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Differential effects of peripheral and brain tumor necrosis factor on inflammation, sickness, emotional behavior and memory in mice

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ABSTRACT

Tumor necrosis factor alpha (TNF) is increased in depression and clinical-trial evidence indicates that blocking peripheral TNF has some antidepressant efficacy. In rodents, peripheral or intracerebroventricular TNF results in sickness e.g. reduced body weight, altered emotional behavior and impaired memory. However, the underlying pathways and responsible brain regions are poorly understood. The aim of this mouse study was to increase understanding by comparing the effects of sustained increases in TNF in the circulation, in brain regions impacted by increased circulating TNF, or specific brain regions. Increased peripheral TNF achieved by repeated daily injection (IP-TNF) or osmotic pump resulted in decreased body weight, decreased saccharin (reward) consumption, and increased memory of an aversive conditioned stimulus. These effects co-occurred with increased plasma interleukin-6 and increased IP-derived TNF in brain peri-ventricular regions. An adenovirus-associated viral TNF vector (AAV-TNF) was constructed, brain injection of which resulted in dose-dependent, sustained and region-specific TNF expression, and was without effect on blood cytokine levels. Lateral ventricle AAV-TNF yielded increased TNF in the same brain regions as IP-TNF. In contrast to IP-TNF it was without effect on body weight, saccharin consumption and fear memory, although it did increase anxiety. Hippocampal AAV-TNF led to decreased body weight. It increased conditioning to but not subsequent memory of an aversive context, suggesting impaired consolidation; it also increased anxiety. Amygdala AAV-TNF was without effect on body weight and aversive stimulus learning-memory, but reduced saccharin consumption and increased anxiety. This study adds significantly to the evidence that both peripheral and brain region-specific increases in TNF lead to both sickness and depression- and anxiety disorder-relevant behavior and do so via different pathways. It thereby highlights the complexity in terms of indirect and direct pathways via which increased TNF can act and which need to be taken into account when considering it as a therapeutic target.

1. Introduction

Major depressive disorder (hereafter depression) is a heterogeneous mental illness with symptoms including sadness, amotivation, helplessness, fatigue, weight change, sleep disturbance, impaired cognition, and suicidality (American Psychiatric Association, 2013; World Health Organization, 1992). Its prevalence is increasing and by 2020 depression is predicted to be the second major cause of disability worldwide (Murray and Lopez, 1996). It has a high co-morbidity with anxiety disorders (American Psychiatric Association, 2013). The etio-pathophysiology of depression remains poorly understood, however, and with current-generation therapies only one third of patients reach full remission (Warden

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et al., 2007). Recently, human studies have linked depression with inflammation, including the pro-inflammatory cytokine network (Dantzer et al., 2008; Maes, 1995; Miller et al., 2009; Smith, 1991). Depression exhibits high comorbidity with chronic inflammatory diseases including rheumatoid arthritis and multiple sclerosis, and is prevalent in individuals undergoing chronic cytokine therapy (Dantzer et al., 2008). The term sickness is often used to describe the weight loss, amotivation, fatigue and impaired cognition that occur in such patients (and indeed those with common pathogen infections), and the overlap with depression – at the symptom level at least – is clear. Tumor necrosis factor alpha (TNF-α, hereafter TNF), a pro-inflammatory cytokine, exhibits increased levels in blood (Dowlati et al., 2010) and brain (in its transmembrane form, (Dean et al., 2010)) in depression patients. Indeed, peripheral blockers of TNF action are under clinical investigation for treatment of depression: with the monoclonal TNF antibody infliximab, efficacy was demonstrated in a sub-group of patients with high baseline plasma CRP and TNF levels (Raison et al., 2013), and with the TNF blocker etanercept, depression and fatigue scores were reduced in psoriasis patients (Tyring et al., 2006).

Animal studies are essential to investigate for causality between increased TNF levels in the circulation or brain, and physical, behavioral and neurobiological changes relevant to sickness and depression. Whilst only a small number of such studies have been conducted to date, they have yielded supportive evidence for important TNF effects. In mice, acute TNF administration in the periphery led to reduced body weight and less social exploration (Bluthé et al., 1994) and reduced operant self-stimulation of lateral hypothalamus (van Heesch et al., 2013). Acute delivery of TNF into the lateral brain ventricles (intra-cerebroventricular, i.c.v.) also reduced social exploration (Bluthé et al., 1994; Palin et al., 2007, 2009). Furthermore, it reduced active responding in the forced swim test and tail suspension test, and decreased consumption of the gustatory reward, sucrose (Kaster et al., 2012). In the latter study, these TNF effects were prevented by i.c.v. co-administration of a TNF antibody (Kaster et al., 2012). Animal manipulations that model infection or chronic inflammatory disease and induce, as one of a constellation of immune responses, increased TNF levels, also lead to sickness and depression- and anxiety disorder-relevant behavioral changes. For example, administering mice with peripheral lipopolysaccharide (LPS) to activate the innate immune system, led to increased plasma TNF (Biesmans et al., 2013; Dantzer et al., 2008) and brain *Tnf* mRNA (Lawson et al., 2013), and loss of body weight, reduced social exploration (Bluthé et al., 2000) and sucrose drinking (Frenois et al., 2007; Lawson et al., 2013). Also in mice, immune activation of the TNF receptor superfamily member CD40, important in the host-pathogen response and autoimmune disorders, induced increased levels of TNF in plasma and of *Tnf* mRNA, although not TNF protein, in brain, whilst reducing body weight and feeding, increasing sleep, reducing sucrose drinking and impairing learning; aspects of the immune response and the behavioral effects were prevented by peripheral TNF blocking with etanercept (Gast et al., 2013; Cathomas et al., 2015b). Murine cytomegalovirus induced increased levels of TNF and interleukins in blood and of their respective genes in brain, with TNF contributing to an observed hyper-anxiety (Silverman et al., 2007). Experimental autoimmune encephalomyelitis (EAE) in mice, a model of multiple sclerosis, led to reduced activity in the center of an open field and impaired learning-memory, concurrent with increased brain TNF levels and signaling; certain of these effects were blocked by i.c.v. etanercept (Acharjee et al., 2013; Haji et al., 2012; Habbas et al., 2015). Chronic psychosocial stress in mice has also been demonstrated to increase TNF and interleukin levels and to lead to depression- and anxiety disorder-relevant behavior (Azzinnari et al., 2014; Fuertig et al., 2016; Kinsey et al., 2008).

Therefore, there is rodent evidence that acute and specific increases in TNF in either the periphery or brain lead to weight loss and specific changes in behavior of relevance to depression and anxiety disorder, with the two routes of TNF administration leading to similar effects. Furthermore, there is rodent evidence that more prolonged increases in TNF in periphery and brain, induced as part of a constellation of immune changes by LPS or CD40-activation, lead to weight loss, other markers of sickness, and depression- and anxiety disorder-relevant behavior. Against this background, the aims of the current mouse study were to investigate and directly compare the effects of (1) continuous/daily-repeated peripheral TNF administration versus (2) continuous brain region-specific viral vector TNF expression on: inflammation in periphery and brain, body weight, emotional behavior, and memory. The behavioral tests utilized were the two-bottle saccharin versus water test for assessment of interest in gustatory-reward; general context or specific auditory-stimulus conditioning to footshock for assessment of fear reactivity, and next-day test of fear expression for assessment of memory; two-way approach-avoidance conflict test for assessment of anxiety. In the research domain criteria (RDoC) framework for mental health research, reward interest is a dimension in the domain Positive valence systems, fear and anxiety are in the domain Negative valence systems, and memory is in the domain Cognitive systems (Cuthbert and Insel, 2013). The observed constellations of immune, physical and behavioral effects induced by TNF were compartment- and brain region-specific. This study adds significantly to the evidence that a sustained increase in either peripheral or central TNF exerts effects on sickness and emotional and cognitive behaviors, with the latter effects being brain region-dependent. It highlights the complexity in terms of indirect and direct pathways via which increased TNF can act and which need to be taken into account when considering it as a therapeutic target for the treatment of sickness and affective pathologies.

2. Materials and methods

2.1. Animals and maintenance

Male C57BL/6J mice (Janvier Labs, Le Genest-Saint-Isle, France) were delivered in littermate pairs and accustomed to the new environment for two weeks. Mice were aged 10 to 12 weeks and weighed 25.0 to 30.0 g at study onset. They were maintained on a reversed 12:12 h light-dark cycle (lights off 07:00–19:00) in an individually-ventilated caging system (IVC) at 20–22 °C and 50–60% humidity. Cages were type 2L and contained woodchips, a sleep igloo and tissue bedding. Complete-pellet diet (Provimi, Kliba Ltd, Kaiseraugst, Switzerland) and water were available continuously. In the week prior to the onset of an experiment, mice were handled on three days. The study was conducted under a permit (170/2012) for animal experimentation issued by the Veterinary Office, Zurich, Switzerland. All efforts were made to minimize the number of mice used and unnecessary stress.

2.2. Experiments with repeated i.p. TNF injection

Daily repeated peripheral injection of recombinant murine TNF (ImmunoTools, Friesoythe, Germany) was performed intraperitoneally (i.p.) in non-anaesthetized mice. TNF was reconstituted in dH_2O vehicle and, based on previous studies (Bluthé et al., 1994; van Heesch et al., 2013), injected daily at a concentration of 20 or 40 ng/g body weight (in a 25 g mouse, equivalent to