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Strain specificities in age-related changes in mechanisms promoting and controlling rat spinal cord damage in experimental autoimmune encephalomyelitis

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ABSTRACT

The study investigated strain specificities in age-related differences in CD8+ T cell- and microglial cell-mediated mechanisms implicated in induction/perpetuation and/or control of neuroinflammation in experimental autoimmune encephalomyelitis (EAE) in Albino Oxford (AO) and Dark Agouti (DA) rats exhibiting age-related changes in the susceptibility to EAE in the opposite direction (increase in relatively resistant AO rats vs decrease in DA rats). In the inductive phase of EAE, the greater number of fully differentiated effector CD8+ T lymphocytes was found in draining lymph nodes (dLNs) from aged rats of both strains than in strain-matched young rats, but this was particularly prominent in AO rats, which exhibited milder EAE of prolonged duration compared with their DA counterparts. Consistently, dLN IFN-γ+ and IL-17+ CD8+ T cell counts were greater in aged AO than in DA rats. Additionally, the magnitudes of myelin basic protein (MBP)-induced rise in the frequency of IFN-γ+ and IL-17+ CD8+ T cells (providing important help to neuroantigen-specific CD4+ T cells in EAE models characterized by clinically mild disease) were greater in dLN cell cultures from aged AO rats. Consistently, the magnitudes of MBP-induced rise in the frequency of both IFN-γ+ and IL-17+ CD8+ T cells were greater in spinal cord mononuclear cell cultures from aged AO rats compared with their DA counterparts. Besides, with aging CD4+CD25+Foxp3+/CD8+CD25+Foxp3+ regulatory T cell ratio changed in spinal cord in the opposite direction. Consequently, in aged AO rats it was shifted towards CD8+CD25+Foxp3+ regulatory T cells (exhibiting lower suppressive capacity) when compared with DA rats. Moreover, the frequency of CX3CR1+ cells among microglia changed with aging and the disease development. In aged rats, in the effector phase of EAE it was lower in AO than in DA rats. This was accompanied by higher frequency of cells expressing IL-1β (whose down-regulation is central for CX3CR1-mediated neuroprotection), but lower that of phagocyting cells among microglia from aged AO compared their DA counterparts. The study indicates the control points linked with strain differences in age-related changes in EAE pathogenesis.

1. Introduction

The clinical course and severity of multiple sclerosis, the most common inflammatory autoimmune disease of the central nervous system (CNS) in humans, is shown to depend on the age of onset (Tullman, 2013). To study different clinical and pathological aspect of multiple sclerosis, a group of experimental models termed experimental autoimmune encephalomyelitis (EAE) is widely used (Gold et al., 2006). In most EAE models, neuroantigen-specific Th1/Th17-polarized CD4+ T cells play a pivotal role in the initiation and perpetuation of CNS inflammation (Fletcher et al., 2010). Although CD4+ T lymphocytes are traditionally considered the main actors in multiple sclerosis and EAE immunopathology, multiple lines of evidence suggest that CD8+ T lymphocytes are also implicated in the pathogenesis (Mars et al., 2011). However, in contrast to abundant studies on the role of Th cells in EAE, those investigating the role of CD8+ T cells in its pathogenesis are rather limited. It has directly and/or indirectly been shown that both the CNS-infiltrating (primed in the periphery) autoreactive conventional CD8+ T cells and the CNS-resident conventional memory CD8+ T cells play a significant role in nervous tissue damage

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in the effector phase of EAE (Ritzel et al., 2016; Sobottka et al., 2009). Additionally, it has been indicated that conventional CD8+ T cells may have an important role in inductive phase of EAE (Camara et al., 2013; Huber et al., 2013; Huber and Lohoff, 2015). They are shown to help neuroantigen-specific CD4+ T cells to fully differentiate into CNS-infiltrating effector cells in draining lymph nodes (dLNs), the phenomenon termed "reverse help" (Camara et al., 2013). This is suggested to be of the utmost importance in EAE models characterized by weak neuroantigen-specific CD4+ T cell response leading to clinically mild EAE (Camara et al., 2013; Huber and Lohoff, 2015).

It should be pointed out that the CNS infiltrating subsets of not only CD4+ cells, but also CD8+ cells have regulatory role in the development of autoimmune inflammation (McGeachy et al., 2005; Sinha et al., 2014). However, the full understanding of interplay between CD4+ and CD8+ regulatory T (Treg) cells, and its relevance for clinical evolution of the disease, is still lacking.

According to the classical paradigm, in EAE pathogenesis a significant role has also activation of resident microglial cells, and the recruitment of blood-derived monocytes/macrophages into the CNS, resulting in production of various pro-inflammatory and neurotoxic mediators (Fletcher et al., 2010; Thompson and Tsirka, 2017). Thus, it is clear that factors controlling microglial cell activation and consequently neurotoxicity, as it is fractalkine (CX3CL1), may also have important role in the restriction of the CNS tissue damage, and severity of subsequent neurological deficit (Cardona et al., 2006).

Susceptibility to autoimmune disorders, including EAE, has a clear genetic component in humans and in rodent models (Lindh and Källén, 1978; Lucas and Lenardo, 2015; Miljkovic et al., 2006). However, the multifactorial nature of organ-specific autoimmunity has limited our understanding of the biology behind the processes that define which organism is affected (Bluestone et al., 2015). Rodent models of autoimmune disease relying on immunization with autoantigens offer the possibility to focus on a defined tissue/cell target and dissect susceptibility factors others than those involved in the original immunological stimuli (Forrester et al., 2013; Morris et al., 2009). Strain differences in the susceptibility to organ-specific autoimmune diseases are suggested to be related to strain specificities in both T effector (Hubert et al., 2006; Siggs et al., 2006) and Treg cell immune responses (Breser et al., 2016). Additionally, in the context of the CNS autoimmune pathology, data indicating strain differences in microglial cell inflammatory properties (Wei and Lin, 2009) suggest that this phenomenon could also contribute to strain-specific differences in the susceptibility to EAE induction and development.

Aging affects both innate and adaptive immune function, but adaptive immune system, particularly T cell-mediated immunity, exhibits more pronounced alterations (Pawelec, 1999). In general, these changes, at clinical level, lead to decrease in the incidence of the majority of autoimmune diseases in the elderly (Vadasz et al., 2013; Vollmer and Waxman, 1991). To explain this phenomenon are data indicating the expansion of many protective mechanisms with aging, which counteract age-related rise in the autoimmune phenomena (Tatari-Calderone et al., 2012; Vadasz et al., 2013). Indeed, concomitant with the increase in the frequency of autoreactive cells with high avidity for self-peptide MHC ligands, the increased generation of Treg cells has been observed in the periphery of aged humans and mice (Tatari-Calderone et al., 2012; Vadasz et al., 2013). Additionally, potentially relevant for the CNS-related autoimmunity, the age-related impairment in CX3CL1 signaling in microglia following LPS challenge has been identified (Fenn et al., 2013; Lyons et al., 2009).

Although age-related strain-specific changes in the immune system may be rather subtle and not of much consequence to animals in normal condition, they are suggested to become very relevant in conditions of disease and stress (Pinchuk and Filipov, 2008). Consistently, the age-associated changes in the incidence and clinical presentation of EAE are shown to be strain-specific (Ditamo et al., 2005; Djikić et al., 2014; Endoh et al., 1990; Stojić-Vukanić et al., 2015; Tatari-Calderone et al., 2012). We have recently shown that with aging female Dark Agouti (DA) rats, which are highly susceptible to EAE induction in young adult age, lose their susceptibility to EAE induction, and show substantial decrease in severity of the disease compared with their younger counterparts (Djikić et al., 2014). On the other hand, Albino Oxford (AO) rats, which are relatively resistant to EAE induction in young adult age (Miljkovic et al., 2006; Stojić-Vukanić et al., 2015), become more susceptible to EAE with aging, and those rats, which develop the clinical disease, exhibit mild neurological deficit of prolonged duration (Stojić-Vukanić et al., 2015). However, the mechanisms underlying strain specificities of age-related changes in the susceptibility to EAE have not been elucidated yet. Therefore, the herein presented study was designed to investigate contribution of (neuro)antigen-specific CD8+ T cells to the development of neuroinflammation, and the cellular and molecular mechanisms contributing to the restriction of the neuroinflammation (specifically, ratio of distinct Treg cell subsets and CX3CR1-mediated control of microglial neurotoxicity) to strain differences in age-related changes in rat susceptibility to EAE, using DA and AO rat EAE models.

2. Materials and methods

2.1. Experimental animals

In two sets of experiments young (2–3-month-old) and aged (22–24-month-old) female DA and AO rats were used. All rats were from breeding colonies at the Immunology Research Centre "Branislav Janković" in Belgrade. The animals were maintained under standard laboratory conditions and their health was monitored on a daily basis by animal care staff and a veterinarian. The animals included in the experiments did not show any clinical signs of neural disorders and none of old animals showed macroscopic signs of illness at necropsy. All experimental procedures and animal care were performed in accordance 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 induction of EAE, rats were administered with 100 μl of an emulsion made of equal volumes of rat spinal cord (SC), viz. SC homogenate in phosphate-buffered saline (PBS) and complete Freund's adjuvant (CFA) containing 1 mg/ml of heat-killed and dried *Mycobacterium tuberculosis* H37Ra (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) followed by an injection of 0.25 ml of 5×10^8 *Bordetella pertussis* (Institute of Virology, Vaccines and Sera "Torlak", Belgrade, Serbia) as previously described in detail (Stojić-Vukanić et al., 2015, 2016). Rats were weighed and graded daily (by two independent experienced observers) for neurological deficit, as follows: 0, no clinical signs; 0.5, distal tail atony; 1, complete tail atony; 2, paraparesis; 3, paraplegia; 4, tetraplegia, moribund state or death. None of the rats reached moribundity during the studies. For those which developed neurological signs of EAE, to facilitate access to food and hydration, mashed food and water were positioned lower. None of rats experienced reduction in body weight greater that 10%. Age- and strain-matched intact (non-immunized) animals served as baseline controls. Additionally, rats injected with CFA and *Bordetella pertussis* were included in the study.

Immunized animals were sacrificed by intracardial perfusion in either the inductive phase of EAE, on the 7th day post-immunization (d.p.i.) or in the effector phase, when the neurological deficit reached the maximum/plateau value, i.e. on the 13th and 16th d.p.i. in DA and AO rats, respectively (Djikić et al., 2014; Stojić-Vukanić et al., 2015, 2016). Prior to the perfusion, the rats were deeply anesthetized with an i.p. injection of ketamine/xylazine anesthetizing cocktail (80 mg/kg