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Genomics at work: the global gene response to enteric bacteria

Hooper LV, Wong MH, Thelin A, *et al*. Molecular analysis of commensal host-microbial relationships in the intestine. *Science* 2001;**291**:881–4.

Abstract
Human beings contain complex societies of indigenous microbes, yet little is known about how resident bacteria shape our physiology. We colonized germ-free mice with Bacteroides thetaiotaomicron, a prominent component of the normal mouse and human intestinal microflora. Global intestinal transcriptional responses to colonization were observed with DNA microarrays, and the cellular origins of selected responses were established by laser-capture microdissection. The results reveal that this commensal bacterium modulates expression of genes involved in several important intestinal functions, including nutrient absorption, mucosal barrier fortification, xenobiotic metabolism, angiogenesis, and postnatal intestinal maturation. These findings provide perspectives about the essential nature of the interactions between resident microorganisms and their hosts.

Comment

The intestinal epithelium is the first point of contact for prokaryotic organisms within the gut and plays a pivotal role in both the recognition of microbial species and coordination of the host response. Research on the molecular cross talk between pathogenic organisms and intestinal epithelial cells has occupied centre stage for many years, as gastroenterologists have actively dissected the major signalling cascades, genes, and protein products that underpin intestinal pathogenesis, with the aim of identifying potential therapeutic targets. In recent years however there has been recognition of the importance of the non-pathogenic (commensal) flora in gut health. These organisms are intimately involved in driving the development and maintenance of digestive and immunological functions of the gut,[1 2] but under predisposing conditions can also trigger inflammatory bowel disease.[3] The study of Hooper *et al* is one of the first to utilise state of the art molecular array technology and laser capture microdissection to investigate the impact of commensal bacteria on intestinal gene expression. Application of these technologies has provided a valuable insight into global gene responses and specific cellular events regulated by the normal intestinal microbiota.

The germ free mouse was used as a model to study the effect of monoassociation with *Bacteroides thetaiotaomicron*, a dominant anaerobic bacterium found in the gut of adult mice and humans. Following 10 days of colonisation, mRNA from ileal tissue was isolated from treated and untreated animals and microarray studies were undertaken. The technology reported in this study involves high density arrays of 25mer oligonucleotides (probes), designed to ~25 000 murine gene sequences. One feature of this technology is that multiple probes (a probe set) are designed to each sequence, which provides a mechanism for robust data analysis and for accurate quantification of gene expression levels. The authors identified 118 probe sets displaying *B thetaiotaomicron* induced gene differences of >twofold where 95 were upregulated and 23 were downregulated. As some DNA sequences were represented by more than one probe set, the studies actually identified 71 known genes and 34 uncharacterised genes and expressed sequence tags. Confirmation of these induced differences was subsequently performed on 12 genes using quantitative real time polymerase chain reaction. Overall the results derived from the two methodologies were very consistent. Those genes modulated by *B thetaiotaomicron* were grouped by cellular function and highlight the diverse gut systems that may be influenced by this organism.

Monoassociation of the germ free intestine with *B thetaiotaomicron* did not induce overt inflammation and immune stimulation. For some bacteria this appears to be the norm but for others such as segmented filamentous bacteria, colonisation of the germ free intestine provides potent stimulation of the immune system.[4] The reasons why bacteria generate differential responses in the intestine are unknown but may be related to their interaction with epithelial cells. Compatible with the findings reported in this study, our data show that exposure of Caco-2 cells to *B thetaiotaomicron* results in negligible inflammatory cytokine gene transcription (fig 1). In the context of a pathogen challenge however, *B thetaiotaomicron* inhibits proinflammatory cytokine expression (fig 1) by a mechanism interfering with nuclear transcription factor κB activation. Similar data describing the anti-inflammatory capability of commensal bacteria have been published recently.[5] In the study of Hooper *et al*, this additional biological activity was not identified in the germ free model. It is worth noting that although the authors reported no effects on immune response genes, increased expression of genes encoding acute phase proteins, serum amyloid A protein, and a homologue of C reactive protein was observed. The significance of these changes is currently unknown but the proteins encoding these genes appear to be multifunctional and capable of regulating innate host resistance as well as exerting specific anti-inflammatory effects.[6 7] The possibility that by exploiting host mechanisms commensal bacteria actively suppress inflammatory responses within the intestine may be important in the development of mucosal tolerance following natural colonisation.[7 8] These bacteria may also have potential as probiotics for the treatment of inflammatory bowel disease.[9]

It is established dogma that the commensal flora is associated with enhanced pathogen resistance and intestinal barrier function. Identification of genes involved in the fortification of the intestinal barrier by Hooper *et al* is therefore potentially very important. One of the most pronounced transcriptional changes was enhanced expression of the small proline rich repeat protein 2a (sprr-2a) mRNA that was localised to the villus epithelium. This gene represented the only anomaly in the study and was shown by quantitative polymerase chain reaction to be upregulated (205-fold) by *B thetaiotaomicron* whereas the two sprr-2a

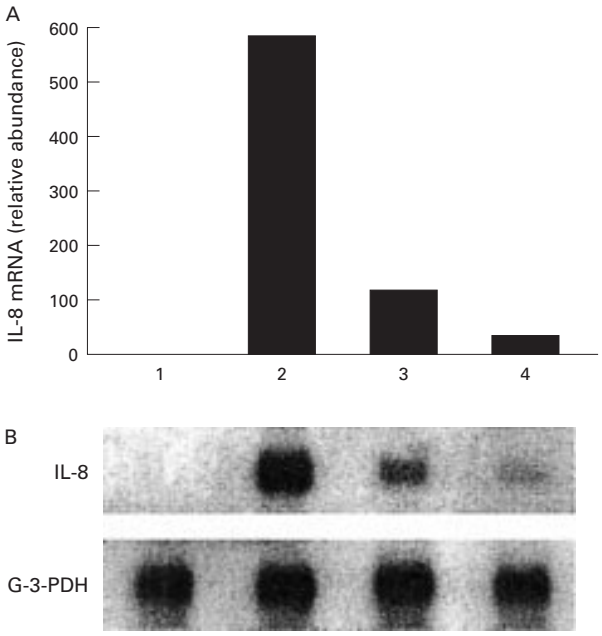

Figure 1 Real time polymerase chain reaction (PCR) (A) and northern blot analysis (B) of interleukin 8 (IL-8) mRNA expression in confluent Caco-2 cells following exposure to pathogenic and commensal bacteria. 1, Unstimulated controls; 2, challenged with Salmonella enteritidis; 3, S enteritidis with Bacteroides thetaiotaomicron; and 4, B thetaiotaomicron alone. mRNA levels were normalised using glyceraldehyde 3 phosphate dehydrogenase (G-3-PDH).

probe sets on the microarray identified upregulation of 102 and 10.6, respectively. Clearly, for the probe set upregulated by 10.6 fold, while being significant, is probably an underestimate of the actual response of sprr-2a. This exemplifies why quantitative methods, such as real time polymerase chain reaction, should be conducted to verify expression profiling studies using microarrays. A recent report describing cDNA microarray analysis of bowel adaptation after intestinal resection also showed significant increases in sprr-2 mRNA expression in the remnant ileum and in response to epidermal growth factor treatment.[10] This gene appears to be important in protein cross bridging in squamous epithelia.[11] The precise role of this gene in the intestinal epithelium is currently unknown but its activation is likely to reinforce the barrier function of the gut. Furthermore, its activation in response to diverse treatments suggests a generic role in adaptation and repair. The reported activation of sprr-2 and genes regulating mucin and trefoil secretion are consistent with defence mechanisms that would minimise bacterial translocation across the gut thereby reducing the level of immune activation in gut tissues.

Colonisation with *B thetaiotaomicron* altered expression of several genes associated with nutrient utilisation, with pronounced effects on colipase, liver fatty acid binding protein, and a novel fasting induced adipose factor (a peroxisome proliferator activated receptor α target that is repressed with chronic fat feeding). The authors postulate that these gene changes may enhance nutrient utilisation and explain the lower energy requirement of conventional animals compared with germ free counterparts. However, both groups of mice utilise similar amounts of metabolisable energy when normally active and fed ad libitum.[12] Perhaps relevant to these findings is the reported activation of genes associated with gut motility. Increase in intestinal transit and nutrient load reaching the ileum in *Bacteroides* treated mice may have promoted dietary adaptations as secondary events. Obviously, microarray analyses of gene responses cannot establish cause and effect relationships and it will require detailed physiological experiments and analysis to disentangle primary and secondary events.

This study raises several intriguing questions concerning the functional relevance of intestinal gene responses and importantly, the mode of action of *B thetaiotaomicron*. With regard to the host, the use of microarrays has provided a picture of the global gene changes and has identified candidates that may be important in relation to immunological and physiological responses in the gut. The methodology is not definitive however, as changes in gene expression may not reflect changes in protein or protein function. Follow up experiments, designed around specific genes, utilising proteomics, dominant negative mutants, or knockouts is the next exciting phase. Linking bacterial genes to changes in host physiology is also very important. It is highly likely that bacteria differentially activate host signalling mechanisms that generate specific gene readouts. This is clearly supported by the study of Hooper *et al*, as an isogenic mutant of *B thetaiotaomicron* lacking the ability to fucosylate ileal mucosa was, like the wild-type, able to stimulate expression of other intestinal genes. The use of transposon mediated mutagenesis represents a powerful means of pursuing the link between bacterial genes and changes in host physiology. In due course, the availability of the *B thetaiotaomicron* genome sequence will permit cDNA microarray experiments designed to identify bacterial genes activated or repressed following contact with gut cells. It is likely that microbial genomics, applied to *B thetaiotaomicron*, will identify bacterial products, including novel ligands for toll-like receptors and other host receptor systems, and possibly confirm the existence of a type III secretion apparatus or an equivalent structure in commensal bacteria.

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