

Escherichia coli and the mucosal immune system

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Arvind Mahajan¹ and David Gally^{1*}

¹Division of Immunity and Infection, Roslin Institute, The University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, UK

*Author for correspondence:

Tel.: +44 131 651 9242

dgally@ed.ac.uk

***Escherichia coli* and the Mucosal Immune System (ECMIS) 2011 Ghent, Belgium, 2–5 July 2011**

The meeting was held in the beautiful city of Ghent, Belgium, to bring together basic scientists and clinicians working on *Escherichia coli* and the mucosal immune system; in particular focusing on cellular interactions, immune modulation and vaccination strategies in humans and animals. The aim was to exchange knowledge on the pathogenicity of different types of *E. coli* and recent advances in the area of mucosal immunity. The meeting was timely given the recent outbreak in northern Germany of an emergent Shiga toxigenic *E. coli* strain that was associated with the deaths of over 45 people and caused hemolytic uremic syndrome in nearly 800 individuals according to the European Centre for Disease Prevention and Control.

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The opening session was introduced by one of the organizing committee, Eric Cox (Ghent University, Belgium) and led into a comprehensive overview of pathogenic *Escherichia coli* by Jaques Mainil (Liege University, Belgium), in particular focusing on the adhesins and toxins that, sometimes in combination with clinical syndromes, account for the different descriptors of *E. coli* pathotypes. These include enterotoxigenic *E. coli* (ETEC), which can produce heat labile (LT) and heat stable (ST) toxins with different adhesins depending on the infected host species; enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC), both types of attaching and effacing *E. coli*, referring to the capacity of these strains to rearrange the host cell cytoskeleton on attachment, forming attaching and effacing lesions. EHEC strains are a subset of Shiga toxigenic *E. coli* (STEC) also known as verotoxigenic *E. coli*, which are associated with clinical disease in humans, and can form attaching and effacing lesions and produce Shiga/Vero cytotoxins. Other pathotypes include enteroaggregative *E. coli*, which have a typical 'stacked brick' adherence phenotype on host cells due to the production of particular adhesins. 'Uropathogenic *E. coli*' refers to strains associated with urinary tract infections.

The 2011 STEC outbreak in Northern Germany was caused by an enteroaggregative *E. coli* strain that had been transduced by a Shiga

toxin-carrying bacteriophage. This produced a 'hybrid' strain with the capacity to colonize the human GI tract effectively in combination with the production of this virulent cytotoxin. This is a clear demonstration that, 100 years after the death of Theodore Escherich, who discovered the bacteria and after whom it is named, this species is constantly evolving and producing new public health threats.

Advances in adaptive and innate mucosal immunity were introduced by Charles Elson (University of Alabama, AL, USA). He highlighted the importance of host interactions with the normal microbiota in health [1]. He discussed research on the mechanisms that maintain homeostasis between the immune system of the host and the resident microbiota; aberration of this balance being associated with different disease syndromes including inflammatory bowel disease. A subset of immune cells in the gut (Treg cells) maintains this homeostasis. The role of Th17 cells in controlling innate mucosal responses, such as MUC2/5 and lipocalin 2 gene expression, was presented along with research demonstrating the importance of Th17 cells in generating mucosal IgA responses. CD25 Treg cells provide a survival signal to IgA-producing B lymphocytes in the gut mucosa to maintain a response. Mick Bailey (University of Bristol, UK) reminded us that there is no substitute for actual studies in

the animal host afflicted by the pathogen and provided examples of where critical differences are found between the innate and adaptive responses of species. In particular, mechanisms of mucosal protection and the regulation of mucosal responses were proposed to be particularly divergent between species. In many respects, the immune system of the mouse is more divergent from the human system than that of the pig and the Th1/Th2 paradigm does not hold up well outside of mammals.

Pathogens, including some of the *E. coli* pathotypes, have evolved to subvert these responses to promote their colonization. In accordance with this subversion strategy, the activity and interplay of EPEC secreted 'effector' proteins was presented by Brendan Kenny (Newcastle University, UK). One strain of EPEC injects over 20 different effectors into host cells through a type III secretion system (molecular syringe). Different effectors target specific host signalling pathways, often converging on NF- κ B regulation. Work was presented on the secreted bacterial effectors, NleC and NleD, which are zinc metalloproteases capable of degrading NF- κ B-I κ B α (RelA) and JNK, respectively, thus blocking NF- κ B and AP-1 activation [2,3]. He speculated that NleE could target TRAF and NEMO adaptors. It was also suggested that the reason some effector proteins may reorganize the cytoskeleton is to facilitate access of other effectors to these signalling pathways. Allied to this, David Gally (Edinburgh University, UK) presented work that Stx also has an immunosuppressive role that may promote the persistence of EHEC/STEC strains in the gut, in addition to the capacity of these toxins to actually upregulate and redistribute cellular receptors for EHEC strains.

Annelie Brauner (Karolinska Institute, Stockholm, Sweden) reviewed uropathogenic *E. coli* and the critical role of the innate response in dealing with urinary tract infections. The importance of the antimicrobial peptides the cathelicidins was discussed, in particular LL37, which is upregulated during infection. This defensin binds to the *E. coli* afimbrial adhesins known as curli to block adherence and biofilm formation. However, it appears that this binding could also limit the activity of the defensin on the bacteria. The link between vitamin D deficiency and reduced innate responses was also being investigated. Avian pathogenic *E. coli* (APEC) was introduced by Catharine Schouler (INRA, Nouzilly, France) and reminded us of the similarity of these strains to those that can cause urinary tract infections and newborn meningitis infections in humans. Cell lines had been developed to study this infection in more detail with the observation that clathrin and caveolin uptake pathways were potentially important in APEC pathogenesis. It was clear that there are still major gaps in our understanding of invasive infections caused by *E. coli* and that APEC studies in poultry can be a good model system to elucidate basic mechanisms. Andreas Muller (Pasteur Institute, Paris, France) presented research that supports the concept that *Salmonella* Typhimurium actually promotes inflammation in the GI tract in order to compete more effectively with the intestinal microflora [4]. *S. Typhimurium* is better evolved to cope with local inflammatory insults at the site of bacterial interaction with the intestinal epithelium. One proinflammatory *S. Typhimurium* effector protein is SopE: a GDP/GTP exchange protein that

activates caspase 1-dependent IL-1 β via RAC1/CDC42 activation. This activation is probably transient, followed by more suppressive effects of other secreted effector proteins.

Research was presented on the *E. coli* fimbrial adhesin F18, which is expressed by some ETEC and STEC strains (Henri De Greve, Vrije University, Amsterdam, The Netherlands). This work examined the specificity of the FedF adhesin tip through structural and site-directed mutagenesis studies. A region for binding to ABH blood group glycans associated with pig enterocytes was defined [5]. Significantly, another region of FedF was identified that interacts with glycosphingolipids and was proposed to be required for initial interactions with lipid bilayers. As such, this additive recognition would make it less likely that the fimbriae would bind to secreted 'decoy' glycan receptors that are released as part of the innate response.

With regard to vaccinology, progress on the use of modified AB₅ bacterial toxins as adjuvants was presented by George Hajishengallis (University of Louisville, KY, USA) [6]. In particular, the use of the B subunits of *E. coli* heat labile toxins was discussed (LT-IIa and LT-IIb). Recombinant B subunits from the two types lack intrinsic enterotoxicity and act as TLR-2 agonists to enhance antibody responses to codelivered antigens. This activity could be enhanced by point mutations in the TLR-2 interacting region. The mechanism(s) of adjuvancy was being investigated, including antigen uptake, migration and antigen presentation by dendritic cells in a TLR-2-dependent manner. With regard to ETEC vaccination in humans, Ann-Mari Svennerholm (University of Gothenburg, Sweden) reviewed different approaches, including use of toxoids, purified fimbria, live-attenuated and inactivated ETEC strains [7]. The research involved testing of an LT toxoid in combination with *E. coli* strains expressing combinations of colonization factors, in particular the CFA/1 and CS1–6 fimbriae. For ETEC vaccination in pigs, Vesna Melkebeek (Ghent University, Belgium) presented immunization with F4 fimbriae to limit ETEC colonization/shedding. These fimbriae induce an IgA response following systemic delivery, as do H7 flagella. Additional work examined the use of vitamin D₃ or vitamin A as adjuvants that help the induction of mucosal IgA responses following systemic immunization. A metabolite of vitamin A, retinoic acid, is produced by dendritic cells, Peyer's patches and mesenteric lymph nodes and was proposed to prime the gut homing capacity of certain lymphocytes, therefore favoring a mucosal adaptive response. Vaccination to protect pigs against STEC strains was also presented (Bruno Goddeeris, Katholieke Universiteit Leuven) based on the use of a Stx2e⁺ toxoid that induces protection in piglets via colostrum following immunization of the mother sow.

Ongoing vaccine research was presented aimed at reducing excretion of EHEC from ruminant reservoir hosts, including cattle and sheep (Eric Cox, Ghent University, Belgium; Arvind Mahajan, Edinburgh University, UK). Strategies include vaccination with type III secretion injection apparatus proteins, outer membrane proteins and flagella. Protection following systemic immunization requires induction of effective mucosal responses, which in turn

depend on antigen combination, concentration and adjuvant choice. Roberto La Ragione (University of Surrey, UK) presented work on the concept of vaccinating animals to limit the emergence of extended-spectrum β -lactamase-producing *E. coli* strains.

Overall, the meeting highlighted the importance of bringing together the two scientific areas of *E. coli* research and the mucosal immune system and that further progress needs to be made in our understanding of how innate and adaptive mucosal responses are controlled before we can manipulate this knowledge to control the threat from rapidly evolving bacterial pathogens such as *E. coli*.

Financial & competing interests disclosure

Both David Gally and Arvind Mahajan have recently entered into a research agreement with Bioniche Animal Health who currently produce an EHEC vaccine with provisional licence approval in Canada. David Gally and Arvind Mahajan also have a patent submission through the University of Edinburgh based on the use of H7 flagellin to generate mucosal responses in cattle. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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