Reactions of Immune System to Immobilization Stress in Inbred Mice of Different Strains

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Mice of different strains can be classified by the intensity of humoral immune reaction to thymus-dependent antigen as high, moderate, and low responders. High and moderate responders (CBA/CaLac, DBA/1, BALB/c) are characterized by high sensitivity of the productive phase of humoral immune response and phagocytic activity of macrophages to immobilization stress. In low responders (C57BL/6, CC57W) stress only slightly affected the productive phase of the humoral immune response, but activity of peritoneal macrophages decreased. These differences in the reactions of the immune system of inbred mice after immobilization stress reflect different reactions of the immune system to extreme factors.

Key Words: *inbred mice; immobilization stress; thymus-dependent antigen; antibody-producing cells; peritoneal macrophages*

Numerous experimental studies in immunology are devoted to evaluation of specific features of behavioral, immune, and other reactions to various stimuli in inbred mice [1,3,8,9]. However, differences in the sensitivity and reactions of the immune system stimulated by thymus-dependent antigen in response to a standard extreme exposure (immobilization stress) were never studied. We made an attempt at systematizing the data obtained on inbred mice.

We previously detected some specific features in the reactions of the immune system in inbred mice to injection of the thymus-dependent antigen. By the reactions to the antigen (count of antibody-producing cells) the animals were divided into high (CBA/CaLac, DBA/2), low (C57BL/6, CC57W), and moderate responders (BALB/c) [5]. Here we investigated the sensitivity of the productive phase of the humoral immune response to immobilization stress in mice of different strains, as genetically determined variability of the response to stress can play a role in susceptibility to inflammatory and infectious diseases.

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MATERIALS AND METHODS

Experiments were carried out on 325 mice of strains CBA/CaLac, DBA/2, BALB/c, C57BL/6, CC57W aged 3 months (18-20 g, 65 animals of each strain, 5 of which were intact controls). The animals were obtained from the collection fund and Breeding Center of Institute of Pharmacology, Tomsk Research Center.

Control mice of each strain were immunized with sheep erythrocytes (single intraperitoneal injection of 0.2 ml 15% suspension). For modeling of immobilization stress (IS) the mice (experimental group 1) were placed in tight cylinders for 16 h. Group 2 animals were immobilized on day 3 after immunization. Material for analysis was collected on days 4, 7, 14, and 21 after immunization and on days 1, 4, 11, and 18 after immobilization. The values were compared with the corresponding values in intact controls (baseline levels) and in animals receiving antigen alone.

Phagocytic activity of peritoneal macrophages (PAPM) [10], the number of antibody-producing cells (APC) in the spleen [7], and specific hemagglutinins [4] were measured.

The results were statistically processed using Student's *t* test.

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RESULTS

Immunization of CBA/CaLac mice led to a significant increase in the absolute count of APC in the spleen on day 4 of the experiment; this parameter remained higher than in intact controls throughout the observation period (Fig. 1, *a*). IS after immunization significantly decreased the absolute count of APC in CBA/CaLac mice in comparison with controls, but on days 4 and 14 this parameter was higher than in intact controls. The maximum content of total hemagglutinins in the serum of mice immunized with sheep erythrocytes was observed on day 7 of the experiment. In group 2 the summary antibody titer remained below the control throughout the observation period. Immunization increased PAPM in CBA/CaLac mice, but later this parameter returned to the baseline level (Fig. 2, *a*). IS decreased phagocytic activity of macrophages on days 4 and 7 in both immunized and nonimmunized animals, but later this parameter surpassed the baseline and control values.

Injection of the antigen to DBA/2 mice induced changes in the absolute count of APC, similar to those in control CBA/CaLac mice (Fig. 1, *b*). APC count in the spleen of mice subjected to IS after immunization decreased below the baseline level on day 14. In contrast to CBA/CaLac mice, in DBA/2 strain APC count after IS returned to normal on day 21. The content of IgM antibodies in immunized mice peaked on day 4; the shift from IgM to IgG antibody production was observed by day 14. Two peaks of serum IgM were observed in animals exposed to IS on day 3 after immunization (days 7 and 21), which can indicate a shift in the production of this hemagglutinin to later periods in comparison with the control. The shift from IgM to IgG production in group 2 was observed on day 14 of the experiment. Immunization of DBA/2 mice with the antigen stimulated activity of peritoneal macrophages, which later returned to normal (Fig. 2, *b*). IS reduced PAPM in both experimental groups on days 4 and 7 of the experiment in comparison with the corresponding controls.

Immunization of BALB/c mice with the thymus-dependent antigen significantly increased the count of splenic APC on days 4 and 14 compared to baseline level (Fig. 1, *c*). Stress exposure after immunization decreased the studied parameter in comparison with the control on days 4-14. The peak of IgM hemagglutinins in BALB/c controls and experimental group 2 was observed on day 4, transition from IgM to IgG antibody production was observed on day 7. Phagocytic activity of macrophages significantly surpassed

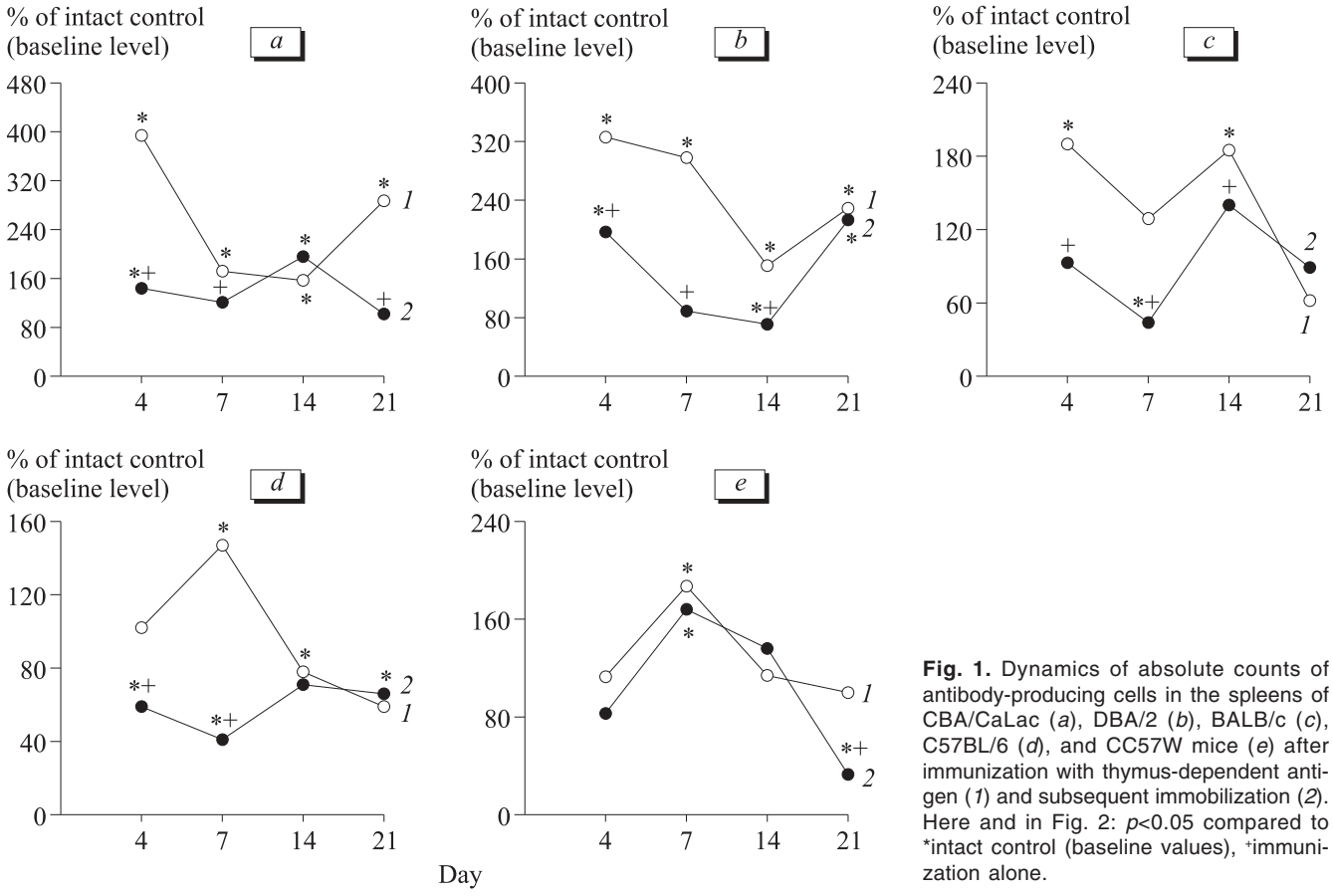

Fig. 1. Dynamics of absolute counts of antibody-producing cells in the spleens of CBA/CaLac (*a*), DBA/2 (*b*), BALB/c (*c*), C57BL/6 (*d*), and CC57W mice (*e*) after immunization with thymus-dependent antigen (*1*) and subsequent immobilization (*2*). Here and in Fig. 2: $p<0.05$ compared to *intact control (baseline values), +immunization alone.

the baseline level in all groups and peaked on day 7 (Fig. 2, *c*).

The increase in the absolute count of spleenic APC in control C57BL/6 mice after immunization with sheep erythrocytes was recorded on day 7 (Fig. 1, *d*). In mice subjected to IS after immunization the APC count decreased below the baseline level throughout the observation period. In control mice the content of IgM antibodies and IgG hemagglutinins peaked on days 4 and 14, respectively. In experimental group 2 the corresponding peaks were recorded on days 4 and 7. Phagocytic activity of macrophages in immobilized mice was below the baseline level throughout the entire observation period (Fig. 2, *d*). In immunized mice IS led to a decrease in PAPM on days 4, 14, and 21 of the experiment in comparison with the control.

In CC57W mice the peak of APC accumulation in the spleens of control and experimental group 2 was recorded on day 7 (Fig. 1, *e*). The maximum titer of IgM antibodies in the sera of mice injected with sheep erythrocytes was recorded on day 4, while the level of IgG hemagglutinins remained high to the end of the study. IS virtually did not modulate the time course of hemagglutinin titers in experimental group 2. Immunization decreased PAPM below the baseline level on day 4 (Fig. 2, *e*). In experimental groups 1 and 2 this parameter decreased in comparison with the baseline level on day 7 of the experiment.

Hence, our studies revealed differences in the reactions of the immune systems of inbred mice to a single 16-h IS after immunization with sheep erythrocytes. When analyzing the data we should take into account the initial differences in the levels of humoral immune response of inbred mouse strains (by the level of splenic APC formation in response to sheep erythrocytes the animals were divided into high (CBA/CaLac, DBA/2), moderate (BALB/c), and low responers (C57BL/6, CC57W), which is in line with previous reports [5]).

The interstrain differences are genetically determined and depend on many factors, *e.g.* on the rate of antigen processing and presentation on the membranes of macrophages and dendritic cells within the MHC II (Ia) histocompatibility complex, which, in turn, plays an important role in productive interactions between immunocompetent cells during the formation of primary immune response [2,6]. For instance, low level of humoral immune response in C57BL/6 and CC57W mice can be determined by disorders in antigen presentation with macrophageal histocompatibility receptors or low concentration of IaR on presenting cells [6]. This fact is indirectly confirmed by low activity

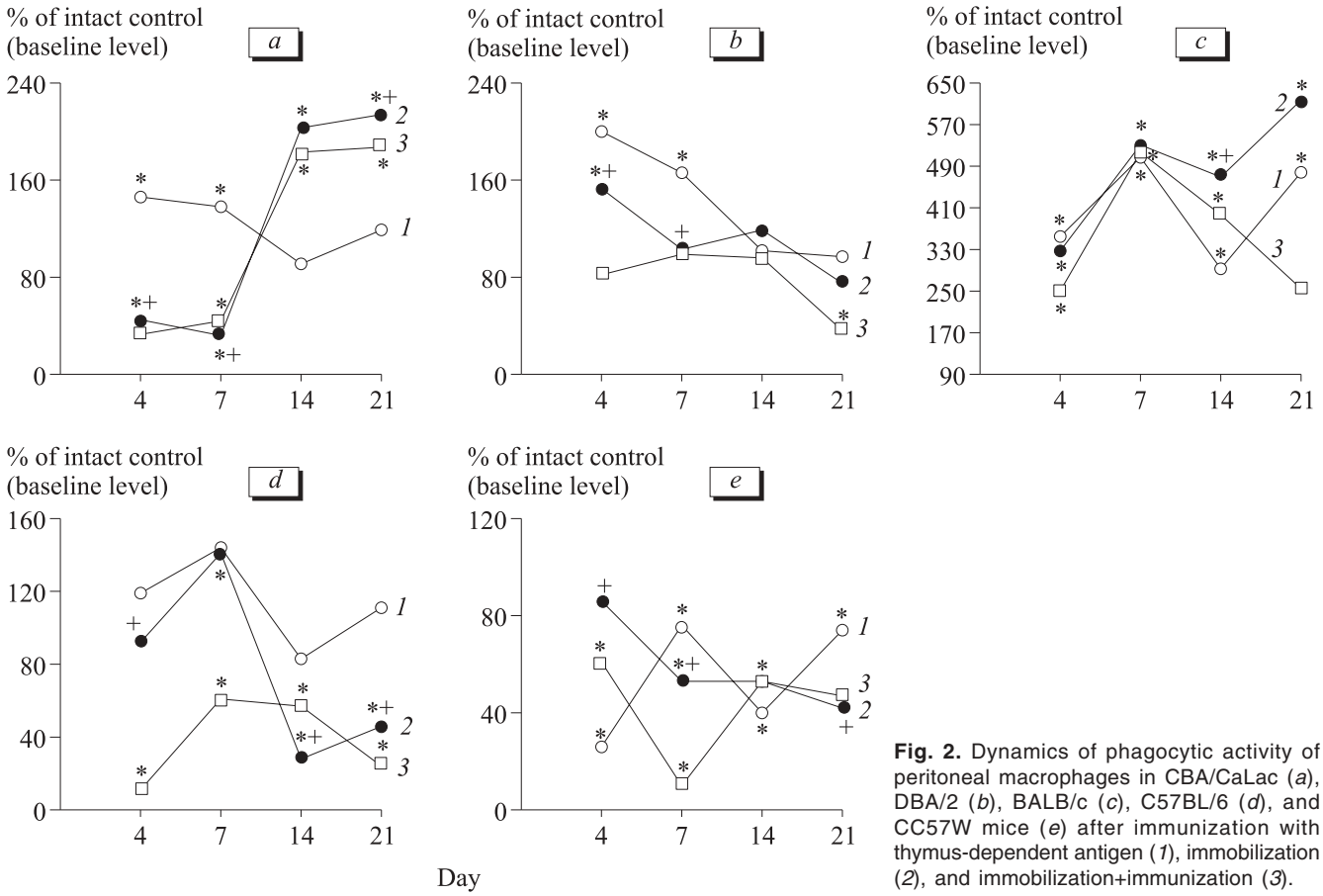

Fig. 2. Dynamics of phagocytic activity of peritoneal macrophages in CBA/CaLac (*a*), DBA/2 (*b*), BALB/c (*c*), C57BL/6 (*d*), and CC57W mice (*e*) after immunization with thymus-dependent antigen (*1*), immobilization (*2*), and immobilization+immunization (*3*).

of peritoneal macrophages in the respective control groups of C57BL/6 and CC57W strains (Fig. 2).

The humoral response in control CBA/CaLac, DBA/2, and BALB/c mice was more intensive compared to that in low responders and was associated with high phagocytic activity of macrophages. The decreased absolute count of APC in experimental groups can be due to stress-induced cytokine and hormone imbalance, more pronounced effects of glucocorticoids and catecholamines on the immune system, and lower presentation of MHC II receptors [11], which, in turn, results in inhibition of the immune response.

The detected differences in the reactions to stress are determined by not only intensive glucocorticoid production, but also differences in the production of cytokines responsible for regulation of the immune response. For example, DBA/2 mice are characterized by higher levels of IL-12 and IF-γ production and more potent induction of MHC II and Th1 response in the 3-cell cooperation component compared to BALB/c animals [8].

Different sensitivity to stress and hence, different levels of the immune response and nonspecific resistance are probably determined by differences in the initial neurotransmitter balance in the studied mouse strains. Serotonin level in the hypothalamus and brain stem of intact C57BL/6 mice is higher than in CBA/CaLac mice [1,3]. The predominance of the serotoninergic influences over dopaminergic decreases the immune response to antigen [3]. S. Hayley *et al.* showed that BALB/c mice are initially more reactive to stress than C57BL/6 and plasma catecholamine level increases more intensely in them than in C57BL/6 strain [9]. These congenital behavioral, metabolic, and other differences between the strains seem to be responsible for differences in the regulation and level of the immune response.

Hence, the detected differences in the reactions of the immune system of inbred mice to IS are genetically determined. The productive phase of the humoral immune response and PAPM are highly sensitive to IS in high and moderate responders (CBA/CaLac, DBA/2, BALB/c), which manifests by suppression of these parameters. By contrast, in low responders (C57BL/6 and C57W mice) the effect of stress on the productive phase of humoral immune response was negligible, activity of peritoneal macrophages only slightly decreased. Hence, it seems that different strategies of response of organism's systems (including the immune system) to stress are realized in inbred mice of different strains.

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