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Attenuation of the cytotoxic T lymphocyte response to lymphocytic choriomeningitis virus in mice subjected to chronic social stress

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ABSTRACT

Chronic stress is suspected to increase the susceptibility to infections but experimental evidence from physiological stress models is scarce. We examined the effects of chronic social stress on virus-specific CTL responses in mice after infection with lymphocytic choriomeningitis virus (LCMV). Mice subjected to social stress on six consecutive days prior to infection showed a significant reduction of IFN-γ producing T_{CD8+} splenocytes and markedly lowered plasma concentrations of IFN-γ. In contrast, the generation of LCMV-specific CTL responses was not altered in mice undergoing the same stress procedure concurrently with infection. Furthermore, stress exposure 6 days before and additional 3 days after LCMV infection profoundly reduced the expansion of T_{CD8+} cells in the spleen, due to diminished *in vivo* proliferation. Pharmacological blockade of glucocorticoid receptors completely abrogated the stress-associated decline of T_{CD8+} expansion. Stressed mice showed a significantly reduced expression of the early T-cell activation marker CD69 as well as impaired *in vitro* cytokine secretion of IFN-γ and IL-2. Additionally, social stress led to an altered migration capacity of T_{CD8+} cells as demonstrated by adoptive T cell transfer experiments. Taken together, this study shows that chronic social stress fundamentally suppresses the functional capacities of T cells during a viral infection.

1. Introduction

Chronic stress increases the susceptibility to infectious diseases by affecting the function of cells of the innate and adaptive immune system. However, only few studies have investigated the effects of chronic stress on T cell-mediated immunity during viral infection. For example, mice exposed to chronic restraint displayed a decreased generation of virus-specific CTLs in response to primary HSV-1 infection (Bonneau et al., 1991a), and altered activation of T_{CD8+} memory cells (Bonneau et al., 1991b; Bonneau, 1996). However, most studies were performed using stressors without relevant behavioral context. Although these studies provided important insights into the immunmodulatory capacities of chronic stress, animal models of social stress are considered to be biologically more relevant, also with respect to stressful situations in humans. In this context, some recent studies examined the impact of social stress on the pathophysiological outcome of viral infections such as Theiler's virus (Johnson et al., 2004, 2006) and influenza virus (Sheridan et al., 2000) or mycobacterial BCG (bacillus Calmette-Guérin)-infection (Merlot et al., 2004) but very little is known about the impact of chronic social stress on T cell responses to viral infections.

A series of studies has investigated the consequences of repeated social stress exposure on T cell number and function. For instance, it has been demonstrated that social stress is accompanied by reduced numbers of T cells in the blood, spleen, and bone marrow of mice (Engler et al., 2004). Moreover, social defeat in rats led to decreased homing of adoptively transferred peripheral blood T cells into lymphoid organs, suggesting altered migration properties (Stefanski et al., 2003). Finally, it has been reported that social stress influences proliferation, cytokine production and T cell mediated cytotoxicity. For example, socially stressed mice exhibit suppressed T cell proliferation responses to the mitogen ConA and increased production of IL-6 and IFN-γ as well as decreased production of IL-10 (Merlot et al., 2004).

Abbreviations: LCMV, lymphocytic choriomeningitis virus; NP, nucleoprotein; GP, glycoprotein; SDR, social disruption stress.

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To analyze the effects of chronic social stress on an anti-viral T cell response, we performed a systemic infection using lymphocytic choriomeningitis virus (LCMV). LCMV is a natural mouse pathogen inducing a strong cytotoxic T cell (CTL) response that is responsible for virus elimination. The CTL response of C57BL/6 mice is focused on the three dominant GP33-41/D^b, GP34-41/K^b and NP396-404/D^b as well as several sub-dominant (GP276-286/D^b, GP92-101/D^b, GP118-125/K^b and NP205-212/K^b) T cell epitopes (Gallimore et al., 1998; vanderMost et al., 2003). In addition, antigen processing and T cell epitope production are well characterized in the LCMV infection model and therefore it represents an optimal model to study potential T cell alterations in response to social stress.

In the present study we provide evidence that chronic social stress compromises the activation, expansion, and migration of LCMV-specific T_{CD8+} cells in the spleen. We further show that glucocorticoids play a fundamental role in these alterations by intrinsically inhibiting T_{CD8+} cell cytokine production and proliferation.

2. Materials and methods

2.1. Experimental animals

Male C57BL/6 mice (H-2^b) as well as B6.SJL-PtprcaPep3b/BoyJ (also referred to as "Ly5.1 congenic mice") used in this study were originally obtained from Charles River Laboratories. B6.PL (Thy1.1) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Experimental mice were used at 7–8 weeks of age and were kept in a specified pathogen-free facility on a 12/12 h light/dark cycle with *ad libitum* access to food and water. Both stressed and control mice were housed in groups of 5 mice per cage. All animal experiments were approved by the reviewing board of the Regierungspräsidium Freiburg.

2.2. Stress procedure

The social disruption (SDR) procedure was previously described (Engler et al., 2005; Avitsur et al., 2001; Stark et al., 2001). The stress paradigm is based on the disruption of an established social hierarchy of group-housed male mice (residents) which is experimentally induced by daily confrontations with an unfamiliar aggressive intruder mouse. For the stress procedure, an intruder was introduced into the residents cage for 2 h daily over a period of six (experiment 1) or nine (experiment 2) consecutive days. To prevent habituation, a different aggressor was used for each stress cycle. The stress procedure always started at the beginning of the dark period when animals display increased activity and naturally rising glucocorticoid levels. Control mice were left undisturbed in their home cages throughout the entire experiment.

2.3. Administration of hormone receptor antagonists

Mice were subcutaneously implanted with time release pellets (Innovative Research of America, Sarasota, FL) containing either 0.5 mg of the non-selective β-adrenergic receptor antagonist nadolol (21-day release) or 30 mg of the glucocorticoid type II receptor antagonist RU486 (12-day release). The pellets were implanted in the neck region of the animals 2 days prior to the stress procedure under ketamine/xylazine anaesthesia. Placebo pellets comprising only the inert carrier substance were used as a control. The optimal drug dosage for RU486 was determined in a preliminary experiment based on the ability to effectively prevent stress-induced thymic atrophy.

2.4. Viruses and media

LCMV-WE was obtained originally from F. Lehmann-Grube (Heinrich Pette Institute, University of Hamburg, Germany) and propagated on the fibroblast line L929. Mice were infected i.v. with 200 pfu of LCMV-WE. All media were purchased from Invitrogen Life Technologies (Karlsruhe, Germany) and were supplemented with 5% or 10% FCS, and 100 U/ml penicillin/streptomycin.

2.5. Intracellular staining (ICS) for IFN-γ and flow cytometry

ICS and flow cytometric analyses were performed as outlined previously (Basler et al., 2006). The following anti-mouse mAbs were used: CD8α (clone 53-6.7), CD69 (clone H1.2F3), Thy1.2 (clone 53-2.1), CD11c (clone HL3), CD86 (clone B7-2), CD80 (clone

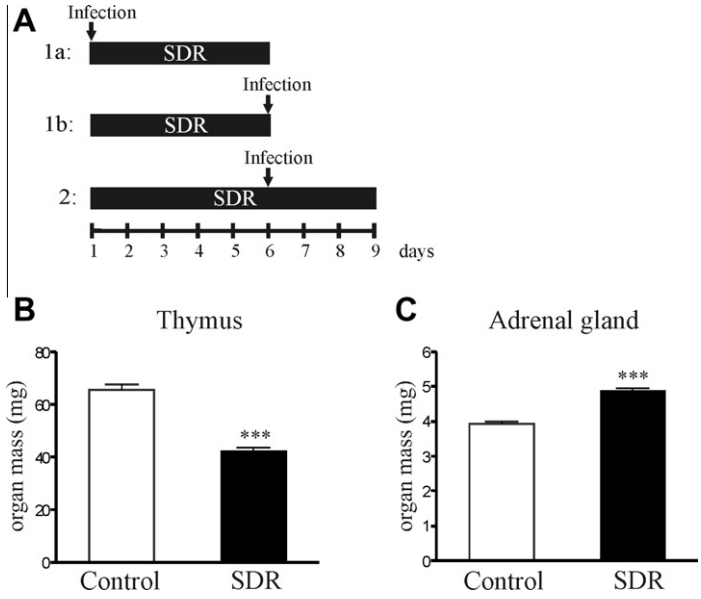

Fig. 1. Social disruption stress (SDR) caused thymic atrophy and adrenal hypertrophy. (A) Scheme of the experimental design. (B and C) Organ masses of thymus (B) and adrenal glands (C) after six consecutive days of SDR. Graphs represent data from two independent experiments with a total of 9–10 mice per group (mean ± SEM).