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Short Article

Gut Microbiota Promote Hematopoiesis to Control Bacterial Infection

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SUMMARY

The commensal microbiota impacts specific immune cell populations and their functions at peripheral sites, such as gut mucosal tissues. However, it remains unknown whether gut microbiota control immunity through regulation of hematopoiesis at primary immune sites. We reveal that germ-free mice display reduced proportions and differentiation potential of specific myeloid cell progenitors of both yolk sac and bone marrow origin. Homeostatic innate immune defects may lead to impaired early responses to pathogens. Indeed, following systemic infection with *Listeria monocytogenes*, germ-free and oral-antibiotic-treated mice display increased pathogen burden and acute death. Recolonization of germ-free mice with a complex microbiota restores defects in myelopoiesis and resistance to *Listeria*. These findings reveal that gut bacteria direct innate immune cell development via promoting hematopoiesis, contributing to our appreciation of the deep evolutionary connection between mammals and their microbiota.

INTRODUCTION

The vast majority of our interactions with bacteria are symbiotic in nature, consisting of colonization by a complex and diverse microbiota that inhabit humans for life. Rather than causing inflammation, commensal microbes largely direct beneficial immune functions and often engender health. In particular, the microbiota shapes global immune cell repertoires, thereby altering host susceptibility to inflammation and infection at sites of colonization (Hill et al., 2012; Kamada et al., 2012; Mazmanian et al., 2008; Naik et al., 2012). Specific gut bacteria or bacterial products have been shown to suppress intestinal inflammation (e.g., colitis) in mice through a variety of immune mechanisms (Atarashi et al., 2013; Round and Mazmanian, 2010; Smith et al., 2013). Furthermore, the impact of commensal microbes on host immune responses is not limited to mucosal interfaces but extends to systemic compartments; gut microbes regulate immune responses that influence organ-specific autoimmunity in animal models of multiple sclerosis, rheumatoid arthritis, and type 1 diabetes (Lee et al., 2011; Markle et al., 2013; Wu et al., 2010). While numerous examples illustrate how the microbiota contributes to immune function at mucosal and systemic sites, little is known about the influences of gut bacteria on cellular development within primary immune tissues.

The immune system begins to develop in utero, but full maturation requires both genetic and environmental signals that further shape immunity after birth. Lymphoid and myeloid cells develop largely from hematopoietic stem cells (HSCs) within primary tissues, where molecular cues orchestrate immune cell differentiation from uncommitted HSCs and progenitor cells via regulation of transcription factors and epigenetic modifications (Weissman, 1994). Additionally, certain phagocyte populations (including Langerhans cells and microglia), derived from embryonic precursors, are maintained independently of HSCs (Sieweke and Allen, 2013). Genetic contributions (i.e., molecular cues encoded by the host genome) to lineage commitment pathways that control the myeloid repertoire are well studied (Georgopoulos, 2002). However, environmental factors that influence hematopoiesis have not been extensively defined. Based on emerging data showing that the microbiota represents an integral environmental factor in shaping numerous features of the immune system, we reasoned that gut bacteria may be controlling central immunity. We report herein that commensal microbes promote the maintenance of both HSC- and embryo-derived myeloid cells during steady-state conditions. The absence of commensal microbes leads to defects in several innate immune cell populations (including neutrophils, monocytes, and macrophages) within systemic sites. By controlling the differentiation of innate immune cells, the gut microbiota prepares the host to rapidly mount immune responses upon pathogen encounter, as germ-free and antibiotic-treated mice are impaired in clearance of systemic bacterial infection. Our study reveals that gut microbes evolved to actively shape immunity at its core—via regulation of hematopoiesis.

RESULTS

Germ-free Animals Display Global Defects in Innate Immune Cells

The commensal gut microbiota profoundly influences cellular proportions, migration, and functions of various immune cell

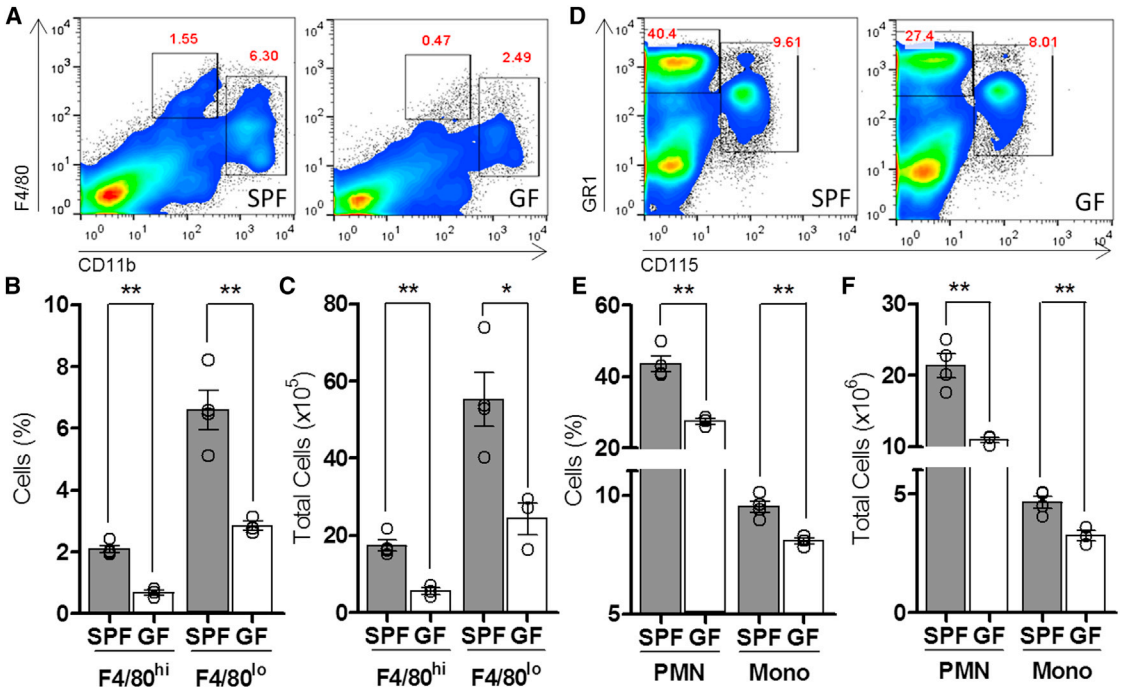

Figure 1. GF Mice Are Deficient in Resident Myeloid Cell Populations in the Spleen and Bone Marrow

(A–C) Splenic phagocyte profile among SPF and GF mice. Representative flow cytometry plots (A), cell proportions (B), and total cell number (C) of CD11b^{lo} F4/80^{hi} and CD11b^{hi} F4/80^{lo} splenic cells in SPF and GF mice. (D–F) Bone marrow populations of neutrophils (Gr1^{hi} CD115^{neg}) and monocytes (Gr1^{hi} CD115^{hi}) among SPF and GF mice. Shown are representative flow cytometry plots (D), cell proportions (E), and total cell number (F) within the bone marrow of SPF and GF mice. For all panels, data are representative of at least three independent trials with n $\geq$ 4 mice/group. Each symbol represents data from a single animal. Error bars represent standard error of mean (SEM). *p < 0.05, **p < 0.01. PMN, polymorphonuclear cells; Mono, monocytes. See also Figure S1.

subsets. Recent studies have provided numerous examples illustrating how gut bacteria modulate innate and adaptive immune responses at mucosal surfaces during infection, inflammation, and autoimmunity (Kamada et al., 2013; Round and Mazmanian, 2009). With such pervasive effects, we reasoned that the microbiota may regulate hematopoiesis—the developmental programming of the immune system. Initially, to determine if the microbiota has global effects on systemic immune cell populations, we profiled myeloid cells in the spleen of colonized (specific pathogen-free, SPF) and germ-free (GF) mice. Indeed, GF animals display reduced proportions and total numbers of F4/80^{hi} and F4/80^{lo} cells compared to SPF mice (Figures 1A–1C). F4/80^{hi} cells are mainly macrophages, while F4/80^{lo} splenocytes are a heterogeneous population of macrophages, monocytes, and neutrophils (Schulz et al., 2012). Intriguingly, all three cell subsets are reduced in GF mice (see Figure S1A available online). Furthermore, treatment of SPF mice with antibiotics also results in diminished myeloid cell populations in the spleen (Figure S1B). Thus, gut bacteria dynamically influence innate immune cell proportions at secondary immune sites in the periphery.

Myeloid cell precursors differentiate into various phagocyte lineages that are stored in the bone marrow and are a major source of cells that populate peripheral tissues (Geissmann et al., 2010). The reduction of splenic macrophages, monocytes, and neutrophils in GF mice suggests that defects in host immunity may include compromised development in primary immune sites. Accordingly, we observed a reduction of myeloid cells within the bone marrow of GF mice (Figures 1D–1F). A similar decrease was observed in the liver, a site of alternative immune cell development (Figure S1C). A global defect in myeloid cell populations in primary immune sites of GF mice demonstrates that gut bacteria shape the architecture of the immune system early in cellular development.

Commensal Microbes Enhance Myelopoiesis

We reasoned that reductions in several phagocytic cell subsets in GF mice may reflect a primary defect in the maintenance of myeloid cell populations. To test if commensal microbes promote myelopoiesis, we pulsed SPF and GF mice with 5-ethynyl-2′-deoxyuridine (EdU), a thymidine analog, to compare the percentage of dividing leukocytes. Both F4/80^{hi} and F4/80^{lo} phagocytes from GF mice show reduced EdU incorporation compared to SPF animals (Figures 2A and 2B). F4/80^{hi} macrophages are largely derived from embryonic yolk sac progenitors and are maintained independently of HSCs (Schulz et al., 2012; Sieweke and Allen, 2013). F4/80^{lo} leukocytes, however, are of hematopoietic origin, and reduced EdU incorporation by these cells in GF mice indicates defects in the expansion and/or differentiation of bone marrow progenitor cells (Schulz et al., 2012). These studies uncover a role for commensal microbes in promoting the maintenance of both splenic yolk sac-derived and HSC-derived myeloid cells.

The reduction of F4/80^{lo} cells in GF mice led us to further investigate the contribution of commensal microbes on HSCs and myeloid progenitor cells in the bone marrow. No differences were detected in the proportion or differentiation potential of