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Age-associated changes in rat immune system: Lessons learned from experimental autoimmune encephalomyelitis

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ABSTRACT

Aging is associated with the decline in immune response to infectious agents and tumors and increasing risk of autoimmunity, but the incidence of autoimmune diseases does not increase in the elderly. To elucidate the cellular and molecular mechanisms influencing clinical expression of autoimmunity in aged animals, the phenotypic and functional characteristics of mononuclear cells isolated from the spinal cords of 3-month-old (young) and 26-month-old (aged) Dark Agouti rats immunized to induce experimental autoimmune encephalomyelitis (EAE) — the model of multiple sclerosis, the most common autoimmune disease of the central nervous system, were examined. Aged rats were less susceptible to EAE induction, and the neurological and histological picture was milder in those rats which developed the clinically manifested disease. At the peak of the disease, several times fewer mononuclear cells and T lymphocytes were isolated from the spinal cords of aged rats compared with the young ones. The frequency of CD4+ cells among TCRαβ+ lymphocytes, as well as that of reactivated CD134(OX40)+ cells within its CD4+ T-lymphocyte subpopulation, was less in spinal cords of aged compared with young rats. Additionally, CD134 surface density on CD4+ lymphocytes was decreased in the spinal cord of aged rats. The changes in CD134 expression most likely reflected in part age-related intrinsic changes in CD4+ lymphocytes as the expression of this molecule was also impaired on *in vitro* stimulated naïve CD4+ splenocytes from aged rats compared with young animals. In addition, greater frequency of CD8+ lymphocytes with regulatory phenotypes could also contribute to impaired CD4+ cell reactivation in aged rats. The increased apoptosis of CD4+ cells from aged rats was consistent with their impaired reactivation and it was accompanied by the greater frequency of CD4+CD11b+CD45$^{int/high}$ cells, which are supposed to be actively engaged in apoptotic cell phagocytosis and to have immunoregulatory properties. Compared with young rats, following short-term PMA and ionomycin stimulation *in vitro*, the frequency of IL-17+ and IFN-γ+CD4+ T lymphocytes among the spinal cord mononuclear cells from aged rats and the cytokine expression density on a *per* lymphocyte basis were reduced. Additionally, the increase in the proportion of autoregulatory IL-17+IL-10+ cells on the account of proinflammatory IL-17+IFN-γ+ cells within IL-17+ lymphocytes suggested their lower pathogenic capacity in aged rats. This most likely reflected alterations in the aged rat spinal cord cytokine milieu, which were mirrored in a diminished expression of IL-1β mRNA followed by an enhanced expression of IL-6 and TGF-β mRNA. Overall, the study points to age-related changes in T lymphocytes and other cells from the spinal cord infiltrate which could contribute to the decreased susceptibility of aged rats to the induction of EAE.

1. Introduction

One of the most recognized, but, yet not fully understood consequences of aging is the decline in immune function termed as immune senescence. At clinical level, this is evident by more frequent and more severe community-acquired and nosocomial infectious diseases and poorer outcomes from these diseases in the elderly when compared with the younger population (Gavazzi and Krause, 2002; Jackson et al., 2008). This partly reflected poor response to vaccination in the elderly (Aspinall et al., 2007; Jackson et al., 2008). In addition, immunosenescence is associated with a greater cancer-related mortality rate (Bulati et al., 2008). On the other hand, in general, despite age-related rise in autoimmunity, the incidence of autoimmune diseases does not increase in the elderly (Vadasz et al., 2013). A number of hypotheses have been proposed to explain the relationship between aging and development of autoimmunity, to mention but a few. It has been suggested that the age-related decline in the thymic T-cell output

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leads to compensatory homeostatic expansion of peripheral T cells, which then contributes to immune system abnormalities, including increased autoimmunity in aged individuals and animals (Ongrádi and Kövesdi, 2010; Tatari-Calderone et al., 2012). In this homeostatic expansion T cells that have higher avidity for self-peptide/MHC complexes enjoy an advantage and expand more than low avidity T cells (Kieper et al., 2004; Tatari-Calderone et al., 2012). This leads not only to the faster disappearance of T cells with naïve phenotype, but also to the accumulation of high avidity T cells and their partial activation (Stacy et al., 2002). Additionally, with aging there is an expansion of CD4+ senescent T lymphocytes that have lost the expression of CD28 (Ongrádi and Kövesdi, 2010). These cells are shown to: i) express molecules specific for natural killer cells, so that they integrate functional properties of rapid, nonspecific immune responses with antigen-specific immunity and ii) have an important role in the pathogenesis of autoimmune diseases (Goronzy and Weyand, 2003; Ongrádi and Kövesdi, 2010).

The discrepancy between the age-related appearance of autoimmune phenomena and the incidence of autoimmune diseases has not been understood yet. A possible explanation may be found in the expansion of protective regulatory mechanisms with aging. This is corroborated by the increased generation of regulatory T-cells in the periphery of aged humans and rodents (Tatari-Calderone et al., 2012; Vadasz et al., 2013). This expansion is suggested to balance the autoimmunity and prevent the development of autoimmune diseases in the elderly. The other side of this coin is that the generation of T-regulatory cells in the periphery occurs on the account of effector cells, so it "requires payment" in terms of higher susceptibility to infections and an increased incidence of cancer in the elderly (Vadasz et al., 2013). Additionally, age-related intrinsic T-cell changes underlying their functional erosion could also contribute to the decreased incidence of clinical autoimmune diseases in aged animals and possibly the elderly (Tatari-Calderone et al., 2012).

The average peak of incidence differs for each individual autoimmune disease. Generally, most autoimmune diseases develop either during puberty (juvenile type diseases) or during mature reproductive life of individuals. Multiple sclerosis (MS), the most common autoimmune disease involving the central nervous system (CNS), typically begins between the ages of 20 and 40, whereas initial symptoms rarely occur before the age of 10 or after the age of 60 (Tullman, 2013). This suggests that the onset of MS is restricted to a rather limited period of life-span (Ditamo et al., 2005). The pathological hallmarks of MS are blood–brain barrier (BBB) disruption, CNS inflammation, demyelination, and neurodegeneration (Lassmann and van Horssen, 2011). The etiopathogenesis of MS is still a matter of discussion. It is believed that MS is an autoimmune disease characterized by proinflammatory T helper (Th)1 and Th17 cell infiltration into the CNS (Fletcher et al., 2010). Despite some criticism it is not disputable that the experimental autoimmune encephalomyelitis (EAE) has been invaluable in identifying or confirming many components of complex pathogenic cascade of events leading to clinical MS (Constantinescu et al., 2011). Briefly, EAE neuropathogenesis results from an induced myelin-specific T cell response, when effector Th cells cross the BBB. In the CNS, on encountering the cognate antigen, the autoreactive T cells are reactivated. Upon reactivation the cells differentiate, produce their signature cytokines (IFN-γ, IL-17 or both the cytokines), which activate the neighboring immune or neural cells, disrupt the BBB and initiate the destructive chain of events in the brain tissue (Shin et al., 2012).

Although data on the influence of aging on susceptibility and development of active EAE could provide insight into age-related restriction in MS development, they are limited and inconsistent. This inconsistency could most likely be ascribed to species and strain differences, as well as to differences in animal chronological (and possibly biological) age and immunization protocol employed (Ben-Nun et al., 1980; Ditamo et al., 2005; Endoh et al., 1990; Källén and Nilsson, 1989; Ludowyk et al., 1993; Matejuk et al., 2005; Tatari-Calderone et al., 2012). Therefore, the study was undertaken in order to examine the influence of aging on the incidence and development of EAE in Dark Agouti (DA) rats and to elucidate the putative cellular and molecular changes at the level of CNS that contribute to age-related changes in pathogenesis of this disease. To this end 3-month-old (young) and 26-month-old (aged) rats were immunized for EAE and at the peak of the disease the spinal cord cells were isolated and examined for their phenotypic characteristics and proinflammatory and immunomodulatory cytokine production.

2. Material and Methods

2.1. Experimental Animals

In the present study 3- and 26-month-old female DA rats from a colony in the animal facility of the Immunology Research Centre "Branislav Jankovic" in Belgrade were used. Animals were maintained in a temperature-controlled environment with a 12-hour light/12-hour dark cycle and were administered standard laboratory food and tap water *ad libitum*. Animal care and use were performed in compliance with the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes (revising Directive 86/609/EEC) and approved by the Institutional Animal Care and Use Committee.

2.2. Induction and Clinical Evaluation of EAE

For EAE rats were immunized by an intradermal injection of a 100 μl emulsion made from a mixture of equal volumes of rat spinal cord homogenate in phosphate-buffered saline (PBS) and complete Freund's adjuvant containing 1 mg/ml of heat-killed and dried *Mycobacterium tuberculosis* H37Ra (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) in the left hind paw followed by a subcutaneous injection of 0.25 ml of saline containing 5×10^8 of *Bordetella pertussis* (obtained from the Institute of Virology, Vaccines and Sera "Torlak", Belgrade, Serbia) on the dorsum of the same paw. All animals were monitored daily for clinical signs of EAE, and scored according to the following scale: 0, no clinical signs; 0.5, distal tail atony; 1, complete tail atony; 2, paraparesis; 3, paraplegia and 4, tetraplegia, moribund state or death. In addition, on 0, 7th and 13th day post-immunization (d.p.i.), animals were weighed. In the preliminary experiment, in nine young and eleven aged rats the clinical course of EAE was monitored daily until the 28th d.p.i. The peak of the disease occurred between the 12th and 14th d.p.i. In four subsequent experiments rats immunized for EAE were killed on the 13th d.p.i. and mononuclear cells were isolated from their spinal cords and brains for further analyses, or their spinal cords and brains were processed for histopathological analysis.

2.3. Histopathological Analysis

The rats with representative clinical disease from both age groups were selected for histopathological analysis. These animals were deeply anesthetized by an i.p. injection of 800 μl/100 g of body weight of anesthetic solution (ketamine, 100 mg/ml Ketamidor, Richter Pharma AG, Wels, Austria; xylasine, 20 mg/ml Xylased, Bioveta, Ivanovice na Hané, Czech Republic and saline, mixed in a 1:0.5:8.5 ratio) and perfused with PBS followed by 4% buffered paraformaldehide (Sigma-Aldrich Chemie GmbH). Brains and spinal cords were removed and fixed in 4% buffered paraformaldehide overnight and then routinely embedded in paraffin wax. Serial 5 μm-thick transverse sections of paraffin embedded tissues were routinely stained with hematoxylin and eosin. Each 50th section from spinal cord and brain tissues was examined for inflammatory signs, with the following grading: 0, no inflammatory cells; 1, a few scattered inflammatory cells; 2, organization of inflammatory infiltrates around blood vessels and 3, extensive perivascular cuffing with extension into adjacent parenchyma, (Wraith et al., 2009). The images were captured and analyzed using an Olympus BH2