Brain, Behavior, and Immunity 64 (2017) 23–32

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Named Series: Brain, Behavior, Immunity and the Microbiome

Individual differences in stress vulnerability: The role of gut pathobionts in stress-induced colitis

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ARTICLE INFO

Article history:
Received 26 August 2016
Received in revised form 15 December 2016
Accepted 16 December 2016
Available online 21 December 2016

Keywords:
Chronic psychosocial stress
Chronic subordinate colony housing (CSC)
Enterohepatic *Helicobacter* species (EHS)
Inflammatory bowel disease (IBD)
Microbiota
Colitis
Stress vulnerability
Stress resilience
Inflammation
Pathobionts

ABSTRACT

Chronic subordinate colony housing (CSC), an established mouse model for chronic psychosocial stress, promotes a microbial signature of gut inflammation, characterized by expansion of Proteobacteria, specifically *Helicobacter* spp., in association with colitis development. However, whether the presence of *Helicobacter* spp. during CSC is critically required for colitis development is unknown. Notably, during previous CSC studies performed at Regensburg University (University 1), male specific-pathogen-free (SPF) CSC mice lived in continuous subordination to a physically present and *Helicobacter* spp.-positive resident. Therefore, it is likely that CSC mice were colonized, during the CSC procedure, with *Helicobacter* spp. originating from the dominant resident. In the present study we show that employing SPF CSC mice and *Helicobacter* spp.-free SPF residents at Ulm University (University 2), results in physiological responses that are typical of chronic psychosocial stress, including increased adrenal and decreased thymus weights, decreased adrenal *in vitro* adrenocorticotropic hormone (ACTH) responsiveness, and increased anxiety-related behavior. However, in contrast to previous studies that used *Helicobacter* spp.-positive resident mice, use of *Helicobacter* spp.-negative resident mice failed to induce spontaneous colitis in SPF CSC mice. Consistent with the hypothesis that the latter is due to a lack of *Helicobacter* spp. transmission from dominant residents to subordinate mice during the CSC procedure, colonization of SPF residents with *Helicobacter typhlonius* at University 2, prior to the start of the CSC model, rescued the colitis-inducing potential of CSC exposure. Furthermore, using SPF CSC mice and *H. typhlonius*-free SPF residents at University 1 prevented CSC-induced colitis. In summary, our data support the hypothesis that the presence or absence of exposure to certain pathobionts contributes to individual variability in susceptibility to stress-/trauma-associated pathologies and to reproducibility of stress-related outcomes between laboratories.

1. Introduction

While some individuals subjected to severe adverse life events develop stress-related pathologies (Aromaa et al., 1994; Duffy et al., 1991; Kiecolt-Glaser and Glaser, 1995; Langgartner et al., 2015; McEwen, 2003, 2004; Tennant, 2001), others do not (Bartolomucci et al., 2004; Castro et al., 2012; Krishnan et al., 2007, 2008; Schmidt et al., 2010; Stiller et al., 2011). However, the underlying mechanisms engendering resilience or vulnerability

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http://dx.doi.org/10.1016/j.bbi.2016.12.019
0889-1591/

to the consequences of adverse life events are far from being understood and, thus, strategies to prevent stress-related pathologies are limited. Consistent with the idea that genetic (DeRijk and de Kloet, 2005; Ising et al., 2008; Kendler et al., 1996, 1994) and/or environmental (Aisa et al., 2007; Eiland and McEwen, 2012) factors modulate individual stress vulnerability, studies in mice suggest that a hyper-responsive immune response plays an important role in determining vulnerability to anxiety- and depressive-like behavioral responses following chronic psychosocial stress (Hodes et al., 2014); specifically, individual differences in interleukin 6 (IL-6) levels from *ex vivo* stimulated leukocytes predict vulnerability versus resilience to stress-induced anxiety and depressive-like behavioral responses, and transplant of bone marrow derived hematopoietic progenitor cells from IL-6 knockout donor mice, or treatment with an anti-IL-6 monoclonal antibody, induce stress resilience (Hodes et al., 2014). Meanwhile, our group showed that preimmunization with a heat-killed preparation of *Mycobacterium vaccae* (NCTC 11659), which has potent immunoregulatory and anti-inflammatory effects, promotes active stress coping and prevents chronic stress-induced colitis and anxiety (Reber et al., 2016). Given that *M. vaccae* is an abundant soil saprophyte with immunoregulatory properties (Zuany-Amorim et al., 2002), these findings suggest that individual differences in immunoregulation, either genetically or environmentally determined, might underlie variations in stress resilience.

The chronic subordinate colony housing (CSC) paradigm results, amongst other stress-related outcomes (Czech et al., 2013; Füchsl and Reber, 2016; Füchsl et al., 2013; Peters et al., 2012, 2013; Reber et al., 2008), in thymic involution, adrenal hypertrophy, decreased adrenal *in vitro* adrenocorticotropic hormone (ACTH) sensitivity, spontaneous colitis, and a long lasting increase in general anxiety-related behavior (Langgartner et al., 2015). Moreover, and in agreement with previous studies (Bassett et al., 2015; Belzer et al., 2014; Lin et al., 2014; Solnick and Schauer, 2001), CSC promotes colitogenic intestinal dysbiosis characterized by an increased abundance of Proteobacteria, including pathobionts (Chow et al., 2011) like *Helicobacter* spp., two unidentified genera within the family of Helicobacteraceae, as well as *Paraprevotella* (Bacteroidetes), in association with decreases in *Mucispirillum* (Reber et al., 2016). In line with these findings, social disruption stress has been shown to severely affect the composition of the gut microbiota, increasing susceptibility to the orally administered enteric pathogen *Citrobacter rodentium* (Bailey, 2012; Galley et al., 2017). Consistent with the idea that CSC-induced spontaneous colitis is causally mediated by the development of this colitogenic microbial milieu, abundance of both Proteobacteria and *Helicobacter* spp. correlated positively with the histological damage in the colon of single-housed control (SHC) and CSC mice (Reber et al., 2016). The latter is in agreement with studies showing that other stress paradigms increase intestinal *Helicobacter* spp. abundance in mice (Guo et al., 2009), and that intestinal *Helicobacter* abundance predicts intestinal inflammation scores in mice with impaired immunoregulation (IL-10−/− mice), whereas unstressed wildtype (WT) mice do not develop intestinal inflammation in response to *Helicobacter* spp. infection (Bassett et al., 2015; Kullberg et al., 2001; Solnick and Schauer, 2001). Of note, *M. vaccae* preimmunization did not prevent these stress/trauma-induced changes in gut microbial composition, but nevertheless provided protection from stress-induced spontaneous colitis and anxiety (Reber et al., 2016). Depletion of regulatory T cells prevented the stress resilience promoting effects of *M. vaccae* preimmunization, suggesting that the balance between inflammatory and immunoregulatory microbial inputs is an important determinant of stress resilience.

Noteworthy in the context of the current study is that C57BL/6N mice used as SHC or CSC mice are generally obtained from Charles River, where they are bred under specific-pathogen-free (SPF) conditions. Therefore, expansion of colitogenic bacteria belonging to the genus *Helicobacter* during CSC exposure can only occur if CSC mice become naturally infected with these pathobionts during CSC housing. Consistent with this hypothesis, these pathobionts were reliably detectable only in CSC mice on days 8 and 15 of CSC exposure, but not on days −21, −14, −7, and 1 before CSC exposure (Reber et al., 2016).

In the current study we are testing the hypothesis that CSC-induced colitis is essentially dependent on the transmission of *Helicobacter* spp. from dominant resident mice to CSC mice, supporting the idea that individual vulnerability to chronic psychosocial stress-associated colitis is strongly determined by the intestinal microbial milieu, including the presence of specific pathobionts.

2. Material and methods

2.1. Animals

Male SPF C57BL/6N (experimental mice weighing 19–22 g) and male SPF CD-1 (dominant resident mice weighing 30–35 g) mice were obtained from Charles River (Sulzfeld, Germany) for studies conducted at both Ulm (University 2) and Regensburg (University 1) University. In both locations, mice were exposed to a 12 h/12 h light dark cycle (lights on at 0600 AM), 22 °C, 60 % humidity, and had free access to tap water and standard mouse diet (Cat. No. V1535-000; SNIFF, Soest, Germany). After delivery to University 2, all mice were individually housed under SPF conditions for at least one week prior to the start of the experiment. After delivery to University 1, all mice were individually housed in a separate and thoroughly cleaned room of the non-SPF animal facility, to avoid colonization by pathobionts present in the colony (="isolated-non-SPF"). All experimental protocols were approved by the Committee on Animal Health and Care of the local government and performed according to the international guidelines on the ethical use of animals.

2.2. Experimental procedures

2.2.1. Ulm University (University 2)

In order to determine if CSC-induced colitis is dependent on the presence of certain pathobionts, CSC and SHC exposures were performed under SPF conditions using SPF CD-1 resident males. A first set of mice (Animal set 1: SHC, n = 16; CSC, n = 16) was tested for anxiety-related behavior in the open-field/novel object (OF/NO) test on day 19 of CSC between 0700 and 1000 AM. A second set (Animal set 2: SHC, n = 9; CSC, n = 12) was euthanized in the morning of day 20 between 0700 and 1000 AM for assessment of physiological and immunological changes. A third set (Animal set 3: SHC, n = 8; CSC, n = 8) was single-housed in the evening of day 19 and euthanized by rapid decapitation following brief CO_2 exposure in the evening of day 20 between 0700 and 1000 PM for assessment of plasma evening corticosterone (CORT) concentrations.

In order to determine if CSC-induced colitis is dependent on colonization of CSC mice with *Helicobacter* spp. originating from the dominant resident mice during CSC exposure, CD-1 dominant resident males were exposed to *H. typhlonius*-positive bedding until their own fecal samples tested positive for *H. typhlonius* by polymerase chain reaction (PCR), prior to the start of CSC exposure. *H. typhlonius*-positive bedding consisted of bedding and stool pellets taken from mice that were verified to be *H. typhlonius*-positive prior to the experiment. Of note, C57BL/6N mice used as CSC subordinate and SHC mice were kept under SPF conditions until the start of CSC. All experimental mice were weighed on days