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The battlefield in the war against attaching-and-effacing bacterial pathogens: Monocytes, macrophages and dendritic cells in action

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ABSTRACT

The recent adoption of a unified nomenclature for the mononuclear phagocyte system has already led to the generation of novel strategies for specifically depleting a single subset of phagocytes in the presence of intact lymphoid structures. Herein, we provide a detailed description of how the various types of tissue phagocyte orchestrate the host's defense against enteric bacterial infections. From a bench-to-bedside perspective, we expect that this paradigm will accelerate the development of novel adjuvants and vaccines in human and veterinary microbiology.

1. Introduction

The attaching and effacing (A/E) family of bacterial pathogens are characterized by (i) intimate attachment to the epithelium, (ii) effacement of microvilli-covered surfaces and (iii) the formation of pedestal-like structures in epithelial cells (due to reorganization of the host's actin cytoskeleton) referred to as "A/E lesions" (Borenshtein et al., 2008; Collins et al., 2014). The locus of enterocyte effacement (LEE) pathogenicity island is required for colonization and the formation of A/E lesions. Enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *Escherichia coli* (EHEC) are A/E pathogens associated with clinically significant morbidity and mortality rates worldwide (Kaper et al., 2004). *Citrobacter rodentium* is a valuable model of EPEC and EHEC infections; in mice, the bacterium colonizes the intestinal epithelium, where it triggers colonic inflammation (characterized by thickening of the mucosa, crypt hyperplasia, and infiltration by inflammatory cells) (Mundy et al., 2005). The mechanisms of colonization and pathogenesis by *C. rodentium* were recently reviewed in detail (Borenshtein et al., 2008; Collins et al., 2014). Protective responses against A/E bacterial pathogens are based on a Th1/17 response in which type 3 innate lymphoid cells (ILC3) trigger the secretion of interferon-gamma (IFNγ) and interleukin-22 (IL-22) (Zheng et al., 2008; Sonnenberg et al., 2011). It is noteworthy that IL-22 is required for the optimal secretion of antimicrobial peptides (Zheng et al., 2008) and for epithelial regeneration after injury (Lindemans et al., 2015). However, the respective roles of monocytes, macrophages (mφ) and conventional dendritic cells (cDCs) are still subject to debate, since most of the studies in the literature relied on relatively non-specific depletion methods based on CD11c and diphtheria toxin (DT) injection (Chow et al., 2011). Indeed, CD11c's lack of specificity may have biased the interpretation of earlier results because in a context of inflammation, the protein is expressed by monocytes, cDCs and certain T and B cell subsets (Hashimoto et al., 2011). Furthermore, it was recently reported that the CD11c.diphtheria toxin receptor mouse models display neutrophilia and monocytosis upon DT injection (van Blijswijk et al., 2013).

The gut mucosa is particularly rich in several subsets of mononuclear cells, which compose the intestinal phagocyte compartment. The resident mφ are non-migratory cells with prominent cytoplasmic vacuoles. The cells specifically express the high-affinity IgG receptor FcγR (also known as CD64) (Zigmond et al., 2012; Grainger et al., 2013). In a physiological context, the pool of intestinal Ly6C$^-$ CD64$^+$ mφ is continually replenished by chemokine receptor CCR2-dependent influx of Ly6C$^+$ CD64$^-$ monocytes from the bloodstream (Tamoutounour et al., 2013; Bain et al., 2014). Intestinal mφ are generated *in situ* via a sequential "monocyte waterfall" process (rather than by the self-renewal of yolk-sac-derived precursor cells) (Bain et al., 2014). Blood monocytes that arrive within the inflamed intestine differentiate into inflammatory mφ (Zigmond et al., 2012), which secreting inducible nitric oxide synthase and the lipid mediator prostaglandin E2 (Grainger et al., 2013). In contrast to intestinal mφ, cDCs are replaced by the differentiation of bone marrow progenitors in a process driven by the growth factor fms-like

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tyrosine kinase 3 ligand (Flt3L) (Bogunovic et al., 2009; Varol et al., 2009). Furthermore, cDCs express the transcription factor Zbtb46 (Meredith et al., 2012; Satpathy et al., 2012) and the alphaE integrin CD103 (Guilliams et al., 2014). Although cDCs are known to migrate from the lamina propria to the mesenteric lymph node (MLN) in a CCR7-dependent manner (Schulz et al., 2009), two ontogenetically distinct subsets of cDCs prime the adaptive immune response in different ways (Guilliams et al., 2014). The $CD103^{+}CD11b^{-}$ subpopulation (also referred to as cDC1 (Guilliams et al., 2014)) is involved in the priming of $CD4^{+}$ Th1 cells by secreting IL-12p40. Development of cDC1 cells depends on the transcription factors Batf3, IRF8, and Id2 (Ginhoux et al., 2009; Edelson et al., 2010). In contrast, the transcription factors Irf4 and Notch2 are required for the development of the $CD11b^{+}$ subset of cDCs (also referred to as cDC2) (Lewis et al., 2011; Persson et al., 2013; Schlitzer et al., 2013). These cDCs help CD4+ T cells to differentiate into Th2 (Gao et al., 2013) and Th17 cells (Bogunovic et al., 2012). However, the transcription factor Zbtb46 is also reportedly expressed on $CD103^{-}CD11b^{+}$ cells (Satpathy et al., 2013) that migrate in the lymph (Cerovic et al., 2013) and are probably derived from DC-committed progenitors (Scott et al., 2015). In the present review, we look at how the use of new mouse models that specifically lack either cDCs or mφ has dramatically improved our understanding of the specific functions of monocytes, mφ and cDCs in the defense against A/E pathogens (Fig. 1).

1.1. Conventional DCs

The involvement of cDCs in the host's defense against *C. rodentium* has been suggested by the fact that Flt3L knockout mice die 8–12 days after being challenged with *C. rodentium* (Satpathy et al., 2013). Likewise, bone-marrow chimera experiments with a mouse expressing the DT receptor (DTR) under the control of the *Zbtb46* promoter (zDC^{DTR}) confirmed that cDCs have a key role in the response to *C. rodentium* (Satpathy et al., 2013). However, the use of the latter mouse models affect both $CD103^{+}CD11b^{-}$ and $CD103^{+}CD11b^{+}$ cells and so do not provide information of the subsets' respective, specific contributions to the response to infection. Furthermore, $Flt3L^{-/-}$ mice display impaired hematopoiesis (McKenna et al., 2000) and have low $CCR2^{+}CX3CR1^{int}CD103^{-}CD11b^{+}$ phagocyte counts (Bogunovic et al., 2009). More recently, Murphy and collaborators studied Batf3 and Notch2 knockout mice, which respectively lack cDC1 cells (Hildner et al., 2008) and cDC2 cells (Lewis et al., 2011). Whereas no signs of morbidity and mortality were observed in $Batf3^{-/-}$ mice infected by *C. rodentium* (Satpathy et al., 2013; Welty et al., 2013), infected $Notch2^{-/-}$ mice displayed abnormally low production of IL-22 by ILCs isolated from the lamina propria (Satpathy et al., 2013). However, it remains to be determined whether the weak IL-22 response may result from sampling of *C. rodentium* through the formation of transepithelial dendrites (Guilliams et al., 2014) (as is observed for non-migrating $CX3CR1^{hi}$ cells) (Rescigno et al., 2001). It is noteworthy that regulation of the Th2 response by cDC2 is not essential for clearance of *C. rodentium* (Satpathy et al., 2013). Further work is now required to determine whether the infected $Notch2^{cKO}$ mice succumb to an IL-12p40-mediated immunopathology (as is observed in $il23a^{-/-}$/CD11c-DTR > WT chimera mice) (Aychek et al., 2015). It is important to note that hematopoietic lineages and the development of cryptopatches (that are lymphoid structures unique for mice) and isolated lymphoid follicles were not affected by deletion of *Notch2* using CD11c-Cre ($Notch2^{cKO}$). Hence, the *Notch2* knock-out mouse is an appropriate system for understanding the cDC2 population's role in the response to enteric bacterial infection. In contrast, hu-LangerinDTA mice, which have a depletion of small intestinal lamina propria cDC2 through lethal expression of the diphtheria toxin A (DTA), showed normal levels of IL22-mediated protection against *C. rodentium* (Welty et al., 2013). Although these

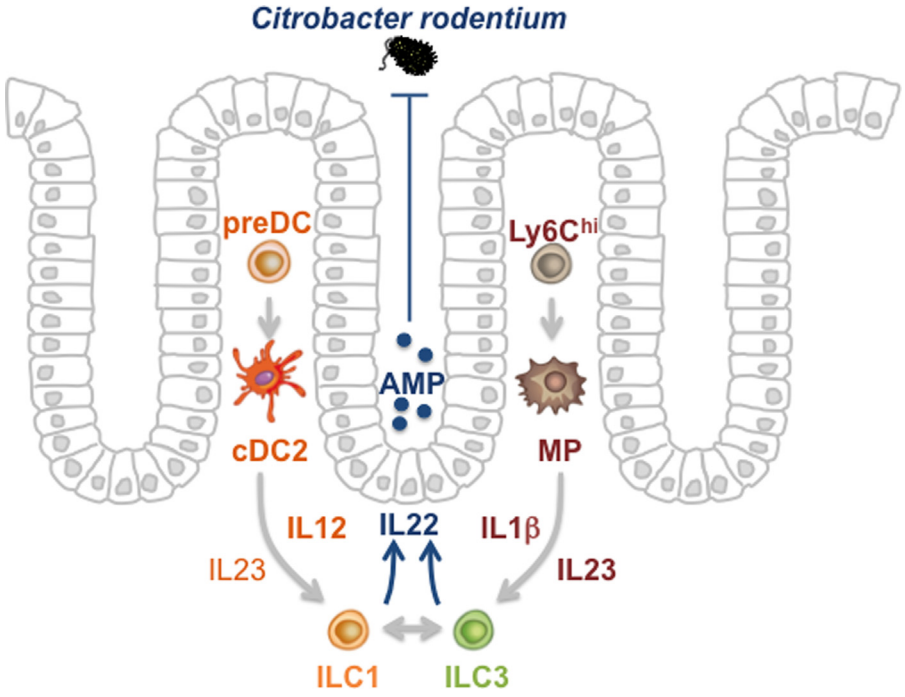

Fig. 1. A model of the intestinal phagocyte compartment's response to enteric bacterial infection.
Interleukin-22 (IL22) produced by type 3 innate lymphoid cells (ILC3) is required to control *Citrobacter rodentium* infection, in part via the induction and release of enterocyte-derived antimicrobial peptides (AMPs). ILC3-derived IL22 production is induced by IL23 secreted by either preDC-derived type 2 cDCs (cDC2) or $Ly6C^{hi}$ monocyte-derived macrophages (MPs). cDC2-derived IL12 and MP-derived IL1β also contribute to the host's response to infection. The observation of ILC3-derived IFNγ production, a ILC1-restricted cytokine, during *C. rodentium* infection raises the possibility of some plasticity between ILC groups.

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