



Tumour necrosis factor receptor deficiency alters anxiety-like behavioural and neuroendocrine stress responses of mice

Ulrike Gimsa^{a,*}, Ellen Kanitz^a, Winfried Otten^a, Margret Tuchscherer^a, Armin Tuchscherer^b, Saleh M. Ibrahim^c

^a Research Unit Behavioural Physiology, Leibniz Institute for Farm Animal Biology, Wilhelm-Stahl-Allee 2, D-18196 Dummerstorf, Germany

^b Research Unit Genetics and Biometry, Leibniz Institute for Farm Animal Biology, Wilhelm-Stahl-Allee 2, D-18196 Dummerstorf, Germany

^c Dept. of Dermatology, University of Lübeck, Ratzeburger Allee 160, D-23538 Lübeck, Germany

ARTICLE INFO

Article history:

Received 18 May 2011

Received in revised form 1 March 2012

Accepted 4 April 2012

Available online 4 May 2012

Keywords:

Transgenic mice

Behaviour

Psychosocial stress

HPA reactivity

TNF receptor

ABSTRACT

Tumour necrosis factor (TNF)-alpha is known to be involved in anxiety and the regulation of the hypothalamic–pituitary–adrenal axis. To examine the role of its receptors in neuroendocrine immunomodulation, we studied behaviour, corticosterone production and T-cell activation in mice with a C57BL/6J background and deficient for one or both TNF receptors (TNFR1^{−/−}, TNFR2^{−/−}, and TNFR1 + 2^{−/−}) compared to wildtype C57BL/6J mice with and without psychological stress. Stress was induced by social disruption (SDR), and anxiety-like behaviour was examined using the elevated plus maze (EPM). Anxiety of unstressed TNFR1 + 2^{−/−} mice was increased compared to C57BL/6J mice as shown by reduced ratios of entries into open arms relatively to total entries. SDR-stressed TNFR1 + 2^{−/−} mice showed reduced ratios of entries into open arms relatively to total entries, reduced ratios of distances walked in open relatively to distances walked in both arms and reduced time in open arms compared to C57BL/6J mice. Locomotor activity of unstressed and SDR-stressed TNFR1^{−/−} and TNFR2^{−/−} mice was reduced. Serum corticosterone concentrations of control mice do not differ between mouse strains. However, TNFR1 + 2^{−/−} mice had significantly higher corticosterone concentrations than C57BL/6J mice after SDR. EPM testing significantly increased corticosterone concentrations in all strains. Mitogen-induced activation-marker expression was reduced in TNFR1^{−/−} T-helper cells under control and stress conditions, while activation marker expression of TNFR2^{−/−} and TNFR1 + 2^{−/−} cells was only slightly affected by stress compared to C57BL/6J T cells. Our study suggests that both TNF receptors contribute to anxiety-like behaviour and corticosterone responses, whereas TNFR1 has a larger impact on T-cell activation.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Tumour necrosis factor-alpha (TNF-α) is an inflammatory cytokine and a key regulator of brain–endocrine–immune interactions. It is able to activate the hypothalamic–pituitary–adrenal (HPA) axis, increase corticosterone levels [1,2], mediate sickness behaviour and depression [2–5] and induce anxiety [6]. The inhibition of TNF-α action in the brain has been shown to reduce HPA axis activation in response to local inflammation [7]. However, to our knowledge, nothing is known about the role of TNF-α signalling during the activation of the HPA axis in response to stress.

* Corresponding author. Tel.: +49 38208 68803; fax: +49 38208 68802.

E-mail addresses: gimsa@fhn-dummerstorf.de (U. Gimsa), ekanitz@fhn-dummerstorf.de (E. Kanitz), otten@fhn-dummerstorf.de (W. Otten), mtuchsch@fhn-dummerstorf.de (M. Tuchscherer), atuchsch@fhn-dummerstorf.de (A. Tuchscherer), saleh.ibrahim@uk-sh.de (S.M. Ibrahim).

TNF-α signals via two receptors: TNFR1 (p55) and TNFR2 (p75). These receptors share most of their extracellular domains but have different intracellular domains, signalling pathways and functions [8–10]. While the disruption of the *TNFR1* gene leads to defective development of germinal centres, the disruption of the *TNFR2* gene has no effect on lymphoid organogenesis [11–13]. It is known that immune functions are modulated by psychosocial stress, including immune functions in mice and rats [14–16].

Because of the behavioural effects of TNF-α, there have been a number of studies on mice with a deletion of the TNF-α gene (TNF-α^{−/−}) or disrupted TNF-signalling (TNFR1^{−/−}, TNFR2^{−/−} or TNFR1/TNFR2 double knockout mice) focusing on anxiety, sickness behaviour, depression and aggressive behaviour [5,17–23]. Interestingly, TNF-α^{−/−} mice showed increased anxiety in the elevated plus maze (EPM) [17]. In contrast, TNFR1^{−/−} and TNFR2^{−/−} mice showed no significant differences in the EPM compared to wildtype C57BL/6J mice or non-deficient littermates [18,19]. TNFR1/TNFR2 double knockout mice (TNFR1 + 2^{−/−}) did not show

altered anxiety-like behaviour in the light/dark box in one study [24] but did show decreased anxiety in the light/dark box and the open field in another study [23].

While numerous studies have investigated the role of TNF in the immune system and in behavioural studies, as of yet there is no investigation of behavioural and immunomodulatory functions of TNF under psychological stress conditions. The aim of this study was to investigate whether TNFR1^{−/−}, TNFR2^{−/−} and TNFR1 + 2^{−/−} mice show alterations in anxiety, neuroendocrine regulation, and immune parameters in response to psychological stress compared to wildtype C57BL/6J mice. We used social defeat (i.e., young male mice losing a confrontation with a physically superior and more aggressive older intruder) as a psychosocial stressor, which has been shown to induce anxiety in mice [25], and combined it with a behavioural test, i.e., the EPM, which is also anxiogenic [26]. We analysed anxiety-like behaviour and serum corticosterone concentrations in response to stress. We investigated adaptive immunity by flow cytometric staining of early (CD69 and CD25) and late (CD71) CD4⁺ T-cell activation markers in response to mitogens.

2. Material and methods

2.1. Mouse strains

The inbred strains C57BL/6J and NZB/B1n were obtained from Jackson Laboratory (Bar Harbor, MA, U.S.A.). Transgenic animals on a C57BL/6J background deficient for TNFR1, TNFR2 or both were provided by A. Fontana, Switzerland (for details see [27]). Six-week old male mice were used for the experiments. Animals were allowed to adapt to the housing conditions at the animal facility at the University of Rostock for at least three weeks prior to experiments. They were housed with their siblings (three males per cage) before and during experiments. All procedures were pre-approved by the local Animal Care Committee and are in accordance with the European Council Directive of 24 November 1986 (86/609/EEC). Wildtype C57BL/6J, TNFR1^{−/−}, TNFR2^{−/−} and TNFR1 + 2^{−/−} mice will further be referred to as B6, R1, R2 and R1 + 2, respectively. Mice were analysed in four experimental groups: control, social disruption stress (SDR), elevated plus maze (EPM) and SDR followed by EPM testing (SDR + EPM). Numbers of individuals in each group are listed in Table 1.

2.2. Social disruption (SDR) stress

Cages of three mice (brothers) were randomly assigned as either control or SDR groups. Control mice remained undisturbed in their home cage. Stressed mice experienced two SDR events over two days; stress was induced by introducing an aggressive intruder, i.e., a retired older breeder male (NZB/B1n > 1 year of age), into the home cage for 6 h (07.00 h to 13.00 h) and again from 06.30 to 09.30 h the following day. Behaviour was observed to ensure that the intruder attacked the residents and that the residents showed submissive postures. All of the attacked mice received small back or tail bite wounds. None of the group-housed control mice were

injured. After the second SDR cycle, mice were either immediately sacrificed by cervical dislocation or tested 20–100 min later on an EPM for 10 min (see Section 2.4) and then sacrificed immediately afterwards.

2.3. Sample collection

After cervical dislocation, the mice were rapidly decapitated, and their trunk blood was collected. Blood was allowed to clot for 2 h and was then centrifuged at 2000g for 10 min. Sera were stored at −20 °C until corticosterone analysis. Spleens were isolated for cell culture.

2.4. Behavioural test

The EPM consists of four arms of 30-cm length and 5-cm width each. Two opposing arms are enclosed in opaque 15-cm high side and end walls. The elevation of the structure is 60 cm. Illumination was 50 lux in the closed arms and 400 lux in the open arms. We tested anxiety based on the following parameters: (i) entries into open arms, (ii) ratio of entries into open arms relatively to total arm entries, (iii) distances the mice walked in open arms, (iv) ratio of distances walked in open arms relatively to distances walked in both arms, (v) time spent in open arms and (vi) defecation numbers. Locomotor activity was determined as (i) total entries into open and closed arms, (ii) entries into closed arms, (iii) distance in closed arms, and (iv) time in closed arms. The conflict between approaching and avoiding risk, i.e., to enter the “dangerous” open arms, was judged from (i) entries into the central position, (ii) time in the central position, (iii) head dips from a closed arm into an open arm, and (iv) returns into closed arms after briefly entering the central position. Mice were put into the central position of the maze and left undisturbed for 10 min. Their behaviour was recorded by video camera and later analysed by a trained observer blind to the treatment. Mice with and without prior SDR stress were alternately tested to minimise influences from time progression after SDR stress and time of day. The test apparatus was thoroughly cleaned between sessions. The EPM test was carried out by an investigator who was blind to the treatment (SDR or control), which was applied in another room. To avoid stress influences on the mice, the mice were sacrificed in a separate laboratory.

2.5. Corticosterone analysis

Serum corticosterone concentrations were measured in duplicate using a commercially available double antibody rat corticosterone ¹²⁵I-RIA kit (DRG Diagnostics, Marburg, Germany) according to the manufacturer's instructions. Cross-reactivities of the antibody with any potentially competing serum steroids were lower than 0.5%. The test sensitivity was 7.2 ng/ml, and intra- and inter-assay coefficients of variation (CV) were 6.9% and 8.1%, respectively.

2.6. Analysis of T-cell activation

Spleen cell suspensions were freshly prepared from sacrificed mice as previously described [28]. Cells were stimulated with

Table 1
Numbers of mice in each experimental group.

Parameter	C57BL/6J (B6)				TNFR1 ^{−/−} (R1)				TNFR2 ^{−/−} (R2)				TNFR1 + 2 ^{−/−} (R1 + 2)			
	Ctrl	SDR	EPM	SDR + EPM	Ctrl	SDR	EPM	SDR + EPM	Ctrl	SDR	EPM	SDR + EPM	Ctrl	SDR	EPM	SDR + EPM
Behaviour			15	15			9	10			8	9			9	7
Corticosterone	15	17	15	15	13	14	10	10	8	5	8	9	13	10	9	9
T-cell activation	8	8	6	9	9	8	6	7	8	5	8	9	7	7	8	9

Total number of mice: 181. ctrl = control; SDR = social disruption; EPM = elevated plus maze.

Download English Version:

<https://daneshyari.com/en/article/2794460>

Download Persian Version:

<https://daneshyari.com/article/2794460>

[Daneshyari.com](https://daneshyari.com)