Journal of Immunological Methods 421 (2015) 1–13

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Purification of dendritic cell and macrophage subsets from the normal mouse small intestine

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ARTICLE INFO

Article history:
Received 23 December 2014
Received in revised form 27 February 2015
Accepted 28 February 2015
Available online 18 March 2015

Keywords:
Gut
Small intestine
Mononuclear phagocytes
Dendritic cells
Macrophages
Flow cytometry

ABSTRACT

Mononuclear phagocytes are essential for protecting against pathogens breaching the intestinal mucosa and maintaining the integrity of the gastrointestinal tract. The mononuclear phagocyte family of the healthy intestine is represented by a small population of hematopoietic cells including dendritic cells and macrophages. Distinct mononuclear phagocyte subsets strategically accumulate within and below the mucosal epithelium and are distributed in the submucosa and muscularis externa. Shaped by its unique microenvironment, each mononuclear phagocyte subset is developmentally and functionally unique and phenotypically distinct. Here we summarize our recent advances on identifying and purifying various intestinal mononuclear phagocyte subsets by flow cytometry in the context of their developmental properties and location within the intestinal tissue.

1. Introduction

Gastrointestinal (GI) mucosa represents the largest surface of the body exposed to the outer environment. Because of the continuous challenge with dietary, microbial and environmental antigens the intestinal immune system accounts for the highest number of immune cells (Mowat and Agace, 2014). Among them are mononuclear phagocytes (MPs), a heterogeneous population of phagocytic hematopoietic cells. Through their ability to ingest the extracellular milieu, MPs sustain tissue homeostasis and repair by scavenging dead and apoptotic cells, induce innate and adaptive immune responses against infection and maintain tolerance to dietary, microbial commensal and self-antigens (Hashimoto et al., 2011b).

Despite its seeming simplicity the intestine is an anatomically complex organ composed of a few tissue layers. The mucosa that faces the inner intestinal space lumen is supported by the submucosa and outlined by the inner longitudinal and outer circular smooth muscles that form the muscularis externa. The intestinal mucosa consists of a simple columnar epithelium that creates an interface with the antigen-rich luminal environment, and the underlying lamina propria that incorporates gut associated lymphoid tissue (GALT) such as large organized lymphoid structures called Peyer's patches and scattered small isolated lymphoid follicles (ILFs) (Hooper and Macpherson, 2010; Mowat and Agace, 2014).

MPs form a continuous cellular network within the mucosa (McDole et al., 2012; Niess et al., 2005) and, based on the combination of developmental and functional parameters, can be further divided into two distinct cell types, dendritic cells (DCs) and macrophages (Mϕs) (Bogunovic et al., 2012; Farache et al., 2013b; Varol et al., 2010; Zigmond and Jung, 2013). DCs are regarded as professional antigen-presenting cells with the exceptional ability to induce adaptive immune responses. Recent advances in the field of MP biology established that mucosal DCs develop from a DC lineage-committed bone marrow progenitor called pre-DC, a process requiring FLT3 ligand (FLT3L) and its receptor FLT3 (Bogunovic et al., 2009; Varol et al., 2009). Mucosal DCs constitutively express a chemokine receptor CCR7 that guides them to the mesenteric

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http://dx.doi.org/10.1016/j.jim.2015.02.013
0022-1759/

lymph nodes (MLNs) that drain the gut to present intestinal antigens to T cells (Bogunovic et al., 2006; Jaensson et al., 2008; Johansson-Lindbom et al., 2005; Schulz et al., 2009). In contrast to DCs, steady state CCR7$^-$ mucosal Mϕs are stationary (MLN-nonmigratory) (Bogunovic et al., 2009; Diehl et al., 2013; Schulz et al., 2009), highly phagocytic cells (Niess et al., 2005; Rivollier et al., 2012) that are thought to be essential for maintaining tissue homeostasis and inducing innate immune responses but playing only a secondary role in adaptive immune responses (Hadis et al., 2011; Mazzini et al., 2014; Schreiber et al., 2013). Mϕs derive from Gr1^{hi} monocytes and their development is regulated by CSF1 and CSF1R (Bain et al., 2014; Bogunovic et al., 2009; Varol et al., 2009).

Intestinal phagocytes express the hematopoietic marker CD45 but lack lymphocyte and granulocyte specific markers such as CD3, B220, Ly6g and SiglecF and selectively express a combination of the integrin CD11c and high levels of the antigen-presenting molecule MHC class II (MHCII) (Bogunovic et al., 2009, 2012; Muller et al., 2014). Based on the cell surface expression of CD103, CD11b and CD8α combined with GFP expression under control of *Cx$_3$cr1* promoter in *Cx$_3$cr1*$^{WT/GFP}$ mice (CX$_3$CR1 stands for CX$_3$C-chemokine receptor 1) mucosal MHCII^{hi}CD11c^{hi} MPs outside of Peyer's patches comprise three main cell populations best characterized as CD11b$^-$CD103$^+$CD8α$^+$CX$_3$CR1$^-$ (5–10% of MHCII^{hi}CD11c^{hi} cells) and CD11b$^+$CD103$^+$CD8α$^-$CX$_3$CR1$^-$ (15–30% of MHCII^{hi}CD11c^{hi} cells) DCs and CD11b$^+$CD103$^-$CD8α$^-$CX$_3$CR1^{hi} (60 − 80% of MHCII^{hi}CD11c^{hi} cells) Mϕs (Bogunovic et al., 2009). Because of phenotypic and developmental similarities with lymph node and splenic CD8α$^+$ DCs, CD11b$^-$CD103$^+$CD8α$^+$ DCs might reside within the ILFs (Bogunovic et al., 2009; Edelson et al., 2010; Ginhoux et al., 2009; Jaensson et al., 2008; McDonald et al., 2010) whereas more numerous CD11b$^+$CD103$^+$ DCs are thought to accumulate mainly in the lamina propria and within the epithelium (Bogunovic et al., 2006; Farache et al., 2013a). CD11b$^+$CD103$^-$CX$_3$CR1^{hi} Mϕs were originally described as CX$_3$CR1$^+$ DCs with a unique ability to phagocytize luminal bacteria using transepithelial protrusions (Niess et al., 2005) as their MHCII^{hi}CD11c^{hi} antigen-presenting phenotype was thought to be a characteristic of DCs. Only recent functional studies together with transcriptome analysis have classified these cells as Mϕs (Farache et al., 2013b; Miller et al., 2012). Mϕs are distributed mainly within the lamina propria, below the epithelium (Zigmond et al., 2012) and around ILFs (Mortha et al., 2014). Comparison of *Cx$_3$cr1*-driven GFP expression in different MP subsets identified an additional population of CD11b$^+$CD103$^-$CD8α$^-$CX$_3$CR1^{int} cells that are reminiscent of both DCs (migrate to the MLNs) (Cerovic et al., 2013) and Mϕs (CCR2-dependent) (Zigmond et al., 2012). CD11b$^+$CD103$^-$CX$_3$CR1^{int} cells represent a minor population of mucosal MPs (less than 5% of MHCII^{hi}CD11c^{hi} cells) but become a dominant population upon inflammation and are often referred to as inflammatory DCs (Zigmond et al., 2012).

Despite the generalized view that the vast majority of intestinal DCs and Mϕs accumulate in the lamina propria to protect the luminal/mucosal interface, MPs are distributed throughout all the layers of the GI tract. Studies of the separated muscularis externa identified the presence of a homogeneous MHCII^{hi}CD11c^{lo} Mϕ population defined as CD11b$^+$CD103$^-$CD8α$^-$CX$_3$CR1^{hi} cells (Bogunovic et al., 2009; Muller et al., 2014) but no equivalents of mucosal DC subsets have been found. When compared to mucosal Mϕs, muscularis Mϕs express a distinct transcriptome, underlying a unique function. We recently found that muscularis Mϕs are positioned along nerve fibers and modulate constitutive GI motility through crosstalk with enteric neurons (Muller et al., 2014). Muscularis Mϕs are also potent antigen-presenting cells in ex vivo studies but their contribution to intestinal immune responses in vivo is unknown (Flores-Langarica et al., 2005). The MP diversity in the submucosa has not yet been studied, although microscopy of intestinal tissues from *Cx$_3$cr1*$^{WT/GFP}$ mice reveals the presence of CX$_3$CR1$^+$ cells, likely Mϕs, in this layer (our unpublished observation).

Here we summarize the accumulated knowledge on intestinal MP diversity and describe our approach to identify and purify DC and Mϕ subsets from the mouse small intestine for transcriptome analysis and ex vivo co-culture experiments.

2. Methods

2.1. Stock solutions

1× Hanks' Balanced Salt Solution (HBSS) without calcium and magnesium, 1× RPMI, 1× phosphate buffered saline (PBS) without calcium and magnesium stock solutions (Gibco, Grand Island, NY) were stored at 4 °C; 0.5 M ethylenediaminetetraacetic acid (EDTA) purchased from Ambion (Grand Island, NY) was stored at room temperature (RT); 0.5 M DTT (Sigma-Aldrich, St Louis, MO) stock was prepared using dH$_2$O and stored at 4 °C. Hyclone Fetal Bovine Serum (FBS; GE Healthcare Life Sciences, South Logan, UT) was heat inactivated at 56 °C for 30 min and stored at − 20 °C; Collagenase Type IV (Gibco) and Trizol Reagent (Ambion) were stored at 4 °C; BCP phase separation reagent (Molecular Research Center, Cincinnati, OH), 2-propanol (Sigma), and 70% ethanol were stored at RT. 4% paraformaldehyde (PFA) stock in 1× PBS was prepared by diluting 16% PFA (Electron Microscopy Sciences, Hatfield, PA) and 10× PBS in distilled water and stock solution was stored at 4 °C.

2.2. Working solutions

Complete Medium (CM) was prepared by supplementing HBSS with 2% FBS. CM/DTT solution was prepared by supplementing CM with 1 mM DTT. CM/EDTA solution was prepared by supplementing CM with 1.3 mM EDTA. Complete RPMI was prepared by supplementing RPMI with 2% FBS. Collagenase solution was prepared by dissolving 2 mg/ml collagenase in complete RPMI. Fluorescence-activated cell sorting (FACS) buffer was prepared by supplementing 1× PBS with 2% FBS and 0.78 mM EDTA.

2.3. Mice

Six–eight weeks old female C57Bl/6J mice purchased from The Jackson Laboratory (Bar Harbor, ME) were used for our experiments. *Batf3*$^{-/-}$ (Hildner et al., 2008), *Flt3*$^{-/-}$ (Mackarehtschian et al., 1995), *Cx$_3$cr1*$^{GFP/WT}$ (Jung et al., 2000) and *Flt3*$^{-/-}$*Cx$_3$cr1*$^{GFP/WT}$ mice were bred and maintained in the animal vivarium at Penn State Hershey. All experimental