Brain, Behavior, and Immunity 51 (2016) 14–28

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Got worms? Perinatal exposure to helminths prevents persistent immune sensitization and cognitive dysfunction induced by early-life infection

Lauren L. Williamson [a], Erin A. McKenney [b], Zoie E. Holzknecht [c], Christine Belliveau [a], John F. Rawls [d], Susan Poulton [c], William Parker [c], Staci D. Bilbo [a,*]

[a] Department of Psychology & Neuroscience, Duke Institute for Brain Sciences, Duke University, United States
[b] Department of Biology, Duke University, United States
[c] Department of Surgery, Duke University Medical Center, United States
[d] Department of Molecular Genetics and Microbiology, Duke University Medical Center, United States

ARTICLE INFO

Article history:
Received 26 May 2015
Received in revised form 6 July 2015
Accepted 6 July 2015
Available online 7 July 2015

Keywords:
Hygiene hypothesis
Developmental programming
Autoimmune
Microglia
Neurodevelopment
Cytokines

ABSTRACT

The incidence of autoimmune and inflammatory diseases has risen dramatically in post-industrial societies. "Biome depletion" – loss of commensal microbial and multicellular organisms such as helminths (intestinal worms) that profoundly modulate the immune system – may contribute to these increases. Hyperimmune-associated disorders also affect the brain, especially neurodevelopment, and increasing evidence links early-life infection to cognitive and neurodevelopmental disorders. We have demonstrated previously that rats infected with bacteria as newborns display life-long vulnerabilities to cognitive dysfunction, a vulnerability that is specifically linked to long-term hypersensitivity of microglial cell function, the resident immune cells of the brain. Here, we demonstrate that helminth colonization of pregnant dams attenuated the exaggerated brain cytokine response of their offspring to bacterial infection, and that combined with post-weaning colonization of offspring with helminths (consistent with their mothers treatment) completely prevented enduring microglial sensitization and cognitive dysfunction in adulthood. Importantly, helminths had no overt impact on adaptive immune cell subsets, whereas exaggerated innate inflammatory responses in splenic macrophages were prevented. Finally, helminths altered the effect of neonatal infection on the gut microbiome; neonatal infection with *Escherichia coli* caused a shift from genera within the Actinobacteria and Tenericutes phyla to genera in the Bacteroidetes phylum in rats not colonized with helminths, but helminths attenuated this effect. In sum, these data point toward an inter-relatedness of various components of the biome, and suggest potential mechanisms by which this helminth might exert therapeutic benefits in the treatment of neuroinflammatory and cognitive disorders.

1. Introduction

Autoimmune and immune hypersensitivity disorders are dramatically on the rise in recent decades, including asthma, allergies, multiple sclerosis, thyroid and gastrointestinal disorders, and many others, a rise that is difficult to account for by changes in genetic susceptibility alone. Numerous environmental factors have been implicated in these increases (e.g., industrial toxins and chemicals), but the associations of single agents with hypersensitive immune diseases have been relatively weak. We and others (Rook et al., 2013; Bilbo et al., 2011; Parker and Ollerton, 2013; Weinstock and Elliott, 2014; Maizels, 2005; Hewitson et al., 2009) have suggested a paradigm shift in the understanding of immune-related disorders, one that points not to individual genes or environmental triggers, but rather to a fundamental disruption of the human "biome" by modern society. This biome is composed of all life associated with the ecosystem of the human body, including microbial communities (the microbiome, which is increasingly disrupted by modern dietary changes and over usage of antibiotics), as well as multicellular organisms such as helminths (intestinal worms), which profoundly modulate the vertebrate immune system and the microbiome (McKenney et al., 2015), and are omnipresent in pre-industrial societies (Bilbo et al., 2011; Parker and Ollerton, 2013; Rook, 2009). Thus, the central

* Corresponding author at: Duke University, 572 Research Drive, Box 91050, Durham, NC 27708, United States.
E-mail address: staci.bilbo@duke.edu (S.D. Bilbo).

http://dx.doi.org/10.1016/j.bbi.2015.07.006
0889-1591/

hypothesis is that immune destabilization due to the loss of helminths and similar organisms (a.k.a. biome depletion) leads to hyperimmune-associated disease in individuals also exposed to environmental triggers and/or genetic susceptibilities, leading to a population-wide increase in the overall incidence of disease associated with immune system dysfunction (see Bilbo et al., 2011 for review).

Immune system dysfunction also affects the brain, due to extensive well-defined interactions between the nervous and immune systems. Importantly, there are parallels between the "sickness behaviors" caused by an acute illness, which are largely adaptive responses, and the behaviors expressed by individuals with certain neurological and neuropsychiatric disorders, which are arguably pathological (Dantzer et al., 2008; Dantzer and Kelley, 2007). For instance, the symptoms of depression are strikingly similar to behavioral changes during acute illness, including decreased food intake, social withdrawal, and increased sleep disturbance; suggesting that many psychiatric disorders may involve a dysregulation of immune function (e.g. chronic inflammation) even in the absence of an overt immune challenge (Pace and Miller, 2009; Dantzer, 2006; Rook and Lowry, 2009; Salim et al., 2012; Waeber and Moskowitz, 2005). Supporting the importance of the biome in inflammation-associated brain dysfunction, supplementation with helminths in humans has recently been found to benefit a number of neuropsychiatric disorders, including major depressive and anxiety disorders (Cheng et al., 2015).

In addition to the vulnerability of the adult brain to inflammation, the brain is particularly susceptible to inflammation during development. Immune molecules are critical for normal brain development, but aberrant expression of these same molecules are increasingly implicated in neuropathology (Bilbo and Schwarz, 2012; Deverman and Patterson, 2009). For instance, autism is associated with a wide range of immune abnormalities, including the presence of auto-antibodies in a subset of autism, and increased incidences of asthma, allergies and other autoimmune disorders in families with autistic children (Ashwood and Van de Water, 2004a,b; Ashwood et al., 2006; Becker, 2007). Notably, functional changes in microglial cells, the primary immunocompetent cells of the brain, have been observed in patients with autism (Pardo et al., 2005; Vargas et al., 2005). In addition to their primary roles in immune defense, microglia are important for several aspects of healthy brain development, including cellular differentiation, axon guidance, and developmental synapse elimination (Schafer et al., 2012; Tremblay et al., 2011; Stevens et al., 2007; Merrill, 1991). Taken together, these data suggest a mechanism in which either a disruption of normal microglial development, or their abnormal activation by immune stimuli and/or environmental factors, leads to aberrant neural development, and thereby behavioral pathology.

One mechanism by which the biome may impact the immune system, and thus the brain, is via the gut microbiome. There is increasing evidence of the importance of the gut-brain axis in behavior (Cryan and Dinan, 2012). For instance, mice raised in germ-free environments exhibit social deficits, which are reversed following bacterial recolonization (Desbonnet et al., 2014). Moreover, in a maternal immune activation (MIA) model that induces autism-like behaviors in male offspring, post-weaning probiotic treatment stabilized the gut, normalized the peripheral immune response, and reversed some of the persistent behavioral abnormalities that occur as a consequence of the early inflammation (e.g., increased anxiety, stereotypy) (Hsiao et al., 2013). Notably, a recent report suggests raising mice germ-free also markedly alters the development and function of microglia within the healthy brain, providing a potential mechanism by which the microbial environment could impact behavioral development (Erny et al., 2015). Importantly, helminth colonization of laboratory rats causes significant shifts in the gut microbiome, affecting approximately 25% of the total (McKenney et al., 2015), the functional consequences of which are currently being explored.

Taken together, we hypothesize the loss of helminths may contribute to the increasing incidence of neurodevelopmental disorders such as autism, via microbiome disruption and immune destabilization in both the periphery and brain. If true, then colonization with helminths should work to prevent hypersensitive immune responses to a challenge such as infection, and thereby protect from downstream neuroinflammatory consequences. We have demonstrated previously that rats infected with bacteria as neonates, during a critical window for microglial cell development, display adult vulnerabilities to cognitive dysfunction, which is specifically linked to long-term hypersensitivity of microglial function within the brain (Bilbo et al., 2005; Bilbo and Schwarz, 2009; Williamson et al., 2011). This experimental model serves as an ideal system in which to test the role of biome depletion in inflammation-associated aberrant brain development, microglial function, and behavior.

2. Materials and methods

2.1. Subjects and experimental overview

Sprague–Dawley rats (Harlan, Indianapolis, IN) were used for all experiments. Experiment 1: we first determined the impact of a naturalistic, "farm-like" environment (including helminth colonization) on microglial activation by neonatal infection in laboratory rats. Rats were housed in: (1) shoebox cages in a Duke satellite facility with "dirty" colony conditions (no water or air filtration beyond temperature control, no laboratory personnel protective (PPE) clothing, in a shared space with wild-caught rats – referred to as "farm-like" for the remainder of the paper); or (2) in standard pathogen-free/"clean" laboratory conditions with individual air- and water-filtered cages and mandatory laboratory personnel PPE, each on a 12/12 h light/dark cycle, with ambient temperature of 22 °C and *ad libitum* food (Lab Diet 5001, St. Louis, MO), and water. Experiment 2: after observing a remarkable attenuation of microglial long-term sensitization in rats in "farm-like" conditions compared to standard laboratory conditions, we next determined the impact of helminths alone, in rats housed in standard, clean laboratory conditions. See Fig. 1 for detailed timeline of experimental procedures. All experiments were conducted using protocols in accordance with and approved by the Duke University Institutional Animal Care and Use Committee.

2.2. Helminth treatment

Adult breeding pairs were orally inoculated with either 4 *Hymenolepsis diminuta* cystercircoids (4 rat tapeworm larvae suspended in a drop of 0.6% sterile saline (n = 12/sex), or with sterile saline alone (n = 12/sex). Weaned pups received the same treatment as their parents on P21. See Fig. 1. The cysticercoids were harvested from mealworm beetles (*Tenebrio molitor*) using a hund Wetzlar Wilovert dissecting microscope. The beetles were previously inoculated with the organisms by feeding them the feces of colonized rats. Colonization with *H. diminuta* was confirmed in feces of rat parents and offspring using the McMaster technique (Sloss et al., 1994).

2.3. Escherichia coli infection

2.3.1. Bacterial culture

E. coli culture (ATCC 15746; American Type Culture Collection, Manassas, VA) vial contents were hydrated and grown overnight