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Mimicking disruption of brain-immune system-joint communication results in collagen type II-induced arthritis in non-susceptible PVG rats

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ABSTRACT

The brain–immune system–joint communication is disrupted during collagen type II (CII) arthritis in DA rats. Since PVG rats are not susceptible to arthritis induction, comparison of hypothalamic and peripheral neuro-endocrine and immune responses between immunized DA and PVG rats might help to explain their different susceptibility to develop the disease. PVG and DA rats were immunized with CII. Corticosterone, neurotransmitters, anti-CII antibodies, and cytokine concentrations in plasma, and hypothalamic neurotransmitters and cytokines were determined by ELISA, Luminex, HPLC and RT-qPCR. Adrenalectomy or sham-operation was performed in PVG and DA rats 14 days before immunization. Basal plasma corticosterone and adrenaline concentrations were significantly higher, and plasma cytokines and hypothalamic noradrenaline were lower in PVG rats than in DA rats. While DA rats developed severe arthritis upon immunization (maximum score 16), only 12 out of 28 PVG rats showed minimal symptoms (score 1–2). The density of sympathetic nerve fibers in arthritic joints of DA rats markedly decreased, but it remained stable in immunized PVG rats. The ratio corticosterone to IL-1β levels in plasma was markedly higher in immunized PVG rats than in arthritic DA rats. Adrenalectomy resulted in severe arthritis in PVG rats upon immunization with CII. While DA rats show an altered immune-brain communication that favors the development of arthritis, PVG rats express a protective neuro-endocrine milieu, particularly linked to the basal tone of the HPA axis. Mimicking disruption of this axis elicits arthritis in non-susceptible PVG rats.

1. Introduction

Similar to other chronic inflammatory diseases, a functional deterioration of the hypothalamic-pituitary-adrenal (HPA) axis can be observed during the course of rheumatoid arthritis (RA), as shown by inadequately low secretion of adrenocorticotropic hormone (ACTH), cortisol, and androgens in relation to inflammation (Crofford et al., 1997; Cutolo et al., 2002; Imrich et al., 2010; Masi et al., 1984; Straub et al., 2002c; Wilder, 1996). The reasons for this phenomenon are not fully understood, but it has been reported that a continuous stimulation of the HPA axis by proinflammatory cytokines such as TNF or IL-6 results in a fast hypothalamic-pituitary adaptation, leading to unresponsiveness of this axis (Harbuz and Jessop, 1999; Mastorakos et al., 1993). Also exposure to IL-1β early in life results in permanent disruption of this axis (del Rey et al., 1996). Moreover, the positive correlation between ACTH and cortisol blood levels observed in healthy controls is weakened or abolished in patients with RA, indicating a dissociation of the interplay between these hormones (Gudbjornsson et al., 1996; Kanik et al., 2000). Thus, although the exact mechanisms are not known, alterations in the activity of the hypothalamus, and/or pituitary and adrenal glands contribute to the functional impairment of the HPA axis during arthritis.

In addition to these alterations, functional defects of the sympathetic nervous system (SNS) have also been described during RA

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and experimental arthritis (reviewed in ref. (Pongratz and Straub, 2013)). This change of function can be characterized by: a) an increased systemic sympathetic tone (Leden et al., 1983), b) a loss of sympathetic nerve fibers in inflamed tissue (Miller et al., 2000), and c) a loss of function of β-adrenoceptors and signaling on/in peripheral immune cells (Baerwald et al., 1992; Lombardi et al., 2002). Additionally, since corticosteroids and noradrenaline (NA) cooperate in an anti-inflammatory fashion, uncoupling of these factors and loss of cooperation as in RA, constitute a proinflammatory signal (Straub et al., 2002a). Such uncoupling has also been described in other chronic inflammatory diseases (Straub et al., 2002b), but the reasons for the disruption are yet not fully understood.

In animal models of acute inflammation, several studies demonstrated that peripheral immune stimulation results in changes in the expression or concentration of central cytokines (e.g. IL-1β) and neurotransmitters (e.g. NA), which in turn influence peripheral immune reaction by activation of the stress axes (Dunn, 1992; Gibb et al., 2011; Maier, 2003; Pitossi et al., 1997; Teeling et al., 2007). Indeed, a bidirectional brain-immune system communication has been demonstrated, which is based on the effect of afferent signals from the immune system to the brain and *vice versa* (Besedovsky and del Rey, 1996; Maier, 2003; Quan and Banks, 2007). These bidirectional pathways are not only activated during acute inflammatory conditions (e.g., endotoxin injection), but also during adaptive immune responses (reviewed in ref. (Teeling et al., 2007)). The question arises whether, or not, comparable interactions occur during chronic inflammatory diseases.

Previous studies in our laboratory demonstrated a severe disruption of the brain – immune system – joint communication in DA rats during collagen type II (CII)-induced arthritis (del Rey et al., 2008). This disruption was not only characterized by an unresponsiveness of the HPA axis, an increased sympathetic tone, and the loss of sympathetic nerve fibers in affected joints, but also by central alterations in hypothalamic cytokines and neurotransmitters (del Rey et al., 2008). Since the reasons for this disruption and consequences for chronic disease are not yet clarified, we compared central and peripheral neuroendocrine and immune parameters in highly susceptible DA rats and non-susceptible PVG rats under basal conditions and following CII immunization.

We hypothesized that: a) CII immunization of non-susceptible PVG rats results in protective changes in central and peripheral neuroendocrine responses that are different from those observed in DA rats; b) experimental disruption of protective stress axes in non-susceptible PVG rats affects the expression of experimental arthritis. Based on the results of descriptive studies performed to test the first part of our hypothesis (a), we recognized the important role of the HPA axis in PVG rats, and performed adrenalectomy experiments to approach the second part (b).

2. Materials and methods

2.1. Animals

Eight-week-old female dark agouti (DA) and Piebald-Viral-Glaxo (PVG) rats were purchased from Harlan Laboratories GmbH (Venray, The Netherlands). Animals were housed under standard conditions as described previously (del Rey et al., 2008). Experiments were conducted according to institutional and governmental regulations for animal use (Government of the Oberpfalz AZ 54-2532.1-06/09).

2.2. Collagen type II – induced arthritis model

DA and PVG rats were immunized with 300 μg bovine CII (Chondrex, Seattle, WA) emulsified in an equal volume of Freund incomplete adjuvant (Sigma, Deisenhofen, Germany). A total of 0.3 ml of this emulsion was injected intradermally at the base of the tail. Control rats received 0.9% NaCl only.

Each limb (front and hind paws) was scored separately for clinical symptoms of arthritis. One point was assigned for each of the following inflamed regions: digits, midfeet and wrists/ankles. One additional point was assigned for limping affecting each extremity. Thus, a maximum arthritis score of 16 points per animal can be reached.

2.3. Determination of specific anti-collagen type II antibodies

DA and PVG rats were killed by decapitation on days 0, 5, 14, 28, 41, or 55 after immunization, trunk blood was collected in EDTA-coated tubes on ice (Sarstedt, Nümbrecht, Germany), and centrifuged at 4 °C (1600 × g, 10 min). Plasma samples were stored at −20 °C until analysis. Anti-CII antibodies were determined using commercially available ELISA kits (MD Biosciences GmnH, Zürich, Switzerland).

2.4. Measurement of plasma cytokines

Plasma samples were obtained as described above and stored at −20 °C until hormone and cytokine determinations were performed. Cytokine (IL-1β, IL-6) concentrations were analyzed by Luminex Multiplex Detection technology using Beadlyte reagents (MILLIPLEX, Millipore, Billerica, MA; detection limit: 2.32 pg/ml). Quantifications of cytokines were performed according to the manufacturer's instructions.

2.5. Determination of brain neurotransmitters, plasma catecholamines and corticosterone

After decapitation, the hypothalamus was collected, divided into left and right sides, quickly frozen and maintained at −80 °C until used for determinations. The right side was used to determine catecholamine and indolamine concentrations by HPLC, as previously described (del Rey et al., 2008). Blood plasma samples underwent a purification step using alumina adsorption prior to HPLC determination. Peaks were quantified by peak height evaluation using evaluation software (Chromeleon version 6.01; Dionex, Sunnyvale, CA). Corticosterone concentrations in plasma were determined by ELISA, using the kits and prescriptions provided by the manufacture (IBL International GmbH, Germany).

2.6. Quantitative analysis of sympathetic nerve fibers

After decapitation of rats on days 0, 5, 14, 28, 41 and 55 after immunization, limbs were harvested and fixed in 4% formalin for 5 days. Limbs were then washed in phosphate buffered saline (PBS), decalcified in RDO Rapid Decalcifier (Apex Engineering Products, Aurora, IL) during 5 days, washed again in PBS and incubated in 20% sucrose/PBS for 24 h. Samples were embedded in protective freezing medium (Tissue-Tek, Sakura, Zoeterwoude, The Netherlands), quick-frozen in liquid nitrogen and stored at −80 °C until use.

Frozen limbs were cut into 12-μm sections, air-dried for 1 h and then rehydrated in PBS. Unspecific binding sides were blocked with PBS containing 10% goat serum, 10% fetal calf serum (FCS) and 10% bovine serum albumin (BSA) for 45 min at room temperature. Sections were washed 10 min with PBS and then incubated with a polyclonal primary antibody against tyrosine hydroxylase for 3 h at room temperature (rabbit anti TH, AB152, dilution: 1:100 in 10% goat serum in 1% BSA/PBS, Chemicon, Temecula, CA). After washing