Cytokine Profile of the Blood in Mice with Normal and Abnormal Heart Rhythm

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Differences in the pools of 10 cytokine were found in blood samples from the caudal vein of mice with normal and abnormal heart rhythm. Both groups were albino mice bred by us and differing from mdx albino mice by the absence of mutation in muscular dystrophin gene. Mice with normal heart rhythm had low IL-17 content and elevated concentrations of proinflammatory cytokines IL-6 and IL-1α in comparison with the normal (according to published data). In mice with bradyarrhythmias, increased blood levels of IL-10, IL-6, IL-5, IL-2, IL-1α, IL-17, IL-4, TNF-α, and granulocyte-macrophage colony-stimulating factor were detected. The relative content of IL-4 and IL-17 in the total cytokine pool increased. The lifespan of mice with bradyarrhythmias and cytokine hyperexpression was shorter by 2-3 months in comparison with mice without heart rhythm disturbances and moderate changes in the cytokine pool.

Key Words: *mdx albino mice; cytokines; heart rhythm; bradyarrhythmia*

According to the current views about cytokine metabolism and function, blood concentrations of these low-molecular-weigh hormone-like proteins should be very low. In cardiology, the elevation of proinflammatory cytokine concentrations accompanying various pathologies, including some arrhythmias [5], is considered to be a proof for the important role of inflammatory processes in the pathogenesis of heart diseases. On order to go out of the scope of the concept of cytokines as exclusively laboratory markers of inflammation, evaluate the contribution of the cytokine system in myocardial damage/reparation, and determine the principles of their therapeutic use in cardiology, animal models of heart diseases are needed.

We previously obtained a new strain of mdx mice, so-called mdx albino mice; genetic marker of this strain is a mutation in the muscular protein dystrophin gene and an additional genetic defect responsible for oculo-cutaneous albinism [1]. Albino mice with the same genetic basis as mdx albino mice, but carrying normal dystrophin gene served as the control. Electrocardiographic analysis revealed animals with bradyarrhythmias among these mice and among mdx albino mice carrying mutation in the dystrophin gene.

Here we compared the pools of circulating cytokines in mice with normal heart rhythm and mice with bradyarrhythmias and evaluated the possibilities of using these animals in cardioimmunology for studying the pathogenesis and development of new medical technologies for the treatment of heart rhythm disturbances.

MATERIALS AND METHODS

Experiments were carried out on 40 albino mice (18 males and 22 females) bred by us and differing from mdx albino mice by the absence of mutation in muscular protein dystrophin gene. Mouse age varied from 1.5 to 2.0 years.

For ECG recording, alert mice were placed onto a horizontal platform in natural posture; the limbs were fixed with soft ties. Needle electrodes were routinely positioned in standard lead I. The electrical signal was

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filtered and amplified using a BioAmp four-channel amplifier (model ML136). A Chart 5.5 module for ECG analysis (ADInstruments Pty Ltd) was used. After the first ECG recording, the animals were not distributed in cages according to their heart rhythm, but were individually labeled and the mice with arrhythmia and controls were kept together in the same cages under conventional conditions maximally far from the common vivarium. ECG was recorded repeatedly with an interval not less than 1 week.

The blood (200 μl) needed for isolation of 60 μl serum was taken from the caudal vein (cut with a scalpel). Quantitative analysis of sera was performed using FlowCytomix kits for simultaneous assay of 10 serum cytokines (mouse Th1/Th2 10plex), the results were recorded using a flow cytometer.

The data were processed statistically using Student *t* test and Kruscal—Wallis test.

RESULTS

In the group of mice with normal sinus rhythm, HR was 600-650 bpm and remained at this level for a long time (Fig. 1). In mice with bradyarrhythmia, heart rate was primarily irregular; the mean HR did not attain 500 bpm and sometimes decreased to 300 bpm. In this group we found no manifest clinical signs of heart pathology (ascitis, dyspnea, cyanosis). Passive behavior of the mice during fixation and low muscular tone are worthy of note. The lifespan of mouse with bradyarrhythmia was somewhat lower than in mice with normal rhythm. These differences (2-3 months)

TABLE 1. Cytokine Concentrations in Serum Samples from Mice with Normal Heart Rhythm and Bradyarrhythmia (Me; Q1-Q3)

Cytokine	Concentration, pg/ml		Significance of intergroup differences by Kruskal–Wallis ANOVA and median tests	Range of normal values in intact mice	Range of high values in toxic shock syndrome
	normal rhythm (*n*=19)	bradyarrhythmia (*n*=21)			
IL-10	28 (20-36)	40 (24-56)	$p \le 0.02$	5-20 pg/ml [4], 80 pg/ml [3], 50 pg/ml [6]	150-500 pg/ml [6]
IL-6	240 (170-280)	310 (260-320)	$p \le 0.04$	5-20 pg/ml [4], 40 pg/ml [3], 30 pg/ml [7]	1000-40,000 pg/ml [6]
IL-5	72 (62-72)	90 (68-140)	$p \le 0.01$	5-20 pg/ml [4], 7 pg/ml [3], 100 pg/ml [6]	100-700 pg/ml [4]
IL-2	32 (24-36)	48 (24-56)	$p \le 0.02$	5-20 pg/ml [4], 12 pg/ml [3]	18,000-80,000 pg/ml [6]
IL-1α	300 (280-320)	560 (188-600)	$p \le 0.009$	25-80 pg/ml [4], 55 pg/ml [6]	400-1500 pg/ml [6]
IL-17	12 (4-20)	48 (16-100)	$p \le 0.03$	100-250 pg/ml [4], 32 pg/ml [3]	1500-7000 pg/ml [6]
IL-4	48 (20-64)	410 (120-580)	$p \le 0.002$	≤1 pg/ml [4], 30 pg/ml [7], 12 pg/ml [6]	500-3000 pg/ml [6]
GM-CSF	40 (32-80)	150 (40-220)	$p \le 0.04$	25-80 pg/ml [4], 11 pg/ml [3]	80-800 pg/ml [6]
TNF-α	20 (0-44)	70 (44-140)	$p \le 0.002$	100-250 pg/ml [4], 10 pg/ml [7], 110 pg/ml [3]	400-4000 pg/ml [6]
IFN-γ	200 (64-860)	640 (188-1520)	$p \le 0.05$	250-400 pg/ml [4], 10 pg/ml [7], 15 pg/ml [3]	500-4500 pg/ml [6]
Total cytokines in 1 ml blood	1126 (818-1486)	2744 (1312-3224)	$p \le 0.04$		

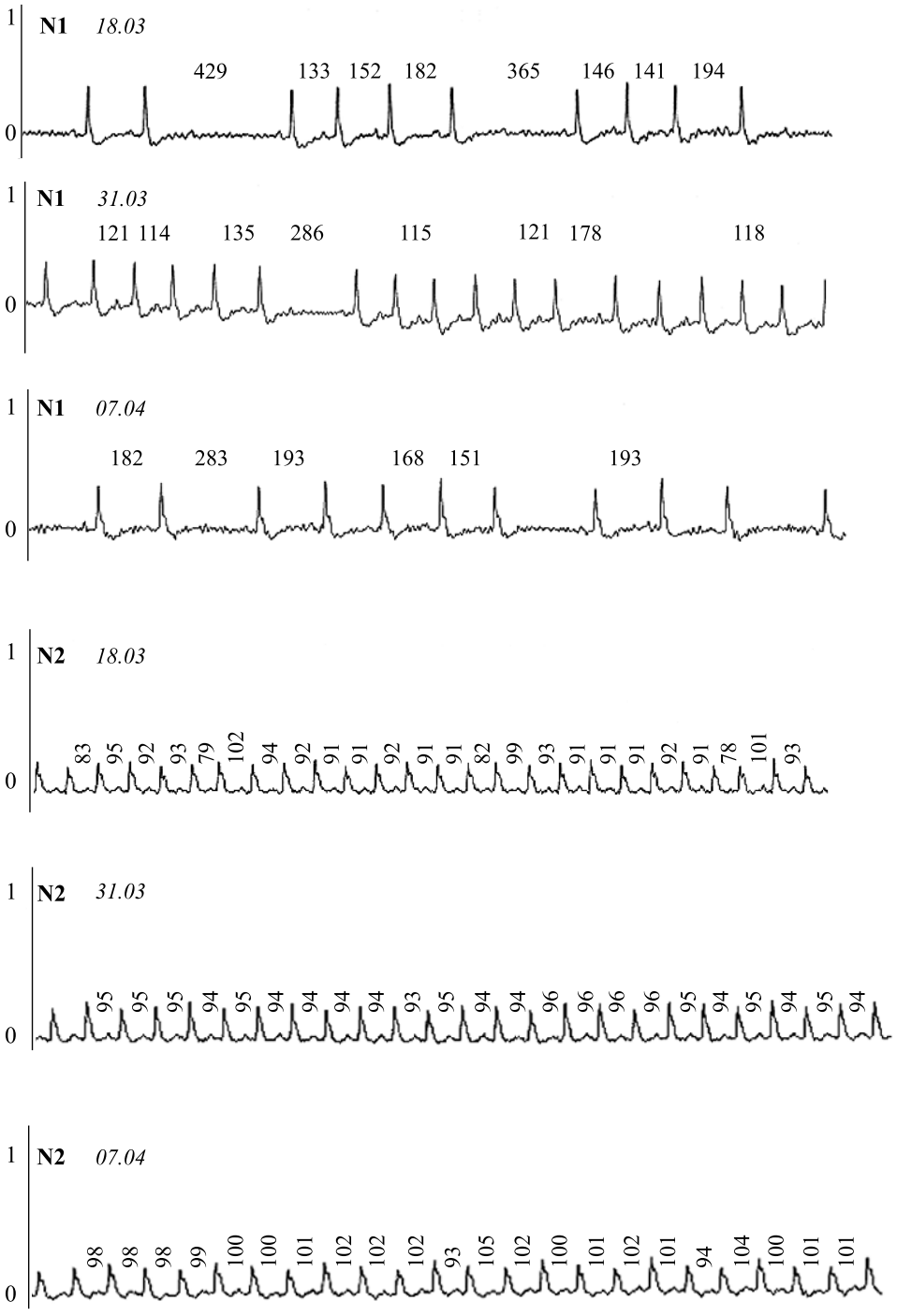

Fig. 1. Dynamic evaluation of electrical activity of the myocardium in a mouse with bradyarrhythmia (N1) and a mouse with normal heart rhythm (N2). Duration of RR intervals is shown in msec.

appeared at the end of life cycle that lasts for about 2.5 years.

The results of quantitative evaluation of the cytokine pool in mice with normal and abnormal heart rhythm and previously published data on cytokine pool in intact mice and animals with toxic shock syndrome are summarized in Table 1. The width of normal range is probably determined by genotypic peculiarities of the used mice, maintenance conditions, the route of blood sampling, cytokine assay methods, *etc*. Despite blurriness of normal range boundaries, we can see reduced content of IL-17 and elevated concentrations of IL-6 and IL-1α in these mice. The concentrations of the latter two cytokines did not attain their levels observed in toxic shock [6], when cytokine hyperexpression leads to grave clinical consequences.

The mice with heart rhythm disturbances differed from mice with normal HR by increased blood content of 9 of 10 studied cytokines. The only exception was IFN-γ; the significance of intergroup differences

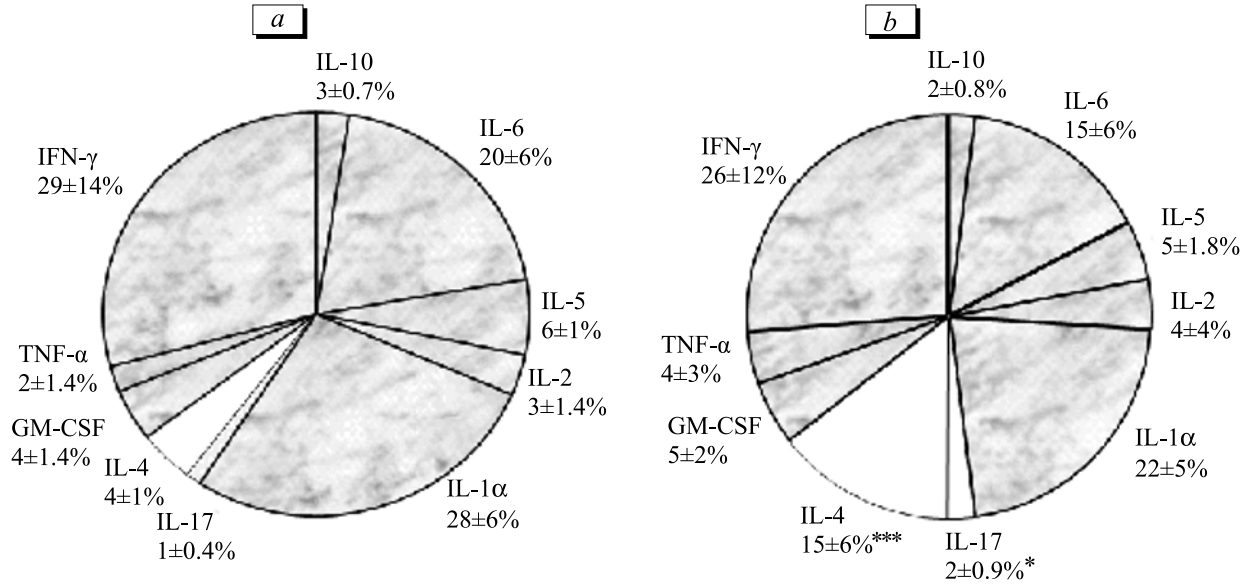

Fig. 2. Relative content of cytokines with different functional activity in the total pool of cytokines in mice with normal heart rhythm (*a*) and bradyarrhythmia (*b*). *$p<0.05$, ***$p<0.001$.

in its concentrations was not proved for this cytokine at the specified number of measurements. Against the background of bradyarrhythmia, the content of IL-1α, IFN-γ, and granulocyte-macrophage colony-stimulating factor (GM-CSF) attained the range of very high concentrations observed in toxic shock syndrome. The content of IL-5 and IL-4 approached the lower limit of this interval.

The total content of all 10 cytokines in 1 ml blood of mice with bradyarrhythmia more than 2-fold surpassed the corresponding parameter in mice with normal heart rate (Table 1).

In mice with bradyarrhythmia, elevated mean relative content of proinflammatory IL-17 and sharply increased content of anti-inflammatory cytokine IL-4 in comparison with those in mice with normal heart rhythm were revealed (Fig. 2).

If we exclude the possibility of cytokine release from skeletal muscles [2] intensively contracting during fixation, the high blood levels of IL-6 and IL-1α in mice with normal heart rhythm can be regarded as an indicator of a latent inflammatory focus that had no electrocardiographic and clinical manifestations. In that case, mice with bradyarrhythmia are at another/next stage of the pathological process in the heart characterized by aggravation of heart failure due to either hemodynamic shifts caused by reduced HR and heart rhythm irregularities or due to impaired morphogenetic (reparative) function of the immune system. The latter conclusion agrees with the data on higher mortality in mice with bradyarrhythmia in comparison with mice with normal heart rhythm and redistribution of cytokines with different functional activity and clear-cut increase in their total content per unit blood volume.

It was previously hypothesized that the pathophysiological mechanisms responsible for the appearance of cytokines in the blood are different in different forms/stages of the pathological process in the heart. It was shown on the mouse model that hyperexpression of cytokines in heart failure manifests only against the background of circulatory congestion, when the liver and intestine become the main sources of cytokines [8]. In our work, the question about the source of cytokines and pathogenetic role of their hyperexpression in mice with bradyarrhythmia remains still open. More substrains of albino mice with the same genetic basis as mdx mice are required as the models for evaluation of the role of the immune system in the pathogenesis of heart diseases and in particular pathologies associated with rhythm disturbances.

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