

# Comparative Characterization of Cytokine Production by Concanavalin A-Activated Splenocytes from BALB/c and C57Bl/6 Mice after Cold Exposure

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The level of cytokines produced by ConA activated splenocytes was studied in male BALB/c and C57Bl/6 mice after single and repeated cold exposure ( $-20^{\circ}\text{C}$ , 3 min). Single cold exposure significantly decreased IL-2, -3, -4, -5, -10, -12, IFN- $\gamma$  production in BALB/c mice and decreased IL-2 content and increased TNF- $\alpha$  level in C57Bl/6 mice. Repeated cold exposure normalized the content of IL-2, -4, -10, -12, and IFN- $\gamma$  in BALB/c mice, which reflects the development of adaptive immune reactions. In C57Bl/6 mice IL-2, -3, -5, -10, -12, and IFN- $\gamma$  production remained significantly decreased, which attested to dysadaptive processes.

**Key Words:** cold exposure; adaptation; cytokines; BALB/c and C57Bl/6 mice

BALB/c and C57Bl/6 mice differ by the resistance to emotional stress, sensitivity to hypoxia, and type of the immune response [1,2]. Antigenic stimulation leads to the development of immune reactions with participation of mainly type 1 T-helpers in C57Bl/6 mice and type 2 T-helpers in BALB/c mice [10]. Differences between the reactions of the immune system to stress in these two strains are intensely studied from the viewpoint of relationship between functional changes in the immune system, adaptive and dysadaptive reactions. This problem was investigated not once [6,7], but it still remains unclear how animals with different types of the immune response react to stress.

It is interesting to investigate the reactions of the immune systems in mice of different strains to stress, for example, to cold. Evaluation of the cytokine profile is often used in studies of the function of the immune system, because it reflects changes in cell and humoral immunity, hemopoietic processes, regenera-

tion, and development of allergic and inflammatory reactions [4].

We studied cytokine production by ConA-activated splenocytes of BALB/c and C57Bl/6 mice after single and repeated cold exposure.

## MATERIALS AND METHODS

The experiments were carried out on 60 male BALB/c and C57Bl/6 mice (18-20 g), 10 animals of each strain per group. Experimental animals were exposed to cold ( $-20^{\circ}\text{C}$ ) for 3 min once or repeatedly (daily for 10 days). Rectal temperature after cold exposure decreased by  $1^{\circ}\text{C}$ , no muscular tremor developed. The terms of investigation (1 and 10 days after cold exposure) correspond to urgent adaptation and phase of transformation of urgent adaptation into long-term adaptation [3]. The mice were sacrificed by hexenal overdose (intraperitoneal injection) 24 h after single or last session of repeated cold exposure.

Splenocyte suspension was cultured for 24 h in RPMI-1640 with 5% fetal serum and ConA in a final concentration of  $5\text{ }\mu\text{g/ml}$ . The concentrations of IL-2, -3, -4, -5, -6, -10, -12, IFN- $\gamma$ , and TNF- $\alpha$  were mea-

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sured in culture medium by enzyme immunoassay using Bio Source Int. kits.

The statistical significance of differences between the means was evaluated by Student's *t* test.

## RESULTS

The levels of IL-2, -4, -10, and TNF- $\alpha$  produced by splenocytes were significantly higher in BALB/c mice compared to C57Bl/6 mice (Table 1). The concentrations of IL-3 and IFN- $\gamma$  were virtually the same in these two strains. The concentration of IL-12 in BALB/c mice was significantly lower ( $p < 0.001$ ) than in C57Bl/6. These data suggest that the function of the immune system in compared mouse strains is normally maintained by different levels of cytokines.

Single cold exposure significantly decreased the levels of IL-2, -3, -4, -5, -10, -12, and IFN- $\gamma$  in BALB/c mice (Table 1). The level of TNF- $\alpha$  did not change after single cold exposure. After repeated cold exposure IL-2, -4, -10, -12, IFN- $\gamma$  production in BALB/c mice returned to normal, while the levels of IL-3 and IL-5 remained low. The cytokine profiles indicate the development of adaptive changes in the immune system of BALB/c mice after repeated cold exposure. Decreased level of IL-3 after repeated cold exposure can be indicative of the absence of normalization of regenerative processes in the hemopoietic system during this period of adaptation (10 days) [8]. Low level of IL-5 produced by activated T-lymphocytes and mast cells and stimulating eosinophil proliferation and differentiation seems to reflect antiallergic effect of repeated cold exposure [4,9].

In C57Bl/6 mice single cold exposure significantly decreased IL-2 level, IL-3 content tended to decrease

(similarly as in BALB/c mice). The concentrations of IL-4, -5, -10, and IFN- $\gamma$  virtually did not change. After single cold exposure the level of TNF- $\alpha$  increased significantly, which probably reflects antiinflammatory effect of single cold exposure. The levels of IL-2, -3, -5, -10, -12, and IFN- $\gamma$  in C57Bl/6 mice repeatedly exposed to cold were significantly below the control. The levels of IL-2, -5, -10, -12, and TNF- $\alpha$  decreased significantly in comparison with the corresponding parameters after single cold exposure (Table 1). Hence, analysis of the cytokine profile of C57Bl/6 mice showed no normalization of the immune status after repeated cold exposure.

The differences in the reaction of the immune system of intact BALB/c and C57Bl/6 mice and in response to cold exposure seem to be genetically determined. BALB/c and C57Bl/6 mice differ by the main histocompatibility complex genes: BALB/c carry H-2<sup>d</sup> haplotype, while C57Bl/6 carry H-2<sup>b</sup> haplotype. Genotypically determined characteristics of the stress-realizing and stress-limiting systems play an important role in the mechanisms of formation of adaptive reactions to cold [3]. Different adaptive and endocrine metabolic reactions to cold exposure were detected in rats of two different strains [6]. C57Bl/6 mice are characterized by active reaction to emotional stress, while BALB/c mice exhibit freezing reaction [2]. Nor-epinephrine level in the hypothalamus of C57Bl/6 mice is higher and that of serotonin lower than in BALB/c mice. Moreover, these mouse strains differ by the type of immune response to antigenic stimulation [6]. However, we failed to detect clear-cut differences in the levels of cytokine production in mice of the two strains, depending on the type of immune response. This is due to the fact that cold exposure is

**TABLE 1.** Levels of Cytokines (pg/ml) Produced by Splenocytes of BALB/c and C57Bl/6 Mice under Normal Conditions and after Cold Exposure ( $M \pm m$ )

Cytokines	BALB/c			C57Bl/6		
	control	cold exposure		control	cold exposure	
		single	repeated		single	repeated
IL-2	4499 $\pm$ 488	2959 $\pm$ 398**	3750 $\pm$ 580	1874 $\pm$ 168	1204 $\pm$ 103**	627 $\pm$ 45*****
IL-3	340 $\pm$ 28	123 $\pm$ 14***	200 $\pm$ 12***	255 $\pm$ 34	186 $\pm$ 7	153 $\pm$ 34*
IL-4	795 $\pm$ 105	424 $\pm$ 78**	648 $\pm$ 128	137 $\pm$ 26	106 $\pm$ 10	92 $\pm$ 7
IL-5	192 $\pm$ 21	108 $\pm$ 20**	100 $\pm$ 10**	185 $\pm$ 17	196 $\pm$ 23	130 $\pm$ 18**
IL-6	1208 $\pm$ 10	1105 $\pm$ 18	1210 $\pm$ 53	1122 $\pm$ 16	1022 $\pm$ 57	1112 $\pm$ 38
IL-10	160 $\pm$ 21	94 $\pm$ 5**	129 $\pm$ 13*	93 $\pm$ 9	93 $\pm$ 7	58 $\pm$ 8****
IL-12	8.24 $\pm$ 1.10	4.0 $\pm$ 1.2**	14.3 $\pm$ 3.0**	16.0 $\pm$ 2.3	14.3 $\pm$ 2.0	9.5 $\pm$ 1.8*
IFN- $\gamma$	1157 $\pm$ 6	829 $\pm$ 50***	1117 $\pm$ 3	1134 $\pm$ 8	1068 $\pm$ 21**	946 $\pm$ 60**
TNF- $\alpha$	860 $\pm$ 75	737 $\pm$ 60	709 $\pm$ 38	597 $\pm$ 28	736 $\pm$ 50**	555 $\pm$ 64**

**Note.** \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to the control; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to single cold exposure.

nonspecific as concerns its effects on the T- and B-immunity systems.

Stress exposure decrease of blood leukocyte counts in humans and animals; functional activities of lymphocyte subpopulation change and the subpopulations are redistributed mainly into the barrier tissues (skin and mucosa) [8]. Hence, decreased production of some cytokines in BALB/c and C57Bl/6 mice in response to single cold exposure is adaptive and reflects activation, proliferation, and migration of immunocompetent cells. Normalization of the parameters and a trend to normalization of the cytokine levels in BALB/c mice after repeated cold exposure indicates that this exposure is physiological.

No significant changes in the levels of the majority of cytokines were detected in C57Bl/6 mice after single cold exposure, except the levels of IL-2 and TNF- $\alpha$ . The production of all cytokines decreased after repeated cold exposure, presumably reflecting quantitative and functional insufficiency of the immune system. It was previously shown that in contrast to BALB/c mice, C57Bl/6 animals developed pronounced inflammatory changes in the periodontium in response single and repeated cold exposure [5]. Hence, the decrease in cytokine levels in C57Bl/6 mice repeatedly

exposed to cold is paralleled by the development of inflammatory changes in the periodontium, which attests to the formation of dysadaptive reactions in this mouse strain.

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