PRIMATOLOGY

Parameters of Cellular Immunity in Primates

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Cellular immunity in clinically healthy *Macaca mulatta* was studied by flow cytometry using monoclonal antibodies (Becton Dickinson). The immune parameters of monkeys were close to those in humans.

Key Words: *immune status; flow cytometry; monoclonal antibodies; primates*

The method of laser flow cytometry was developed about 30 years ago. Cytometry at rest, or flow cytometry, is used for description of the phenotype of analyzed cell populations by morphological and molecular characteristics, differing in lymphocytes, monocytes, and granulocytes. At present analysis by flow cytometry is a highly effective method for the diagnosis, prediction, and monitoring of the immune status of patients.

According to WHO recommendations (1988), lymphocyte phenotyping is diagnostically significant in primary and acquired immunodeficiencies and lymphoproliferative diseases. The diagnostic criteria and phenotypical characteristics of lymphoid cells are clearly defined for immunodeficiencies and lymphoproliferative diseases.

By the present time, the studies of lymphocyte phenotype in inflammatory diseases are at the stage of data accumulation. Specific shifts in the proportion of lymphocyte subpopulations were defined for some diseases, but are still unclear for others [7,8].

The phylogenetic relations of representatives of the primate tribe makes the monkeys an adequate model for reproduction of human diseases, including immunopathological conditions. The parameters of some physiological systems of monkeys were studied and compared with the corresponding parameters in humans [1-4]. However, the data on the status of cellular immunity are contradictory [1,12].

We studied cellular immunity of clinically healthy *Macaca mulatta* and validated the possibility of using these animals for simulation of human immunopathological states.

MATERIALS AND METHODS

The method is based on the reaction of monoclonal antibodies (MAb) labeled with fluorescent label with surface antigens on lymphocytes and subsequent analysis of specimens on a flow cytometer (Beckman Coulter Epics X1-MCL). Double label of the cells was used, because two MAb differing by specificity of recognized antigens and carrying different fluorescent stains, FITC and phycoerythrin (PE), were added to blood samples simultaneously. Due to this, lymphocytes binding two MAb types could be detected in one sample.

Monoclonal antibodies (Becton Dickinson) were used for phenotyping in the following combinations:

CD3–FITC/CD4–PE
CD3–FITC/CD8–PE
CD3–FITC/CD16–PE
CD3–FITC/CD20–PE
CD25–FITC/CD3–PE

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CD3–FITC/CD45RA–PE
CD3–FITC/CDHLA-DR–PE

The study was carried out on 66 *Macaca mulatta* monkeys of both sexes aged 2-14 years. The animals were divided into 3 groups. Group 1 consisted of 34 animals aged 2-4 years, group 2 consisted of 29 animals aged 4.1-7 years, and group 3 consisted of 3 males aged 9-14 years.

The blood for immunophenotyping was collected after overnight fasting. Monoclonal antibodies (20 µl) were added to 100 µl blood stabilized with heparin (20-U/ml, Microgen). The blood was thoroughly mixed with MAb and incubated for 20 min in the darkness at ambient temperature.

Lysis and fixation of samples were carried out on a TQ-Prep automated system for sample preparation (Beckman Coulter) or manually. In the latter case, 2 ml lysing solution was added to the sample and incubated for 10-15 min in the darkness at ambient temperature until clarification of the sample. The sample was then centrifuged (5 min, 1500 rpm), after which 2 ml PBS was added, the sample was centrifuged, and PBS (500 µl) was added to the sample. Analysis was carried out on the same day. The cell population gate was determined by combination of forward and side light scatter. The number of cells in a gate was 5000. The samples were analyzed on an Epics XL flow cytometer (Beckman Coulter).

The results were compared with the standard values for humans [5,6,9-11].

RESULTS

The level of common T-cell marker ($CD3^+$) was the same in animals of both sexes in all 3 groups (Tables 1, 2). T-helper ($CD4^+$) population did not change much with age and did not depend on animal sex. However, all animals exhibited a trend to an increase in the percentage of T-cytotoxic/suppressor cells with age. This trend was more pronounced in males. The trend observed for B-lymphocytes was opposite: the parameters somewhat decreased by the age of 9-14 years. The content of $CD20^+$ cells was higher in females. The content of natural killers did not change much and did not depend on sex or age.

Analysis of immunograms showed a wide range of values for all studied parameters (Table 1). The minimum and maximum values in some animals in

TABLE 1. Content of Lymphocytes with Different Membrane Markers in the Peripheral Blood of Clinically Healthy Monkeys (% of Cells)

Age, sex		Number of animals	CD3	CD4	CD8	CD16	CD20	CD25	CD45RA	HLA-DR
2-4 years	males	28	50.0-77.7	22.0-52	17.5-36	4.5-21	12.0-38.5	0.0-3.7	45.0-80	7.4-39.5
	females	6	40.0-77.6	18.5-37.2	16.2-48.3	3.0-23.9	13.0-47.2	Not determined	53.0-59.9	16.5-32.1
4.1-7 years	males	23	53.6-69.3	22.0-42.5	17.5-38.9	3.9-24.2	12.6-28.5	1.0-3.2	44.1-73.2	15.0-32.5
	females	6	43.8-69.8	20.0-39.2	25.6-35.8	6.0-24.0	12.7-47.4	1.0-2.2	44.5-63.0	16.8-50.5
9-14 years	males	3	51.0-68.1	28.5-34.3	25.2-46.5	11.8-21.3	16.0-20.0	1.0-1.3	63.4-68.6	25.0-26.3

TABLE 2. Mean Content of Different Lymphocyte Subpopulations in Monkeys of Different Sex and Age ($M \pm m$)

Age, sex		Number of animals	CD3	CD4	CD8	CD16	CD20	CD25	CD45RA	HLA-DR
2-4 years	males	28	58.3±6.8	30.5±6.9	23.5±5.1	12.4±4.8	22.7±8.0	1.6±1.1	57.0±10.4	20.4±10.0
	females	6	56.1±17.2	26.7±7.6	27.8±14.2	12.1±8.6	28.0±12.5	Not determined	56.5±4.9	24.3±11.0
4.1-7 years	males	23	61.9±5.0	34.0±5.3	28.4±7.4	13.5±6.4	21.4±5.2	2.1±0.8	57.3±9.2	24.0±5.5
	females	6	56.8±9.8	27.9±7.0	30.1±3.8	12.8±7.2	27.1±13.2	1.4±0.7	51.9±9.8	31.6±14.4
9-14 years	males	3	59.7±8.6	31.3±2.9	37.2±10.9	16.3±4.8	18.0±2.8	1.2±0.2	66.0±3.7	25.7±0.9

TABLE 3. Content of Lymphocytes with Different Membrane Markers in the Peripheral Blood of Clinically Healthy Humans (Flow Cytometry Data)

Author, source	Membrane markers, % of cells						
	CD3	CD4	CD8	CD16	CD20	CD45RA	HLA-DR
A. A. Yarilin [11]	65-80	–	–	5-20	8-20	–	–
R. M. Khaitov *et al.* [9]	61-84	32-58	11-36	6.6-30	5-16	–	–
B. A. Nikulin [6]	58-76	36-55	17-37	6-26	8-19	32-45	12-20
S. V. Khaidukov *et al.* [10]	61-85	35-55	19-35	8-18	7-17	–	–

Note. "-": no data.

the groups could differ significantly, this presumably reflecting individual features of each animal and was in line with the data (Table 3) on cellular immunity in humans [6,9-11].

The data on normal values of cellular immunity in humans, obtained by different authors, do not differ much, though the upper and lower threshold values of the normal range are somewhat different [5,6,9-11]. This can be explained by the ambiguity of the notion of the "norm" for the immune system. The normal status implies not only the status "at rest", when an individual is healthy, but also active work of the immune system during an acute inflammatory process. Hence, normal characteristics are highly individual; not all deviations from the normal range should be considered as a pathology. In addition, the individual norm should be taken into consideration when evaluating the dynamics of a disease. V. M. Dilman showed that the formation of the norm during the development of the organism, "optimal norm" intrinsic of an adult human, is individual and is completed in different humans at different age, from 20 to 25 years [5,6].

Hence, we evaluated the content of the main lymphocyte subpopulations in the blood of clinically healthy *Macaca mulatta* and showed that animal immunogram does not differ from that of humans, which fact once more confirms the importance of monkeys as the model for modeling and studies of immunopathological conditions in humans.

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