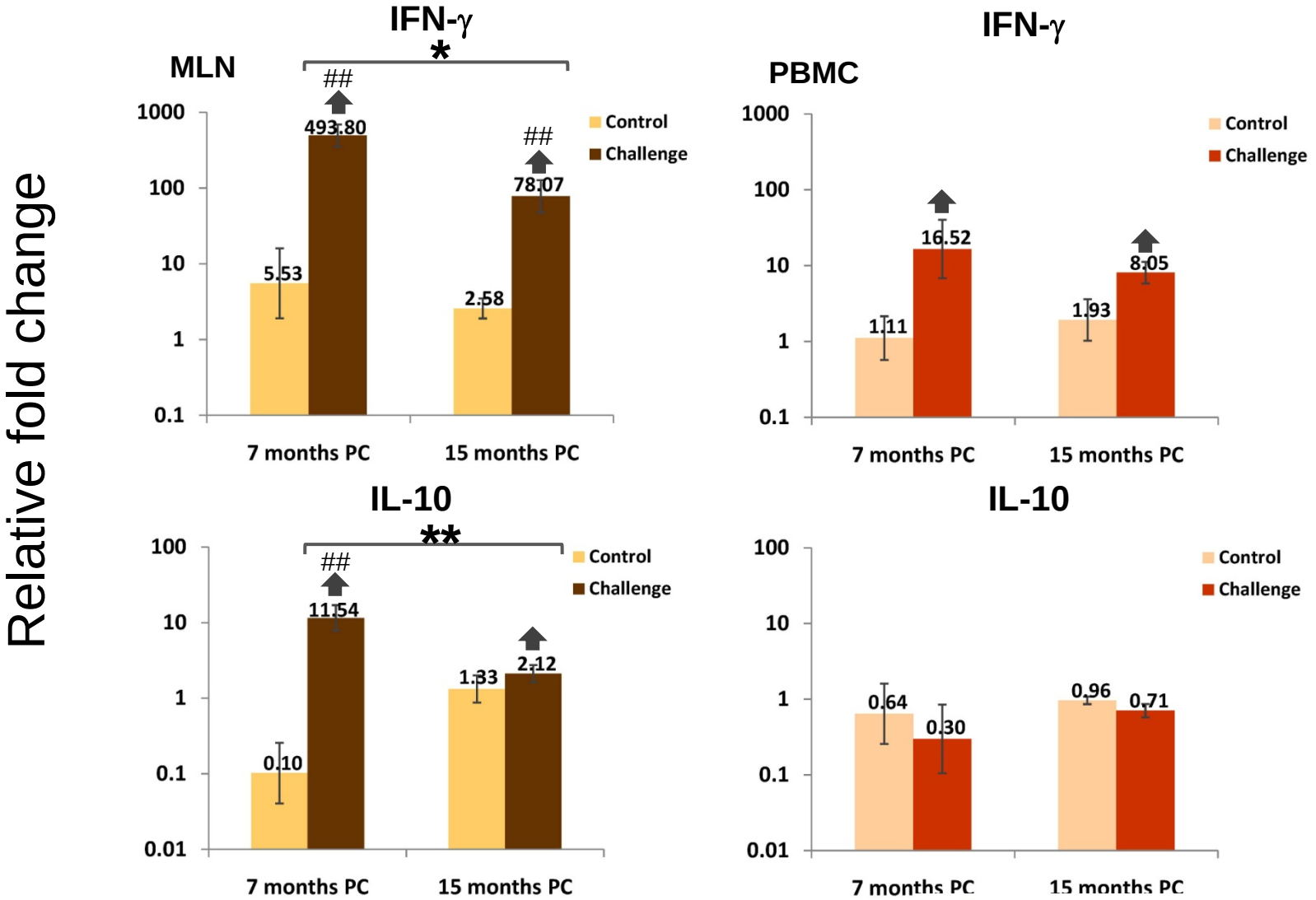
Gene expression from MAP-stimulated MLN and PBMC cultures calibrated to non-stimulated



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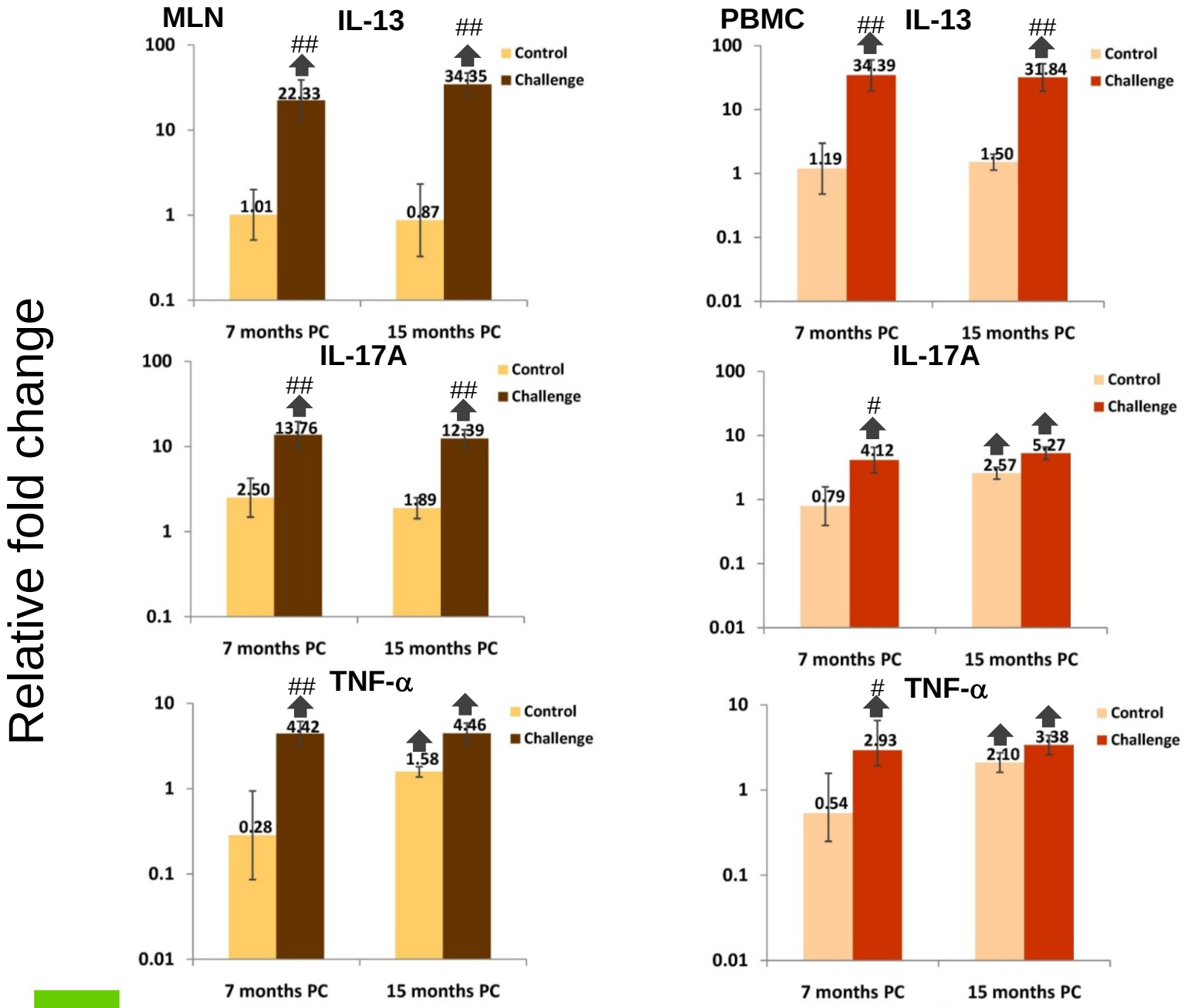

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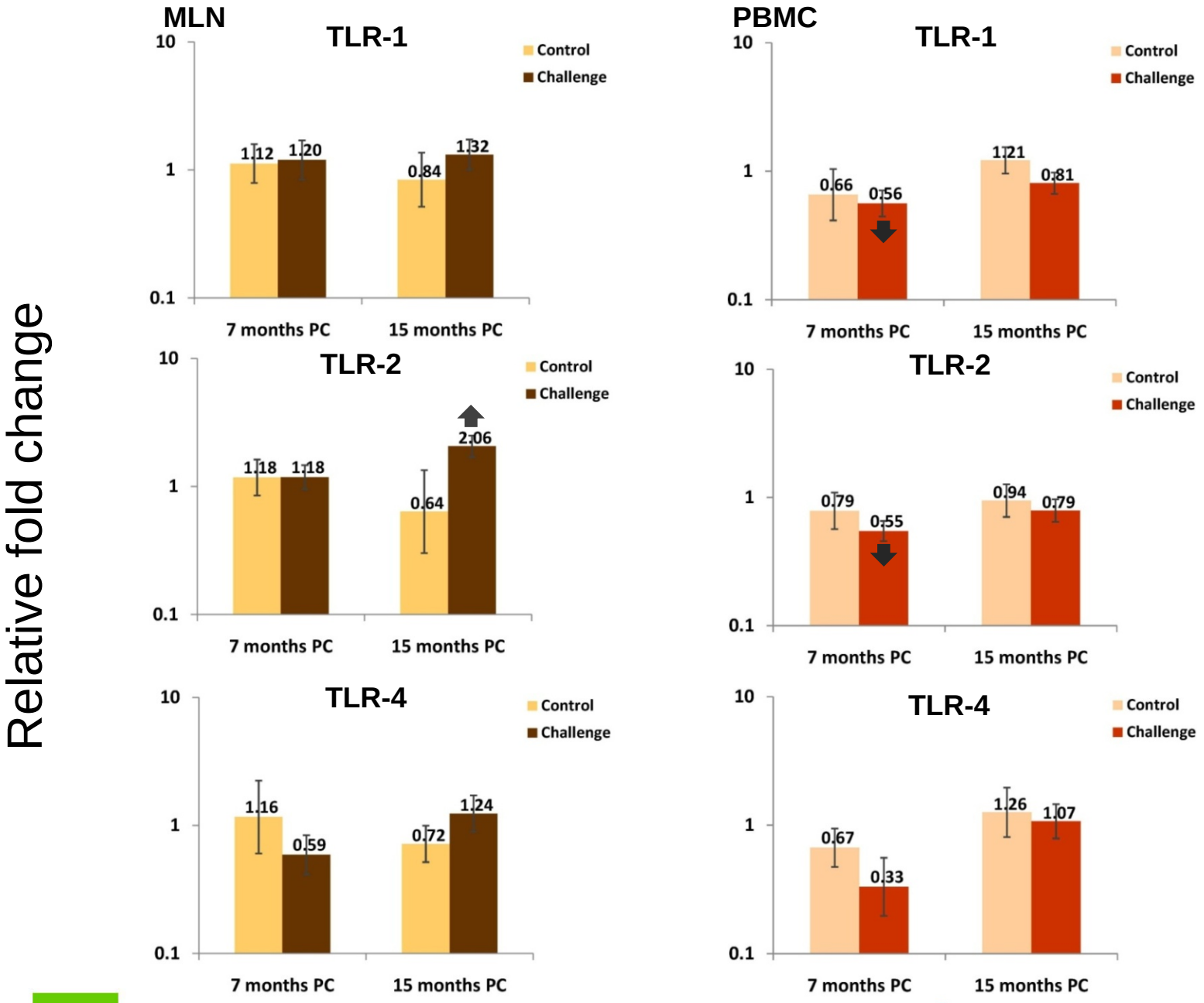

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Conclusions

- Culture of MAP from faeces & tissue peaked at early stage then declined
- MAP-specific whole blood IFN-γ peaked 6 months PC and remained high, No MAP antibody response
- MAP challenged calves
  - ↑ IFN-γ and IL-10 release (MLN and PBMC cultures)
  - ↑ IFN-γ, IL-10, IL-13, IL-17A and TNF-α gene expression (MLN and/or PBMC cultures)
  - ↓ TLR1 and TLR2 gene expression (PBMC cultures, 7 months PC)

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Summary

- Successful bovine MAP experimental infection model
- Challenged calves partially controlled infection by 15 months PC
- Dis-regulation of immune response, both stimulatory and inhibitory cytokines released & gene expression in challenged animals
- No clear association between histopathology/culture and immune responses in challenged calves

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