Morphofunctional Characteristic of the Immune System in BALB/c and C57Bl/6 Mice

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 1, pp. 112-115, January, 2011
Original article submitted March 24, 2010

Inbred animals serve as an adequate model to study the role of genetic factors in adaptive, disadaptive, and pathological processes. Morphofunctional study of the immune system was performed on intact BALB/c and C57Bl/6 mice. The structural and functional parameters of the immune system in BALB/c and C57Bl/6 mice differ under physiological conditions. In BALB/c mice, volume density of T zone in the spleen and production of IL-2, IL-3, IL-4, IL-10, and TNF-α were much higher than in C57Bl/6 mice. However, IL-12 production in BALB/c mice was lower than in C57Bl/6 mice. C57Bl/6 mice were characterized by higher cytostatic activity of splenic NK cells. The observed interstrain differences are genetically determined and contribute to the type of adaptive processes and different sensitivity of these mice to pathogenic agents.

Key Words: *BALB/c; C57Bl/6; immune system; Th/1; Th/2; cytokines*

The degree and directionality of adaptive reactions are determined on the one hand by the severity and duration of stress exposure and on the other hand by the genotype and phenotype [5]. Therefore, studying the factors for individual resistance to stress is an urgent problem. Inbred animals serve as an adequate model for these researches [2].

Previous experiments revealed interstrain differences in the resistance of BALB/c and C57Bl/6 mice to emotional stress. The emotional and stress response is determined by specific features of the brain neurotransmitter systems in BALB/c and C57Bl/6 mice [3,13]. The humoral immune reactions mediated by type 2 T helper cells (Th2) were shown to prevail in BALB/c mice under physiological conditions and during stress or antigenic stimulation. By contrast, C57Bl/6 mice are genetically predisposed to the predominance of cellular immunity (Th1) [16]. The baseline differences in immunological reactivity of BALB/c and C57Bl/6 mice contribute to various sensitivities of these animals to pathogenic agents, tumor growth, and autoimmune diseases [1]. The mice of these strains are extensively used to evaluate the dependence of pathological processes on genetically determined characteristics of the immune system. However, little is known about morphofunctional parameters of the immune system in BALB/c and C57Bl/6 mice.

Here we studied morphofunctional characteristics of the immune system in BALB/c and C57Bl/6 mice under physiological conditions.

MATERIALS AND METHODS

Experiments were performed on 40 adult male BALB/c and C57Bl/6 mice weighing 18-20 g. The mice were euthanized by cervical dislocation under ether anesthesia. The spleen and thymus were sampled, fixed in Bouin's fluid, treated with ascending alcohols, and embedded into paraffin. Histological sections were stained with hematoxylin and eosin. Volume density of functional zones in the spleen and thymus was evalua-

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0007-4888/11/1511-0099

ted under a light microscope (×200) by the method of point counting.

Spleen cells of BALB/c and C57Bl/6 mice were isolated using a Potter homogenizer to evaluate the production of cytokines and cytotoxic activity of cells. The suspension of splenic cells was cultured in a growth medium with 5% fetal serum and concanavalin A (final concentration 5 μg/ml) to induce the synthesis and secretion of cytokines. The concentrations of IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, IFN-γ, and TNF-α in the supernatant of a one-day-old culture were measured by EIA (Bio Source International).

Cytotoxic activity of NK cells from mouse spleen was studied calorimetrically. The cytotoxic reaction was evaluated by biotransformation of 3-(4,5-dimethylthiazole-2-il)-2,5-diphenyltetrazolium bromide (MTT tetrazolium, Sigma) [8]. Mouse splenocytes and YAC-1 mouse lymphoma cells were used as the effector and target cells, respectively.

The results were analyzed by methods of variation statistics. The differences were evaluated by Student's *t* test.

RESULTS

Morphofunctional characteristics of the immune system in BALB/c and C57Bl/6 mice under physiological conditions were evaluated by a morphological and morphometric study of the thymus and spleen. The thymus was shown to have normal structure in mice of both strains. A greater part of the thymus was presented by the cortex. Hassall bodies (phase 1-2 of development) were identified in the medulla. Histological analysis and morphometry showed that the red and white pulp of the spleen were similar in these mice (Table 1). The volume density of periarterial lymphoid sheaths (PALS) in the spleen of C57Bl/6 mice was much lower than in BALB mice (Table 1). PALS belong to the T zone of the spleen. No interstrain differences were found in the volume density of lymphoid follicles in the marginal follicular zone and red pulp (Table 1).

The mice of these strains differed by cytokine production by splenocytes. The ability of mitogen-stimulated splenocytes to produce cytokines was shown to differ in these animals. Production of IL-2, IL-3, IL-4, IL-10, and TNF-α in BALB/c mice was much higher than in C57Bl/6 mice. However, IL-12 production in BALB/c mice was lower than in C57Bl/6 mice (Table 2). These animals did not differ by production of IL-5, IL-6, and IFN-γ (Table 2).

Cytotoxic activity of splenic NK cells in BALB/c mice was much lower than in C57Bl/6 mice (36.6±8.2 and 57.8±5.5, respectively; $p<0.01$).

Morphometry of the thymus and spleen and evaluation of the cytokine profile suggest that normal physiological processes in the immune system of BALB/c and C57Bl/6 mice are provided by various structural and functional parameters. Under physiological conditions, the volume density of spleen structures with T lymphocytes (PALS) in BALB/c mice is much higher than in C57Bl/6 mice. Published data show that these mice differ by the ratio between T lymphocyte populations of the thymus and peripheral lymphoid organs. BALB/c mice are characterized by higher percentage of $CD4^+CD25^+$ (regulatory T cells) and $CD4^+$ cells and lower percentage of $CD8^+$ cells than C57Bl/6 mice [14].

The ability of activated splenocytes to produce cytokines was shown to differ in BALB/c and C57Bl/6 mice. Previous studies revealed the existence of significant differences in cytokine production in specimens of the same population, which is related to genotypic characteristics of the body [4]. Production of IL-2, IL-3, and TNF-α was much higher in BALB/c mice than in C57Bl/6 mice (Table 2). The ability of splenic T cells in BALB/c mice to produce a considerable amount of IL-2 was shown to correlate with increased proliferative activity of T cells in response to mitogens [10]. C57Bl/6 mice are characterized by reduced production of IL-2 and low proliferative activity of splenic T cells.

IL-2 is one of the major T cell growth factors, which plays a role in induction and suppression of specific immune response and mediates fine mechanisms of regulation of effector T cells and regulatory T cells. A correlation was found between the production of IL-2 and number of regulatory $CD4^+CD25^+$ T cells in the thymus and peripheral lymphoid organs of BALB/c and C57Bl/6 mice. As differentiated from C57Bl/6 mice, BALB/c mice are characterized by increased count of $CD4^+CD25^+$ T cells, which shifts the Th1/Th2 balance toward Th2 cells [10].

TABLE 1. Comparative Morphofunctional Characteristics of the Spleen in BALB/c and C57Bl/6 Mice under Normal Conditions (*M*±*m*)

Strain	Volume density of functional zones in the spleen, %		
	white pulp		red pulp
	lymphoid follicles	PALS	
BALB/c	34.6±1.4	20.0±0.8	45.4±1.3
C57Bl/6	37.4±2.0	12.1±1.8*	46.4±1.1

Note. *$p<0.01$ compared to BALB/c mice.

TABLE 2. Cytokine Production by Splenocytes of BALB/c and C57Bl/6 Mice under Normal Conditions (*M*±*m*)

Strain	Cytokines, pg/ml								
	IL-2	IL-3	IL-4	IL-5	IL-6	IL-10	IL-12	IFN-γ	TNF-α
BALB/c	4499±488***	340±28*	795±105***	192±21	1208±10	160±21**	8.2±1.1**	1157±6	860±75***
C57Bl/6	1874±168	255±34	137±26	185±17	1122±16	93±9	16.0±2.3	1134±8	597±28

Note. *p<0.05, **p<0.01, and ***p<0.001 compared to C57Bl/6 mice.

IL-4 and IL-12 are the key cytokines that direct differentiation of antigen-stimulated naive $CD4^+$ T cells to Th1 and Th2, respectively. Splenocytes of BALB/c and C57Bl/6 mice were shown to differ by the ability to produce these cytokines. Under normal conditions, the production of IL-4 in BALB/c mice was much higher than in C57Bl/6 mice. Studying the mechanisms of genetic regulation of immunological reactivity provides indirect support to the results of our experiments. As differentiated from cells of C57Bl/6 mice, antigen-stimulated naive $CD4^+$ T cells of BALB/c mice produce a considerable amount of IL-4 [16]. It contributes to a shift in the Th1/Th2 balance toward Th2 cells.

IL-12 and IFN-γ serve as the antagonists of IL-4 in peripheral immune organs. Naive T cells (Th0) differentiate into Th1 cells in the presence of these cytokines. Activated antigen-presenting cells, B cells, and NK cells are the main producers of IL-12 in peripheral immune organs. We found that the production of IL-12 by splenocytes in BALB/c mice under normal conditions is much lower than in C57Bl/6 mice. These differences are probably associated with the fact that immunocompetent organs and tissues of intact C57Bl/6 mice contain more mature subpopulations of dendritic cells (as compared to BALB/c mice) [11]. Mature dendritic cells are characterized by high expression of class II major histocompatibility complex molecules and costimulatory molecules. Moreover, these cells produce a greater amount of IL-12. The resistance of these strains to intracellular parasites depends on the production of IL-12 and IFN-γ by stimulated dendritic cells and macrophages [9]. It should be emphasized that the mice of these strains differ by sensitivity of Th0 to IL-12.

IL-10 is an anti-inflammatory cytokine suppressing Th1 cells and macrophages. This cytokine is mainly produced by Th2 cells and macrophages. Considerable amounts of IL-10 are produced by $CD4^+CD25^+$ T cells. Our experiments showed that splenocytes from BALB/c mice produce a greater amount of IL-10 as compared to cells from C57Bl/6 mice. The main producers of IL-10 in the spleen of these animals probably differ by functional and quantitative characteristics. Our hypothesis is confirmed by published data. Under normal conditions, the total counts of $CD4^+CD25^+$ T cells, $CD4^+CD25^+Foxp3^+$ cells, and macrophages in the spleen of BALB/c mice are higher than in C57Bl/6 mice [15]. Moreover, IL-10 production by these populations of spleen cells in BALB/c mice is much higher than in C57Bl/6 mice.

Our results are consistent with published data that BALB/c and C57Bl/6 mice differ by the humoral (Th2) and cellular (Th1) components of the immune response. These differences are genetically determined. BALB/c and C57Bl/6 mice differ by the H2 haplotype and carry various alleles of immunoregulatory genes in the H2 region. They regulate a variety of immunological parameters, including the rate of antigen processing, expression and affinity of receptors for cytokines and hormones, and cytokine production by immunocompetent cells [12].

Recent studies of the cytotoxic activity of splenic NK cells confirm the existence of mouse strains with genetically determined high or low activity of NK cells [6]. C57Bl/6 mice belong to the former group. BALB/c mice are characterized by intermediate cytotoxic activity of NK cells.

The results of our study and published data indicate that normal physiological processes in the immune system of BALB/c and C57Bl/6 mice are provided by various morphofunctional parameters. The volume density of PALS and production of IL-2, IL-3, IL-4, IL-10, and TNF-α in the spleen of BALB/c mice are much higher than in C57Bl/6 mice. C57Bl/6 mice are characterized by higher production of IL-12 and greater cytotoxic activity of splenic NK cells. These genetically determined interstrain differences determine the type of adaptive reactions and different sensitivity of BALB/c and C57Bl/6 mice to stress and infections.

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