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Kinetic characteristics of euflammation: The induction of controlled inflammation without overt sickness behavior

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ABSTRACT

We found recently that controlled progressive challenge with subthreshold levels of *E. coli* can confer progressively stronger resistance to future reinfection-induced sickness behavior to the host. We have termed this type of inflammation "euflammation". In this study, we further characterized the kinetic changes in the behavior, immunological, and neuroendocrine aspects of euflammation. Results show euflammatory animals only display transient and subtle sickness behaviors of anorexia, adipsia, and anhedonia upon a later infectious challenge which would have caused much more severe and longer lasting sickness behavior if given without prior euflammatory challenges. Similarly, infectious challenge-induced corticosterone secretion was greatly ameliorated in euflammatory animals. At the site of *E. coli* priming injections, which we termed euflammation induction locus (EIL), innate immune cells displayed a partial endotoxin tolerant phenotype with reduced expression of innate activation markers and muted inflammatory cytokine expression upon *ex vivo* LPS stimulation, whereas innate immune cells outside EIL displayed largely opposite characteristics. Bacterial clearance function, however, was enhanced both inside and outside EIL. Finally, sickness induction by an infectious challenge placed outside the EIL was also abrogated. These results suggest euflammation could be used as an efficient method to "train" the innate immune system to resist the consequences of future infectious/inflammatory challenges.

1. Introduction

Peripheral inflammation and the resultant release of cytokines have long been realized to be one of the main culprits in the pathogenesis of many CNS related disorders including, fatigue (Arnett and Clark, 2012), hyperalgesia (Sommer and Kress, 2004), anorexia (Langhans, 2007), and anhedonia (Salazar et al., 2012). Collectively these have been termed "sickness behavior" (Kelley et al., 2003). These centrally mediated sequelae are consistent features of systemic inflammation and are often associated with the presence of inflammagens or increased inflammatory cytokines in the blood. However, depending upon the level of the inflammatory challenge, sickness behavior may or may not manifest after localized peripheral inflammation. For example, well contained localized inflammation, such as those that occur during the healing of minor wounds, do not cause sickness behavior, but exhibit apparent local inflammatory histopathology including the infiltration of leukocytes and increased expression of inflammatory cytokines at the site of inflammation (Horan et al., 2005). We have recently simulated the type of inflammation that is unaccompanied with overt concomitant sickness behaviors by local administration of subthreshold levels of LPS or live *E. coli*. Interestingly, the kinetic responses to consecutive daily administration of subthreshold levels of LPS and *E. coli* differed dramatically. We found prior exposure to subthreshold levels of LPS sensitized mice to display a greater sickness behavior response upon subsequent LPS challenges (Tarr et al., 2012). However, following repeated *E. coli* administration, increased host resistance to the induction of sickness behavior by *E. coli* was evident if mice received prior challenges with subthreshold levels of *E. coli* (Chen et al., 2013). We have thus termed a peripheral inflammation that does not cause overt sickness behavior, yet primes the immune system to provide more resistance to a subsequent inflammatory stimulation as "euflammation." By using this definition we have restricted the training of innate immune activity within the boundary of "absence of overt sickness behavior", thereby preventing changes in the innate immunity from reaching hyper-inflammation.

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Additionally, we define the highest level of inflammagen that causes euflammation at a given time point without inducing decreased movement in the open field as maximal euflammatory potential (MEP).

Further investigation of euflammation needs to consider the dynamic characteristics of the inflammatory response. Depending on the dose level of the bacterial challenge, the time point for maximal sickness behavioral responses may vary. In addition, cells that express receptors important in the recognition of pathogens and the propagation of the immune response (e.g., MHCII, TLR4, and CD86) are recruited to the site of infection (Albiger et al., 2007). Higher expression of these receptors is indicative of an "activated" cellular phenotype. Associated with the activated immune phenotype, inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), and interleukin-10 (IL-10) that are important in innate immune function and communication are also increased through NF-κB signaling mechanisms (Lawrence, 2009). Upon TLR4 activation, these cytokines are released and bactericidal mechanisms are activated (e.g., nitric oxide) to help eradicate the pathogen (Wei et al., 1995). Furthermore, once the opsonization of bacteria and the subsequent antibody binding has occurred, activated macrophages phagocytize the bacteria as an additional mechanism of host defense (Aderem and Underhill, 1999). In addition, the hypothalamic–pituitary–adrenal (HPA) axis is activated upon bacterial challenge (Zimomra et al., 2011) which is well known to play critical a role in inflammatory-induced immunological and behavioral effects.

Recent research shows following repeated administration of bacteria or bacterial components (i.e., LPS), endotoxin tolerance (ET) can emerge (Biswas and Lopez-Collazo, 2009) or a short-term innate memory (trained immunity) which might last for days to months (Netea, 2013) can be generated. However, the majority of the studies examining these phenomena has used high levels of inflammagen and/or has used intravenous administration that causes a systemic response. In our euflammation model we give progressive subthreshold doses of bacteria in the peritoneal cavity (i.e., euflammatory induction locus [EIL]) which could yield substantially different results. We refer to the peritoneal cavity as the EIL because repeated exposure of *E. coli* in the present study occurred only at this location during euflammation induction as opposed to ET models which cause systemic inflammation.

In light of our previous report describing the beneficial effects of progressive euflammatory injections on sickness behavior (Chen et al., 2013), this report sought to further characterize the immunological, behavioral, and neuroendocrine changes during the kinetic induction of euflammation. Specifically, studies were designed to: (1) assess the kinetic nature of our euflammatory paradigm, (2) evaluate the extrapolation potential of euflammation to additional sickness behaviors, (3) determine if innate immune activity and function inside and outside the EIL in euflammatory animals follow the pattern of ET and/or trained immunity, and (4) assess the ability of euflammation to regulate neuroendocrine responses.

2. Methods

2.1. Subjects

Subjects were 6–8 week-old male FVB mice purchased from Charles River Laboratories (Wilmington, MA). Upon arrival, animals were separated according to experimental design and allowed to acclimate in the animal facility for ~1 week prior to the start of experimental procedures. Mice were kept in standard polycarbonate mouse cages and maintained on a 12 h light/dark cycle with lights being turned on at 0600 in an AAALAC (American Association of Accreditation of Laboratory Animal Care) facility. Food and water was available *ad libitum* unless experimental manipulations were being conducted. Animals were treated in compliance with the *Guide for the Care and Use of Laboratory Animals*, and experiments were carried out in accordance with a protocol approved by the Institutional Laboratory Animal Care and Use Committee (ILACUC) at The Ohio State University.

2.2. Euflammation induction

To obtain a robust euflammatory induction, *E. coli* cultures, strain, and injections were similar to procedures previously described by our laboratory (Chen et al., 2013). Briefly, animals were given intraperitoneal (i.p.) injections of GPF labeled *E. coli* (LT004; kindly provided by Dr. Monica Rydén Aulin of the Karolinska Institute, Solna, Sweden). Animals that received an *E. coli* injection(s) on the first day, second day, and/or third day (i.e., 2.0×10^7 on day 1, 25×10^7 on day 2, and 100×10^7 CFUs of *E. coli* on day 3) were designated as 1d-EU, 2d-EU, or 3d-EU groups, respectively. These progressive doses have reliably been shown to not cause overt sickness behavior in the open field box (Chen et al., 2013).

2.3. Experimental designs

2.3.1. Experiment 1: Time-course assessment of open field locomoter activity following a single bolus injection of E. coli or 3d-EU

To determine what time following injection with *E. coli* caused the maximal behavioral sickness response, three groups of animals ($n = 10$ per group) were tested in the open field for locomoter activity 1, 3, and 6 h following a single i.p. 25×10^7 *E. coli* injection. In addition, to assess if progressive euflammatory doses caused a shift in locomotor deficits another three groups of animals were given 3d-EU injections ($n = 8$–9/group). On day 3, each one of the groups was tested for locomoter activity in the open field 1, 3, or 6 h following the last injection.

2.3.2. Experiment 2: Food/water intake and sucrose preference following 3d-EU and single bolus E. coli administrations

To evaluate if euflammation caused alterations in sickness behaviors other than locomoter deficits, three groups of animals were given PBS, 3d-EU, or a single dose of 100×10^7 *E. coli* on day 3 following PBS on days 1 and 2 which served as a positive control (designated PC in Figs. 2 and 3; cage $n = 3$ with 3 animals/cage). Both food and water intake was measured 5 and 24 h following the last corresponding injection time point. In addition, additional signs of sickness behavior (i.e., anhedonia) were evaluated in 3 separate groups of animals (groups were the same as they were for food and water intake) for their preference of a sucrose solution 5 and 24 h following the last corresponding injection time point (cage $n = 4$–5 with 2 animals/cage).

2.3.3. Experiment 3: Phenotypic alterations in CD11b+ cells inside and outside of the EIL

To examine changes in the activation phenotype of myeloid cells that euflammation may cause, CD11b+ cells inside and outside of the EIL were examined by flow cytometry for their activation status in 2 groups of animals given PBS or 3d-EU ($n = 6$–7/group). Peritoneal cells inside the EIL, and blood and spleens outside the EIL, were harvested 24 h following the last round of injections. Cells were first identified for their CD11b positivity, then activation status was further determined based on MHCII, TLR4, and CD86 expression.