УДК 616.24-002.5:578.233.22
https://doi.org/10.20538/1682-0363-2022-4-140-149

Expression of scavenger receptors CD163, CD204, and CD206 on macrophages in patients with pulmonary tuberculosis

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ABSTRACT

The aim of the study was to evaluate the expression of scavenger receptors (CD163, CD204, CD206) on macrophages in patients with pulmonary tuberculosis, depending on the clinical form of the disease and sensitivity of the pathogen to anti-tuberculosis drugs.

Materials and methods. 64 patients with pulmonary tuberculosis (TB) were examined: 26 patients with disseminated pulmonary tuberculosis (DTB) and 38 patients with infiltrative pulmonary tuberculosis (ITB). Of these, 42 patients secreted *Mycobacterium tuberculosis* (MBT) sensitive to basic antituberculosis drugs (ATBD), and 22 patients secreted MBT resistant to first-line anti-TB drugs. Material for the study was venous blood.

To isolate monocytes from the whole blood in order to transform them into macrophages, Ficoll density gradient centrifugation with a density of 1.077 g / cm^3 was used followed by immunomagnetic separation of CD14+ cells. Monocytes were cultured in the X-VIVO 10 medium with gentamicin and phenol red with the addition of macrophage colony-stimulating factor (M-CSF) (5 ng / ml) at a concentration of 1×10^6 cells / ml with stimulators: interleukin (IL)-4 (10 ng / ml) and interferon (IFN) γ (100 ng / ml). Immunophenotyping of macrophages was performed using monoclonal antibodies to CD163, CD204, and CD206 on the Beckman Coulter CytoFLEX LX Flow Cytometer. The analysis of the obtained data was carried out using the CytExpert 2.0 software. The results were analyzed using statistical methods.

Results. Switching the phenotype of macrophages from the M1-like proinflammatory phenotype to M2-like anti-inflammatory one contributes to the chronic course of pulmonary TB, dissemination, and persistence of infection. In the present study, we analyzed the features of the expression of CD163, CD204, and CD206 scavenger receptors on macrophages in patients with pulmonary TB. An increase in the number of macrophages carrying markers of the M2 subpopulation (CD163, CD204, and CD206) on their surface was noted, regardless of the clinical form of pulmonary TB and drug resistance of M. tuberculosis.

Conclusion. Studying the mechanisms underlying M1 or M2 activation of macrophages is necessary for a deeper understanding of the immunopathogenesis of TB and the role of innate immunity cells in protecting the body from mycobacteria. The analysis of the expression of scavenger receptors CD163, CD204, and CD206 on macrophages allowed to conclude that, in pulmonary TB, especially in patients with drug resistant *M. tuberculosis* and infiltrative TB, regulatory mechanisms that suppress the activation of innate immunity are implemented together with polarization of macrophage differentiation towards the M2 phenotype. It may be the cause of immune deficiency induced by the pathogen.

Keywords: macrophages, pulmonary tuberculosis, innate immunity, immune response, scavenger receptors, IL-4, IFNγ, CD163, CD204, CD206

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

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Source of financing. The reported study was funded by the Council for Grants of the President of the Russian Federation for leading scientific schools (SS-2690.2018.7) and the RFBR grant, project number 19-315-90018.

Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Ethics Committee at Siberian State Medical University (Protocol No. 5648 of 27.11.2017).

For citation: Churina E.G., Popova A.V., Urazova O.I., Patysheva M.R., Kolobovnikova Ju.V., Chumakova S.P. Expression of scavenger receptors CD163, CD204, and CD206 on macrophages in patients with pulmonary tuberculosis. *Bulletin of Siberian Medicine.* 2022;21(4):140–149. https://doi.org/10.20538/1682-0363-2022-4-140-149.

Экспрессия скавенджер-рецепторов CD163, CD204 и CD206 на макрофагах у больных туберкулезом легких

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РЕЗЮМЕ

Цель работы – оценка экспрессии скавенджер-рецепторов (CD163, CD204, CD206) на макрофагах у больных туберкулезом легких в зависимости от клинической формы заболевания и чувствительности возбудителя к противотуберкулезным средствам.

Материалы и методы. Обследованы 64 пациента с туберкулезом легких (ТБ): 40 мужчин и 24 женщины, из которых 26 человек с диссеминированным туберкулезом легких (ДТБ) и 38 – с инфильтративным туберкулезом легких (ИТБ). Из них было 42 пациента, выделяющих *Mycobacterium tuberculosis* (МВТ), чувствительные к основным противотуберкулезным средствам (ПТС), и 22 пациента, выделяющих МВТ, устойчивые к лекарственным средствам основного ряда противотуберкулезной терапии. Материалом исследования являлась венозная кровь.

Для выделения моноцитов из цельной крови с целью их трансформации в макрофаги использовали метод центрифугирования в градиенте фиколла плотностью 1,077 г/см^3 с последующей иммуномагнитной сепарацией CD14+ клеток. Моноциты культивировали в полной питательной среде X-VIVO 10 with gentamicin and phenol red с добавлением колониестимулирующего фактора макрофагов (M-CSF) (5 нг/мл) в концентрации 1 × 10^6 клеток/мл со стимуляторами: интерлейкином (IL) 4 (10 нг/мл) и интерфероном (IFN) γ (100 нг/мл). Иммунофенотипирование макрофагов проводили с использованием моноклональных антител к CD163, CD204, CD206 на проточном цитометре Beckman Coulter CytoFLEX LX. Анализ полученных данных осуществляли при помощи программного приложения CytExpert 2.0. Полученные результаты анализировали статистическими методами.

Результаты. Переключение фенотипа макрофагов с провоспалительного М1 на противовоспалительный М2, установленное нами в ходе настоящего исследования, способствует хроническому течению туберкулеза легких, диссеминации и персистенции инфекции. Мы проанализировали особенности экспрессии скавенджер-рецепторов CD163, CD204 и CD206 на макрофагах у больных туберкулезом легких. Анализ экспрессии скавенджер-рецепторов на макрофагах показал значимое увеличение численности CD163, CD204 и CD206-позитивных клеток у больных ТБ независимо от клинической формы заболевания и лекарственной чувствительности *M. tuberculosis* к ПТС по сравнению с группой здоровых доноров.

Заключение. Исследование механизмов, лежащих в основе М1- или М2-активации макрофагов, необходимо для более глубокого понимания иммунопатогенеза туберкулезной инфекции и роли клеток врожденного иммунитета в защите организма от микобактерий. Анализ экспрессии скавенджер-рецепторов CD163, CD204 и CD206 на макрофагах позволил нам прийти к заключению, что при туберкулезе легких, особенно у больных с лекарственной устойчивостью *M. tuberculosis* и при инфильтративной форме заболевания, реализуются механизмы регуляции, подавляющие активацию врожденного иммунитета, с поляризацией

дифференцировки макрофагов в направлении М2-фенотипа, что, вероятно, является причиной формирования иммунодефицита, индуцированного возбудителем.

Ключевые слова: макрофаги, туберкулез легких, врожденный иммунитет, иммунный ответ, скавенджер-рецепторы, IL-4, IFNγ, CD163, CD204, CD206

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Исследование выполнено при финансовой поддержке Совета по грантам Президента Российской Федерации для ведущих научных школ (НШ-2690.2018.7) и РФФИ в рамках научного проекта № 19-315-90018.

Соответствие принципам этики. От каждого обследованного было получено добровольное информированное согласие на проведение исследования. Исследование одобрено локальным этическим комитетом СибГМУ (протокол № 5648 от 27.11.2017).

Для цитирования: Чурина Е.Г., Попова А.В., Уразова О.И., Патышева М.Р., Колобовникова Ю.В., Чумакова С.П. Экспрессия скавенджер-рецепторов CD163, CD204 и CD206 на макрофагах у больных туберкулезом легких . *Бюллетень сибирской медицины.* 2022;21(4):140–149. https://doi.org/10.20538/1682-0363-2022-4-140-140.

INTRODUCTION

Macrophages play a crucial role in the defense of the body from *Mycobacterium tuberculosis.* They are involved in both innate and adaptive immune responses and also regulate remodeling and repair processes in damaged tissues [1, 2]. Macrophages are characterized by versatility and plasticity; they are capable of switching a functional phenotype in tissues [3–6]. This heterogeneity is determined by the ability of macrophages to implement different activation programs in response to different stimuli, such as cytokine signals, damage-associated molecular patterns, or pathogenicity patterns in the body.

According to the recent WHO Global Tuberculosis Report, there was a decline in deaths from tuberculosis (TB) in 2018: 1.5 million people died in comparison with 1.6 million in 2017. However, the incidence of TB remains high: about 10 million people worldwide were diagnosed with TB in 2018 [7]. *M. tuberculosis* develops resistance to anti-tuberculosis drugs (ATBD), which is an important problem. A variant of extensively drug-resistant tuberculosis (XDR-TB), when *M. tuberculosis* does not respond to any of the existing antibiotics, has been registered in 117 countries [8].

Dysregulation of the immune response during the development of pulmonary tuberculosis (TB) occurs even at its earliest stages, primarily at the stage of macrophage activation and antigen presentation to T-helper cells. Macrophages play an important role in successful immune defense when *M. tuberculosis* components penetrate into mucous membranes of the respiratory tract. M1 macrophages trigger acute lung inflammation and rapidly activate the mechanisms of innate immunity, inflammatory and cytotoxic T-cell responses. It causes the development of acute destructive, clinical and pathogenetic forms of pulmonary TB, such as infiltrative and disseminated TB [9]. Later an immune control of the *M. tuberculosis* infection depends on the direction of the macrophage differentiation and the effectiveness of the inflammatory response implemented by CD4+ type 1 T-helper cells (Th1) [10–12]. Switching the phenotype of macrophages to the M2-like anti-inflammatory one leads to chronic and persistent TB infection. Mechanisms of innate immunity in pulmonary TB require more detailed consideration, first of all, through analyzing the receptor profile of macrophages. The scavenger receptors of monocytes and (or) macrophages, which include CD206 mannose receptor, scavenger receptor A – SR-A (CD204), and CD163 membrane marker, are of the greatest interest [12, 13–16]. Many questions remain open related to the plasticity, polarization, and activation of macrophages in various clinical forms of pulmonary TB and depending on the resistance or sensitivity of *M. tuberculosis* to ATBD.

Therefore, the aim of the study was to evaluate the expression of scavenger receptors (CD163, CD204, CD206) on macrophages in patients with pulmonary TB depending on its clinical form and sensitivity of the pathogen to ATBD.

MATERIALS AND METHODS

We examined 64 patients (40 men and 24 women) aged 23–50 years (average age 43.10 ± 10.00 years) with newly diagnosed pulmonary TB. The disease was diagnosed at Tomsk Phthisiopulmonology Medical Center. The diagnosis was established based on the past medical history, clinical presentation of the disease, as well as the results of the X-ray examination of the lungs and bacteriological and microscopic examination of the sputum. All patients were examined before the initiation of anti-TB chemotherapy. The clinical form of the disease was diagnosed using an X-ray examination of the lungs. In most patients, both lungs were affected by the pathological process. In ITB, the examination detected one or more heterogeneous infiltration shadows (3–6 cm in diameter) in the lungs. In DTB, multiple small- and medium-sized foci with heterogeneous structures were detected. Bacterial excretion (MTB+) was registered in all patients. *Mycobacterium tuberculosis* (MTB) was identified by Ziehl – Neelsen stained sputum smear microscopy as well as by fluorescence microscopy of auramine-stained sputum smears. To determine the species of *M. tuberculosis* and MBT sensitivity to ATBD, the absolute concentration method and sputum culture in Lowenstein – Jensen and Finn-2 solid nutrient media were used.

TB patients were divided into two groups depending on the clinical form of the disease: 26 patients with disseminated pulmonary tuberculosis (DTB) and 38 patients with infiltrative pulmonary tuberculosis (ITB). In all examined TB patients, the causative agent had drug sensitivity to basic ATBD. This criterion was used to identify 42 patients secreting *M. tuberculosis* (MBT), sensitive to the basic ATBD, and 22 patients secreting MBT resistant to the first-line drugs (isoniazid, rifampicin, streptomycin, ethambutol). Exclusion criteria for TB patients to participate in the study were: 1) cancer, diabetes mellitus, allergies and autoimmune diseases, viral hepatitis, and HIV; 2) treatment with ATBD and immunosuppressants. The comparison group consisted of 30 healthy donors (20 men and 10 women) aged 23–50 years (average age 41.31 ± 7.47 years) without past medical history of pulmonary TB. All patients signed an informed consent to participate in the study.

Immunomagnetic separation of blood monocytes. The material for the study was venous blood taken from healthy donors and patients with pulmonary TB. Blood sampling was carried out once, during the most intense phase of the disease, before the initiation of the anti-TB chemotherapy. To isolate monocytes from the whole blood for their further transformation into macrophages, the method of magnetic separation of CD14+ monocytes (MACS MultiStand, Germany) was used according to the manufacturer's instructions for the Monocyte isolation kit, Miltenyi Biotec GmbH (Germany). 30 ml of whole venous blood was collected into vacuum blood collection systems with an anticoagulant (K3-EDTA). The blood was diluted with phosphate – buffer saline (PBS) at a ratio of 1:1 and layered on 15 ml of the Ficoll cushion with a density of 1.077 g / cm^3. The samples were centrifuged for 30 min at 0.016 g. The resulting mononuclear fraction was collected and washed from PBS twice. After that, 5 ml of PBS was added and mixed; then the number of mononuclear cells was counted using the automated cell counter Scepter 2.0 (Merck Millipore, Germany). The cell suspension was centrifuged, the supernatant was removed, and the appropriate volumes of MACS Separation Buffer (containing bovine serum albumin (BSA), EDTA, and 0.09% sodium azide) and CD14+ magnetic particles (Micro Beads, Germany) were added based on the number of cells, and the suspension was incubated for 40 min. The resulting suspension underwent positive magnetic separation according to the manufacturer's protocol (Miltenyi Biotec, Germany).

Cultivation of macrophages in vitro. Monocytes were cultivated in the X-VIVO 10 complete growth medium with gentamicin and phenol red (Lonza, Switzerland) at a concentration of 1×10^6 cells / ml with the addition of macrophage colony stimulating factor M-CSF (5 ng / ml; R&D Systems, USA). Recombinant cytokines IL-4 (10 ng / ml; PeproTech, USA) (for M2 macrophage activation) and IFNγ (100 ng / ml; PeproTech, USA) (for M1 macrophage activation) were used for additional cell induction. The samples were cultured for 6 days in the CO_2-incubator set to 7.5% CO_2 at 37 °C without additional stimulation and with the addition of cytokines for M1 and M2 macrophage activation.

Immunophenotyping of macrophages. Macrophage phenotyping was performed on day 6 of cultivation. To collect cells, a plate with a cell culture was placed on ice and held for 10 minutes, and then the cells were harvested using a cell scraper (Cell-scraper, USA). For immunophenotyping of macrophages, monoclonal antibodies to CD163, CD204, and CD206 (eBioscience, USA) were added.

Cell suspensions were measured on the Beckman Coulter CytoFLEX flow cytometer (Beckman Coulter, USA). The obtained data were analyzed using the CytExpert 2.0 software application (Beckman Coulter, USA).

SPSS Statistics 17.0 and Microsoft Excel were used for statistical analysis of the obtained results. The data were presented as the median and the interquartile range *Me* (Q_1–Q_3). To perform a comparative analysis, the non-parametric Mann – Whitney test with the Benjamini – Hochberg correction was applied. The results of statistical analysis were considered significant at $p < 0.05$.

RESULTS

Expression of CD163, CD204, and CD206 scavenger receptors on macrophages transformed *in vitro* from CD14+ blood monocytes in TB patients depending on the clinical form of the disease

The analysis of the expression of scavenger receptors on macrophages showed a significant increase in the number of CD163- and CD206-positive cells in TB patients compared with the group of healthy donors, regardless of the clinical form of the disease and sensitivity of *M. tuberculosis* to ATBD (Table 1, 2; Fig. 1, 2).

Table 1

The expression of scavenger receptors on macrophages in patients with pulmonary tuberculosis depending on the clinical form of the disease, %, *Me* (Q_1–Q_3)

Markers of macrophages	Groups of examined persons	Conditions for *in vitro* cultivation of macrophages		
		No stimulation	IL-4 stimulation	IFNγ stimulation
CD163	Healthy donors	12.43 (6.51–22.33)	4.11 (2.17–8.34) $p_3 = 0.011$	13.24 (7.41–16.71) $p_3 = 0.511$ $p_4 = 0.014$
	ITB patients	44.23 (24.14–64.35) $p_1 = 0.012$	48.55 (27.31–59.54) $p_1 = 0.015$	26.70 (14.74–38.02) $p_1 = 0.010$ $p_3 = 0.011$ $p_4 = 0.027$
	DTB patients	40.81 (25.42–61.27) $p_1 = 0.010$	26.30 (17.11–41.72) $p_1 = 0.025$ $p_2 = 0.027$ $p_3 = 0.011$	27.83 (16.01–34.73) $p_1 = 0.010$ $p_3 = 0.014$
CD204	Healthy donors	11.31 (6.75–20.14)	8.05 (4.11–17.76)	10.26 (7.11–19.33)
	ITB patients	24.52 (14.27–34.36) $p_1 = 0.041$	40.83 (24.35–59.21) $p_1 = 0.017$ $p_3 = 0.037$	32.19 (16.14–50.36) $p_1 = 0.010$ $p_3 = 0.013$
	DTB patients	9.56 (6.02–20.33) $p_2 = 0.014$	8.91 (5.63–21.30) $p_2 = 0.025$	19.62 (11.38–35.17) $p_1 = 0.017$ $p_2 = 0.011$ $p_3 = 0.045$ $p_4 = 0.037$
CD206	Healthy donors	17.16 (9.17–28.43)	13.4 (6.35–22.45)	4.41 (2.15–9.37) $p_3 = 0.017$ $p_4 = 0.035$
	ITB patients	57.59 (28.12–68.18) $p_1 = 0.014$	58.27 (27.01–66.22) $p_1 = 0.037$	46.31 (26.45–61.27) $p_1 = 0.020$
	DTB patients	33.01 (18.34–52.43) $p_1 = 0.021$ $p_2 = 0.021$	29.37 (19.17–44.36) $p_1 = 0.012$ $p_2 = 0.021$	23.44 (13.16–37.46) $p_1 = 0.037$ $p_2 = 0.014$ $p_3 = 0.012$

Note: the level of statistical significance of differences compared with healthy donors – p_1; in ITB patients – p_2; in *in vitro* cell cultivation with no stimulation – p_3; in *in vitro* cell cultivation with IL-4 (M2 stimulation) – p_4.

After adding IL-4 to the cell culture (M2 macrophage activation), the expression of CD163 in ITB patients did not significantly change in comparison with its value in the absence of cytokine stimulation. In the group of healthy donors, the number of CD163-positive macrophages in M2 macrophage activation was 3.2 times lower relative to the number of cells in M1 macrophage activation (when cells were induced

by IFNγ). In DTB patients, the CD163 expression on macrophages was almost 1.5 times lower than that in the absence of stimulation both in M1 and M2 macrophage activation (Table 1, Fig. 1).

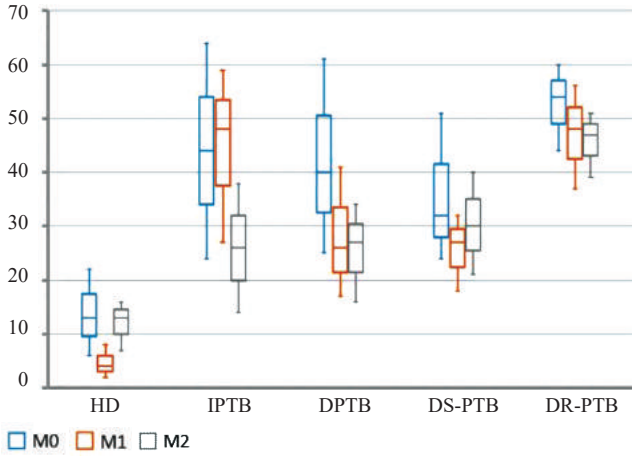

Fig. 1. The expression of the CD163 scavenger receptor on macrophages in patients with pulmonary tuberculosis, *Me* (Q_1–Q_3): HD – healthy donors, ITB – infiltrative pulmonary tuberculosis, DTB – disseminated pulmonary tuberculosis, DR TB – drug-resistant pulmonary tuberculosis, DS TB – drug-sensitive pulmonary tuberculosis, M0 – cell culture of macrophages without stimulation by cytokines, M1 – cell culture of macrophages stimulated by IFNγ, M2 – cell culture of macrophages stimulated by IL-4 (here and in Fig. 2, 3)

In cytokine stimulation, the number of CD206-positive macrophages in ITB patients did not change significantly in comparison with its basal level. In healthy donors and DTB patients, the expression of the CD206 molecule on macrophages significantly decreased in response to the IFNγ stimulation of cells, compared with the intact culture (Table 1, Fig. 2).

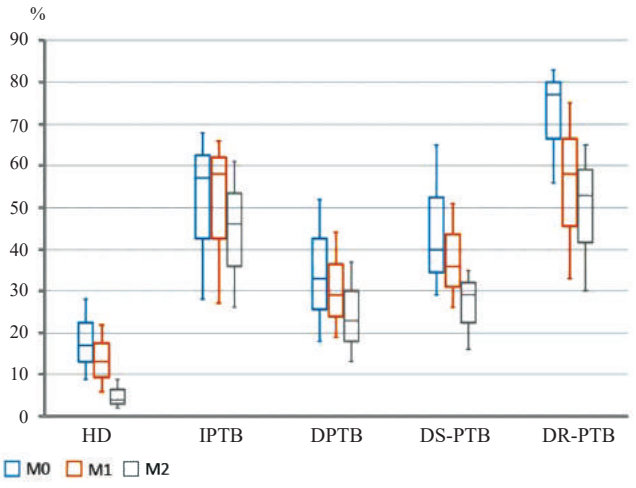

Fig. 2. The expression of the CD206 scavenger receptor on macrophages in patients with pulmonary tuberculosis, *Me* (Q_1–Q_3)

In patients with ITB, the induced expression of the CD204 scavenger receptor on macrophages more significantly increased after IL-4 stimulation of the cell culture than after IFNγ stimulation, compared with that in the intact cell culture. In the case of IL-4 stimulation, it increased by 5.1 times compared with its value in healthy donors and by 4.6 times compared with DTB patients. In DTB patients, the number of CD204-positive macrophages increased to a greater extent (by more than 2 times) in response to IFNγ stimulation of cells compared with that in the control group and after IL-4 stimulation (Table 1, Fig. 3).

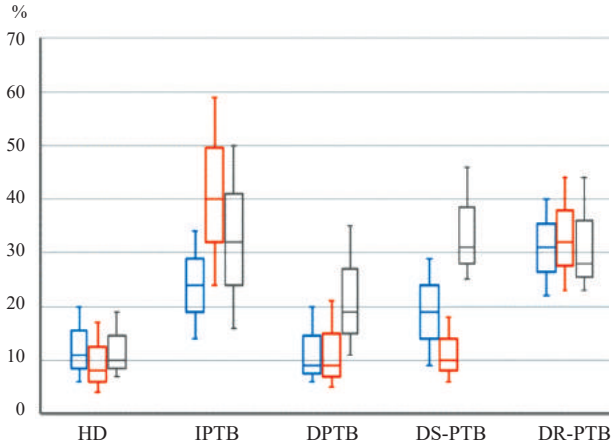

Fig. 3. The expression of the CD204 scavenger receptor on macrophages in patients with pulmonary tuberculosis, *Me* (Q_1–Q_3)

Expression of CD163, CD204, and CD206 scavenger receptors on macrophages transformed *in vitro* from CD14+ blood monocytes in TB patients depending on drug sensitivity of the pathogen to ATBD

The expression of M2 macrophage activation markers in TB patients, depending on the sensitivity of the pathogen to ATBD, was analyzed. It was found that the highest expression (basal and with stimulation by both cytokines) of the CD163 molecule on macrophages was observed in patients with DR TB. In DS TB patients, the cytokine-stimulated CD163 expression did not significantly change compared with the basal level, but it was higher (as in DR TB patients) than in healthy donors (Table 2, Fig. 1).

In cell cultivation without stimulation and with stimulation by cytokines, patients with DR TB also had the maximum number of CD206-positive macrophages. It was higher than in the control group and in patients with DS TB. However, in DR TB in both M1 and M2 stimulation by cytokines, the number of CD206+ macrophages was smaller than in the unstimulated cell culture (Table 2, Fig. 3).

Similarly, the highest expression of CD204 on macrophages was registered in DR TB. When cells were cultured without stimulation, the expression was higher than in the control group and in patients with DS TB. At the same time, it remained at the same level when cells were induced by cytokines. However, after adding IFNγ to the macrophage culture (M1 macrophage activation), patients with DS TB showed an increase in the expression of the CD204 receptor – by 1.6 times compared with its basal level and by 3.1 times compared with M2 macrophage activation and the control group (Table 2, Fig. 2).

Table 2

The expression of the scavenger receptors on macrophages in patients with pulmonary tuberculosis depending on the sensitivity of *M. tuberculosis* to ATBD, %, *Me* (Q_1–Q_3)

Markers of macrophages	Groups of examined persons	Conditions for *in vitro* cultivation of macrophages		
		No stimulation	IL-4 stimulation	IFNγ stimulation
CD163	Healthy donors	12.43 (6.51–22.33)	4.11 (2.17–8.34) $p_3 = 0.012$	13.24 (7.41–16.71) $p_4 = 0.015$
	DS TB patients	32.52 (24.45–51.23) $p_1 = 0.031$	27.25 (18.12–32.65) $p_1 = 0.012$	30.56 (21.65–40.28) $p_1 = 0.024$
	DR TB patients	54.23 (44.23–60.56) $p_1 = 0.021$ $p_2 = 0.012$	48.77 (37.56–56.44) $p_1 = 0.024$ $p_2 = 0.043$	47.32 (39.11–51.22) $p_1 = 0.035$ $p_2 = 0.044$
CD204	Healthy donors	11.31 (6.75–20.14)	8.05 (4.11–17.76)	10.26 (7.11–19.33)
	DS TB patients	19.23 (9.54–29.11) $p_1 = 0.032$	10.26 (6.23–18.25)	31.33 (25.4–46.12) $p_1 = 0.031$ $p_3 = 0.012$ $p_4 = 0.032$
	DR TB patients	31.23 (22.56–40.12) $p_1 = 0.034$ $p_2 = 0.011$	32.44 (23.56–44.36) $p_1 = 0.025$ $p_2 = 0.023$	28.56 (23.54–44.2) $p_1 = 0.037$
CD206	Healthy donors	17.16 (9.17–28.43)	13.40 (6.35–22.45)	4.41 (2.15–9.37) $p_3 = 0.017$ $p_4 = 0.035$
	DS TB patients	40.13 (29.14–65.45) $p_1 = 0.012$	36.45 (26.17–51.45) $p_1 = 0.027$	29.03 (16.54–35.47) $p_1 = 0.015$ $p_3 = 0.014$
	DR TB patients	77.36 (56.45–83.12) $p_1 = 0.031$ $p_2 = 0.022$	58.36 (33.47–75.16) $p_1 = 0.010$ $p_2 = 0.025$ $p_3 = 0.013$	53.27 (30.45–65.44) $p_1 = 0.014$ $p_2 = 0.042$ $p_3 = 0.014$

Note: the level of statistical significance of differences compared with values in healthy donors – p_1; in DS TB patients – p_2; in *in vitro* cell cultivation with no stimulation – p_3; in *in vitro* cell cultivation with IL-4 (M2 stimulation) – p_4.

DISCUSSION

High efficiency of innate immunity activation in TB plays a crucial role in the development of the disease and its outcomes. Abnormalities in the immune response induction are often associated with the development of tolerance to the antigen already at the stage of its presentation. In this case, instead of macrophage activation and differentiation into M1 cells, a tolerogenic and anti-inflammatory M2 phenotype is formed. Mobilization of monocytes and their entry into the systemic circulation from the bone marrow are always caused by an increased antigenic load and a need for resident macrophages for the immune system during lung inflammation. More and more studies on the macrophage population heterogeneity indicate that

a timely switch of the macrophage phenotype from the M1-like proinflammatory phenotype to the M2-like anti-inflammatory one and vice versa affects the clinical outcome of TB [17–20].

The analysis of the expression of scavenger receptors on macrophages showed, in general, an increase in the number of cells carrying markers of the M2 phenotype (CD163, CD204, and CD206) on their surface, regardless of the clinical form of the disease and sensitivity of *M. tuberculosis* (Table 1, 2, Fig. 1–3).

We registered the largest number of CD163-positive macrophages in ITB patients, especially in M2 macrophage activation. In case of M1 macrophage activation, on the contrary, the number of CD163+ macrophages decreased compared with that in the absence of cytokine stimulation of cells. In DR TB, the number of CD163-positive macrophages was higher than in DS TB in both the intact culture of cells and in the cells stimulated with IL-4 and IFNγ (Table 1, 2, Fig. 1). It is known that the hemoglobin scavenger CD163 receptor is expressed by monocytes and mainly by M2 macrophages [21]. The surface CD163 receptor on macrophages functions as an innate immunity receptor for recognizing patterns of bacterial pathogenicity. Its overexpression may be a mechanism to reduce an acute severe inflammatory response [22]. It is logical that macrophages expressing CD163 should have a regulatory and regenerative potential in order to timely suppress the immune response that damages tissues.

When evaluating the CD204 expression on macrophages in TB patients, we determined its highest intensity in ITB compared with DTB. In DTB, the CD204 expression increased only in M1 macrophage activation. Drug sensitivity or drug resistance of MBT did not affect the expression of the CD204 molecule – it increased in both cases (Table 1, 2, Fig. 2).

CD204 is a scavenger receptor A (SR-A). It is mainly expressed on macrophages, dendritic cells, and epithelial cells of the respiratory tract. It is a multifunctional receptor with a big ligand-binding potential [23, 24]. The CD204 molecule recognizes modified lipid proteins, apoptotic cells, and pathogen-associated molecules [25]. Studies of CD204 knockout mice have shown that the CD204 expression plays an important role in polarizing the differentiation of macrophages towards the M2 phenotype by inhibiting TLR signaling [26].

The CD206 molecule is a C-type lectin or mannose receptor type 1 (MR1), which is usually expressed on tissue macrophages, dendritic cells, and endothelial cells. It binds structures with a high mannose content on the surface of potentially pathogenic bacteria, viruses, and fungi [27]. The CD206 molecule plays an essential role in immune homeostasis. Cells in the microenvironment of malignant tumors have high expression of this receptor. An increase in CD206-positive tumor-associated macrophages is associated with a poor disease prognosis and indicates the development of chronic inflammation in metastatic niches [28]. In the examined patients, the CD206 expression on macrophages was the most significant in ITB and DR TB. However, in the latter, the number of CD206-positive cells decreased when the culture was induced with both M1 and M2 stimulators (Table 1, 2, Fig. 3).

There is evidence in the literature that the population of macrophages involved in the fight against *M. tuberculosis* is heterogeneous [29, 4]. Studies have examined various mechanisms by which the antigen transforms M1 macrophages into M2 macrophages with immunoregulatory activity, creating a favorable environment for its existence. The study conducted on a model of the staphylococcal lung infection in mice concluded that *Staphylococcus aureus* induced the Akt1 signaling pathway (Akt1 – protein kinase B), shifting the phenotype of macrophages from the anti-microbial M1 phenotype towards the functionally dormant M0 phenotype [30]. Another work showed that *M. tuberculosis* secretes LAM (lipoarabinomannan) and ESAT-6 virulence factors, which inhibit M1 macrophage activation by blocking maturation of phagolysosomes and activation of nuclear factor κB (NF-κB) [17].

Therefore, high expression of scavenger receptors on macrophages in pulmonary TB may be associated with predisposition of patients to the implementation of regenerative and anti-inflammatory functions of macrophages and their polarization towards the M2 phenotype.

CONCLUSION

Studying the mechanisms underlying the M1 or M2 macrophage activation is necessary for a deeper understanding of TB immunopathogenesis and the role of innate immune cells in protecting the body from MBT. The analysis of the expression of CD163, CD204, and CD206 scavenger receptors on macrophages allowed to make the following conclusion. In pulmonary TB, especially in DR TB and ITB, there are regulatory mechanisms that suppress the activation of innate immunity with polarization

of macrophages towards the M2 phenotype. It might cause immune deficiency induced by the pathogen.

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Authors contribution

Churina E.G. – design of the study, review of the literature, statistical processing and interpretation of research results, drafting of the manuscript. Popova A.V. – preparation of samples, carrying out of immunomagnetic separation and flow cytometry, drafting of the manuscript. Urazova O.I. – material and technical assistance in carrying out the laboratory research, interpretation of results, drafting and translation of the manuscript. Patysheva M.R. – carrying out of immunomagnetic separation and flow cytometry, consulting assistance in designing the study. Kolobovnikova Yu.V. – interaction with patients, consultations on phthisiological and pulmonological aspects of the study. Chumakova S.P. – interaction with patients, collection of the biomaterial.

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Received 24.04.2022;
approved after peer review 06.05.2022;
accepted 09.06.2022