УДК 616.24-002.5-078.75- 092.4
https://doi.org/10.20538/1682-0363-2020-4-179-188

Expression of pro-inflammatory and co-stimulatory molecules on the surface of macrophages in vitro in patients with pulmonary tuberculosis

Churina E.G.[1, 2], Sitnikova A.V.[1], Urazova O.I.[1, 2], Novitskiy V.V.[1, 2], Patysheva M.R.[2, 4], Golubchikov P.N.[5], Stepanova E.P.[5]

[1] Siberian State Medical University
2, Moscow Trakt, Tomsk, 634050, Russian Federation

[2]National Research Tomsk State University
36, Lenina Av., Tomsk, 634050, Russian Federation

[3]Tomsk State University of Control Systems and Radioelectronics
40, Lenina Av., Tomsk, 634050, Russian Federation

[4] Cancer Research Institute, Tomsk National Research Medical Center, the Russian Academy of Sciences
5, Kooperativny Str., Tomsk, 634009, Russian Federation

[5] Tomsk Phthisiopulmonological Medical Center
17/1, R. Luksemburg Str., Tomsk, 634009, Russian Federation

ABSTRACT

The aim of this study was to identify features of the expression of pro-inflammatory and co-stimulatory molecules on the surface of macrophages *in vitro* in patients with pulmonary tuberculosis, based on the clinical form of the disease and sensitivity of the pathogen to anti-TB drugs.

Materials and methods. 40 patients (36 men and 4 women) with pulmonary tuberculosis (TB) were examined: 18 patients (16 men and 2 women, average age (44.56 ± 8.10) years) with disseminated tuberculosis (DTB) and 22 patients (20 men and 2 women, average age (46.54 ± 5.24) years) with infiltrative tuberculosis (ITB). Of those, 30 patients had *Mycobacterium tuberculosis* (MBT) sensitive to the basic anti-TB drugs (ATBD), and 10 patients had MBT resistant to first-line anti-TB drugs. Venous blood was the study material. To isolate monocytes from the whole blood in order to transform them into macrophages, ficoll density gradient centrifugation with gradient density of 1.077 g/cm^3 was used followed by immunomagnetic separation of CD14$^+$ cells. Monocytes were cultured in a complete culture medium X-VIVO 10 with gentamicin and phenol red with the addition of the macrophage colony-stimulating factor (M-CSF) (5 ng/ml) at a concentration of 1×10^6 cells/ml with the following stimulators: interleukin (IL) 4 (10 ng/ml) and interferon (IFN) γ (100 ng/ml). Immunophenotyping of macrophages was performed using monoclonal antibodies to CD80, CD86, and HLA-DR on a Beckman Coulter CytoFLEX LX flow cytometer (Beckman Coulter, USA). The analysis of the obtained data was carried out using the CytExpert 2.0 software application. The results were analyzed using statistical methods.

Results. The number of intact and cytokine-stimulated (IL-4 and IFNγ) CD80-positive macrophages in patients with ITB and drug-resistant TB (DR TB) exceeded their number not only in healthy donors, but also in patients with DTB and drug-sensitive TB (DS TB), respectively. In addition, an increase in CD86 expression on the surface of macrophages was registered in patients with ITB and DR TB after adding IFNγ (M1-activation inducer) to the suspension culture. In contrast, in patients with DTB and DS TB, the number of macrophages with expression of B7 family co-stimulating molecules decreased or remained within the normal values in the absence of a reaction to cytokines during cytokine induction. Deficiency of HLA-DR-positive macrophages was found in all TB patients. The minimal number of macrophages expressing HLA-DR was found in patients with DTB and DS TB after cell incubation with IL-4 (M2-activation inducer).

Conclusion. Evaluation of the expression of B7 (CD80/86) and HLA-DR membrane molecules on macrophages in TB patients allows to conclude that anti-TB immune response is impaired at stages of antigen presentation (in all

✉ *Churina Elena G.,* e-mail: Lena1236@yandex.ru.

examined patients with TB) and co-stimulation (in DTB and DS TB). An increase in the expression of macrophage surface molecules CD80 (with M1- and M2-stimulation) and CD86 (with M1-stimulation) in patients with ITB and DR TB indicates an increase in cell reactivity in these forms of TB. In addition, deficit of expression of HLA-DR (a key marker of pro-inflammatory cell activation) on the surface of macrophages in TB can be considered as a general (independent of the clinical form of the disease and drug sensitivity of the pathogen) pathogenetic factor of immune imbalance in pulmonary tuberculosis.

Key words: macrophages, pulmonary tuberculosis, innate immunity, immune response, co-stimulating molecules, IL-4, IFNγ, CD80, CD86, HLA-DR.

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

Source of financing. The reported study was funded by Council for Grants of the President of the Russian Federation for leading scientific schools (No. SS-2690.2018.7) and the RFBR, project No. 19-315-90018.

Conformity with the principles of ethics. All patients participating in the study signed an informed consent. 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., Sitnikova A.V., Urazova O.I., Novitskiy V.V., Patysheva M.R., Golubchikov P.N., Stepanova E.P. Expression of pro-inflammatory and co-stimulatory molecules on the surface of macrophages *in vitro* in patients with pulmonary tuberculosis. *Bulletin of Siberian Medicine.* 2020; 19 (4): 179–188. https://doi.org/10.20538/1682-0363-2020-4-179-188.

Экспрессия провоспалительных и костимулирующих молекул на макрофагах *in vitro* у больных туберкулезом легких

Чурина Е.Г.[1, 2], Ситникова А.В.[1], Уразова О.И.[1, 3], Патышева М.Р.[2, 4], Новицкий В.В.[1, 3], Голубчиков П.Н.[5], Степанова Е.П.[5]

[1] *Сибирский государственный медицинский университет (СибГМУ)*
Россия, 634050, г. Томск, Московский тракт, 2

[2] *Национальный исследовательский Томский государственный университет (НИ ТГУ)*
Россия, 634050, г. Томск, пр. Ленина, 36

[3] *Томский государственный университет систем управления и радиоэлектроники(ТУСУР)*
Россия, 634050, г. Томск, пр. Ленина, 40

[4] *Научно-исследовательский институт (НИИ) онкологии, Томский национальный исследовательский медицинский центр (НИМЦ) Российской академии наук*
Россия, 634009, г. Томск, пер. Кооперативный, 5

[5] *Томский фтизиопульмонологический медицинский центр*
634009, г. Томск, ул. Р. Люксембург, 17

РЕЗЮМЕ

Цель работы – установить особенности экспрессии провоспалительных и костимулирующих молекул на макрофагах *in vitro* у больных туберкулезом легких в зависимости от клинической формы заболевания и чувствительности возбудителя к противотуберкулезным лекарственным средствам.

Материалы и методы. Обследованы 40 пациентов (36 мужчин и 4 женщины): 18 пациентов с диссеминированным туберкулезом легких (ДТБ) (16 мужчин и 2 женщины, средний возраст (44,56 ± 8,10) лет) и 22 пациента с инфильтративным туберкулезом легких (ИТБ) (20 мужчин и 2 женщины, средний возраст (46,54 ± 5,24) лет) с туберкулезом легких (ТБ). Из них было 30 пациентов, выделяющих *Mycobacterium tuberculosis* (МВТ), чувствительные к основным противотуберкулезным средствам (ПТС), и 10 пациентов, выделяющих МВТ, устойчивые к лекарственным средствам основного ряда противотуберкулезной терапии. Группу сравнения составили 15 здоровых доноров с сопоставимыми характеристиками по полу и возрасту.

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 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 CD80, CD86, HLA-DR на проточном цитометре Beckman Coulter CytoFLEX LX (Beckman Coulter, США). Анализ полученных данных осуществляли при помощи программного приложения CytExpert 2.0 (Beckman Coulter, США). Полученные результаты анализировали статистическими методами.

Результаты. Количество интактных и стимулированных цитокинами (IL-4 и IFNγ) CD80-позитивных макрофагов у больных ИТБ и с лекарственно-устойчивым ТБ (ЛУ ТБ) превышало их число не только у здоровых доноров, но и у больных ДТБ и с лекарственно-чувствительным ТБ (ЛЧ ТБ) соответственно. Кроме того, у больных ИТБ и ЛУ ТБ регистрировалось повышение экспрессии CD86 на макрофагах после добавления в суспензионную культуру IFNγ (индуктор M1-активации). У больных ДТБ и ЛЧ ТБ количество макрофагов с экспрессией костимулирующих молекул семейства B7 при индукции цитокинами, напротив, снижалось или сохранялось в пределах нормы в отсутствие реакции на цитокины. Дефицит HLA-DR-позитивных макрофагов обнаруживался у всех больных ТБ. Минимальное число макрофагов, экспрессирующих HLA-DR, установлено у больных ДТБ и ЛЧ ТБ после инкубации клеток с IL-4 (индуктор M2-активации).

Заключение. Оценка экспрессии мембранных молекул B7 (CD80/86) и HLA-DR на макрофагах у больных ТБ позволяет сделать вывод о нарушениях противотуберкулезного иммунного ответа на стадии презентации антигена (у всех обследованных больных ТБ) и костимуляции (при ДТБ и ЛЧ ТБ). Увеличение экспрессии макрофагами поверхностных молекул CD80 (при M1- и M2-стимуляции) и CD86 (при M1-стимуляции) у больных ИТБ и ЛУ ТБ свидетельствует о повышении реактивности клеток при данных формах течения ТБ. Наряду с этим дефицит экспрессии на макрофагах HLA-DR (ключевого маркера провоспалительной активации клеток) при ТБ можно рассматривать как общий (не зависящий от клинической формы болезни и лекарственной чувствительности возбудителя) патогенетический фактор иммунного дисбаланса при туберкулезе легких.

Ключевые слова: макрофаги, туберкулез легких, врожденный иммунитет, иммунный ответ, костимулирующие молекулы, IL-4, IFNγ, CD80, CD86, HLA-DR.

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. Пациенты подписали информированное согласие. Исследование одобрено локальным этическим комитетом СибГМУ (протокол № 5648 от 27.11.2017).

Для цитирования: Чурина Е.Г., Ситникова А.В., Уразова О.И., Патышева М.Р., Новицкий В.В., Голубчиков П.Н., Степанова Е.П. Экспрессия провоспалительных и костимулирующих молекул на макрофагах *in vitro* у больных туберкулезом легких. *Бюллетень сибирской медицины.* 2020; 19 (4): 179–188. https://doi.org/10.20538/1682-0363-2020-4-179-188.

INTRODUCTION

It is known that immunity is a complex of factors of life-sustaining activity of an organism aimed at maintaining body homeostasis. Acquired resistance to tuberculosis is a result of complex immune responses with involvement of macrophages, dendritic cells, lymphocytes, and granulocytes. At the same time, macrophages and human lymphatic system serve as a "phylogenetic cradle" for the causative agent of tuberculosis infection and promote the formation of symbiotic relationships between *Mycobacterium tuberculosis* (MBT) and a host organism [1]. Macrophages play a crucial role in the successful realization of innate immunity mechanisms in during pathogen invasion into mucous membranes of the respiratory tract (including MBT).

Macrophages are the most ancient immunocompetent cells. They represent a heterogeneous population of resident professional antigen presenting cells. The macrophage is the main effector cell in protecting the host from pathogens and regulating the innate and adaptive immune responses. Macrophages are involved in remodeling and restoration of damaged tissues [2, 3]. Versatility and plasticity of macrophages make it

possible for a rapid conversion of their functional phenotype in the focus of inflammation. This heterogeneity is determined by the ability of macrophages to implement different programs of activation in response to various stimuli, such as cytokine signals and signals associated with cell damage or penetration of the pathogen- and damage-associated molecular pattern molecules (DAMPs/PAMPs) into the body. In classic activation, macrophages maintain the course of an acute inflammatory Th1-dependent immune response while simultaneously performing the effector function (M1-activation). In alternative activation, macrophages acquire an anti-inflammatory phenotype resulting in their functional realignment and they start performing a tolerogenic function promoting fibrogenesis and enhanced cell proliferation (M2-activation) [4, 5].

The classic macrophage activation leading to polarization of their maturation towards M1-cells is induced by interferon (IFN) γ produced by type 1 T-helpers (Th1) and natural killers (NK) cells as well as tumor necrosis factor (TNF) α and bacterial lipopolysaccharides (LPS) [6]. Interleukins (IL) 4 and 10 are the main differentiation factors for alternatively activated M2-macrophages [7, 8]. Toll-like receptors (TLR) on the surface and inside macrophages recognize patterns of pathogenicity and, thus, trigger the activation of innate immunity. Molecules of the major histocompatibility complex (MHC) (HDL-DR) and co-stimulating molecules of the B7 group form a functionally important group of surface macrophage molecules. The expression of MHC-II molecules is enhanced by cell activation; CD80 and CD86 act as co-stimulating molecules. The former appears on the macrophage surface only after activation, the latter is expressed constitutively, but in antigen induction the intensity of expression increases [9–11].

Inflammation is the major effector mechanism of innate immunity which is implemented in the lungs in response to *M. tuberculosis* invasion into alveolar macrophages. This occurs if macrophages do not perform a phagocytic function for some reason. The effectiveness of inflammatory and regenerative potential of macrophages is determined, first of all, by their functional phenotype and the intensity of pro-inflammatory molecule expression on the cells.

Thus, the aim of this study was to identify the features of the expression of pro-inflammatory and co-stimulatory molecules on the surface of macrophages *in vitro* in patients with pulmonary tuberculosis, depending on the clinical form of the disease and sensitivity of the pathogen to anti-TB drugs.

MATERIALS AND METHODS

We examined 40 patients (36 men and 4 women): 18 patients with disseminated tuberculosis (DTB) (16 men and 2 women, average age (44.56 ± 8.10) years) and 22 patients with infiltrative tuberculosis (ITB) (20 men and 2 women, average age (46.54 ± 5.24) years). The diagnosis was established on the basis of medical history data, clinical findings, results of an X-ray examination of the lungs, and bacteriological and microscopic examination of the sputum.

Drug sensitivity of the pathogen to the basic ATBD was examined in all patients with TB. According to this criterion, 30 patients secreted MBT sensitive to the basic ATBD and 10 patients secreted MBT resistant to the first line ATBD (isoniazid, rifampicin, streptomycin, ethambutol). The exclusion criteria were under the age of 20 years and over 55 years, the presence of allergies, and severe concomitant diseases of infectious and non-infectious origin. 15 healthy donors with comparable characteristics by age and gender composed the comparison group.

The material for the study was venous blood collected from healthy donors and TB patients. Blood sampling was carried out once, in the middle of the disease course, before the start of anti-TB chemotherapy. To isolate monocytes from the whole blood with the aim of their following transformation into macrophages, the method of magnetic separation of $CD14^+$ monocytes (MACS MultiStand, Germany) was used according to the manufacturer's instructions (Miltenyi Biotec GmbH, Germany).

20 ml of the whole venous blood was collected into vacuum blood collection systems with an anticoagulant (K_3-EDTA). The blood was diluted with phosphate-buffer saline (PBS) at a 1:1 ratio and layered over 15 ml of ficoll 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 the mixture was stirred; then the number of mononuclear cells was counted using an automatic cell counter Scepter 2,0 (Merck Millipore, Germany). The cell suspension was centrifuged, the supernatant was removed, and the appropriate volume of MACS Separation Buffer (containing bovine serum albumin (BSA), EDTA, and 0.09% azide) and $CD14^+$ magnetic particles (Micro Beads, Germany) were added based on the number of cells, followed by incubation for 40 min. The resulting suspension underwent positive magnetic separation according to the manufacturer's protocol (Miltenyi Biotec, Germany).

IN VITRO MACROPHAGE CULTIVATION

Monocytes were cultivated in X-VIVO 10 complete growth-supporting medium with gentamycin and phenol red (Lonza, Switzerland) at a concentration of 1×10^6 cell/ml with the addition of M-CSF (5 ng/ml; RnD Systems, USA). Recombinant cytokines IL-4 (10 ng/ml; PeproTech, USA) (for M2-cell activation) and IFNγ (100 ng/ml PeproTech, USA) (for M1-cell activation) were used for additional cell induction. The samples had been cultivated in the CO_2-incubator for 6 days at 37 ° C and 7.5% of CO_2 without additional stimulation and with the addition of M1- and M2-activation cytokines.

IMMUNOPHENOTYPING OF MACROPHAGES

Macrophage phenotyping was performed on the sixth day of cultivation. To collect cells, a plate with the cell culture was placed on ice and held for 10 minutes, then the cells were collected using a cell scraper (Cell-scraper, USA). Monoclonal antibodies to CD80, CD86, and HLA-DR (eBioscience, USA) were added for immunophenotyping of macrophages. Measurement of cell suspensions was implemented using Beckman Coulter CytoFLEX flow cytometer (Beckman Coulter, USA). The analysis of obtained data was performed using CytExpert 2.0 software application (Beckman Coulter, USA).

SPSS Statistics 17.0 and Microsoft Excel were used for statistical analysis of the obtained data. The data were presented as the median (Me) and 25th and 75th percentiles (1st and 3rd quartiles: Q_1 and Q_3 respectively). 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

When studying the expression of co-stimulating B7 molecules (CD80 / CD86) and HLA-DR activation marker on the surface of macrophages, it was found that the number of macrophages expressing CD80 molecules in patients with ITB was higher than in patients of the control group and patients with DTB. In DTB patients, it was lower than in healthy donors (Table 1, Fig. 1). The addition of IFNγ to cell cultures in patients with ITB was accompanied by an increase in the expression of CD80. The addition of IL-4 to the cell culture, on the contrary, was accompanied by a decrease in the CD80 expression, compared with its value in the absence of stimulation (Table 1). In DTB, the level of cytokine-induced expression of CD80 did not significantly differ from normal values, but in the absence of stimulation it was significantly lower than in healthy patients (Table 1).

Table 1

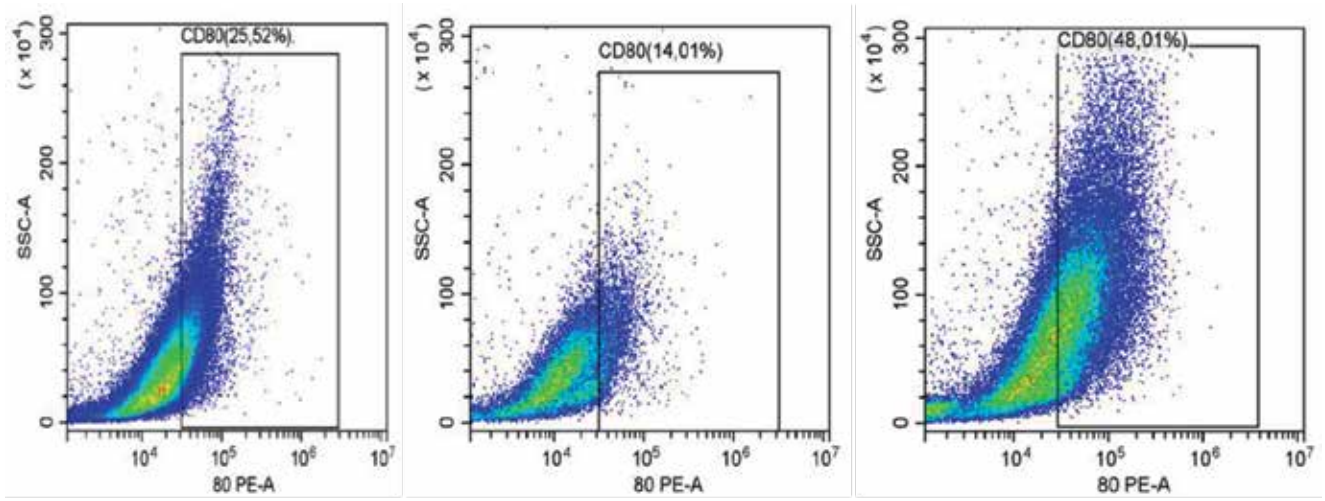
Expression of pro-inflammatory markers on the surface of macrophages depending on the clinical form of the disease in patients with TB, %, *Me* (Q_1–Q_3)

Markers of macrophages	Groups of comparison	During cultivation without stimulation	During cultivation with IL-4 (M2-stimulation)	During cultivation with IFNγ (M1-stimulation)
CD80	Healthy donors	23.11 (15.14–27.11)	15.25 (7.53–25.14)	20.32 (10.91–31.44)
	Patients with DTB	12.23 (8.42–25.13) $p_1 = 0.012$	11.65 (8.01–26.13)	18.70 (9.34–28.27)
	Patients with ITB	48.60 (24.17–51.14) $p_1 = 0.014$ $p_2 = 0.022$	41.61 (20.15–53.23) $p_1 = 0.015$ $p_2 = 0.031$	58.50 (28.73–70.35) $p_1 = 0.021$ $p_2 = 0.012$ $p_4 = 0.014$
CD86	Healthy donors	11.12 (8.52–28.01)	43.51 (32.53–54.55) $p_3 = 0.012$	23.22 (10.01–31.14) $p_3 = 0.016$ $p_4 = 0.025$
	Patients with DTB	14.14 (9.37–21.52)	13.48 (4.73–19.04) $p_1 = 0.012$	15.52 (7.14–25.37) $p_1 = 0.013$
	Patients with ITB	16.54 (9.22–27.63)	19.12 (8.56–23.14) $p_1 = 0.021$	27.02 (15.23–39.14) $p_2 = 0.012$ $p_3 = 0.015$ $p_4 = 0.034$
HLA-DR	Healthy donors	95.61 (76.66–98.73)	97.33 (85.41– 98.43)	96.66 (76.32–99.32)
	Patients with DTB	71.12 (51.33–83.72) $p_1 = 0.021$	57.71 (33.62–77.71) $p_1 = 0.022$ $p_3 = 0.025$	74.16 (42.74–84.23) $p_1 = 0.017$ $p_4 = 0.014$

Table 1 (continued)

Markers of macrophages	Groups of comparison	During cultivation without stimulation	During cultivation with IL-4 (M2-stimulation)	During cultivation with IFNγ (M1-stimulation)
HLA-DR	Patients with ITB	75.44 (51.51–87.53) $p_1 = 0.012$	67.51 (45.63–78.42) $p_1 = 0.013$	62.51 (44.72–83.43) $p_1 = 0.024$

Note: p_1 – the level of statistical significance of differences compared to the value of the indicator in healthy donors; p_2 – compared to the value in patients with DTB; p_3 – compared to the value during in vitro cell culture without stimulation; p_4 – compared to the value during *in vitro* cell culture with IL-4 (M2-stimulation).



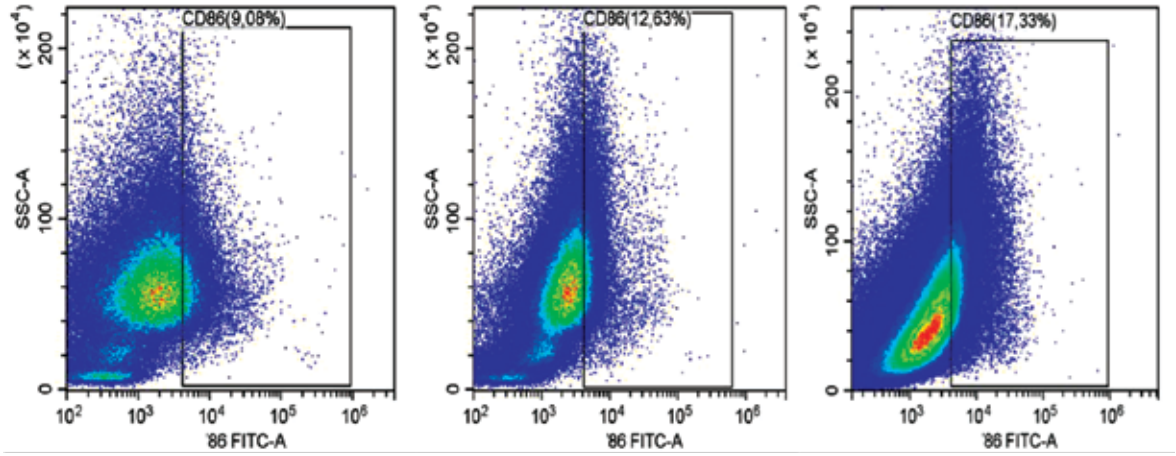
a *b* *c*

Fig. 1. The expression of CD80 on macrophages in patients with pulmonary tuberculosis depending on the clinical form of the disease, %: *a* – in healthy donors; *b* – in patients with DTB; *c* – in patients with ITB

The analysis of CD80 expression in TB patients depending on sensitivity of MBT to ATBD revealed the maximum number of CD80-positive macrophages in patients with DR TB compared to their number in healthy donors and patients with DS TB (Table 2). Herewith, in patients with DR TB, the expression of CD80 on the surface of macrophages stimulated by IL-4 and IFNγ cytokines was significantly higher than that on unstimulated cells (Table 2). In patients with DS TB after IFNγ induction of cells (M1-activation), the number of CD80-positive macrophages corresponded to that in healthy patients but was 1.9 times higher than with IL-4 stimulation (M2-activation) and without the addition of cytokines (Table 2).

In patients with TB, there were no differences in the expression of CD86 molecules by macrophages in the absence of stimulation by recombinant cytokines, regardless of the clinical form of the disease. When adding cytokines to the cell culture, the expression of CD86 on the surface of macrophages in healthy donors increased by 3.9 times in response to IL-4 induction (M2-activation) and 2.1 times in response to IFNγ induction (M1-activation), as opposed to values without stimulation. In patients with ITB, the number of CD86-expressing macrophages upon IFNγ induction was higher than in DTB patients and compared with their number in the absence of stimulation and with IL-4 induction of cells (it decreased, on the contrary) (Table 1). In addition, under IFNγ effects, an increase in CD86 expression by macrophages was observed in DR TB in comparison with patients with DS TB and in healthy donors, as well as in comparison with expression of the marker by unstimulated macrophages and during induction of cells by IL-4 (Table 2).

The analysis of the expression of HLA-DR-activating marker on the surface of macrophages revealed its decrease in patients with TB compared with the group of healthy donors, regardless of the clinical form of the disease and sensitivity of the pathogen to ATBD (Table 1, 2, Fig. 3). The maximum decrease in the number of macrophages expressing HLA-DR was observed in patients with DTB and DR TB after incubation of cells with IL-4, as opposed to their number in cell culture without stimulation and during IFNγ induction (Table 1, 2).



a *b* *c*

Fig. 2. The expression of CD86 on the surface of macrophages in patients with pulmonary tuberculosis depending on the clinical form of the disease, %: *a* – in healthy donors; *b* – in patients with DTB; *c* – in patients with ITB

Table 2

The expression of pro-inflammatory markers on the surface of macrophages depending on drug sensitivity of the pathogen to anti-TB drugs, %, *Me* (Q_1–Q_3)

Markers of macrophages	Groups of comparison	During cultivation without stimulation	During cultivation with IL-4 (M2-stimulation)	During cultivation with IFNγ (M1-stimulation)
CD80	Healthy donors	23.11 (15.14 –27.11)	15.25 (7.53 –25.14)	20.32 (10.91–31.44)
	Patients with DS TB	11.01 (9.21–26.63) $p_1 = 0.041$	12.22 (10.02–28.41)	23.55 (11.5–34.24) $p_3 = 0.025$ $p_4 = 0.017$
	Patients with DR TB	51.22 (23.11–68.33) $p_1 = 0.015$ $p_2 = 0.022$	62.33 (37.21–71.42) $p_1 = 0.037$ $p_2 = 0.025$ $p_3 = 0.027$	61.22 (32.45–70.66) $p_1 = 0.026$ $p_2 = 0.022$ $p_3 = 0.011$
CD86	Healthy donors	11.12 (8.52–28.01)	43.51 (32.53–54.55) $p_3 = 0.010$	23.22 (10.01–31.14) $p_3 = 0.015$ $p_4 = 0.024$
	Patients with DS TB	14.02 (8.51–21.44)	13.54 (10.25–25.11) $p_1 = 0.031$	17.23 (10.32–28.55)
	Patients with DR TB	18.22 (9.25–30.45) $p_1 = 0.030$	25.23 (14.01–36.12) $p_1 = 0.042$ $p_2 = 0.010$	34.45 (18.23–41.56) $p_1 = 0.024$ $p_2 = 0.014$ $p_3 = 0.012$ $p_4 = 0.021$
HLA-DR	Healthy donors	95.61 (76.66–98.73)	97.33 (85.41–98.43)	96.66 (76.32–99.32)
	Patients with DS TB	69.23 (56.25–86.12) $p_1 = 0.012$	55.12 (43.22–75.23) $p_1 = 0.022$ $p_3 = 0.011$	66.23 (42.5–84.23) $p_1 = 0.031$ $p_3 = 0.015$
	Patients with DR TB	80.23 (59.12–94.54) $p_1 = 0.044$ $p_2 = 0.012$	76.12 (49.52–90.13) $p_1 = 0.034$ $p_2 = 0.012$	72.12 (57.32–86.42) $p_1 = 0.035$ $p_2 = 0.014$

Note: p_1 – the level of statistical significance of differences compared to the value of the indicator in healthy donors; p_2 – in patients with DS TB; p_3 – in in vitro cell culture without stimulation; p_4 – in in vitro cell culture with IL-4 (M2-stimulation).

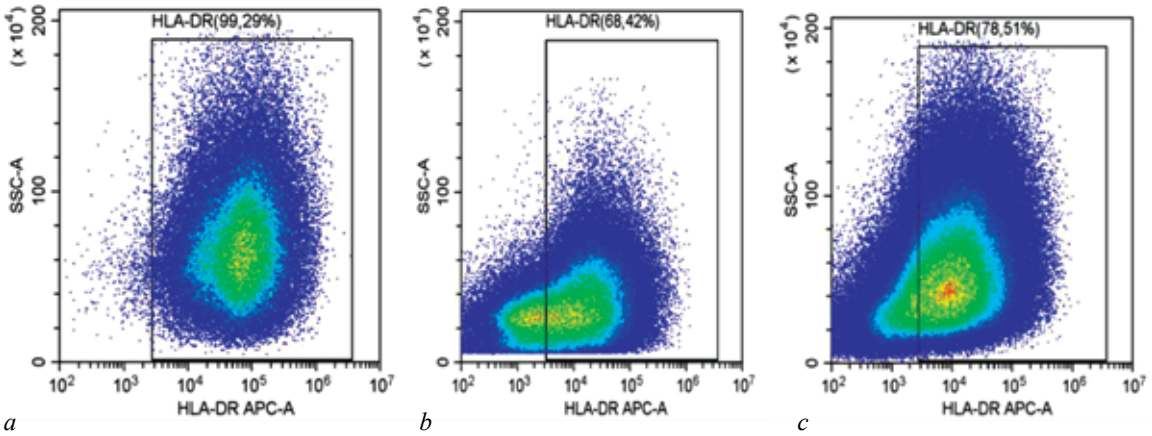


Fig. 3. The expression of HLA-DR on the surface of macrophages in patients with pulmonary tuberculosis depending on the clinical form of the disease, %: *a* – in healthy donors; *b* – in patients with DTB; *c* – in patients with ITB.

DISCUSSION

Analysis of the expression of pro-inflammatory markers, namely B7 co-stimulatory molecules (CD80, CD86) and HLA-DR marker of activation on the surface of macrophages, showed that the number of macrophages expressing HLA-DR, which is necessary for implementation of their antigen-presenting function, was reduced in patients with DTB and ITB, especially during M2-activation of cells. Interestingly, macrophage expression of CD80 and CD86 molecules in patients with TB was multidirectional. For instance, we observed a significant increase in the number of CD80-positive cells in patients with ITB, especially during stimulation with IFNγ (at M1-activation), while in patients with DTB, the number of $CD80^+$ macrophages decreased (Table 1, Fig. 1). It should be noted that in DR TB, the expression of the CD80 molecule sharply increased both during M1- and M2-activation of macrophages (Table 2).

The expression of the CD86 molecule on the surface of unstimulated macrophages did not generally differ from that in the control group. However, it increased during M1-activation of cells in patients with infiltrative and drug resistant TB (Table 1, 2). It may be assumed that intensive expression of CD86 co-stimulating molecule during *in vitro* differentiation of cells into M1-macrophages in ITB and DR TB was caused by the preservation of their pro-inflammatory potential. At the same time, natural expression of CD86 on the surface of macrophages regardless of the antigen load and mediator stimulation should be taken into account.

It is known that co-stimulating CD80 and CD86 molecules are the members of the B7 family [12]. CD80 and CD86 markers were found not only on the surface of dendritic cells, activated B-lymphocytes, and macrophages [13], but also on the surface of non-professional antigen-presenting cells [14]. The CD80 molecule, often in the tandem with CD86, plays an important role in the regulation of both adaptive and innate immune responses. These molecules are ligands for the CD28 receptor on the surface of naive T-lymphocytes, and their interaction is an important co-stimulating signal in immunological synapse between a macrophage and a T-cell, which leads to the activation, proliferation, and differentiation of T-lymphocytes in a necessary direction [15]. CD80 is a key marker of the activation of macrophages and it is not expressed on the surface of cells in the absence of the antigen load [16]. In inflammation, interaction of the macrophage with the receptor on the surface of the T-lymphocyte via MHC-II leads to the activation of CD80 [13].

HLA-DR is constitutively expressed on the surfaces of monocytes, macrophages, and dendritic cells. Monocytes of a healthy human also express HLA-DR molecules on their surface at high density. Previously, while investigating in vitro dendritic cells transformed from blood monocytes in patients with TB, we had showed enhanced generation of tolerogenic dendritic cells (HLA-DR-negative) associated with an imbalance of their cytokine secretory activity [17].

HLA-DR expression on the surface of monocytes and macrophages is crucial for the presentation of microbial peptides by T-cells, which contributes to the initiation of the adaptive immune response [18]. The negative role of decreased expression of HLA-DR on the surface of macrophages has been shown. Mono-

cytes and macrophages with reduced or no expression of HLA-DR are not able to perform their antigen-presenting function. The change in the expression of HLA-DR on the surface of monocytes/macrophages is considered to be an informative marker of the dynamics of the immune response in critical conditions [19]. A decrease in the number of HLA-DR-positive monocytes has been described in severe injuries, in the postoperative period, in acute pancreatitis, and burn injury [20, 21]. In the development of hospital-acquired infection, a decrease in the expression of HLA-DR on the surface of monocytes determines the development of sepsis [22].

Thus, a significant decrease in the number of HLA-DR-positive cells (especially in patients with DTB) established by us indicates impairment of the mechanism of classic activation of macrophages and their antigen-presenting and effector functions.

CONCLUSION

According to the obtained findings, changes in *in vitro* expression of CD80/CD86 co-stimulating molecules on the surface of macrophages in patients with TB are multidirectional. In case of DTB and DS TB, the number of intact (unstimulated) CD80-positive macrophages is lower than the normal value; and in ITB and DR TB, the number of such macrophages is higher than the normal value, both in the absence of stimulation and with M2- and especially M1-activation of macrophages. The latter in association with an increase in the expression of CD80 during IFNγ-mediated M1-induction of macrophages in ITB and DR TB patients indicates an increase in pro-inflammatory reactivity of cells in these forms of TB. The absence of increase or, on the contrary, a decrease in expression of CD80 and CD86 on the surface of macrophages in response to cytokine stimulation in patients with DTB and DS TB along with a deficiency of the expression of HLA-DR may be considered as a pathogenetic factor of the immune imbalance, a manifestation of secondary immune deficiency in pulmonary tuberculosis, and an indicator of an unfavorable prognosis of the disease.

REFERENCES

1. Novitskiy V.V., Urazova O.I., Strelis A.K., Voronkova O.V., Filinyuk O.V., Shilko T.A. Pathology of the immunity: a cause or consequence of TB infection? *Bulletin of Siberian Meedicine*. 2006; 5 (2):70–74 (in Russ.).
2. Murray P., Wynn T. Protective and pathogenic functions of macrophage subsets. *Nat. Rev. Immunol.* 2011; 11 (11): 723–737. DOI: 10.1038/nri3073.
3. Churina E.G., Sitnikova A.V. *Urazova O.I.,* Chumakova S.P., *Vins M.V.,* Beresneva A.E.., Novitskiy V.V. Macrophages in bacterial diseases of the lungs: phenotype and functions (review). *Bulletin of Siberian Mdicine.* 2019; 18(1): 142–154 (in Russ.). DOI: 10.20538/1682-0363-2019-1-142-154.
4. Swirski F.K., Nahrendorf M. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. *Science*. 2013; 339 (6116): 161–166. DOI: 10.1126/science.1230719.
5. Possamai L.A., Thursz M.R., Wendon J.A., Antoniades C.G. Modulation of monocyte/macrophage function: a therapeutic strategy in the treatment of acute liver failure. *J. Hepatol*. 2014; 61 (2): 439–445. DOI: 10.1016/j.jhep.2014.03.031.
6. Cassetta L., Cassol E., Poli G. Macrophage polarization in health and disease. *Sci. World J*. 2011; 11: 2391– 2402. DOI: 10.1100/2011/213962.
7. Hussell T., Bell T. Alveolar macrophages: plasticity in a tissue-specific context. *Immunol*. 2014; 14 (2): 81–93. DOI: 10.1038/nri3600.
8. Hoeffel G., Ginhoux F. Ontogeny of tissue-resident macrophages. *Immunology*. 2015; 6: 486. DOI: 10.3389/fimmu.2015.00486.
9. Schenk M., Fabri M., Krutzik S.R., Lee D.J., Vu D.M., Sieling P.A., Montoya D., Liu P.T., Modlin R.L. Interleukin-1β triggers the differentiation of macrophages with enhanced capacity to present mycobacterial antigen to T cells. *Immunology*. 2014; 141 (2): 174–180. DOI: 10.1111/imm.12167.
10. Gordon S., Martinez F.O. Alternative activation of macrophages: mechanism and functions. *Immunity*. 2010; 32 (5): 593–604. DOI: 10.1016/j.immuni.2010.05.007.
11. Martinez F.O., Sica A., Mantovani A., Locati M. Macrophage activation and polarization. *Front. Biosci.* 2008; 1 (13): 453–461. DOI: 10.2741/2692.
12. Peyravian N., Gharib E., Moradi A., Mobahat M., Tarban P., Azimzadeh P., Nazemalhosseini-Mojarad E., Asadzadeh Aghdaei H. Evaluating the expression level of co-stimulatory molecules CD 80 and CD 86 in different types of colon polyps. *Curr. Res. Transl. Med.* 2018; 66 (1): 19–25. DOI: 10.1016/j.retram.2017.11.003.
13. Owen J., Punt J., Stranford S., Jones P., Kuby J. Kuby Immunology. New York: W.H. Freeman & Co., 2013: 574.
14. Scarpa M., Brun P., Scarpa M., Morgan S., Porzionato A., Kotsafti A., Bortolami M., Buda A., D'Incà R., Macchi V., Sturniolo G.C., Rugge M., Bardini R., Castagliuolo J., Angriman I., Castoro C. CD80-CD28 signaling controls the progression of inflammatory colorectal carcinogenesis. *Oncotarget*. 2015; 6(24): 20058–20069. DOI: 10.18632 / oncotarget.2780.
15. Ganesan A., Moon T.C., Barakat K.H. Revealing the atomistic details behind the binding of B7–1 to CD28 and CTLA-4: A comprehensive protein-protein modelling study. *Biochimica et Biophysica Acta (BBA)*. 2018; 1862 (12): 2764–2778. DOI: 10.1016/j.bbagen.2018.08.010.
16. Wang L.X., Mei Z.Y., Zhou J.H., Yao Y.S., Li Y.H., Xu Y.H., Li J.X., Gao X.N., Zhou M.H., Jiang M.M., Gao L., Ding Y., Lu X.C. Low dose decitabine treatment induces CD80 expression in cancer cells and stimulates tumor specific cytotoxic T lymphocyte responses. *PLoS One.* 2013; 8 (5): e62924. DOI: 10.1371/journal.pone.0062924.

17. Urazova O.I., Churina E.G., Hasanova R.R., Novitskiy V.V., Poletika V.S. Association between polymorphisms of cytokine genes and secretion of IL-12p70, IL-18, and IL-27 by dendritic cells in patients with pulmonary tuberculosis. *Tuberculosis*. 2019; 115: 56–62.
18. Zhuang Y., Peng H., Chen Y., Zhou S., Chen Y. Dynamic monitoring of monocyte HLA-DR expression for the diagnosis, prognosis, and prediction of sepsis. *Front. Biosci. (Landmark Ed).* 2017; 22: 1344–1354. DOI: 10.2741/4547.
19. Venet F., Lukaszewicz A.C., Payen D., Hotchkiss R., Monneret G. Monitoring the immune response in sepsis: a rational approach to administration of immunoadjuvant therapies. *Curr. Opin. Immunol.* 2013; 25 (4):477–483. DOI: 10.1016 / j. coi.2013.05.006.
20. Monneret G.,Venet F.,Pachot A., Lepape A. Monitoring immune dysfunctions in the septic patient: a new skin for the old ceremony. *Mol. Med*. 2008; 14 (1-2): 64–78. DOI: 10.2119/2007-00102.
21. Monneret G., Lepape A.,Voirin N., Bohe J.,Venet F., Debard A.L. Persisting low monocyte human leukocyte antigen-DR expression predicts mortality in septic shock. *Intensive Care Med.* 2006; 32 (8): 1175–1183. DOI: 10.1007/s00134-006-0204-8.
22. Grimaldi D., Louis S., Pène F., Sirgo G., Rousseau C., Claessens Y. E., Vimeux L., Cariou A., Mira J. P., Hosmalin A., Chiche J. D. Profound and persistent decrease of circulating dendritic cells is associated with ICU-acquired infection in patients with septic shock intensive care. *Med*. 2011; 37 (9): 1438–1446. DOI: 10.1007/s00134-011-2306-1.

Authors contribution

Churina E.G. – design of the study, analysis of literature, statistical processing and interpretation of research results, drafting of the manuscript. Sitnikova A.V. – sample preparation of biomaterial, implementation of immunomagnetic separation and flow cytometry, methods, drafting of the manuscript. Urazova O.I. – material and technical support of laboratory research, interpretation of results, drafting, design and translation of the manuscript. Patysheva M.R. – implementation of immunomagnetic separation and flow cytometry, consulting assistance in designing the study. Novitskiy V.V. – consultations on hematological aspects of the study, editing of the manuscript. Stepanova E.P. – interaction with patients, collection of the biomaterial. Golubchikov P.N. – interaction with patients, consultations on phthisiological and pulmonological aspects of the study.

Authors information

Churina Elena G., Dr. Sci. (Med.), Professor, Department of Pathophysiology, Siberian State Medical University; Professor, Department of Organic Chemistry, Leading Researcher at the Laboratory of Translational Cellular and Molecular Biomedicine, National Research Tomsk State University, Tomsk, Russian Federation. ORCID 0000-0002-8509-9921.

Sitnikova Anjelika V., Post-Graduate Student, Department of Pathophysiology, Siberian State Medical University, Tomsk, Russian Federation.

Urazova Olga I., Dr. Sci. (Med.), Professor, Corresponding Member of RAS, Head of the Pathophysiology Division, Siberian State Medical University; Professor, Department of Complex Information Security of Computer Systems, Tomsk State University of Control Systems and Radioelectronics, Tomsk, Russian Federation. ORCID 0000-0002-9457-8879.

Novitskiy Vyacheslav V., Dr. Sci. (Med.), Professor, Academician of RAS, Honored Scientist of Russia, Professor of the Pathophysiology Division, Siberian State Medical University; Professor of the Department of Complex Information Security of Computer Systems, Tomsk State University of Control Systems and Radioelectronics, Tomsk, Russian Federation. ORCID 0000-0002-9577-8370.

Patysheva Marina R., Junior Researcher, Laboratory of Tumor Progression Biology of the Cancer Research Institute, Tomsk NRMC; Junior research scientist, Laboratory of Translational Cellular and Molecular Biomedicine, NR TSU, Tomsk, Russian Federation. ORCID 0000-0001-5758-7330.

Golubchikov Petr N., Deputy Chief Physician for Medicine, Tomsk Phthisiopulmonological Medical Center, Tomsk, Russian Federation.

Stepanova Ekaterina P., TB Specialist, Pulmonologist, Head of the Department for patients with respiratory tuberculosis, Tomsk Phthisiopulmonological Medical Tomsk, Russian Federation.

(✉) **Churina Elena G.,** e-mail: Lena1236@yandex.ru.

Received 18.06.2020
Accepted 29.09.2020