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Research Report

Sympathetic innervation of the spleen in male Brown Norway rats: A longitudinal aging study

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ABSTRACT

Aging leads to reduced cellular immunity with consequent increased rates of infectious disease, cancer, and autoimmunity in the elderly. The sympathetic nervous system (SNS) modulates innate and adaptive immunity via innervation of lymphoid organs. In aged Fischer 344 (F344) rats, noradrenergic (NA) nerve density in secondary lymphoid organs declines, which may contribute to immunosenescence with aging. These studies suggest there is SNS involvement in age-induced immune dysregulation. The purpose of this study was to longitudinally characterize age-related change in sympathetic innervation of the spleen and sympathetic activity/tone in male Brown Norway (BN) rats, which live longer and have a strikingly different immune profile than F344 rats, the traditional animal model for aging research. Splenic sympathetic neurotransmission was evaluated between 8 and 32 months of age by assessing (1) NA nerve fiber density, (2) splenic norepinephrine (NE) concentration, and (3) circulating catecholamine levels after decapitation. We report a decline in NA nerve density in splenic white pulp (45%) at 15 months of age compared with 8-month-old (M) rats, which is followed by a much slower rate of decline between 24 and 32 months. Lower splenic NE concentrations between 15 and 32 months of age compared with 8M rats were consistent with morphometric findings. Circulating catecholamine levels after decapitation stress generally dropped with increasing age. These findings suggest there is a sympathetic-to-immune system dysregulation beginning at middle age. Given the

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Abbreviations: 3,4-dihydroxybenzylamine, DHBA; adrenergic receptors, AR; Brown Norway, BN; BN X F344 (F1), BNF_1; central nervous system, CNS; delayed type hypersensitivity, DTH; epinephrine, EPI; ethylenediaminetetraacetic acid, EDTA; Fischer 344, F344; glyoxylic acid condensation method, SPG method; high-performance liquid chromatography with electrochemical detection, HPLC; interleukin, IL; month-old, M; natural killer, NK; nerve growth factor, NGF; neurotrophin-3, NT-3; New Zealand black, white, and black and white mice, respectively, NZB, NZW, and NZBW; noradrenergic, NA; norepinephrine, NE; periarteriolar lymphatic sheath, PALS; perchloric acid, $HClO_4$; sympathetic–adrenal medullary system, SAM; sympathetic nervous system, SNS; T-helper-1, Th1; T-helper-2, Th2; Wistar-Kyoto, WK

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unique T-helper-2 bias in BN rats, altered sympathetic-immune communication may be important for understanding the age-related rise in asthma and autoimmunity.

1. Introduction

The central nervous system (CNS) regulates immune function, at least in part, via noradrenergic (NA) sympathetic nerves that innervate primary and secondary immune organs (reviewed by Bellinger et al., 2008b). Norepinephrine (NE) released by sympathetic nerves interacts with adrenergic receptors (AR) expressed on the surface of cells of the immune system (reviewed by Kin and Sanders, 2006; Bellinger et al., 2008b). The sympathetic nervous system (SNS) modulates many aspects of immune function. Based on *in vitro* and *in vivo* studies in young adult rodents, the roles ascribed to the SNS include (1) limiting the magnitude of both acute and chronic inflammatory responses by shifting the cytokine balance from a pro-inflammatory towards an anti-inflammatory cytokine profile (Vizi and Elenkov, 2002); (2) promoting T-helper-2 (Th2)-driven antibody responses via β_2-AR signaling of B cells by up-regulating B cell accessory molecule expression and increasing B cell responsiveness to interleukin (IL)-4 (reviewed by Kin and Sanders, 2006); (3) enhancing cell-mediated responses, such as a delayed-type hypersensitivity (DTH) reaction to a contact sensitizing agent through direct interaction with both T cells and antigen-presenting cells (Madden et al., 1989; Li et al., 2004); and (4) influencing Th1-driven antibody responses through β_2-AR stimulation of Th1 cells (Madden et al., 1995; Kruszewska et al., 1995; reviewed by Kin and Sanders, 2006; Bellinger et al., 2005). In general, agents that activate the SNS tend to reduce T cell responses (Madden et al., 1989; Sanders et al., 1997; reviewed by Kin and Sanders, 2006), anti-viral immune reactivity (Dobbs et al., 1993), and natural killer (NK) cell activity (Irwin et al., 1988). Collectively, studies performed in young adult rodents demonstrate that genetic background, gender, site of immunization, type of immune cells involved in the immune response, and timing of exposure to catecholamines during the immune response all contribute to the complexity of SNS interactions with the immune system and affect the outcome of SNS modulation of the immune response (Madden et al., 1995; Kin and Sanders, 2006; Bellinger et al., 2008b). Furthermore, these complexities likely reflect an important role of the SNS in fine-tuning an immune response with the goal of effectively eliminating the threat to the host and restoring immune system homeostasis.

NA innervation of, and NE content in, secondary lymphoid organs, such as the spleen, can be affected by physiological changes (i.e., immune challenge or immunosuppression) (Yang et al., 1998; Lorton et al., 2009), immunodeficiency virus infection (Kelley et al., 2003; Sloan et al., 2008), psychosocial stress (Sloan et al., 2007), hypertension (Purcell and Gattone, 1992), pregnancy and parturition (Bellinger et al., 2001), and advancing age (Bellinger et al., 1987, 2001, 2002). Age-related changes in sympathetic innervation of the spleen are species- and/or strain-specific. For example, sympathetic NA innervation of spleens from aged C57Bl/6J and BALB/cJ mice is preserved (Madden et al., 1997; Bellinger et al., 2001), but declines with advancing age in male C3H, MRL-lpr/lpr (Breneman et al., 1993), and New Zealand mice (NZB, NZW, and NZBW) (Bellinger et al., 1989). In murine strains that develop autoimmune diseases (MRL-lpr/lpr, NZB, and NZBW) the onset of sympathetic nerve loss in the spleen occurs with, or slightly precedes, the onset of the autoimmune disease (Breneman et al., 1993; Bellinger et al., 1989).

In a previous study from our laboratory (Bellinger et al., 2002), we compared sympathetic innervation of the spleen in four strains of young (3 months old (M)) and old (21 M) rats. These strains of rats were chosen for study because they are commonly used as models for human aging and/or used to study neural-immune interactions. We reported that NA innervation of spleens from male Fischer 344 (F344) and Lewis rats declines in normal aging, whereas NA innervation was preserved in age-matched Brown Norway (BN) and BN X F344 (F1; BNF_1) rats. The reason for this strain difference is unclear, but maybe a result of the BN and BNF_1 rats having a longer life span (median age, 32 M) than F344 and Lewis rats (median age, 24 M) (Nadon, 2006). Unlike F344 rats, the most commonly used rat model for aging, in BN rats there is low morbidity from pituitary adenomas or glomerulonephrosis with increasing age (Lipman et al., 1996, 1999; Nadon, 2006). BN and F344 rats also differ in their behavior (Rex et al., 1996; Ramos et al., 1997), learning and memory (van der Staay and Blokland, 1996), immune function (Sado et al., 1986; Koch 1976; Stankus and Leslie 1976; Festing, 1998; Lipman, 1996, 1999), and stress responses (Segar et al., 2009; Duclos et al., 2005; Sarrieau et al., 1998; Gómez et al., 1998), which may affect NA nerve integrity in the spleen with advancing age.

The purpose of the present study was to longitudinally examine the effect of age on sympathetic NA innervation of spleens from BN rats. Since age-related changes in SNS and sympatho-adrenal medullary system (SAM) reactivity may contribute to age-induced changes in NA nerve integrity and splenic NE content via increased local catecholamine concentrations influencing uptake into the nerve terminals from the circulation, local sympathetic nerve activity, and/or affecting leukocyte migration (Bellinger et al., 2008b; Elenkov et al., 2000; Madden et al., 1995), SNS and SAM reactivity to decapitation stress was also assessed by measuring circulating catecholamine levels (NE and epinephrine (EPI), respectively). We report here an age-related decline in NA nerve density in the splenic white pulp, evident by morphometric analysis at 18 months of age, and reduced splenic NE concentration between 18 and 32 months of age. Decapitation stress-induced plasma catecholamine levels were diminished in middle aged and aged rats. Given the well documented role of the SNS in immune modulation, altered sympathetic innervation likely affects immune competence in aging BN rats.