OC02 & P01 - **Systemic Dissemination of Commensal Bacteria is CX_3CR1-dependent and is Mediated by $CD11c^+$ $CD103^+$ Dendritic Cells**

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Bifidobacteria are one of the earliest colonisers of the human gut. Previously, our group has shown that certain surface molecules of bifidobacteria, such as exopolysaccharide (EPS) and Tad pili, are able to modulate colonisation and influence host immunity[1,2]. Specifically, surface EPS seems to be important for the immunomodulatory and host protective effects of bifidobacteria[3,4]. However, the precise mechanisms through which EPS contributes to these effects remain poorly understood. To investigate the effects of EPS on host-microbe interaction at the interface of the intestinal mucosal immune system we colonised the murine gastro intestinal tract with *Bifidobacterium breve* UCC2003 wild type strain (*B.breve* EPS^+) and its isogenic mutant lacking EPS (*B.breve* EPS^-). To facilitate tracking of the bacteria, both strains stably expressed the red fluorescent protein mCherry. We observed that *B.breve* EPS^+ colonised the gut to a higher level compared to *B.breve* EPS^-, indicating that EPS is important for initial colonisation of the host possibly due to the fact that it provides stress tolerance to low pH and bile acids. Interestingly, we found that, after colonisation and in the absence of overt inflammation, live colonies of *B.breve* EPS^+ but not *B.breve* EPS^- could be recovered from several systemic tissues (liver, kidney, spleen, thymus, adipose tissue) in addition to intestinal tissues and faeces. FACS analysis of these tissues from colonised mice, indicates that a specific subset of colonic dendritic cells (DC), the migratory $CD11c^+CD103^+$ DC, can carry *B.breve* EPS^+ but not *B.breve* EPS^- to the draining mesenteric lymph node (MLN) and also to different systemic tissues. Furthermore, sorting of these specific DC subsets from dissociated systemic tissues of colonised mice, allowed for recovery of live colonies of *B.breve* EPS^+. Considering the role that CX_3CR1 mononuclear phagocytes play in sampling and delivery of luminal antigen to migratory DC[5,6], we investigated if CX_3CR1^+ cells might be involved in the systemic spread of *B.breve* EPS^+. Our data demonstrate that CX_3CR1 mononuclear phagocytes facilitated the colonisation of *B. breve* in the murine gut and were required for systemic dissemination of the bacteria. In addition, in vitro experiments showed that *B.breve* EPS^+ but not *B.breve* EPS^- could persist inside a pure population of sorted bone marrow derived dendritic cells (BMDDC) for 72 hours in the absence of inflammatory cytokine production. These data indicate that bifidobacteria may specifically target $CD11c^+CD103^+$ DC to facilitate their systemic dissemination and to mediate their diverse effects on host immunity and physiology.

References

1. *Bifidobacterial surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection.* PNAS February 7, 2012. 109 (6) 2108-2113; https://doi.org/10.1073/pnas.1115621109. Saranna Fanning, Lindsay J. Hall, Michelle Cronin, Aldert Zomer, John MacSharry, David Goulding, Mary O'Connell Motherway, Fergus Shanahan, Kenneth Nally, Gordon Dougan, and Douwe van Sinderen

2. *Functional genome analysis of Bifidobacterium breve UCC2003 reveals type IVb tight adherence (Tad) pili as an essential and conserved host-colonization factor.* PNAS July 5, 2011. 108 (27) 11217-11222; https://doi.org/10.1073/pnas.1105380108. Mary O'Connell Motherway, Aldert Zomer, Sinead C. Leahy, Justus Reunanen, Francesca Bottacini, Marcus J. Claesson, Frances O'Brien, Kiera Flynn, Patrick G. Casey, Jose Antonio

Moreno Munoz, Breda Kearney, Aileen M. Houston, Caitlin O'Mahony, Des G. Higgins, Fergus Shanahan, Airi Palva, Willem M. de Vos, Gerald F. Fitzgerald, Marco Ventura, Paul W. O'Toole, and Douwe van Sinderen

3. *Bifidobacterium breve reduces apoptotic epithelial cell shedding in an exopolysaccharide and MyD88-dependent manner.* Open Biol. 2017 Jan;7(1). pii: 160155. doi: 10.1098/rsob.160155. Hughes KR, Harnisch LC, Alcon-Giner C, Mitra S, Wright CJ, Ketskemety J, van Sinderen D, Watson AJ, Hall LJ.

4. *The Surface-Associated Exopolysaccharide of Bifidobacterium longum 35624 Plays an Essential Role in Dampening Host Proinflammatory Responses and Repressing Local TH17 Responses.* Appl Environ Microbiol. 2016 Nov 21;82(24):7185-7196. Print 2016 Dec 15. Schiavi E, Gleinser M, Molloy E, Groeger D, Frei R, Ferstl R, Rodriguez-Perez N, Ziegler M, Grant R, Moriarty TF, Plattner S, Healy S, O'Connell Motherway M, Akdis CA, Roper J, Altmann F, van Sinderen D, O'Mahony L.

5. *CX_3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance.* Science. 2005 Jan 14;307(5707):254-8. Niess JH, Brand S, Gu X, Landsman L, Jung S, McCormick BA, Vyas JM, Boes M, Ploegh HL, Fox JG, Littman DR, Reinecker HC.

6. *Oral tolerance can be established via gap junction transfer of fed antigens from CX_3CR1^+ macrophages to $CD103^+$ dendritic cells.* https://doi.org/10.1016/j.immuni.2013.12.012 Elisa Mazzini, Lucia Massimiliano, Giuseppe Penna, Maria Rescigno.