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

Drs Robert Bentley/Dylan Glubb/Richard G erry

Autoimmune Diseases Research Group
Department of Pathology
University of Otago, Christchurch
NEW ZEALAND
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

1. **Patient Blood collection**
   - ATG16L1 (AA or GG)
   - NOD2 (het)
2. **Cultured monocytes**
3. **Human MAP isolate or (control) heat-killed MAP**
4. **Culture**
5. Multiplex ELISA (measurement of 13 cytokines to give inflammatory profile)

Time points: 0h, 24h, 48h, 72h, 96h
Patient genotypes

<table>
<thead>
<tr>
<th>ATG16L1</th>
<th>NOD2**</th>
<th></th>
<th></th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>6</td>
<td>0</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>GG*</td>
<td>6</td>
<td>6</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Sum</td>
<td>12</td>
<td>6</td>
<td></td>
<td>18</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Time  (h)</th>
<th>NOD2 Het $^1$</th>
<th>Hom wt $^2$</th>
<th>95% CI</th>
<th>p-value</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.58</td>
<td>4.30</td>
<td>-0.16 to 0.73</td>
<td>0.18</td>
<td>4.24</td>
<td>4.47</td>
</tr>
<tr>
<td>24</td>
<td>4.63</td>
<td>4.60</td>
<td>-0.47 to 0.53</td>
<td>0.89</td>
<td>4.62</td>
<td>4.60</td>
</tr>
<tr>
<td>48</td>
<td>4.88</td>
<td>4.66</td>
<td>-0.11 to 0.55</td>
<td>0.18</td>
<td>4.67</td>
<td>4.77</td>
</tr>
<tr>
<td>72</td>
<td>5.05</td>
<td>4.88</td>
<td>-0.26 to 0.61</td>
<td>0.40</td>
<td>4.79</td>
<td>5.01</td>
</tr>
<tr>
<td>96</td>
<td>5.42</td>
<td>5.12</td>
<td>-0.09 to 0.69</td>
<td>0.12</td>
<td>5.08</td>
<td>5.29</td>
</tr>
<tr>
<td>Overall $^a$</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>0.04</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

$^1$Het=heterozygous for NOD2 R702W and P268S or 1007fs alone; $^2$Hom 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 - IFNγ, IL10, IL12p40, IL12p70, IL17, IL1β, IL2, IL4, IL5, IL6, IL8, TNFα, TNFβ.

IL10 Viable bacteria <3.2 = 3.2

IL6 Viable bacteria <3.2 = 3.2

IL8 Viable bacteria <3.2 = 3.2

TNFα Viable bacteria <3.2 = 3.2
Genotype effect on cytokine expression

• Analysed independently of time effects

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>NOD2</th>
<th>ATG16L1</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10</td>
<td>0.558</td>
<td>0.047</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.317</td>
<td>0.019</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.413</td>
<td>0.758</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.970</td>
<td>0.289</td>
</tr>
</tbody>
</table>

- 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

• Canterbury IBD Patients who participated in this study.

• Upper South B Regional Ethics Committee approval number URB/08/05/024