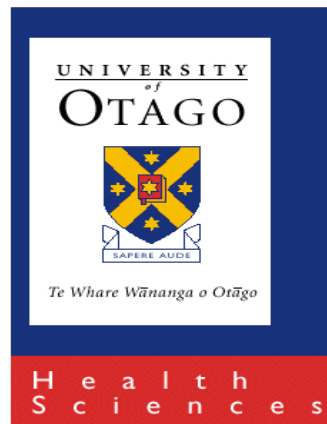


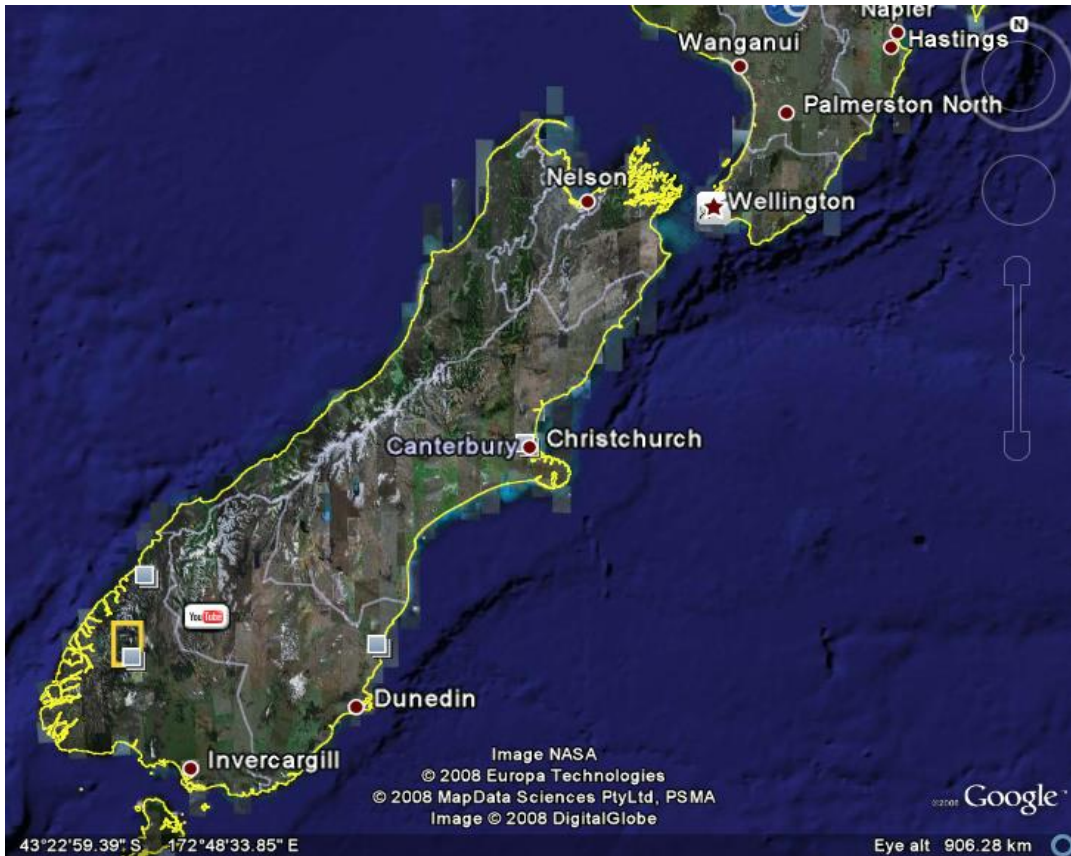
A cellular model to assess the role of *Mycobacterium avium* subsp. *paratuberculosis* in the pathogenesis of Crohn's disease

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CD in NZ



Prevalence per 100,000

Canada (Manitoba) 199

NZ (Canterbury) 155

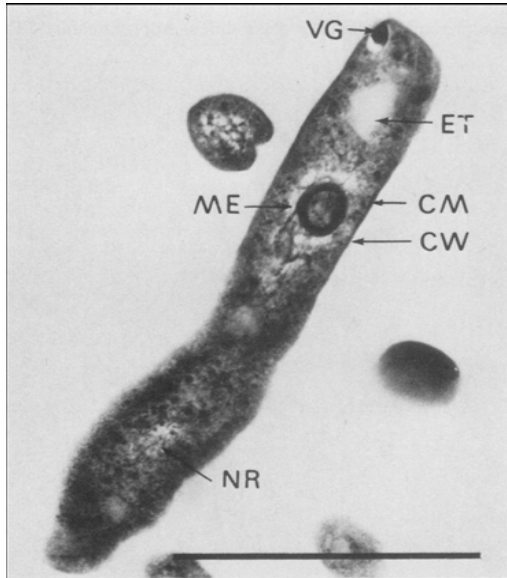
Britain (Aberdeen) 147

USA (Minnesota) 133

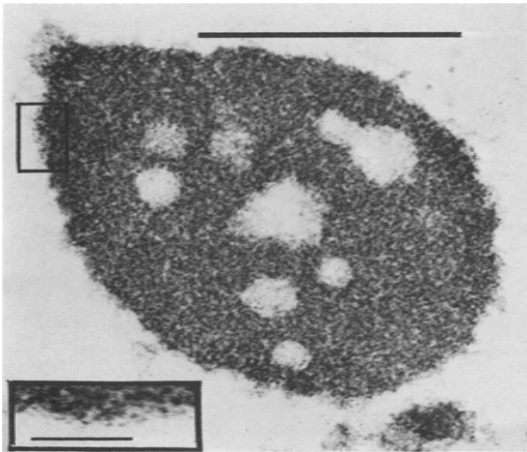
Causes of CD

- **Genetics** > 35 genes.
- Innate immune system
- Altered bacterial recognition/handling ?
- **Bacteria** – Symbionts or pathobionts
e.g. *Mycobacterium avium* subspecies
paratuberculosis.

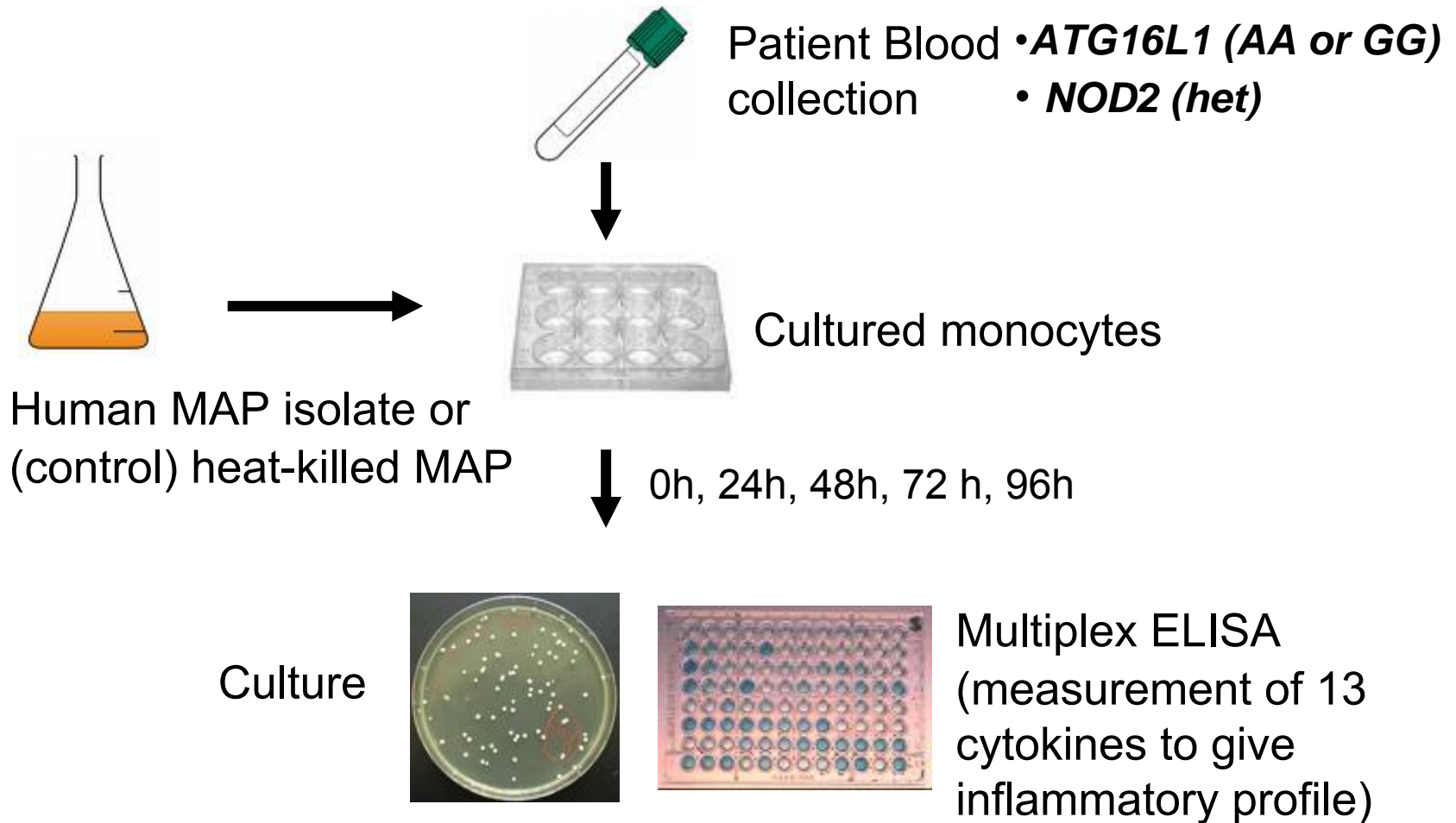
M. avium paratuberculosis - MAP



- Acid-fast rods also spheroplast form
- Intracellular survival
- Persistent but slow
- Ruminants, monogastrics, primates
- Zoonotic ??
- Association with CD ?



Cellular Model of CD pathogenesis



Patient genotypes

ATG16L1	NOD2**		
	-	+	Sum
AA	6	0	6
GG*	6	6	12
Sum	12	6	18

* G is major allele of ATG16L1 and confers risk

** NOD2 het for R702W and P268S or 1007fs alone

• *IRGM controlled - WT*

Genotype effect on growth of MAP in monocytes

<u>Time (h)</u>	<i>NOD2</i>				<i>ATG16L1</i>			
	<u>Het</u> ¹	<u>Hom</u> <u>wt</u> ²	<u>95% CI</u>	<u>p-value</u>	<u>AA</u>	<u>GG</u>	<u>95% CI</u>	<u>p-value</u>
0	4.58	4.30	-0.16 to 0.73	0.18	4.24	4.47	-0.68 to 0.22	0.29
24	4.63	4.60	-0.47 to 0.53	0.89	4.62	4.60	-0.59 to 0.63	0.93
48	4.88	4.66	-0.11 to 0.55	0.18	4.67	4.77	-0.61 to 0.42	0.68
72	5.05	4.88	-0.26 to 0.61	0.40	4.79	5.01	-0.74 to 0.30	0.36
96	5.42	5.12	-0.09 to 0.69	0.12	5.08	5.29	-0.81 to 0.39	0.45
Overall ^a	n/a	n/a	n/a	0.04	n/a	n/a	n/a	0.18

¹Het=heterozygous for *NOD2* R702W and P268S or 1007fs alone; ²Hom wt=homozygous wildtype.

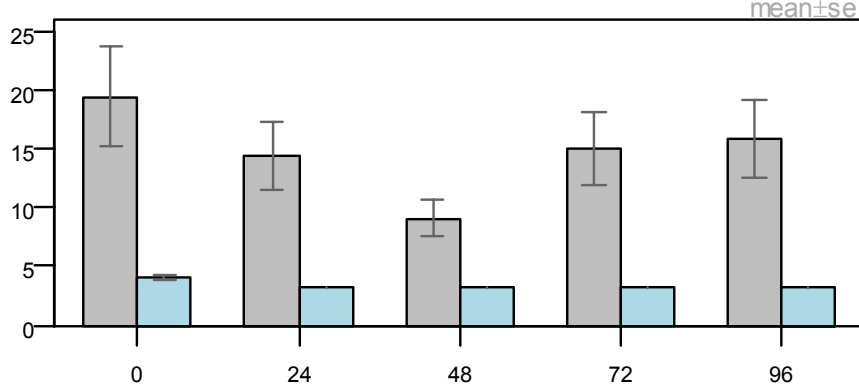
Refer to Table 1 for further genotype details

^a ANOVA effect of genotype independent of effect of time.

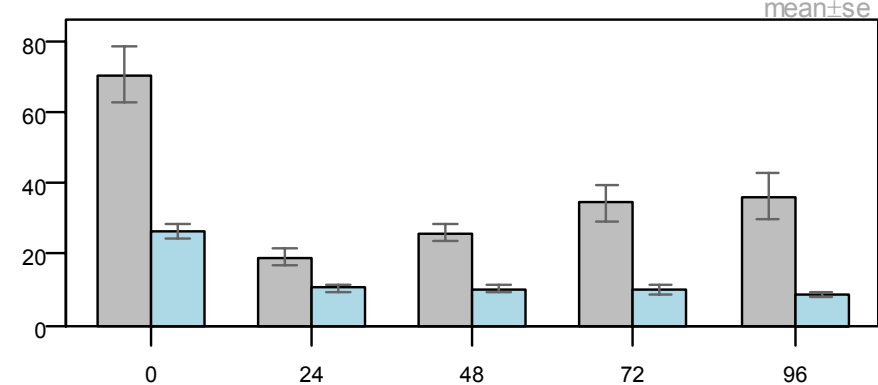
ATG16L1 genotype and cytokine expression

Cytokines: 13 assayed - IFN γ , IL10, IL12p40, IL12p70, IL17, IL1 β , IL2, IL4, IL5, IL6, IL8, TNF α , TNF β .

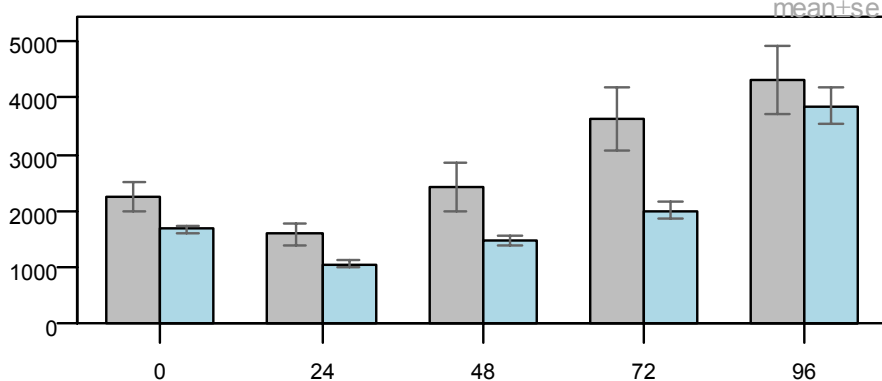
IL10 Viable bacteria <3.2 = 3.2



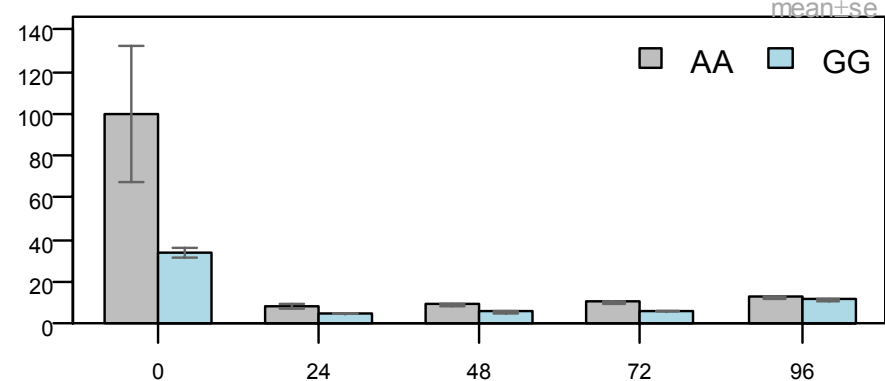
IL6 Viable bacteria <3.2 = 3.2



IL8 Viable bacteria <3.2 = 3.2



TNF α Viable bacteria <3.2 = 3.2



Genotype effect on cytokine expression

- Analysed independently of time effects

<i>Cytokine</i>	<i>NOD2</i>	<i>ATG16L1</i>
IL-10	0.558	0.047
IL-6	0.317	0.019
IL-8	0.413	0.758
TNF- α	0.970	0.289

- Risk genotype GG of *ATG16L1* associated with lower cytokine expression for IL10 and IL6.

Conclusions

***NOD2* mutations**

- impair MAP elimination
- do not affect cytokine production wrt MAP challenge.
- increase susceptibility to prolonged intracellular bacterial infection ?

Conclusions

***ATG16L1* mutation T300A**

- influences expression of IL-10 and IL-6 after MAP challenge
- does not affect autophagic clearance of this putative CD pathogen.
- **Relevance of disease model**
- SNP versus gene silencing, and in human cells.
- Platform for investigating host/bacteria in IBD

Acknowledgements



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