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ScienceDirect
Immunobiology 212 (2007) 613–627
Immunobiology
www.elsevier.de/imbio

Stress-induced alterations in the programmed natural cycles of post-natal lymphoid organ development in C57BL/6 mice: Evidence for a regulatory feedback relationship between bone marrow and thymus

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Received 2 August 2006; received in revised form 12 March 2007; accepted 24 April 2007

Abstract

This study investigated some effects of weaning and immobilization stress in C57BL/6 mice aged 22–68 days, i.e., over a period including activation of the hypothalamus–pituitary–adrenal (HPA) axis and puberty. Specifically, the study evaluated the evolution, over the referred age interval, of a set of variables (body, thymus, spleen and axillary lymph nodes weights, the proportion of lymphoid cells in the bone marrow, the relative chemoattraction capacity of thymic supernatants for lymphoid cells and the migratory capacity of bone marrow lymphoid cells) in either weaned mice or weaned mice subjected to immobilization stress, compared to "non-stressed" unweaned mice. Cyclic patterns, observed for most variables in unweaned mice, were especially pronounced in two cases: the relative migratory capacity of bone marrow lymphoid cells collected at different ages towards neonatal thymic supernatant, and the relative chemoattraction capacity of thymic supernatants of different ages as tested against a sample of bone marrow lymphoid cells from mice aged 35 days. Weaning stress tended to intensify the involution stages of the cycles in thymus, spleen and lymph node weight, but increased the relative proportion of lymphoid cells in the bone marrow cell population. Both types of exogenous stress tended to affect cycle phase, i.e., cycle peaks and troughs were shifted in time. Correlations were observed between patterns seen in the thymus and bone marrow, suggesting the existence of an autoregulatory feedback loop governing pre-T cell migration and bone marrow/thymus homeostasis. These results also suggest that exogenous stress acts as a non-programmed regulator, modulating the naturally programmed cyclic patterns.

Keywords: Stress; Wean; Puberty; Thymus; Bone marrow; Migration; Chemoattraction

Introduction

The growth and maintenance of living organisms is achieved by highly coordinated body systems, often "natural clocks" regulated by a variety of substances including neurotransmitters and hormones. A centrally important regulatory system of this type is the hypothalamus–pituitary–adrenal (HPA) axis. Feedback mechanisms are important for the control of this system and other similar systems. However, many aspects of the function of regulatory systems of this type remain little understood.

The immune system was previously thought to be basically autonomous, but it is now evident that its function is strongly influenced by nervous and endocrine

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0171-2985/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.imbio.2007.04.005

signals associated with regulatory systems like the HPA axis. Equally, components of the immune system influence the function of the nervous and endocrine systems. In this connection, it has been proposed that the concept of HPA axis should be expanded to include the immune system (“the HPA–immune axis”), given that cytokines have important regulatory effects on the HPA axis (Besedovsky and del Rey, 1991; Besedovsky et al., 1991; Daneva et al., 1995). In fact, interactions between the immune, nervous and endocrine systems seem to be of key relevance for understanding diverse categories of diseases, including autoimmune diseases and stress-related diseases (Bartolomucci, 2005; De Kloet and Derijk, 2004; Vanitallie, 2002).

Organisms are continuously faced with stresses, both endogenous and exogenous. Reported responses to stress include organ-, cell- and molecular-level alterations of the nervous, endocrine, immune, reproductive and cardiovascular systems. These responses are highly complex and remain poorly understood. For example, in the immune system it is known that chronic stress typically leads to involution of lymphoid tissues and to immunosuppression (Kubera et al., 1998; Murray et al., 2001; Silberman et al., 2003). However, for acute stress, studies reporting either reinforcement of the immune system (Viswanathan and Dhabhar, 2005) or immunosuppression (Kizaki et al., 1996; Ruiz et al., 2003) have been published. Alterations in migration patterns under stress were reported for different cell types, for instance for granulocytes, macrophages and leukocytes (Mizobe et al., 1997; Zhang et al., 1998). For the thymus, the observed involution was associated with an increase of thymocyte apoptosis due to an increase of glucocorticoids (Jondal et al., 2004), and also with alterations in migration patterns of precursor-T cells to the thymus (Bauvois et al., 1989; Bomberger and Haar, 1992a, b; Dominguez-Gerpe and Rey-Mendez, 2003). Chemoattractant factors and bone marrow pre-T cells play important roles in the thymic involution (Dominguez-Gerpe and Rey-Mendez, 2000), but additional factors, such as lactoferrin (Artym et al., 2005; Zimecki et al., 2005) and alpha lactalbumin (Markus et al., 2000; Matsumoto et al., 2001; Orosco et al., 2004), seem to be also involved in the maintenance and restoration of homeostasis under stress or other conditions of immunosuppression. Although β2-microglobulin was reported to be a chemoattractant factor for avian pre-T cells (Dargemont et al., 1989; Deugnier et al., 1989; Dunon et al., 1990), its role in mouse is not clear, since β2-microglobulin-deficient mice normally developed thymus (Zijlstra et al., 1990). Chemokines CCL25 (TECK) and CCL21 (SLC) were reported to be involved in recruiting precursor T cells to the fetal thymus (Liu et al., 2005); however, for mice deficient for both CCR9 and CCR7, which are receptors for CCL25 and CCL21, respectively, colonization was deficient only in the prevascular fetal thymus (Liu et al., 2006). All these observations point towards other factors being responsible for the colonization of the postvascularized thymus by precursor T cells.

As part of an ongoing effort to further understand the immune system by studying its complex relationship with stress, we have now investigated the influence of weaning stress and immobilization stress in C57BL/6 mice aged 22–68 days, i.e., over the period including activation of the HPA axis and subsequently puberty. Specifically, the study comparatively evaluated the differences in the time-course evolution of a selected group of variables (body weight, the weights of the thymus, spleen and axillary lymph nodes, the proportion of lymphoid cells in the bone marrow cell population, the chemoattraction capacity of thymic supernatants and the migratory capacity of bone marrow cells) in three groups of mice: (a) weaned mice (i.e. mice separated from their dams at age 21 days), (b) weaned mice subjected to regular immobilization stress and (c) “non-stressed” unweaned mice, under exactly the same experimental conditions.

Materials and methods

Animals

Male and female C57BL/6 mice, bred in our animal facilities, were used in all experiments. Animals were kept under minimal-stress conditions, with lights on at 8:00 a.m. and off at 8:00 p.m., and free access to food and water.

Wean stress

Weaned mice were separated from their mothers at age 21 days. Mice were sexed at age 30 days. Unweaned mice were kept with their mothers all the time.

At regular intervals (i.e. at 18, 21, 22, 23, 24, 25, 26, 27, 28, 32, 35, 38, 42, 46, 49 and 53 days of age) subgroups of weaned and control unweaned mice were sacrificed (with CO_2) and weighed. Thymuses, spleens and axillary lymph nodes were then extracted and likewise weighed. Femurs and tibias were collected to extract bone marrow cells.

Immobilization stress protocol

Seven groups of 35 ± 1-day-old mice (total number of mice per group $n = 29$–42) were subjected to immobilization stress for 1 h per day for 1, 2, 3, 5, 8, 11 or 14 consecutive days. Immobilization was achieved by placing the animal inside a Plexiglas tube (length 120 mm, internal diameter 30 mm); this procedure is