Formation and Some Cytophysiological Characteristics of Polynuclear Macrophages in Primary Cultures of Peritoneal Cells

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We studied the formation and cytophysiological characteristics of polynuclear macrophages in primary cultures of peritoneal cells from C57Bl/6 mice. Production of reactive oxygen species and phagocytic activity in polynuclear macrophages were higher than in mononuclear macrophages. The formation of polynuclear macrophages in cultures of peritoneal cells is realized via amitosis.

Key Words: *polynuclear macrophages; amitosis; phagocytosis; NBT test; lysosomes*

Some nosological types of granulomatous diseases are accompanied by the formation of granulomas with giant polynuclear cells [2,12,14]. The formation of polynuclear cells in chronic inflammation is mediated by various mechanisms, including fusion of mononuclear cells, mitosis, endomitosis, karyokinesis, and amitosis [7,10,13]. It remains unclear which factors determine the major mechanism for the formation of polynuclear cells under certain conditions (granulomatous disease; and *in vivo* or *in vitro* experimental models). Under the influence of infectious granulomatous factors, granulomatosis is considered as a reaction to localize the infectious agent and to prevent dissemination of infection [5,6,8,10]. The role of polynuclear cells in this process is poorly understood. These cells have high hydrolytic activity, which determines the risk of destructive processes at the site of granulomatous inflammation in case of cell damage or death [5,6, 10,13]. It is important not only to evaluate the mechanisms for induction and formation of polynuclear cells, but also to study variations in some cytophysiological characteristics related to changes in cell ploidy.

Here we studied the formation and cytophysiological characteristics of polynuclear macrophages (MP) in primary cultures of mouse peritoneal cells.

MATERIALS AND METHODS

In vitro experiments were performed on peritoneal transudate cells from male C57Bl/6 mice aging 2 months, weighing 21-22 g, and obtained from the nursery of the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Sciences, Novosibirsk). The animals were killed by cervical dislocation under ether anesthesia to obtain PC [1,2].

Phagocytic activity of peritoneal MP was studied with zymosan granules [2]. Zymosan granules were added to cultures by the 48th hour of culturing. Phagocytic activity of MP was evaluated 1 h after addition of zymosan granules. We calculated the phagocytic index (number of phagocytic MP) and mean number of particles engulfed by one MP.

PC were cultured on coverslips (10^6 cells in 2 ml medium 199 with 10% fetal bovine serum)

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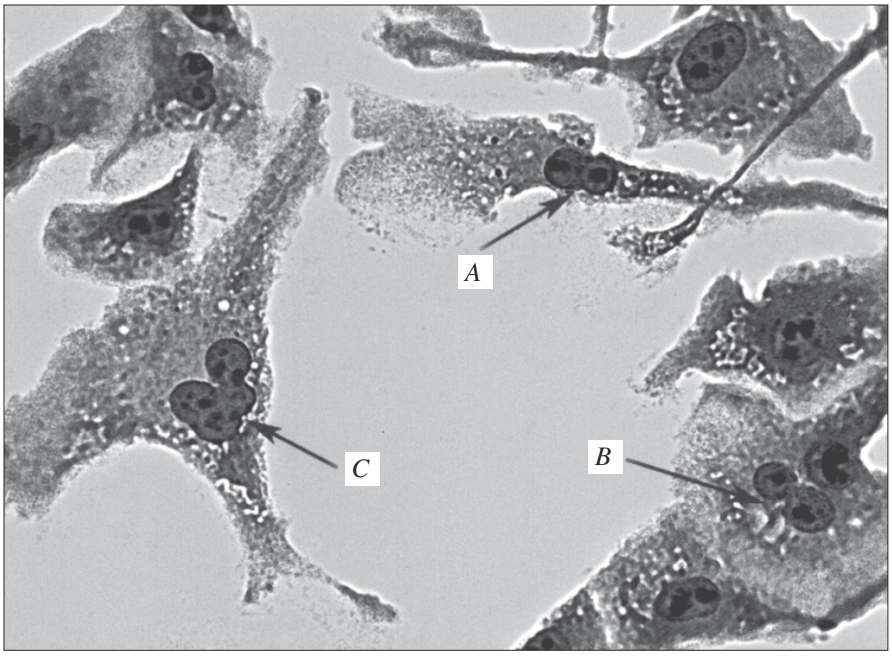

Fig. 1. Culture of PC from C57Bl/6 mice on day 2 of culturing. Arrows: MP in various phases of mitotic activity. (*A*) Initial formation of binucleated MP; (*B*) complete formation of trinucleated MP; and (*C*) initial formation of the trinucleated cell and, probably, of the quadrinucleated cell. Here and in Figs. 2 and 3: azure-eosin staining, ×1000.

using glass flasks at 37°C. The samples were fixed in 2% solution of formaldehyde in phosphate buffered saline (pH 7.3) and stained with azure and eosin (Romanovsky method). The study was performed after incubation for 2, 24, 48, and 72 h.

Production of reactive oxygen species (ROS) by MP was studied in the nitroblue tetrazolium (NBT) test. PC were incubated in the medium with 0.1% NBT at 37°C for 1 h. Cell nuclei were stained with a aqueous solution of safranin (1%) [4]. ROS generation in the activated NBT test was evaluated 1 h after addition of zymosan granules [2]. The amount of formazan in cells was estimated by means of computer morphometry with Video-Test-Morpho 3.2 software. Digital images of cells were binarized by formazan color. The sum of areas (μ^2) for all binary images of formazan granules served as the arbitrary index of their total intracellular content.

The amount of lysosomes in MP was studied after vital fluorochroming with acridine orange [3]. Digital images of acridine orange-stained cells were binarized by the color of lysosome fluorescence. The sum of areas for all binary images of lysosomes served as the conditional criterion for the total content of lysosomes in cells. The cells were photographed with a Nicon Coolpix 5000 camera under a Mikmed-2 fluorescence microscope. Vital examination of cells was conducted in Maksimov microchambers with cover glasses. The significance of differences between the mean values was evaluated by nonparametric Mann—Whitney test.

RESULTS

The ratio of binucleated MP in primary cultures of PC (before culturing) did not exceed 0.5%. The number of these cells reached maximum by the 2nd hour of culturing (Table 1). Trinucleated MP were revealed by the 2nd hour of culturing. The number of trinucleated MP reached maximum by the 48th hour of culturing. Quadrinucleated and pentanucleated MP were found on day 1. The num-

TABLE 1. Number of Polynuclear MP in the Culture of PC from C57Bl/6 Mice ($M \pm m$)

Number of MP nuclei	Time of culturing, h				
	0 (n=7)	2 (n=7)	24 (n=7)	48 (n=6)	72 (n=5)
2	4.9±0.8	66.7±6.7***	56.4±6.1***	43.5±5.3***	5.7±4.2
3	0	8.2±1.5**	10.1±1.4**	19.0±2.5***	10.6±1.6**
4	0	0	1.1±0.3*	2.3±0.4*	3.5±0.6*
5	0	0	1.0±0.3*	2.2±0.2*	3.3±0.3*

Note. Here and in Table 2: *p<0.05, **p<0.01, and ***p<0.001 compared to the control.

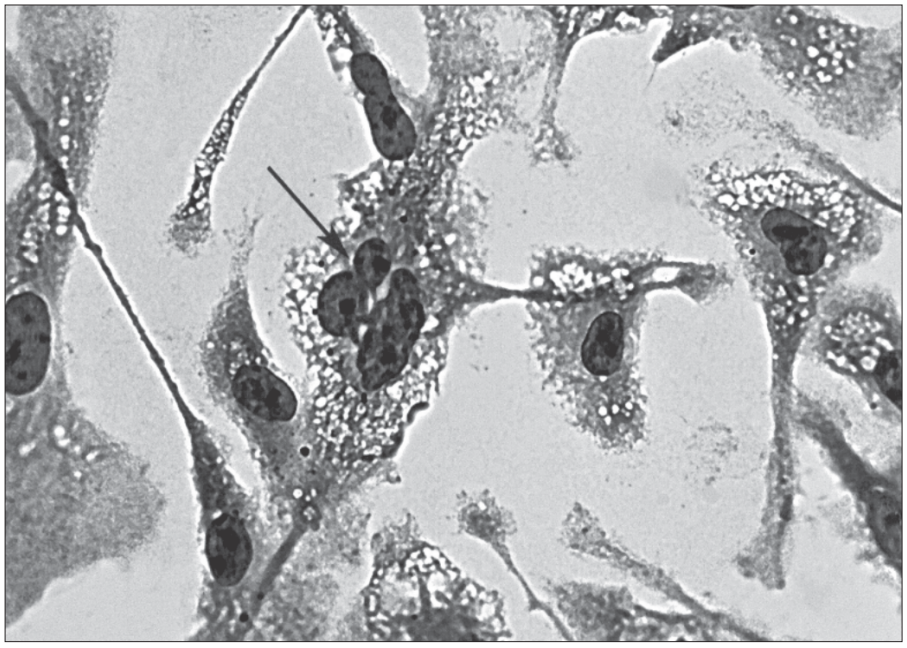

Fig. 2. Culture of PC from C57Bl/6 mice on day 2 of culturing. Formation of trinucleated MP due to synchronous amitosis. Arrow: division of the MP nucleus into 3 nuclei of different size (asymmetric amitosis). Chromatin "bridges" between the nuclei.

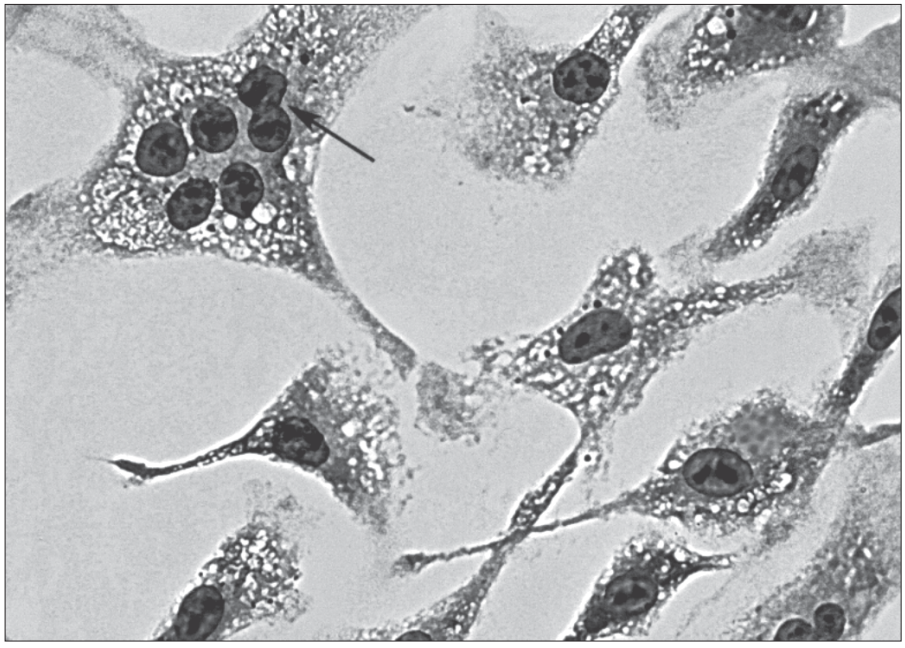

Fig. 3. Culture of PC from C57Bl/6 mice on day 3 of culturing. Formation of hexanucleated MP due to amitotic activity of pentanucleated MP. Arrow: MP nucleus at the stage of amitotic division.

ber of these cells progressively increased with an increase in the time of culturing.

Mitotic figures were not identified in the culture of PC. Cytomorphological signs for MP fusion were absent. The formation of polynuclear MP in PC cultures is related to direct symmetric and asymmetric nuclear division with no cytotomy. The cells with signs of amitotic nuclear division were found at all stages of culturing. Some MP had a dumbbell-shaped nucleus of different polarization (complete division of the nucleus into 2 nuclei with preservation of a thin "bridge"). The nucleus of some cells was characterized by budding-like division with strangulation at the division site (Figs. 1-3). Vital examination of cultured MP under a phase contrast microscope confirmed the formation of polynuclear MP due to amitotic activity.

On day 2 of culturing, the phagocytic number in trinucleated MP was higher than in mononuclear MP (by more than 4 times, Table 2). ROS generation in the NBT test was higher for polynuclear MP than for mononuclear MP. This parameter increased after cell stimulation with zymosan. The total volume of the lysosomal apparatus increased similarly (fluorescence assay). Our results confirm the hypothesis that the increase in the number of

TABLE 2. Cytophysiological Characteristics of Mono-, Bi-, and Trinucleated MP in the Culture of PC from C57Bl/6 Mice (*M*±*m*)

Number of MP nuclei	1 (control)	2 nuclei	3 nuclei
Phagocytic index, %	85.0±9.6	97.0±9.1	100.0±0.0
	(*n*=7)	(*n*=7)	(*n*=6)
Phagocytic number	3.5±0.3	7.6±0.8*	15.9±1.7***
	(*n*=7)	(*n*=7)	(*n*=6)
Conditional index of the spontaneous NBT test	75.4±10.3	109.5±9.1*	162.3±15.1**
	(*n*=7)	(*n*=7)	(*n*=7)
Conditional index of the induced NBT test	102.8±10.4	145.6±19.5*	270.9±34.2**
	(*n*=6)	(*n*=6)	(*n*=6)
Index of lysosome content in macrophages	530.7±45.8	760.4±87.3*	1050.5±80.4**
	(*n*=7)	(*n*=7)	(*n*=7)

MP nuclei is associated with functional activation of these cells [9]. The formation of polynuclear cells during infectious granulomatosis probably increases the ability of cells to kill the pathogenic agent and, therefore, to prevent the spread of live bacilli from infected cells and further dissemination of the infection. However, the increase in hydrolytic activity due to the formation of these cells is a risk factor for destructive processes and neoplasia.

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