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Systemic TNF-α produces acute cognitive dysfunction and exaggerated sickness behavior when superimposed upon progressive neurodegeneration

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ABSTRACT

Inflammation influences chronic neurodegeneration but its precise roles are not yet clear. Systemic inflammation caused by infection, trauma or co-morbidity can alter the brain's inflammatory status, produce acute cognitive impairments, such as delirium, and drive new pathology and accelerated decline. Consistent with this, elevated systemic TNF-α is associated with more rapid cognitive decline over 6 months in Alzheimer's disease patients. In the current study we challenged normal animals and those with existing progressive neurodegeneration (ME7 prion disease) with TNF-α (i.p.) to test the hypothesis that this cytokine has differential effects on cognitive function, sickness behavior and features of underlying pathology contingent on the animals' baseline condition. TNF-α (50 μg/kg) had no impact on performance of normal animals (normal brain homogenate; NBH) on working memory (T-maze) but produced acute impairments in ME7 animals similarly challenged. Plasma TNF-α and CCL2 levels were equivalent in NBH and ME7 TNF-challenged animals but hippocampal and hypothalamic transcription of IL-1β, TNF-α and CCL2 and translation of IL-1β were higher in ME7 + TNF-α than NBH + TNF-α animals. TNF-α produced an exaggerated sickness behavior response (hypothermia, weight loss, inactivity) in ME7 animals compared to that in NBH animals. However a single challenge with this dose was not sufficient to produce *de novo* neuronal death, synaptic loss or tau hyperphosphorylation that was distinguishable from that arising from ME7 alone. The data indicate that acutely elevated TNF-α has robust acute effects on brain function, selectively in the degenerating brain, but more sustained levels may be required to significantly impact on underlying neurodegeneration.

1. Introduction

It has become clear that inflammation contributes to chronic neurodegeneration but its precise roles are not yet clear and there remains no effective treatment for slowing the progression of chronic conditions such as Alzheimer's and Parkinson's disease. There is substantial epidemiological evidence that inflammatory co-morbidities are significant risk factors for dementia (Yaffe et al., 2004; Dunn et al., 2005; Reitz et al., 2011) and taking non-steroidal anti-inflammatory drugs protects against subsequent development of AD (Etminan et al., 2003). Consistent with a role of co-morbid inflammation, we and others have shown that systemic inflammation can robustly alter brain inflammatory status, inducing a switching of microglial phenotype from 'primed' to activated or from M2 to M1, with the consequence of acutely elevated brain levels of IL-1β (Cunningham et al., 2005; Godbout et al., 2005; Pott-Godoy et al., 2008). The pro-inflammatory cytokine IL-1β has been shown to contribute to impaired cognitive function and decreased neuronal viability (Relton and Rothwell, 1992; Vereker et al., 2001; Balosso et al., 2008; Pott-Godoy et al., 2008; Vezzani et al., 2011) in a number of model systems. In animal models of chronic neurodegeneration, such superimposed inflammatory activation can produce *de novo* pathology and has been shown to exacerbate the progression of neurodegenerative disease (Sheng et al., 2003; Kitazawa et al., 2005; Lee et al., 2008; Cunningham et al., 2009; Field et al., 2010; Krstic et al., 2012). However the mechanisms by which systemic inflammation exacerbate neurodegeneration remain unclear.

While many systemic inflammatory molecules may exacerbate cognitive decline, the role of systemic TNF-α has been of particular interest. It has been shown in a clinical population of AD patients

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that Systemic Inflammatory Events (SIEs) were associated with more rapid cognitive decline over 6 months (approximately 2-fold) and when those patients with SIE also had elevated plasma TNF-α levels, this was associated with a 10-fold greater rate of cognitive decline over the 6 month observation period (Holmes et al., 2009). Analysis of the Neuropsychiatric inventory (NPI) from the same cohort showed that elevated systemic TNF-α was associated with a 2-fold increase in symptoms characteristic of sickness behavior including apathy, anxiety, depression and agitation (Holmes et al., 2011). Elevated baseline TNF-α has also been associated with lower hippocampal volume (Sudheimer et al., 2014) and with greater likelihood of mild cognitive impairment (MCI) patient conversion to AD (Tarkowski et al., 2003) suggesting increased systemic TNF-α may have roles in hippocampal neurodegeneration. Neuroscientific studies of TNF-α have revealed a potentially complex biphasic role: administration of anti-TNF-α antibodies i.c.v. decreased Aβ, tau hyper-phosphorylation and memory deficits in AD-related models (Medeiros et al., 2007; Shi et al., 2011) but crossing of 3xTgAD mice with TNFR1/R2 double knockout mice actually exacerbated amyloid and Tau pathology and microglia from these mice had impaired phagocytic ability (Montgomery et al., 2011).

We sought to specifically investigate whether exogenously added systemic TNF-α has deleterious effects on cognitive function, inflammation, sickness behavior and neurodegeneration on a background of robust chronic neurodegeneration. In this study we used the ME7 model of prion disease, which shows robust synaptic loss, extracellular amyloidosis and a sequence of behavioral and cognitive impairments (Betmouni et al., 1999; Cunningham et al., 2003) and which we have previously shown to show acute neuronal death and more rapid decline after systemic challenge with LPS (Cunningham et al., 2005, 2009). We assessed the cognitive-disrupting effects of acute systemic TNF-α in ME7 and normal brain homogenate-injected controls (NBH) using a T-maze working memory test. We then examined hippocampal and hypothalamic mRNA, plasma proteins and hippocampal IL-1β protein induced by systemic TNF-α. The interaction of systemic TNF-α with existing pathology was also measured in ME7 and NBH animals using standard measures of sickness behavior: core body temperature, weight loss and open field activity. Finally the occurrence of *de novo* neuronal pathology after a single (i.p.) challenge with TNF-α was explored using quantification of apoptotic cell death, synaptic loss and tau hyperphosphorylation post-TNF-α in ME7 and NBH animals.

2. Methods

2.1. Animals and stereotaxic surgery

Female C57BL/6 mice (Harlan, Bicester, United Kingdom) were housed in cages of 5 at 21 °C with a 12:12 light, dark cycle. Food and water access was *ad libitum*. Mice were anaesthetized intraperitoneally (i.p.) with Avertin (2,2,2-tribromoethanol 50% w/v in tertiary amyl alcohol, diluted 1:40 in H_2O; 20 ml/kg, i.p.; Sigma) and positioned in a stereotaxic frame (Kopf instruments). Holes were drilled at −2.0 mm (anteroposterior) and ±1.6 mm (lateral, either side of the midline) from Bregma and 1 μl 10% w/v ME7-infected C57BL/6 brain homogenate was injected into the hippocampus using a Hamilton microsyringe (Reno, Nevada, USA) to a depth of −1.7 mm. The needle was left in situ for 2 min before slow withdrawal. Control animals were administered 1 μl 10% w/v normal brain homogenate (NBH). Mice were placed in an incubator at ~25 °C for recovery. When returned to their home cage they were administered Sucrose (5% w/v) and Carprofen (0.05% v/v; Rimadyl, Pfizer, Ireland) in their drinking water for 2 days post-surgery. Animals were monitored for recovery from surgery. All animal experimentation was performed under a license granted by the Minister for Health and Children, Ireland, with approval from the TCD Animal Research Ethics Committee and in compliance with the Cruelty to Animals Act, 1876 and the European Community Directive, 86/609/EEC. Every effort was made to minimize stress to the animals.

Animals were intraperitoneally administered recombinant mouse TNF-α (Peprotech, Rocky Hill, NJ, USA) at doses of 50 μg/kg or 250 μg/kg prepared in sterile saline (Sigma, Poole, UK). Lower doses were used for the T-maze experiments and higher doses for the pathology experiment. Our previous studies using these 2 TNF doses (Skelly et al., 2013) suggested that sickness at the earliest times points assessed was not terribly different between these 2 doses. Therefore we performed our cognitive experiments with the minimum dose that was practical (50 μg/kg i.p.) since more robust sickness may suppress the mouse behavioral repertoire too significantly to allow robust assessment of cognitive function (due to motivational and motor confounders). However, it has been our experience with LPS effects on cognition and *de novo* pathology that more intense systemic inflammation was required to produce damage (500 μg/kg LPS; (Cunningham et al., 2005)) versus that required to produce measureable cognitive dysfunction (100 μg/kg; (Murray et al., 2012)). This five-fold difference in potency guided our choice of doses in the current experiments with TNF-α. Control animals were injected using sterile saline (Sigma, Poole, UK) 200 μl per 20 g body weight. In additional experiments ME7 animals were challenged with LPS (Salmonella equine abortus, 500 μg/kg, Sigma, Poole, UK) in the presence or absence of the dominant negative TNF inhibitor XPRO1595 (a generous gift from David Szymkowski, Xencor, Monrovia, CA) at 30 mg/kg, i.p. 1 h before treatment with LPS. Appropriate action of XPRO1595 was assessed by administration of XPRO1595 (30 mg/kg i.p.) 1 h before TNF-α (50 μg/kg) to block TNF-induced hypothermia.

These TNF-α challenges were made at 16 weeks for T-maze experiments since we have previously established that ME7 animals at 18 weeks can no longer perform the T-maze at baseline (ie before acute inflammatory challenge). However our prior demonstrations of *de novo* neuropathology induced by systemic inflammation in ME7 animals has been exclusively at 18–19 weeks, which is the period when neuronal cell soma loss is determined to be occurring in this prion model (Cunningham et al., 2003, 2005).

2.2. Working memory

Hippocampal-dependent working memory was assessed in mice by observing alternation behavior in a novel water T-maze task (Murray et al., 2012). The T-maze, constructed from black perspex, was of the following dimensions (cm); long axis 67, short axis 38, depth 20 and arm width 7. Single 4 cm diameter holes were at the end of each choice arm, 2 cm from the floor and black exit tubes were fitted into these holes. Solid or permissive exit tubes could be fitted into these holes as appropriate, to facilitate/block escape at any location. A "guillotine" door was inserted to prevent access to one or other choice arm on the first run of each trial. Water to a depth of 2 cm at a temperature of 20 °C was poured into the maze; at this height a mouse must paddle and thus is motivated to leave the maze through an exit tube. Animals were taken with their cage mates to a holding cage. Each mouse was individually placed in the start arm of the maze with 1 arm blocked, such that they were forced to make a left or right turn. The arm sequences were chosen in a pseudo-random manner, with equal numbers of left and right turns in any one session; moreover, there were no more than 2 consecutive runs to the same arm. On making the turn the mouse could escape from the water by entering the exit tube and walking to a transit tube, in which it was carried to