Unravelling the molecular mechanisms underlying the therapeutic effects of Bifidobacterium breve MRx0004

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Introduction

Members of the genus Bifidobacterium are associated with health-promoting effects. The effects of bifidobacteria on the host are largely mediated through surface components including exopolysaccharide (EPS) and surface-associated proteins¹. EPS has functional roles in bacterial survival² and persistence³, and modulates the host immune response in a strain-specific manner⁴.

Bifidobacterium breve MRx0004 was isolated from the intestinal tract of a healthy human donor and is a promising next generation live biotherapeutic for the treatment of asthma. MRx0004 has demonstrated efficacy in a murine model of severe asthma, by reducing pulmonary infiltration of neutrophils and eosinophils and increasing levels of Foxp3+ T cells⁵.

Objective: To investigate the immunoregulatory capacity of MRx0004, by targeting its EPS as a potential immunogen

Study Design

Approach:

- Transcriptional profiling of candidate MRx0004 effectors
- Characterisation of the adaptive immune response
- Innate immune response analysis
- Surface and secretome analysis of MRx0004

Results

Identification of candidate host response effectors in MRx0004

Table 1: Predicted moonlighting proteins and adhesins were detected in MRx0004 cell surface shavings and culture supernatants using nanoLC-MU/MS.

<table>
<thead>
<tr>
<th>Cell shavings</th>
<th>Supernatant</th>
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<tbody>
<tr>
<td>DeaK</td>
<td>✓</td>
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<tr>
<td>Efts</td>
<td>✓</td>
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<tr>
<td>Enoylase</td>
<td>✓</td>
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<td>GAPDH</td>
<td>✓</td>
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<tr>
<td>GpxRS</td>
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</tr>
<tr>
<td>Pullulanase</td>
<td>✓</td>
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<tr>
<td>Transaldolase</td>
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fig. 1: Six out of ten predicted MRx0004 effector genes (including the priming glycantransferase [pGTF] of the EPS locus) were significantly upregulated between late log phase growth in vitro and contact with intestinal epithelial cells.

Properties of an EPS-negative strain of MRx0004

fig. 2: Absence of EPS in EPS⁻ⁿ was confirmed by TEM. Absence of EPS was associated with increased adhesion and autoggregation, increased detection of surface-associated proteins post-ICE contact, and increased detection of proteins in culture supernatant.

fig. 3: Undetectable cell surface in EPS⁻ⁿ resulted in increased TLR2 activation by culture supernatants, and increased NFκB activation by both live bacteria and supernatants, in comparison to MRx0004.

fig. 4: MRx0004 EPS was directly associated with CD8+ and Th1 IL-12p70 responses from PBMCs, but did not impact activation of CD8⁺ cells. EPS⁻ⁿ treatment increased Tregs and IL-10 secretion to a greater extent than MRx0004, possibly due to increased cell surface exposure as a result of EPS deficiency.

Key Findings

- MRx0004 mediated the host response via modulation of TLR2/NFκB signalling, and activation of CD8⁺, Th1 and Treg responses
- Regulation of CD8⁺ and Th1 responses was directly associated with the presence of EPS
- EPS⁻ⁿ treatment resulted in an increased Treg response, which may be attributable to unshielding of surface components

Future Direction

- Further studies are underway to determine the roles of additional MRx0004 predicted effectors and surface components
- MRx0004 is currently in development for a first-in-man clinical trial in asthma patients