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In Vitro Study of the Expression of CD1, CD14, CD25, CD30, CD35, CD95 Receptors by Macrophages of Mice Infected with *Mycobacterium tuberculosis*

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In cultures of peritoneal macrophages (MP) of male BALB/c mice infected with *Mycobacterium tuberculosis* from the BCG vaccine, the expression of CD1, CD14, CD25, CD30, CD35, and CD95 receptors was studied *in vitro* 3 months after infection. In MP cultures from intact and infected mice, mononuclear MP predominated (96 and 92%, respectively). Bi- and trinuclear MP in MP cultures from control and infected mice constituted 4 and 8.3% of all MP, respectively. In the cultures of both groups, no obvious correlations between the number of MP expressing CD-receptors and number of nuclei in these cells were found, but the expression of CD14 receptor was more often noted. In cultures from infected animals, hypertrophied MP and enhanced (by several times) expression of all CD-receptors were observed. The increase in the expression of CD-receptor can be determined by activation of plastic processes in hypertrophied MP (in epithelioid and in numerically insignificant polynuclear MP), which is due to the phenomenon of prolonged *M. tuberculosis* persistence in the vacuolar apparatus of these cells.

Key Words: *tuberculosis granulomatosis; macrophages; CD-receptor expression*

Macrophages (MP) express various receptors and are involved in diverse processes in the mammalian body under normal and pathological conditions. In tuberculosis and a number of other granulomatoses (about 70 nosologies) [6], granulomas determined by the phenomenon of prolonged persistence (PPP) of various non-biodegradable or slowly degradable factors are formed from MP [5]. During granuloma formation, not only their size increases, but also the cellular composition of granulomas changes [4,6]. Thus, during the formation period (about 90 days after infection of mice with mycobacteria from BCG vaccine), the "core" of the granuloma consists of large MP (~80%) — epithelioid cells and polynuclear MP (Langhans cells) [6]. This is associated with the development of PPP of *Mycobacterium tuberculosis* (MBT) in the vacuolar apparatus of MP and, as a result, with non-specific stimulation of plastic processes in MP (epithelioid cells and Langhans cells) [6]. However, the biological role of these processes is not clear, in particular, how this phenomenon (hypertrophy) affects the MP receptor system. These changes in the cellular composition of granulomas are a "turning point" in the pathogenesis of tuberculous granulomatosis. PPP is associated with transformation of the adaptive protective reaction of the body (isolation of MBT in granulomas MP) into a pathological reaction, which is determined

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by the adaptive reaction of MBT, *i.e.* transformation into L-forms that block their fusion with MP lysosomes and initiate PPP. In this regard, the hydrolytic potential and the size of granulomas increase at the expense of hypertrophied MP [6].

The combination of PPP with the absence of microcirculatory structures in granulomas is associated with a high risk of spontaneous ischemic necrosis, because the only mechanism of hypertrophied cell trophism in "growing" granulomas (1-3 mm in diameter) is diffusion. However, diffusion is effective at a much smaller diameter of granulomas (~200 μm); ischemic necrosis leads to loss of a significant part of MP in granulomas and, as a result, their receptors.

In the model of BCG granulomatosis, spontaneous necrosis never develops due to small size of granulomas (35-65 μm in diameter) [6], so it allows us to study the expression of CD-receptors in hypertrophied (epithelioid and multinucleated) MP [6] during the transformation of the cellular composition of granulomas associated with the risk of spontaneous ischemic necrosis [3].

Here we studied *in vitro* expression of CD1, CD14, CD25, CD30, CD35, and CD95 receptors that mediate participation of MP in multinucleation, apoptosis, antigen presentation, phagocytosis, and other processes using MP cultures from mice infected with MBT.

MATERIALS AND METHODS

The experiments were performed on peritoneal MP of male BALB/c mice weighing 20-22 g. MP cultures were isolated from intact animals (control) and animals infected with MBT from the BCG vaccine (experimental). MBT was administered to mice intraperitoneally in a dose of 0.5 mg. The animals were kept under conditions of free access to water and food and euthanized by cervical dislocation under ether anesthesia 3 months after MBT administration. MP cultures were incubated *in vitro* for 48 h [1]. The fractions of mono-, bi-, and polynuclear (three or more nuclei) MP in cultures were evaluated by light microscopy (expressed in % of all MP in each cell culture).

Cytological and immunocytochemical analysis of cultures was performed under an AxioStar Plus (Carl Zeiss) light microscope at ×400. The expression of CD1, CD14, CD25, CD30, CD35, and CD95 receptors in cultured MP was studied by indirect immunocytochemical method using diagnostic kits of monoclonal antibodies (BD Pharmingen). The frequency of expression of CD-receptors in MP with different numbers of nuclei was determined, taking the number of all MP as 100%.

Statistical processing of the results was carried out using Statistica 8.0 software (StatSoft, Inc.). The significance of the differences between the means was determined using the nonparametric Mann—Whitney test. The data are presented as $M \pm m$. The differences were considered significant at $p<0.05$.

RESULTS

In 3 months after infection with MBT from the BCG vaccine, we observed an increase in MP size (hypertrophy) and number of polynuclear MP (to 8.3% of all MP). In the control, the number of all polynuclear MP was 4% (Table 1). In a similar *in vivo* experiment in mice infected with MBT from the BCG vaccine [8], approximately 80% granuloma cells were so-called epithelioid cells (hypertrophied MP). Hypertrophy is realized via activation of plastic processes in MP, including in the lysosomes, as in other granulomatosis [6,9]. We believe that the mono- and polynuclear cells we observed *in vitro* are predominantly hypertrophied MP with PPP [6,8,9] induced by the persistence of L-forms of MBT in their vacuolar apparatus.

In mice infected with MBT, the number of bi- and trinuclear MP slightly increased (in total 8.3%) in comparison with mononuclear MP (Table 1). However, infection significantly increased the number of MP expressing the studied receptors (Table 1). These data indicate that in hypertrophied MP, the plastic processes initiated by MBT PPP are also realized in the receptor system. We observed no obvious dependence of the frequency of expression of any receptors on the number of nuclei in the control and experimental MP cultures (Table 1). The only exception was mononuclear MP expressing CD30 receptor (for TNF), which mediates MP multinucleation [13]. This can determine the increase in the number of multinuclear MP in infected mice. Infection of animals significantly increased the frequency of MP expressing most of the studied receptors. The number of MP expressing CD14 receptor increased slightly relative to the control MP. The number of MP from infected animals expressing CD25 receptor increased to a far greater extent (Table 1). This indicates the persistence of MBT in MP and possible hyperfunction of MBT during implementation of complement-mediated phagocytosis [10] and presentation of MBT antigens to T and B lymphocytes [15]. The increase in the expression of CD35 and CD1 receptors in experimental MP cultures confirm these processes (Table 1).

In MP cultures from infected animals, we observed increased frequency of MP expressing CD30 receptor for TNF [13] probably determining a certain increase in the number of multinuclear MP [1] (Table 1). An increase in the number of MP expressing CD95 receptor involved in the regulation of apoptosis [14] in experimental cultures can be regarded as

TABLE 1. Proportion of Mono- and Multinuclear MP in BALB/c Mice Expressing CD1, CD14, CD25, CD30, CD35, and CD95 Receptors *In Vitro* (%; *M±m*)

Parameter	Control MP cultures (from intact mice)			Experimental MP cultures (from BCG-infected mice)		
	number of nuclei in MP					
	1	2	≥3	1	2	≥3
Number of MP in culture	96.0±4.0	3.5±0.2^{+}	0.5±0.1^{+o}	92.0±7.0	6.0±0.5^{*+}	2.3±0.2^{*+o}
CD1	6.8±0.8	27.5±2.5^{+}	14.3±1.4^{+o}	41.5±3.8*	74.3±5.6^{*+}	76.9±8.0^{*+}
CD14	72.8±3.1	84.7±1.7^{+}	81.8±8.0	93.8±1.5*	96.5±2.4*	93.3±4.7
CD25	22.5±2.6	31.8±3.0^{+}	22.2±2.0o	92.3±1.8*	84.4±3.2^{*+}	90.4±3.3*
CD30	13.8±1.5	34.4±3.1^{+}	63.6±6.0^{+o}	93.0±2.2*	81.8±7.8*	94.3±3.4*
CD35	14.5±1.1	14.8±1.3	14.3±1.0	95.0±3.1*	92.3±5.0*	96.2±3.0*
CD95	5.3±0.4	10.7±1.6^{+}	9.1±1.0^{+}	82.3±2.3*	93.6±3.7^{*+}	93.5±6.5*

Note. Both groups included the same number of samples of MP cultures (n=6). p<0.05 in comparison with *the control, $^{+}$mononuclear MP, ^{o}binuclear MP.

a positive factor (Table 1), because this can contribute to a decrease in the risk of spontaneous ischemic necrosis of granulomas.

An increase in the expression of CD35 receptor involved in the implementation of complement-mediated phagocytosis [10] observed in MP cultures from infected mice (Table 1) can contribute to MBT clearance from the body. On the other hand, transformation of MBT into L-forms that prevent fusion of primary phagosomes containing MBT with lysosomes in MP can contribute to intracellular persistence [6] and increase the risk of spontaneous ischemic necrosis. The probable risk of developing spontaneous ischemic necrosis is confirmed by enhanced expression of CD25 receptor in experimental MP cultures (Table 1), which indicates infection of MP [12].

In addition, a slight increase in CD14 expression in mono- and binuclear MP in experimental cultures should be taken into account (Table 1), as it indicates their intracellular infection [11] and antigenic stimulation. The observed increase in the number of MP with CD1 expression (Table 1) indicates their participation in the presentation of MBT antigens to various subpopulations of lymphocytes [15], which is important for implementation of subsequent immune responses involved in granulopoiesis.

Thus, the expression of the studied CD-receptors increases in MP as a result of prolonged persistence of MBT in their vacuolar apparatus due to intensification of plastic processes in hypertrophied MP, a small part of which becomes multinuclear due to multinucleation. Understanding of the processes of MBT persistence in MP [7], MP multinucleation [1], their apoptosis [4], implementation of phagocytosis [2], and presentation of MBT antigens by MP to lymphocyte subpopulations [15] is important for studying the pathogenesis of tuberculosis.

The study confirms the need for the earliest possible treatment of tuberculosis and effective suppression of MBT to exclude the development of PPP (granulomatosis, adaptive reaction of the microorganism) and the formation of L-forms of MBT (adaptive reaction of MBT).

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