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RESEARCH HIGHLIGHT

Traffic control at the "Gut-GALT crossroads"

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In mice treated with antibiotics to deplete commensal microbiota, there is a significant overhaul of host cellular disposition and function with CX3CR1+ mononuclear phagocytic cells carrying pathogenic and non-pathogenic administered bacteria to the (messenteric lymph node) MLN, resulting in T cell stimulation and IgA production.

Intestinal homeostasis in the gut mucosa is a critical process in deciding the fate of immune stimuli, which lead to either pro-inflammatory or anti-inflammatory immune responses. The ultimate decision on the direction of the immune response is determined to a great extent by subsets of intestinal dendritic cells (DCs) bearing chemokine receptor CX3CR1 or CD103 molecules [1]. Luminal antigens have been shown to be sampled by the CX3CR1+ DCs in the lamina propria of the mammalian gut via formation of transepithelial dendrites [2]. In principle, DCs pick up antigen from the gut lumen, get activated and migrate to lymphoid tissues to initiate naïve T cell responses. However, some doubt has been cast on the immunomodulatory behavior of CX3CR1+ cells particularly because of their reported inability to migrate in response to Toll-like receptor (TLR) ligands. This response has been attributed to the CD103+ DCs [3].

Commensal microbiota residing in the mammalian gut provide a number of benefits to the host immune system. Host species-specific microbiota contribute to immunologic maturation [4]. Control of host immune maturation by the commensal microbiota may, in part, be related to the interaction between commensal microbes and pattern recognition receptors [5]. Given the high expression of of pattern recognition receptors (PRRs) on mononuclear phagocytes, a rationale for the role of mononuclear phagocytes in influencing the impact of commensal microbiota on the immune system can be imagined. However, the mechanisms responsible for this regulation need to be elucidated.

Diehl *et al*. [6] showed that when commensal bacteria were decreased in number by antibiotic treatment, passage of non-pathogenic or pathogenic bacteria (non-invasive *Salmonella enterica*) to the messenteric lymph node (MLN) occurred. Interestingly, only in the antibiotic-treated mice were *Salmonella*-specific T cells in MLN and *Salmonella*-specific IgA titre in the feces observed, indicating that bacterial disposition in the MLN has far-reaching immunomodulatory consequences. When mice were treated with antibiotics, the MLN localization of microbes was not limited to *Salmonella*, but was also seen with orally administered *E. coli* K 12. Without antibiotic treatment, administered microbes did not localize to the MLN. Thus, the relative virulence of the orally administered microbe was not a factor governing its MLN localization.

The selective use of antibiotics in this study highlighted the nature of the commensal microbial species-restricting the MLN localization of the administered bacteria. The localization of administered *Salmonella* in the MLN and the finding of *Salmonella*-specific IgA in the feces were seen by treatment with either vancomycin or ampicillin alone and not by metronidazole. This suggests a prominent role for Gram-positive bacterial species, and likely not strict anaerobes or Gram-negatives.

Non-invasive *Salmonella* was further utilized to unravel the cellular mechanism underlying localization to the MLN. After oral *Salmonella* administration Myd88-deficient mice (Myd88 KO) but not wild-type, NOD2-deficient or NALP3-deficient mice, had significantly increased numbers of *Salmonella*, *Salmonella*-specific T cells and *Salmonella*-specific IgA in MLN. Myd88, NOD2 and NALP3 are all vital molecules involved in intracellular signaling following engagement of PRRs. Myd88 determines a major pathway in TLR signaling that leads to activation of mononuclear phagocytes [7]. Taken together, antibiotic treatment studies and results from Myd88 KO mice strongly suggest participation of commensal bacterial recognition by PRRs in the trafficking of *Salmonella* to the MLN. Activation of mononuclear phagocytes through TLRs would lead to upregulation of migratory molecules. Using the CD11c-DTR mice (in which human diphtheria toxin (DT) receptor is incorporated and CD11c+ DCs can be specifically ablated following DT injection) and CCR7-deficient mice (CCR7 is an important migratory molecule on DCs allowing DCs to traffic to lymph nodes from periphery towards ligands CCL19 or CCL21), Diehl *et al*. showed that MLN migration of *Salmonella* was highly restricted. In the steady state MLN also contains DCs coming from lamina propria and Peyer's patches [8]. Since DC subsets have different functions that affect homeostasis, it is intuitive that homeostasis necessitates

selection of DC subsets in the MLN. In the antibiotic-treated mice, compared to the control group with no antibiotics, especially after infection with *Salmonella*, Diehl *et al*. observed expansion of CD103-CXRCR1+MHCII+CD11c+ DCs. These CX3CR1+ cells differentiate from monocyte precursors and hence are not conventional DCs as CD103+ cells that develop from classical DC precursors [1].

The investigators created a mouse strain wherein the CX3CR1 locus contained a knock-in of a stop cassette upstream of DTR-coding region so that application of DT would result in specific ablation of CX3CR1+CD11c+ DCs. In this mouse they observed no-MLN localization of *Salmonella*, confirming that the CX3CR1+CD11c+ cells were carrying *Salmonella* to the MLN. CX3CR1+MHCII+CD11c+ cells from the MLN of antibiotic-treated, *Salmonella*-infected animals showed significantly higher numbers of *Salmonella* when compared to all other subsets based on CX3CR1, CD103, MHCII and CD11c phenotypic marking. Importantly, a similar cellular phenotype was observed in afferent lymph of antibiotic-treated and *Salmonella*-infected mice, confirming the migratory nature of these cells. Interestingly, the observation that CX3CR1+ cells promote bacterial translocation to MLN is in apparent contrast to an earlier report that a deficiency of CX3CR1 led to translocation of commensal microbiota into the MLN [9]. Thus, distinct roles for CX3CR1+ cells appear in the absence of commensal microbial conditioning and this is demonstrated elegantly by the work of Diehl *et al*.

Salmonella is a classical example of an enteric pathogen whose study in mouse models has shed light on human enterocolitis [10]. Diehl *et al*. while choosing this model organism avoided Peyer's Patches, thus allowing them to examine the MLN as the lone lymph node draining the gut. It would be interesting to perform a similar study using pathogenic bacteria that localize to the Peyer's patches like the Crohn disease-associated adherent-invasive *E. coli* [11]. It would be also interesting to see these experiments using mice that have not seen commensals from birth, i.e., where CX3CR1+ DCs or other host cells are never conditioned with the microbiota. The study by Diehl *et al*. also opens avenues to study CX3CR1+ cells in higher details. Factors governing diseases like inflammatory bowel disease, can be investigated more deeply by knowing the interaction of a pathogen, a critical host cell and commensal microbiota, which leads to the control of mucosal immunity as elucidated by the investigation of Diehl *et al*.

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