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Changes in innate and acquired immune responses in mice with targeted deletion of the dopamine transporter gene

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Abstract

The dopamine transporter (DAT) is responsible for the re-uptake of dopamine into presynaptic nerve terminals and thereby controls dopaminergic neurotransmission. Deletion of DAT results in a hyperdopaminergic phenotype and $DAT^{-/-}$ mice are characterized by pituitary hypoplasia, impaired maternal behavior, and increased locomotion. From earlier studies, we have evidence that the activity of the central dopaminergic system may play a role in determining immune reactivity and disease susceptibility. To further explore the functional relation between the dopaminergic system and the immune system, we investigated the activity of the immune system in $DAT^{-/-}$ mice. We show that in vitro, splenocytes from $DAT^{-/-}$ mice displayed reduced natural killer cell activity and reduced mitogen-induced cytokine responses. In contrast, LPS-induced cytokine production by macrophages was enhanced. In vivo, the cellular response to immunization with ovalbumine (OVA-induced delayed type hypersensitivity response) was significantly reduced. Interestingly, the OVA-induced humoral response (anti-OVA IgG) was increased in $DAT^{-/-}$ mice compared to wild-type animals. Plasma levels of catecholamines and corticosterone did not differ significantly between $DAT^{-/-}$ and wild-type animals.

In conclusion, we show in the present study that interfering with the dopaminergic system has major consequences for both the acquired and the innate immune response.

Keywords: Dopamine transporter; Immune response; Innate immunity; Adaptive immunity; Neuro–endocrine immune interaction

1. Introduction

It is now well-established that there is a mutual communication between the nervous and immune system. The two most important routes via which the central nervous system and the immune system can interact are the hypothalamus–pituitary–adrenal axis and the sympathetic nervous system (Elenkov et al., 2000; Besedovsky and del Rey, 1996). Immune organs are innervated by sympathetic nerves and thus several neurotransmitters and neuropeptides are released in the immediate vicinity of immune effector cells. The expression of receptors for a large number of neuropeptides and neurotransmitters allows a direct interaction with these substances. The capacity of the noradrenergic system and its neurotransmitters epinephrine and norepinephrine to modulate the immune response have been studied extensively (reviewed in (Kohm and Sanders, 2001)). However, little is known about the immunomodulatory role of the dopaminergic system. The neurotransmitter dopamine is widespread in the brain and the dopaminergic system affects mood, motor activity, learning, and emotion (Carlsson, 1987; Jackson and Westlind-Danielsson, 1994). Imbalance of the central dopaminergic system is associated with mental illnesses such as schizophrenia, Parkinson's disease, and attention deficit hyperactivity disorder (Nieoullon, 2002). There is evidence that lymphocytes express dopamine receptors and high concentrations of dopamine in vitro can inhibit mitogen-induced proliferation and induce apoptosis of lymphocytes (Caronti et al., 1998; Amenta et al., 1999; Ricci et al., 1999). The effect of in vivo administration of dopaminergic

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agonists is controversial and both stimulatory as well as inhibitory effects on lymphocyte function have been reported (Tsao et al., 1997; Ilani et al., 2004; Cook-Mills et al., 1995; Basu and Dasgupta, 2000).

Previously, we have presented evidence that individual differences in the activity of the dopaminergic system in vivo can play a role in determining susceptibility to infections, autoimmunity and tumor growth (Teunis et al., 2002; Kavelaars et al., 1997). For example, in two lines of Wistar rats that were selected based on their central dopaminergic reactivity, we showed that rats with a hyperdopaminergic phenotype (APO-SUS rats) are resistant to the autoimmune disease experimental autoimmune encephalomyelitis. Consistent with the resistance to the Thelper-1 (T_H1) cytokine mediated autoimmune disease, these animals have a reduced T_H1/T_H2 cytokine balance when compared to their hypodopaminergic counterparts (APO-UNSUS rats) who are susceptible to EAE.

In addition, Alaniz et al. (1999) showed that mice with a targeted deletion of the dopamine β-hydroxylase gene, resulting in hyperdopaminergia and the absence of norepinephrine and epinephrine, are more susceptible to infections. In addition, these mice showed impaired T cell function and decreased T_H1 cytokine-dependent IgG_{2a} antibody production (Alaniz et al., 1999).

In this study, we further investigated whether changes in the dopaminergic system have consequences for the immune response. As a model system, we used mice that lack the DAT gene ($DAT^{-/-}$) (Giros et al., 1996). These animals display a central hyperdopaminergic phenotype characterized by increased locomotion, rearing, and stereotypic behavior (Gainetdinov et al., 1999). In addition, $DAT^{-/-}$ mice are characterized by neuro–endocrine dysfunction revealed by the inability of females to lactate (Bosse et al., 1997). Furthermore, $DAT^{-/-}$ mice are significantly growth retarded, but have normal circulating levels of growth hormone (Bosse et al., 1997).

We compared the activity of the innate (natural killer cell activity and macrophage cytokine production) and acquired (T cell function, cellular and humoral response to vaccination) immune system in $DAT^{-/-}$ and wild-type mice. Our data demonstrate that perturbation of the dopaminergic system by deletion of DAT has consequences for both innate and acquired immunity.

2. Material and methods

2.1. Animals

$DAT^{-/-}$ mice were created through genetic deletion of the DAT by homologous recombination (Giros et al., 1996). Wild-type and homozygous $DAT^{-/-}$ mice were derived from crossing $DAT^{+/-}$ animals in a mixed C57Bl6/129SvJ background. In all experiments female WT and $DAT^{-/-}$ littermates were used. Animals were kept at the Utrecht University animal facility, fed a standard diet (Hope Farms, Woerden, The Netherlands) and water ad libitum.

2.2. Determination of NK activity in splenocytes

Splenocytes from naive animals were cultured in flat bottom microtiter wells (Costar Corp., Cambridge, MA) at different effector-to-target ratios (50:1, 100:1, and 200:1) for 4 h with radiolabeled NK-sensitive ^{51}Cr-YAC-1 target cells ($1*10^4$ YAC-1 cells/well). Maximal (MR) and spontaneous release (SP) of ^{51}Cr was determined by incubating labeled YAC-1 cells with 1.5% Triton and medium, respectively. Specific killing was calculated using the formula: (X-SP)/(MR-SP)*100%, whereby X is the release of ^{51}CR in the experimental sample. All measurements were performed in triplicate.

2.3. Stimulation of peritoneal macrophages

Naive animals were killed by cervical dislocation and 2 ml of ice-cold RPMI 1640 was injected into the peritoneum. Peritoneal macrophages were collected after gentle massage of the peritoneum (5 min). Cells were seeded in 24-well plates (5×10^5/well) in culture medium containing 5% FBS. After 1 h, nonadherent cells were removed and adherent macrophages were cultured in the presence of LPS (10 ng/ml final concentration). Culture supernatants were collected after 24 h and stored at −80 °C until assay.

2.4. T cell cytokine production and proliferation

Splenocytes from naive animals were cultured in quadruplicate in 200 μl flat bottom microtiter wells (Costar Corp., Cambridge, MA) at 2.5×10^5 cells per well with either plate-bound anti-CD3 (2 μg/ml final concentration) or PMA/ionomycin (10 and 400 ng/ml final concentration, respectively). Cultures were incubated for 72 h at 37 °C in a humidified atmosphere, 5% CO_2. For the final 24 h cultures were pulsed with 1 μCi/well [^{3}H] thymidine (Amersham, Bucks, UK). [^{3}H] thymidine uptake was measured using a liquid scintillation β counter. The results are expressed as stimulation index: the mean counts per minute (cpm) of cells cultured with mitogen divided by the mean cpm of cells cultured with medium alone.

2.5. Flow cytometry

CD4 (monoclonal antibody [mAb] L3T4-phycoerythrin), CD8 (mAb Ly-2-biotin), CD19 (mAb 1D3-fluorescein isothiocyanate), and streptavidin-CyChrome were purchased from PharMingen. Splenocytes were stained with anti-CD4, anti-CD8, anti-CD19 and flow cytometric analysis was performed with FACSCalibur® (Becton Dickinson, San Jose, CA). Fluorescence data are expressed as percentage of positive cells.