Morphofunctional Characteristics of the Immune System in CBA and C57Bl/6g Mice

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We studied peculiarities of morphofunctional organization of the immune system in C57Bl/6g and CBA mice differing by their susceptibility to various types of infectious agents. The revealed differences in the structure of lymphoid organs, T lymphocyte subpopulation ratio and their differentiation into Th1/Th2 cells after mitogen stimulation drove us to a conclusion on genetically determined regularities in the development of the immune response in these animal strains.

Key Words: *opposite mouse strains CBA and C57Bl/6g; antiviral immunity; Th1/Th2 cells*

The mechanisms of sensitivity and resistance to various types of infectious agents and the role of phenotype in determining the response to infections in mammals are urgent problems of modern immunology and biology. Comparative analysis of the peculiarities of the immune response and infectious processes in inbred animals is widely used for studies in this field [1]. A correlation was found between the resistance of experimental animals with different genotypes to *Porphyromonas gingivalis* and expression of immune response genes [4]. Some authors revealed interstrain differences in the response of CBA and C57Bl/6g to bacterial and fungal agents *M. tuberculosis* [3] and *C. albicans* [2]. It is known that C57Bl/6g mice are less susceptible to most intracellular parasites compared to CBA mice, which is related to predominance of Th1-dependent immune response and intensive elimination of the agent by macrophages [8,9,10]. However, the role of phenotype in determination of the immune response to infectious pathogens, especially viruses [5,6], is still poorly studied.

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Here we studied morphofunctional peculiarities of the immune system in CBA and C57Bl/6g mice for evaluation of the role of genotype in determination of the immune response to virus infection in mammals.

MATERIALS AND METHODS

The study was performed on 2-month-old male CBA and C57Bl/6g mice weighing 20-25 g obtained from the nursery of Institute of Clinical Immunology, Siberian Division of Russian Academy of Sciences. The mice were decapitated under ether narcosis. The blood was collected into tubes with 40 U heparin and the thymus and spleen were isolated.

The samples for histological examination were fixed in 10% neutral formalin, dehydrated in ascending alcohols and xylenes, and embedded in paraffin. The sections (4-5 μ) were stained with hematoxylin and eosin. For immunophenotyping of thymus and splenic cells and evaluation of their proliferative activity, immunohistochemical analysis was performed by indirect streptavidin-biotin method using specific monoclonal antibodies CD4, CD8, CD20, CD68 (DBS), and PCNA (Novocastra). Visualization was carried out on Axio-

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Imager A1 microscope (AxioCam MRc camera, Carl Zeiss). Morphometry of tissue structural elements was performed using ocular grid (100 points, 3.64×10^4 μ^2) and AxioVision 4.7 software.

Immunophenotype of cell subpopulations in the studied organs was more precisely determined by flow cytometry. The thymus and spleen were homogenized in a Potter–Elvehjem homogenizer and lymphocyte suspension was collected after sedimentation of the stroma. Erythrocytes were lyzed with 10-fold lyzing buffer (0.8% NH_4Cl, 0.08% EDTA, 0.08% $NaHCO_3$). After washout, the cells in phosphate buffer with 10% FCS and sodium azide (10^7/ml, 100 μl) were transferred into tubes for cytofluorometry and incubated for 20 min at 25°C in the dark with fluorochrome-labeled fluorochrome (BioLegend) in the following combinations: CD3-FITC/CD8-PE/CD45-PerCP/CD4-APC (for evaluation of T cell subpopulations) and CD45-PerCP/CD19-APC (for B cells) with subsequent washout. Fluorescence was analyzed on a FACSCalibur flow cytofluorometer (Becton Dickinson) using CellQuest software. Type 1 and type 2 T helpers were analyzed by flow cytometry. Splenocytes were cultured in a 24-well plate (Greiner Bio-One) in a concentration 10^6/ml in RPMI-1640 medium containing 10% FCS, 0.03% L-glutamine, 100 U/ml streptomycin, 100 μg/ml penicillin, 25 ng/ml phorbol 12-myristate 13-acetate (PMA), and 1 μM ionomycin. Twenty-four hours after stimulation, the cells were washed from the culture medium and immunotyped as described above using CD3-FITC/CD45-PerCP antibodies. After washout the cells were fixed, permeabilized using eBioscience kits, and stained with antibodies to intracellular markers GATA3-PE and Tbet-AlexaFluor647 (eBioscience).

The data were processed statistically using parametric Student's *t* test (the differences were significant at $p<0.05$).

RESULTS

Peripheral blood test revealed no interstrain differences in the content of neutrophils, monocytes, and $CD3^+$T- and $CD19^+$B lymphocytes, but the content of cytotoxic $CD3^+CD8^+$ T lymphocytes was higher (by 2.4 times) in C57Bl/6g mice and the content of T helpers ($CD3^+CD4^+$) was higher (by 1.6 times) in CBA mice. Therefore, mice of the opposite strains significantly differed by CD4/CD8 ratio: in CBA mice this parameter was higher by 3.3 times than in C57Bl/6g mice (Table).

Histological examination of thymus samples showed that numerical density of Hassall bodies (presumably playing a role in elimination of pathogens via phagocytosis) in the thymus cortex of C57Bl/6g mice was 2-fold higher than in CBA mice. The number of macrophages in the thymus of CBA mice 1.5-fold surpassed that in C57Bl/6g. Numerical density of lymphocytes in the thymus of C57Bl/6g mice was higher by 31%. In CBA mice, the number of proliferating $PCNA^+$-lymphocytes was 2-fold higher and apoptotic lymphocytes 1.5-fold lower than in C57Bl/6g mice. Thus, we can hypothesize that selection with elimination of autoreactive thymocytes is more active in C57Bl/6g mice.

Analysis of cell composition of the thymus in mice of the opposite strains by the method of flow cytometry revealed no differences in the content of $CD3^+$-thymocytes; the number of cells carrying B lymphocyte marker did not exceed 1% in all mice, other cells were most likely early T lymphocyte precursors ($CD3^-$), non-lymphoid stromal cells, and cells of adjacent tissues. CBA mice were characterized by higher content of monopositive T cells ($CD4^+$ or $CD8^+$), while the content of double positive cells ($CD4^+CD8^+$) was higher in C57Bl/6g mice, which also confirms more intensive negative selection of T cells. The opposite strains did not differ by the content of double negative thymic T lymphocytes ($CD4^-CD8^-$). The CD4/CD8 ratio among most mature (monopositive) cell subpopulation was also similar in mice of the compared groups (Table 1).

Analysis of cytoarchitectonics of the spleen revealed predominance of the volume density of the white pulp (61.6% in CBA and 54.6% in C57Bl/6g) presented by primary lymphoid follicles, dense clusters of small lymphocytes. The diameter of splenic follicles in CBA mice surpassed the corresponding parameter in C57Bl/6g by 15.4% (490.55 *vs.* 425.06 μ, Table 1). The number of proliferating $PCNA^+$-lymphocytes in the spleen of CBA mice was higher than in C57Bl/6g mice by 27.3%. These findings agree with the data of other researchers who consider CBA and C57Bl/6g as strains with high and low levels of spontaneous splenocyte proliferation, respectively [5]. B lymphocytes ($CD20^+$) of primary follicles were phenotypically identical to blood B cells. The T/B lymphocyte ratio in C57Bl/6g mice was considerably higher than in CBA mice (1.46 and 1.1, respectively). High T/B ratio typical of both mouse strains attests to their high potential in the realization of cell-mediated immune reactions playing the key role in antiviral defense [7]. Numerical density of $CD68^+$ monocytic cells in C57Bl/6g mice was higher by 1.4 times than in CBA mice (Table 1).

Analysis of cell immunophenotype by the method of flow cytometry showed that cytotoxic lymphocytes predominate among splenic T cells in C57Bl/6g mice (20.5% all splenocytes) and T helpers in CBA mice (13.85%). Therefore, T lymphocyte CD4/CD8 index was also considerably higher in CBA mice compared to that in C57Bl/6g mice (2.0 and 0.7, respectively,

TABLE 1. Immunomorphological Analysis of Cells from the Blood, Spleen, and Thymus of Male Mice of Opposite Strains C57Bl/6g and CBA (*M±m*)

Studied parameters		Opposite mouse strains	
		C57Bl/6g	CBA
Peripheral blood			
Relative content	T lymphocytes, %	18.5±2.3	25.0±8.0
	$CD4^+$ T lymphocytes, %	36.7±14.5	59.6±2.1*
	$CD8^+$ T lymphocytes, %	44.6±18.4	18.4 ±3.6*
	B-lymphocytes, %	30.1±12.1	20.0±6.5
	monocytes, %	7.0±2.4	7.9±1.1
	neutrophils, %	21.2±4.2	23.0±1.4
CD4/CD8		1.0±0.6	3.3±0.8*
Thymus			
Nai of Hassall bodies, μ^0/μ^2		2.0±0.2	1.2±0.1*
Nai of lymphocytes, μ^0/μ^2		190.8±0.8	131.5±0.6*
	of them $PCNA^+$ thymocytes, %	30.3±0.4	63.1±0.6*
	of them apoptotic thymocytes, %	5.4±0.3	3.2±0.1*
Nai of macrophages, μ^0/μ^2		1.4±0.1	1.9±0.1*
Relative content	$CD4^+$ lymphocytes, %	29.0±11.6	36.4±5.5
	$CD8^+$ lymphocytes, %	10.9±1.3	12.6±3.1
	$CD4^+CD8^+$ lymphocytes, %	51.4±13.6	38.5±4.3
	$CD4^-CD8^-$ lymphocytes, %	7.8±2.9	11.2±4.3
CD4/CD8		2.6±0.8	3.1±1.2
Spleen			
Vv of red pulp, %		43.8±2.1	36.1±2.2*
Vv of white pulp, %		54.6±2.0	61.6±2.4*
Diameter of splenic follicle, μ		425.1±17.5	490.6±21.3*
Nai of $CD68^+$ cells, μ^0/μ^2		38.0±2.9	26.8±2.4*
Nai of T lymphocytes, μ^0/μ^2		105.7±5.1*	92.67±2.7
	of them $PCNA^+$ lymphocytes, %	12.3	8.9
Nai of $CD20^+$ cells, μ^0/μ^2		72.4±3.4	83.9±3.9*
T/B lymphocytes, %		1.46	1.1
Relative content	$CD4^+$ T lymphocytes, %	34.8±11.9	59.0±8.2*
	$CD8^+$ T lymphocytes, %	56.5±14.9	30.0±1.9*
	$CD8^+CD4^+$ T lymphocytes, %	3.5±2.8	0.5±0.3*
CD4/CD8		0.7±0.5	2.0±0.4*

Note. *$p<0.05$ between the strains. Nai: numerical density, Vv: volume density. Relative number of T and B lymphocytes, monocytes, and neutrophils is presented in percents of the total number of nucleated cells. Relative content of T lymphocyte subpopulations is presented in percents of the total number of $CD3^+$ cells.

Table 1). Among splenic lymphocytes of C57Bl/6g mice, a subpopulation of T cells carrying CD4 and CD8 membrane molecules ($CD4^{int}CD8^{int}$) was found, these cells constituted 1.1% all splenocytes. In the spleen of CBA mice, the number of cells with this phenotype was minimum (0.1%). Cells with this phe-

notype can be activated T lymphocytes possessing cytolytic potential and secreting cytokines of the Th1 and Th2 profiles (secretion of Th1 associated cytokines predominated) and IL-17 and expressing differentiation markers typical of regulatory T lymphocytes (T-reg) [12].

After mitogenic stimulation *in vitro* (PMA+ionomycin), splenocytes of CBA mice expressed GATA3 marker, that acts as a transcription factor triggering expression of genes determining Th2-dependent differentiation of lymphocytes. In CBA mice, T lymphocyte population contained 10% $GATA3^+$ cells, which surpassed the corresponding parameter in C57Bl/6g mice by 3.2 times (3.1% $GATA3^+$ lymphocytes). These findings suggest that T helpers in CBA mice after activation primarily differentiate into Th2 cells with specific phenotypic and functional characteristics. This cell type can stimulate humoral immune response and participate in the realization of the immune response against some extracellular parasites, whereas the role of Th2 immune reactions in antiviral defense in many cases is disputable [12].

Thus, we revealed interstrain differences in morphofunctional organization of the thymus and spleen. Higher numerical density of mononuclear phagocytes, T/B ratio, predominance of cytotoxic T lymphocytes typical of C57Bl/6g mice attest to their high potential in the realization of cell-mediated immune reactions playing the key role in antiviral defense [7]. The existence of a considerable population of peripheral double-positive lymphocytes ($CD4^+CD8^+$) and a tendency to Th1-type polarization of the immune reactions in C57Bl/6g mice can affect activity of antiviral immunity. It can be hypothesized that functional capacities of effector cells aimed at elimination of the pathogen are higher in C57Bl/6g mice and determine more effective antiviral immunity. These findings characterize C57Bl/6g and CBA mice as opposite strains by the parameters of immune reaction development, which allow us to recommend them as the models for evaluation of genetically determined peculiarities of the immune response in mammals.

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