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Leptin is involved in age-dependent changes in response to systemic inflammation in the rat

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ABSTRACT

Obesity contributes to a state of subclinical peripheral and central inflammation and is often associated with aging. Here we investigated the source and contribution of adipose tissue derived cytokines and the cytokine-like hormone leptin to age-related changes in lipopolysaccharide (LPS)-induced brain-controlled sickness-responses. Old (24 months) and young (2 months) rats were challenged with LPS or saline alone or in combination with a neutralizing leptin antiserum (LAS) or control serum. Changes in the sickness-response were monitored by biotelemetry. Additionally, ex vivo fat-explants from young and old rats were stimulated with LPS or saline and culture medium collected and analyzed by cytokine-specific bioassays/ELISAs. We found enhanced duration/degree of the sickness-symptoms, including delayed but prolonged fever in old rats. This response was accompanied by increased plasma-levels of interleukin (IL)-6 and IL-1ra and exaggerated expression of inflammatory markers in brain and liver analyzed by RT-PCR including inhibitor κBα, microsomal prostaglandin synthase and cyclooxygenase 2 (brain). Moreover, for the first time, we were able to show prolonged elevated plasma leptin-levels in LPS-treated old animals. Treatment with LAS in young rats tended to attenuate the early- and in old rats the prolonged febrile response. Fat-explants exhibited unchanged IL-6 but reduced IL-1ra and tumor necrosis factor (TNF)-α release from adipose tissue of aged compared to young animals. In addition, we found increased expression of the endogenous immune regulator microRNA146a in aged animals suggesting a role for these mediators in counteracting brain inflammation. Overall, our results indicate a role of adipose tissue and leptin in "aging-related-inflammation" and age-dependent modifications of febrile-responses.

1. Introduction

Aging is associated with a complex array of physiological and behavioral changes, which can lead to an impaired immune response with an increased frequency of infection and increased mortality in the elderly population (Laupland et al., 2003). These age-linked alterations contribute to the development of a chronic low-grade inflammation in the brain and periphery (Bruunsgaard et al., 2001; Godbout and Johnson, 2009). Experimental treatment of aging rodents with the bacterial lipopolysaccharide (LPS) a potent immunogen, activates an array of sickness like responses such as fever (Dantzer et al., 2008), which are significantly exacerbated and prolonged in these animals compared with young adults (Godbout et al., 2005). These changes were associated with higher than normal concentrations of a number of inflammatory mediators including interleukin (IL)-6 (Godbout et al., 2005; Wei et al., 1992), IL-1β (Alvarez-Rodriguez et al., 2012), tumor necrosis factor (TNF)-α (Bruunsgaard et al., 1999), IL-1 receptor antagonist (IL-1ra) (Ferrucci et al., 2005) and IL-10 (Alvarez-Rodriguez et al., 2012) both in the periphery and the brain. These cytokines are pivotal mediators of brain inflammation and the induction of sickness symptoms (Dantzer et al., 2008). They act by genomic activation of cells via inflammatory transcription factors including the signal transducer and activator of transcription 3 (STAT3) or nuclear factor (NF)-IL6 activated by IL-6, or NFκB stimulated by IL-1β (Damm et al., 2011, 2013; Nadjar et al., 2005; Rummel et al., 2006, 2011a). Studies to date have been limited to investigating the changes in the levels of these mediators in both humans and animal models and in general have not addressed the immunoregulatory pathways during aging in depth. In the current study we aimed to perform a more detailed investigation that encompasses the analysis of changes in signaling pathways of cytokines (transcription factors) as well as additional relevant inflammatory markers such as

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the rate-limiting enzymes of the PG-synthesis pathway, cyclooxygenase 2 (COX2), microsomal prostaglandin (PG) E synthase (mPGES), and the inducible form of nitric oxide synthase (iNOS) all of which are important signals for fever induction (Kozak and Kozak, 2003; Roth et al., 2009). In addition, microRNA146a, a member of endogenous regulatory molecules potentially involved in age related immunological changes, was investigated (Quinn and O'Neill, 2011; Sonkoly et al., 2008).

Other than the increase in the basal levels of inflammatory mediators, aging has been reported to be also associated with elevated circulating leptin levels in rats (Scarpace et al., 2000). High plasma leptin levels are often but not necessarily coupled with a steady weight gain that is described as late-onset obesity (Gabriely et al., 2002; Scarpace and Tumer, 2001) although also increased (Baumgartner et al., 1999), unchanged or reduced leptin levels have been reported in elderly humans (Isidori et al., 2000) potentially age and gender dependent (Schautz et al., 2012). Leptin, is mainly known as a regulator of energy homeostasis by suppressing appetite and stimulating energy expenditure via activation of hypothalamic neurons (Halaas et al., 1995) but has recently been associated with a number of other physiological systems including immunity (Fantuzzi and Faggioni, 2000). Indeed we, and others demonstrated that this hormone is a neuro-immune mediator (Inoue et al., 2006) and is involved in mediating the fever response to LPS in rats (Harden et al., 2006; Luheshi et al., 1999; Sachot et al., 2004). Hyperleptinemia in elderly individuals often fails to exert the modulatory functions on energy homeostasis to the expected degree, for which full implication and exact mechanism are still not completely understood (Carrascosa et al., 2009).

Similar to aged rodents, obese rats respond to an immune stimulus with a prolonged fever response and an enhanced induction of central and peripheral inflammatory markers indicative of the proposed underlying systemic inflammation during obesity (Pohl et al., 2009). We and others have shown that one important source of this increase in systemic inflammation i.e. circulating cytokines during obesity is white adipose tissue (WAT) (Pohl et al., 2009; Trayhurn and Wood, 2005). Research on a contribution of presumptive adipose tissue inflammation to the increased inflammatory state in old individuals has revealed promising insights in previous studies in mice (Wu et al., 2007) but remains to be further elucidated.

In the present study, we wanted to obtain further insight into the contribution of new inflammatory candidate molecules to the age-related changes in the immune- and sickness response. In addition, age-associated conditions like hyperleptinemia and late-onset obesity i.e. WAT as potential source for increased systemic inflammation was investigated. For this purpose, we analyzed alterations in brain controlled sickness behavior and immunological signaling pathways in young and old rats after LPS-stimulation including mPGES, mir146a and markers of oxidative stress. To investigate the possible contribution of age-associated hyperleptinemia to the immunological alterations of the LPS-induced sickness response in old rats, we used a specific leptin antiserum (LAS) raised against rat leptin in young and old animals. In a last step, the presumed impact of adipose tissue on the peripheral chronic inflammation in elderly individuals was assessed by comparing the LPS-induced cytokine secretion from WAT-explants of young and old rats.

2. Materials and methods

2.1. Animals

Young (2 months) and old (23.17 ± 0.28 months) male Wistar rats (Rattus sp.) with a body weight (BW) of 200–250 g (young) or 637 ± 24 g (old) were used, originating from an in-house breeding colony with parent animals obtained from Charles River WIGA (Sulzfeld, Germany). Animal care, breeding, and experimental procedures were conducted according to guidelines approved by the local ethics committee (ethics approval number GI 18/2 Nr. 1/2011 and V54-19, c20/15c GI18/2). Eight days before the experiment, animals were implanted with intra-abdominal biotelemetry transmitters (VM-FR TR-3000; Mini-Mitter, Sunriver, OR) for measuring core body temperature and activity. Surgery was performed under general anesthesia using 60 mg/kg ketamine hydrochloride (Albrecht, Aulendorf, Germany) and medetomidin 0.25 mg/kg (CP Pharma Handelsgesellschaft GmbH, Burgdorf, Germany). Analgesic treatment was ensured pre- and post-surgery using Meloxicam (5 mg/kg, s.c. Boehringer Ingelheim, Ingelheim, Germany). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available. During the experiment (3 days before surgery, during recovery and for the injections; in total ~2 weeks) young and old rats were housed individually in a temperature- and humidity-controlled climatic chamber (Weiss Umwelttechnik GmbH, Typ 10'US/+5 to +40 DU, Germany), adjusted to 28 °C and 50% humidity on a 12:12-h light–dark cycle (lights off at 1900 h), with constant access to water and powdered standard lab chow. The 28 °C ambient temperature was chosen based on previous reports showing that old rats fail to mount a fever response to LPS at an ambient temperatures of 21 °C (Buchanan et al., 2003). Moreover, 29 °C has been reported to be thermoneutral for rats (Romanovsky et al., 2002). Output (frequency in HZ) of the biotelemetry transmitters was monitored at 5 min intervals by an antenna placed under each cage (RA-1000 radio receivers; Mini-Mitter). A data acquisition system (Vital View; Mini-Mitter) was used for automatic control of temperature and locomotor activity data collection and analysis. Water bottles and food supply dishes on scales in combination with the respective software (Accudiet 1.20; AccuScan Instruments, Columbus, OH, USA) allowed for continuous recording of food and water intake. At least 3 days before injections, animals were handled extensively for habituation to confinement and experimental procedures.

2.2. Treatment and experimental protocols

Experiment 1: Age-dependent differences in the LPS-induced sickness response.

On the day of the experiment, rats were injected intraperitoneally (i.p.) with LPS (100 μg/kg BW; derived from *Escherichia coli*, serotype 0128:B12; Sigma, Deisenhofen, Germany) or with an equal volume of sterile pyrogen-free 0.9% PBS (1 ml/kg; Dulbecco's phosphate-buffered saline; PAA, D-Cölbe). All injections took place between 1100 and 1300 h. After 24 h, animals were deeply anesthetized with sodium pentobarbital (160 mg/kg i.p., Narcoren; Merial, Hallbergmos, Germany), and blood samples collected via cardiac puncture for the analysis of circulating cytokines. Subsequently, rats were transcardially perfused with 200–300 ml ice-cold 0.9% saline, brains and liver quickly removed, frozen in powdered dry ice, and stored at −55 °C until analysis.

Experiment 2: Age-dependent effects of leptin antiserum on the LPS-induced sickness response.

On day 1, rats were injected i.p. with neutralizing sheep anti-rat leptin antiserum (LAS) (S4159B1, NIBSC, Potters Bar, UK) or normal sheep serum (NSS) (Sigma–Aldrich, München, Germany) at 1 ml/kg BW in combination with PBS-injection. This procedure enabled the recording of control values for physiological data without sacrificing additional animals. On day 2, rats received a second dose of LAS or NSS in combination with LPS-injection (100 μg/kg BW). A previous study (Sachot et al., 2004) in young animals had shown, that this moderate dose of LAS has an attenuating effect on LPS-induced