PRIMATOLOGY

Immunophenotypical Characteristics of Permanent Cultures of Lymphoid Cells from *Papio Hamadryas* and *Macaca Arctoides*

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Immunophenotypical characteristics of primate cells were studied by enzyme immunoassay and flow cytofluorometry using a panel of monoclonal antibodies to human B- and T-lymphocyte antigens. Specific features of immunophenotype of cultured cells were detected. Simian lymphoid cultures consist of a mixture of B- and T-cells with mosaic antigenic structure expressing markers of B and T cell specificity.

Key Words: *simian lymphoid cell cultures; B and T lymphocytes; immunophenotyping; mixed lymphoid cell cultures; flow cytofluorometry*

Institute of Medical Primatology has a collection of permanent cell cultures derived from *Papio hamadryas* and *Macaca arctoides* by culturing blood and hemopoietic tissue cells from monkeys with malignant lymphoma and clinically healthy animals [1-3]. These cultures are characterized by many parameters, but their immunophenotypical characteristics remain little studied. We therefore investigated the immunophenotypical characteristics of *in vitro* cultured lymphoid cells of baboons and macaques using a wide panel of monoclonal antibodies (Mab) to differentiation antigens of human B- and T-lymphocytes.

MATERIALS AND METHODS

Suspension cultures of lymphoid cell derived from *Papio hamadryas* (KM93, C42, SPG5, E5) and *Macaca arctoides* (MAL1-3, MB20) were used in the study. The following Mab were used: to T-lymphocyte differentiation antigens (CD2, CD3, CD4, CD5, CD7, CD8) and to those of B-lymphocytes (CD10, CD19, CD20, CD21, CD23, CD24, CD72), IgM, light chains of human κ and λ immunoglobulins (DAKO and Med-BioSpektr). Culturing, cryopreservation, storage in liquid nitrogen, and defrosting were carried out as described previously [1,2].

Cell immunophenotype was determined by two methods: indirect enzyme immunoassay (EIA) with cells fixed in acetone (10 min at 4°C) or 2% glutaraldehyde (10 min at 4°C) with peroxidase-antiperoxidase complex (200 cells were analyzed) and flow cytofluorometry (FCF) of live cells on an automated cytofluorometer (Becton Dikinson MV). Up to 300,000 cells were analyzed in each specimen. Antibodies were labeled with phycoerythrin and FITC. The percentage of positive cells and mean intensity of staining in each sample were evaluated routinely. The results were presented as histograms reflecting the number of cells reacting with each Mab.

RESULTS

Culturing characteristics of suspension lymphoid cultures virtually did not change after long (1-25 years)

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cryopreservation. Proliferative activity and morphological structure of cells completely recovered 48-72 h after defrosting. The main structural elements of cultures, irrespective of their origin, were lymphoblastoid cells with sharply pronounced polymorphism with an admixture of macrophages (5-10% of the entire cell population) (Fig. 1).

Immunophenotyping of baboon cell cultures originating from hemopoietic tissues of animals with malignant lymphoma by EIA showed their pronounced antigenic mosaicity irrespective of the method of cell fixation (Table 1). Cells carrying B- and T-cell markers were found in all cultures. B-lymphocyte population predominated in KM93 culture. As for T-cell markers, they were detected in lesser number of cells. After fixation with glutaraldehyde the rate of detection of B- and T-lymphocyte markers was about the same.

Virtually the same picture was observed in other baboon lymphoid cultures (E5, SPG5, C42). After acetone fixation B-cell markers were detected most often, particularly CD20 ones and light chains of κ immunoglobulins. The same baboon cultures contained rather high percentage of CD2 and CD3 antigens on cells fixed with acetone. After fixation with glutaraldehyde the highest percentage of CD20 marker was detected in E5 culture (72%) and 61% of CD19 was detected in SPG5 culture. After similar fixation the highest percentage of cells expressing T-cell markers was detected in C42 culture (CD2 - 40.4%), while in other cultures the percentage of cells with these markers reached 25%. Hence, according to EIA, all baboon lymphoid cultures consist of cells expressing B- and T-cell markers in different degree.

Phenotypical characteristics of lymphoid cultures from *Macaca arctoides* were similar to those observed in cell cultures from baboons (Table 2). After fixation of MAL1 and MAL3 in both fixatives both B- and T-cell antigens were detected on cells; after acetone fixation MAL2 and MB20 cultures presented as mixed-cell cultures, while after fixation with glutaraldehyde an appreciable fraction of B-cells was detected in these cultures.

These seemingly contradictory results can be explained by different methods of cell fixation. Acetone damages cell membranes and under these conditions Mab detect surface and cytoplasmic antigens. Glutaraldehyde fixation does not impair cell membranes and only surface antigens are detected. However we cannot rule out the expression of markers of different cell specificity on the same cells, as was shown for *Papio hamadryas* lymphomas, when B-cell specificity antigens (CD40 and Bgp 95) were expressed on T-cells [4,6]. The expression of CD2 antigen on peripheral B-cells and B-cellular mouse strains was demonstrated: common lymphoid precursors coexpressed CD7 and CD19. Other authors observed in general a similar picture [5].

FCF showed a different picture, in comparison with EIA. Only B-cell markers were detected in KM93 culture (Table 3). Antigenic characteristics of the cells in this culture are to a certain measure monotonous. By immunological parameters SPG5 culture approximated KM93 and differed much from C42. High percentage of two immunological cell types (CD2 and CD23) was detected in C42 culture, and hence, this culture is a mixed cell one. E5 culture proved to be strictly B-cellular.

The study of lymphoid cell cultures from baboons and macaques by the FCF method showed more definite type of cultures than that shown by EIA. For

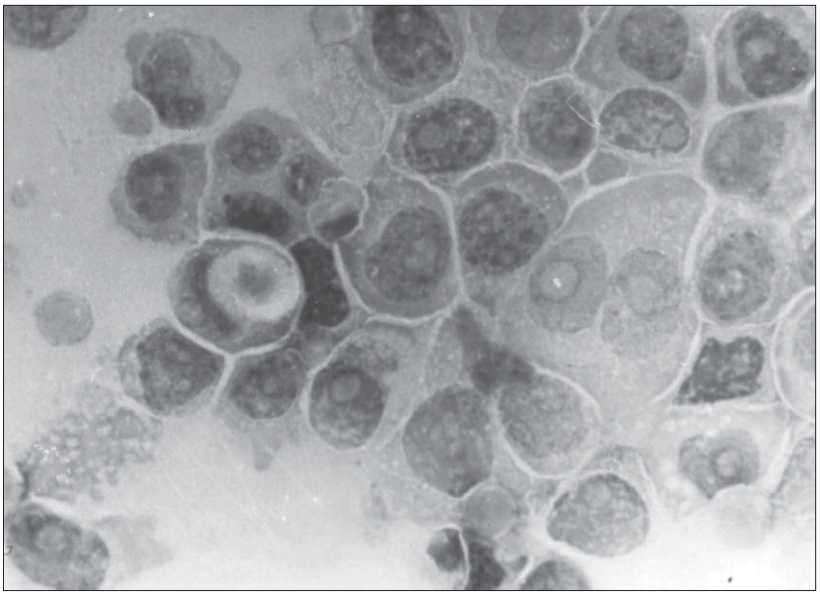

Fig. 1. E5 cell culture. Romanowskii—Giemza staining, ×600.

TABLE 1. Immunophenotypical Characteristics of Cells in *Papio hamadryas* Permanent Lymphoid Cultures (*M*±*m*)

Mab	Lymphoid cell cultures, fixation by							
	KM93		E5		SPG5		C42	
	acetone	GA	acetone	GA	acetone	GA	acetone	GA
CD 2	24.7±1.3	21.4±1.6	54.7±0.3	25.0±1.0	64.4±2.6	25.0±2.0	53.0±2.0	40.4±1.6
CD 3	19.4±1.6	11.4±3.6	34.0±2.0	20.0±4.3	53.7±2.3	19.8±1.2	60.4±1.6	16.0±2.0
CD 4	27.0±2.0	17.0±4.0	37.4±0.6	23.0±1.9	51.7±2.3	8.7±1.3	54.7±1.3	18.3±2.7
CD 8	31.0±3.0	13.9±6.1	36.0±2.0	15.0±2.7	30.7±1.3	6.3±3.7	43.0±1.0	11.5±3.5
CD 10	79.7±2.3	16.4±1.6	72.7±2.3	44.0±1.3	61.7±4.3	27.4±2.3	65.4±2.6	19.6±3.4
CD 19	70.0±4.0	12.4±4.6	68.7±0.3	42.0±2.0	42.7±2.3	61.0±4.0	59.7±2.3	15.7±5.3
CD 20	82.0±3.0	19.0±3.0	94.4±4.4	72.0±5.2	77.4±2.4	43.0±5.0	73.7±2.3	26.3±3.7
κ	71.0±2.0	21.3±4.7	46.4±1.6	—	87.0±2.0	21.5±4.5	71.7±2.3	—
λ	21.3±1.7	5.6±1.4	28.6±1.4	19.0±3.0	51.7±2.3	2.2±3.8	58.0±2.0	—

Note. Here and in Table 2: GA: glutaraldehyde.

TABLE 2. Immunophenotypical Characteristics of Cells in *Macaca arctoides* Permanent Lymphoid Cultures (*M*±*m*)

Mab	Lymphoid cell cultures, fixation by							
	KM93		E5		SPG5		C42	
	acetone	GA	acetone	GA	acetone	GA	acetone	GA
CD 2	57.4±1.3	27.3±3.7	32.6±9.3	11.7±3.2	57.4±1.3	27.3±3.7	32.6±9.3	11.7±3.2
CD 3	47.5±2.3	23.9±2.7	33.7±4.0	13.3±9.4	47.5±2.3	23.9±2.7	33.7±4.0	13.3±9.4
CD 4	48.7±3.4	24.1±5.2	44.7±5.7	16.2±7.5	48.7±3.4	24.1±5.2	44.7±5.7	16.2±7.5
CD 8	44.2±1.0	35.0±8.1	27.5±7.2	14.8±2.9	44.2±1.0	35.0±8.1	27.5±7.2	14.8±2.9
CD 10	29.6±4.3	10.7±4.3	17.4±1.2	4.8±4.2	29.6±4.3	10.7±4.3	17.4±1.2	4.8±4.2
CD 19	17.1±1.9	7.0±1.9	24.6±1.3	11.0±1.3	17.1±1.9	7.0±1.9	24.6±1.3	11.0±1.3
CD 20	74.7±2.7	34.8±2.7	62.7±3.7	31.2±7.7	74.7±2.7	34.8±2.7	62.7±3.7	31.2±7.7
κ	19.9±7.2	3.7±1.4	13.0±1.5	1.5±0.5	19.9±7.2	3.7±1.4	13.0±1.5	1.5±0.5
λ	16.0±3.0	5.9±3.7	47.3±3.8	31.9±3.8	16.0±3.0	5.9±3.7	47.3±3.8	31.9±3.8

TABLE 3. Immunophenotyping of Cultured Lymphoid Cells on a Flow Cytofluorometer

Specificity; Mab	*Papio hamadryas*				*Macaca arctoides*			
	E5	C42	SPG5	KM93	MAL1	MAL2	MAL3	MB20
Isotypical control								
IgG PE	1.8	1.3	1.4	3.5	0.4	0.7	0.1	1.3
IgG F	1.0	0.4	2.0	2.4	0.4	0.8	0.3	1.8
Negative control								
$F(ab)_2$ F	2.5	0.8	1.1	2.8	0.85	1.1	0.4	2.7
T-cell markers								
CD2	3	46.7±12.0	2.6	1.5	29.7±5.0	1.8	1	2.5
CD3	1	0.4	0.3	2.0	0.2	0.6	0.2	1.4
CD5	2.1	0.9	1.7	1.1	0.3	0.4	0.4	2.6
CD7	2.2	0.3	1.1	2.5	0.4	0.7	0.8	2.3
CD4	0.9	0.7	0.3	1.4	0.3	0.3	0.4	2.2
CD8	0.9	0.5	1.3	1.4	0.3	0.6	0.5	2.7
B-cell markers								
CD10	0.9	0.4	0.9	1	2.1	0.6	0.9	2.1
CD19	0.4	0.3	1.4	2.8	0.1	0.6	0.5	2.5
CD20	11.2±6.0	32±1	3.9	7.4±3.0	2	1.8	0.6	3.1
CD21	4.1	2.8	1.6	3.3	4	2.5	1.4	2.8
CD24	0.8	1	1.1	1.3	1.3	0.9	0.4	2.9
CD72	1	0.5	0.8	2.3	1.8	0.7	0.8	2.6
CD23	78.6±9.0	63.3±10.0	11.4±9.0	18.9±9.0	30.9±10.0	19.5±8.0	14.9±11.0	15.9±4.0
IgM	1.8	2.7	2.8	2	0.5	0.9	0.9	0.3
κ	1	0.6	25.3±5.0	20.8±3.0	1.2	3.6	1.6	1
λ	21.5±4.0	1.3	2.0	2.3	42.5±16.0	29.8±16.0	36.2±5.0	10.9±4.0

example, T-cell markers were virtually absent in baboon cultures, except C42 culture, where 46% cells were CD2 positive. As for B-cell markers, they were expressed mainly at the expense of CD23. Restriction by the k light chain of immunoglobulins was detected in baboon cultures SPG5 and KM93, by l light chain in all macaque cultures (MAL1-3 and MB20) and baboon culture E5. No restriction by κ or by λ light chains was detected in C42 culture.

In macaque lymphoid cultures T-cellular CD2 antigen was present only in MAL1, but not in any other culture. As for B-cell markers (CD23), they were present in all macaque cultures (MAL1-3, MB20) at the level of 15-30%, while in baboon cultures their levels varied greately (11-78%). This study showed that MAL1 culture is a mixed-cell one.

Hence, immunophenotypical characteristics of lymphoid cell cultures originating from *Papio hamadryas* and *Macaca arctoides* were detected for the first time. The composition of the studied cell cultures was mixed B- and T-cell (C42 and MAL1) and markers of different cell specificity were expressed on the same lymphoblasts.

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