Tuberculosis 115 (2019) 56–62

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: www.elsevier.com/locate/tube

Immunological Aspects

Association between polymorphisms of cytokine genes and secretion of IL-12p70, IL-18, and IL-27 by dendritic cells in patients with pulmonary tuberculosis

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ARTICLE INFO

Keywords:
Pulmonary tuberculosis
Immune response
Cytokine gene polymorphism
Dendritic cells
Cytokines

ABSTRACT

Proinflammatory cytokines are known to play a crucial role in the pathogenesis of tuberculosis, and gene polymorphisms in the promoter regions of cytokine genes were shown to substantially influence the secretory capacity of immune cells. In the present study, we analyzed the association between polymorphisms of the *IL12B*, *IL18,* and *IL27* genes and the secretion of the proinflammatory cytokines IL-12p70, IL-18, and IL-27 by myeloid dendritic cells (mDCs) in pulmonary tuberculosis (PTB) patients. The study enrolled 334 patients with newly diagnosed infiltrative and disseminated PTB. Cultivation of mDCs was performed from non-proliferating progenitors of $CD14^+$ blood monocytes. Cytokine secretion was evaluated by measuring cytokine concentration in the mDC culture supernatants using enzyme-linked immunosorbent essay (ELISA). To study cytokine gene polymorphisms, a polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis (RFLP) were performed. Reduced secretion of IL-18 and IL-27 by mDCs in PTB patients was associated with 105A/C polymorphisms of the *IL18* gene, and 2905T/G, 4730T/C, and −964A/G of the *IL27* gene, respectively. Polymorphism IL12B/insertion had a bidirectional influence on the secretion of IL-12p70, being associated with decreased levels of the cytokine in infiltrative PTB and increased levels in disseminated PTB patients.

1. Introduction

Tuberculosis (TB) is a multifactorial disease that develops as a result of the interaction of a multitude of genes, many of which are involved in the regulation of immune response. Polymorphisms in such genes may substantially influence the degree of host resistance to mycobacterial infection, as well as the severity and duration of the disease. Thus, a number of studies have shown the association of cytokine gene polymorphisms with susceptibility to tuberculosis infection [1–4].

Most individuals infected with *Mycobacterium tuberculosis* (MBT) develop a full-fledged immune response involving realization of innate and adaptive mechanisms, and only in 3–5% patients the immune response against tuberculosis infection turns out to be ineffective, resulting in the reactivation of a latent infection and the development of active forms of TB. An initiation of adequate anti-tuberculosis immune response relies heavily on the activity of dendritic cells (DCs), specialized antigen-presenting leukocytes that present *Mtb* antigen to naive $CD4^+$ T-lymphocytes during the primary invasion of the pathogen into the host organism [5–7].

It is generally accepted that DC represent a crucial link between the innate and adaptive immunity, since they are responsible for the activation of antigen-specific immune response, and are critically involved in whether T cell anergy or an active immune response to an antigen will be induced [8,9]. Moreover, it was shown that in the context of anti-tuberculous immune response, an adequate secretion of proinflammatory cytokines by activated DCs is necessary for the activation of naive T-lymphocytes and their subsequent differentiation into T helper cells type 1 (Th1), while a reduced secretion of these cytokines might diminish the host immune defense against *Mtb* [8,9]. At the same time, whether the pattern of DC-derived cytokine secretion in PTB is genetically predetermined, or arises as a consequence of the immune system dysregulation induced by the causative agent, remains to be fully elucidated [10,11].

Since the exon sequences of cytokine genes are very conservative,

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https://doi.org/10.1016/j.tube.2019.02.003
Received 17 June 2018; Received in revised form 30 January 2019; Accepted 3 February 2019

and given the central role of promoter regions in the regulation of gene expression, the aim of the study was to investigate the association of polymorphisms of promoter regions of cytokine genes *IL12B, IL18*, and *IL27* with the mDC-derived cytokine secretion in patients with pulmonary tuberculosis.

2. Materials and methods

2.1. Study population

The study population included 334 patients (220 men and 114 women) aged 23–50 (average age 43,10 ± 10) with newly diagnosed TB attending the Tomsk Phthisiopulmonological Medical Center. The PTB diagnosis was confirmed based on a culture and/or smear positive for *Mtb*, and clinical and radiographic presentation consistent with PTB. All participants were examined prior to specific anti-tuberculous chemotherapy. Enrolled patients were classified into two groups according to the clinical form of the disease: 177 with infiltrative TB and 157 with disseminated TB. Individuals with the following conditions were excluded from the study: 1) cancer, diabetes mellitus, bronchial asthma, autoimmune and allergic diseases, hepatitis virus infection, human immunodeficiency virus; 2) treatment with anti-tuberculosis drugs and immunosuppressive drugs. Also enrolled were 183 healthy controls aged 23–50 (130 men and 53 women) without active TB, without history of TB and matched with cases by gender and age (average age 41.31 ± 7.47). Written informed consent was obtained from each subject.

2.2. Cultivation of dendritic cells

The mDCs were cultured from non-proliferating progenitors of $CD14^+$blood monocytes. The whole venous blood collected from TB patients was used to isolate mononuclear cells by gradient centrifugation at 1500 rpm for 20 min using ficoll urografin gradient ($\rho = 1.077$ g/sm^3; PanEco, Russia). Then, the monocytes were isolated from the acquired mononuclear cell suspension by gradient centrifugation at 4000 rpm for 45 min using standard isotonic Percoll solution. An expression of CD14 receptors on monocytes was evaluated via flow cytometry using CD14-PE monoclonal antibodies according to the manufacturer's protocol (BD Pharmingen™, USA).

Cultivation of mDCs from acquired suspension of blood monocytes was performed as previously described [12]. The 1×10^6 cell suspension containing monocytes was added to each well of the 24-well flat-bottomed plate in combination with 1 ml of complete nutrient medium. The plates with cell suspension were then placed in a CO_2 incubator for 1 h at 37 °C and 5% CO_2, and then the complete nutrient medium was re-added to the suspension in the same amount, along with 20 ng/ml of GM-CSF and IL-4 (Sigma, USA). On the third day of incubation, 75% of the medium was removed and an equal amount of the complete culture medium with recombinant cytokines was added to the suspension.

After two to three days of monocytes stimulation with GM-CSF and IL-4 in the culture medium, immature round mDCs with abundant cytoplasm and poorly developed dendrocytes were identified in the cell culture (Fig. 1a). On the fifth day of incubation, the nutrient medium was renewed, and 5 ng/ml of lipopolysaccharide (Sigma, USA) was added. On the seventh day of incubation, the phenotype of mature mDCs (Fig. 1b) and the concentration of cytokines in the supernatants were evaluated. The viability of mDCs was examined by the combined staining with annexinV-FITC (Beckman Coulter, USA) and intranuclear vital dye 7-AAD (7 amino-actinomycin) (BDPharmingen, USA) using FACSCanto™ II flow cytometer (BD, USA) The viable population of mDCs generated under *in vitro* conditions on the seventh day of cultivation varied between 83 and 89%. The phenotype of obtained DCs was studied using flow cytometry. Cells were stained with fluorochrome-labeled monoclonal antibodies to CD 209, TLR-2, HLA-DR, CD80, and CD86 («eBioscience», USA) and analyzed on FACSCanto™ II flow cytometer («BD», USA) (Suppl. 1: Fig. 1-1 – 1-3).

2.3. Evaluation of cytokine levels in supernatants of culture suspensions of dendritic cells generated in vitro from blood monocytes

To measure the concentrations of IL-18, IL-12p70, and IL-27 in supernatants of mDC culture suspensions, an enzyme-linked immunosorbent assay (ELISA) was performed according to manufacturer's recommendations (for IL-18 and IL-12p70 – Bender MedSystems, Austria; for IL-27 – eBioscience, USA). The optical density of the plate well content was registered using photometer analyzer Multiscan EX (Finland) at the 450 nm wave length. The concentrations of cytokines (pg/ml) were calculated on the basis of the calibration curve.

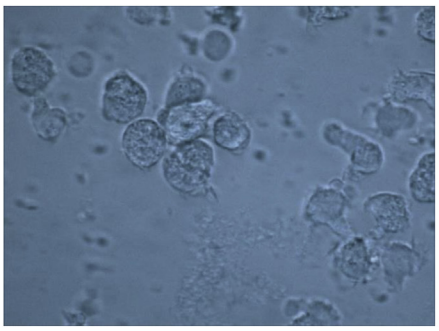
2.4. DNA extraction and cytokine gene polymorphism genotyping

DNA was isolated from 20 ml of the whole venous blood using the phenol-chloroform method according to the manufacturer's instructions (InterLabService, Russia).

The samples of extracted DNA were typed for the following single nucleotide polymorphisms (SNPs): *IL12B* gene polymorphism (IL12B/insertion); *IL18* gene polymorphism 105C/A (rs549908); *IL27* gene polymorphisms 2905T/G (rs17855750), 4730T/C (rs181206), and −964A/G (rs153109) (Table 1).

The SNPs were selected using the Online Mendelian Inheritance in Man database (OMIM) and the database of the National Center for Biotechnological Information. DNA amplification was performed via polymerase chain reaction (PCR) on the Tercik MC2 amplifier (DNA-technology, Russia). The identification of chosen cytokine genes polymorphisms was performed via PCR and restriction fragment length polymorphism (RFLP) analysis according to manufacturer's recommendations (Syntol, Russia; Sibenzyme, Russia; New England Biolabs, UK). The structure of primers, temperature of anneal, restriction enzymes, and lengths of hydrolysis fragments are presented in Table 1 [13–16].

Amplification products were separated in 1% agarose gel. For RFLP analysis, amplification products were cleaved by endonucleases (Table 1). The products of restriction were separated with


a)

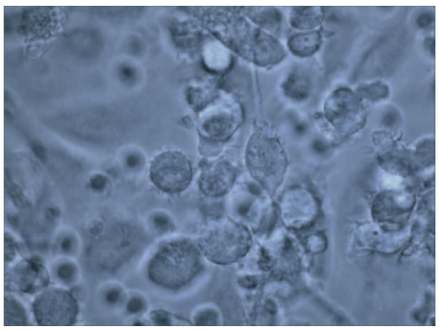

b)

Fig. 1. Generation of dendritic cells from blood monocytes (× 250 magnification) *in vitro*: a – immature dendritic cells in complete growth medium on the third day of cultivation; b – mature dendritic cells in complete nutrient medium on the sixth day of cultivation. The analysis of the cell population was performed via FACS analysis using FACSCanto™ II flow cytometer (BD, USA).

Table 1
Primer sequences and conditions of detection of cytokine gene polymorphisms.

Gene	Mutation (method)	Primers, 5′-3′	Annealing t, °C	Restriction enzyme	Fragments
IL12B	*IL12B/insertion* (PCR)	TGGATTGTGAAGTGGGACAT TAATGTGGTCATTGGCAGGT	55	–	a, no insertion: 158 bp A, insertion: 162 bp
IL18	*rs549908* (RFLP)	CCTCTACAGTCAGAATCAGT TGTTTATTGTAGAAAACCTGGAATT	50	*Taq*I	A: 148 bp C: 123 + 25 bp
IL27	*rs17855750* (RFLP)	ATCTCGCCAGGAAGCTGCGC CTGTTAGTGGGGGCCAGAAGGGA	67	*Bst*UI	T: 120 bp G: 100 + 20 bp
	rs181206 (RFLP)	GCTTCAGCCCTTCCATGCCC TCTACCTGGAAGCGGAGGTGCC	62	*Msp*I	T: 132 bp C: 111 + 21 bp
	rs153109 (RFLP)	CTGATCCTGACCTCACTCAACGC CTGACTGGGACTGGGACTCAGC	61	*Bst*UI	A: 468 bp G: 343 + 125 bp

Note: PCR – polymerase chain reaction, RFLP – restriction fragment length polymorphism.

electrophoresis in 8% polyacrylamide gel at 200 V for 2 h and visualized in the ultraviolet spectrum (Suppl. 2: Fig. 2-1 – 2-4). The pUC19 plasmid cleaved by *Msp*I restrictase («Sibenzym», Russia) was used as a marker of DNA length. The lengths of hydrolysis fragment are presented in Table 1.

2.5. Statistical analysis

To assess the normality of the distribution of sample data, the Kolmogorov–Smirnov test was performed. Since the quantitative characteristics in the comparison groups did not have a normal distribution, the non-parametric Mann–Whitney test for comparison of independent samples was used. For all quantitative characteristics in the compared groups, the median and the 25% and 75% quartiles were calculated.

The genotype distribution of each SNP was analyzed for Hardy-Weinberg equilibrium. In the healthy controls, all genotypic frequencies were in Hardy-Weinberg equilibrium. To compare the allele frequencies between the groups, the $\chi 2$ test with Yates correction for continuity was used. The association of polymorphisms with the PTB was concluded based on the value of odds ratio (OR) with the calculation of 95% confidence interval. An OR < 1 was considered as a negative association, and OR > 1 was considered as a positive association between the evaluated parameters. Results were considered significant when $P < 0.05$.

3. Results

3.1. Association of IL18 105 A/C polymorphism with IL-18 secretion by mDCs in PTB

There was a statistically significant difference in the genotype frequencies of the *IL18* 105 A/C polymorphism between patients with infiltrative PTB and healthy donors ($\chi^2 = 20.53$ ($p < 0.001$)). On the other hand, in patients with disseminated PTB, the genotype frequencies did not differ significantly compared to control group, but differed from infiltrative PTB patients ($\chi^2 = 18.73$ ($p < 0.001$)). There was also an association of the homozygous CC genotype (OR = 1.50 (0.83–2.70)) and C allele (OR = 1.13 (0.83–1.53)) of the 105A/C polymorphism with the incidence of disseminated form of PTB. Additionally, the carriage of homozygous AA genotype (OR = 2.61 (1.70–4.00)) and A allele (OR = 1.75 (1.28–2.39)) of 105A/C polymorphism was a predisposing factor of infiltrative PTB (Suppl. 3, Table 3-1).

In carriers of the C allele and the AC and CC genotypes of the *IL18* 105 A/C polymorphism with disseminated PTB, the level of mDC-derived secretion of IL-18 was lower (30.2% ($p_1 < 0.05$), 29.4% ($p_1 < 0.05$) and 25% ($p_1 < 0.05$), respectively) compared to the healthy donors (Fig. 2). In patients with infiltrative PTB, regardless of the carriage of alleles and genotypes of 105A/C polymorphism, the level of IL-18 secretion (32.56 (16.51–34.54) pg/ml) was comparable to that in healthy donors.

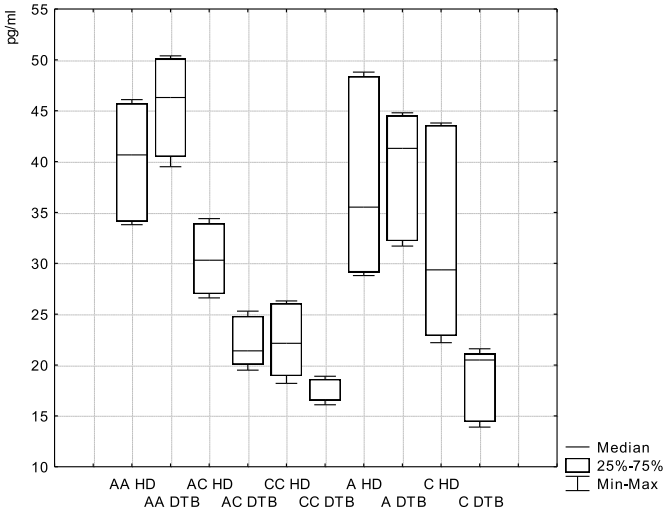


Fig. 2. Association between IL-18 secretion by mDCs and IL18 gene polymorphism 105A/C in healthy donors and patients with disseminated PTB. Note: HD – healthy donors, ITB – infiltrative tuberculosis, DTB – disseminated tuberculosis. Results are presented as median, lower and upper quartiles. In legend: AA, AC, CC – genotypes of 105A/C polymorphism; A, C – alleles of 105A/C polymorphism.

3.2. Association of IL12 gene polymorphism IL12B/insertion with IL-12p70 secretion by mDCs in PTB

Genotyping for *IL12* polymorphism IL12B/insertion showed that in patients with infiltrative PTB, there was an increase in the Aa genotype frequency ($\chi^2 = 24.35$ ($p < 0.001$)), while in patients with disseminated PTB, the aa genotype was observed more frequently ($\chi^2 = 36.49$ ($p < 0.001$)). Heterozygous Aa genotype (OR = 2.08 (1.36–3.20)) and a allele (OR = 1.07 (0.79–1.46)) were associated with infiltrative PTB, while the homozygous aa genotype (OR = 3.30 (2.11–5.16)) and a allele (OR = 2.91 (2.01–4.21)) were associated with disseminated PTB (Suppl. 3, Table 3-2).

In patients with infiltrative PTB, IL-12p70 levels in carriers of both genotypes (Aa and aa) and alleles (A and a) were decreased in comparison with healthy donor group. In contrast, in disseminated PTB patients, level of IL-12p70 secretion was higher than in controls ($p_1 < 0.05$–0.001) (Fig. 3). Among infiltrative PTB patients carrying Aa genotype and A allele, concentration of IL-12p70 was significantly lower compared to the carriers of aa genotype and a allele of the IL12B/insertion polymorphism (41.2% ($p_{Aa/aa} < 0.05$) and 47.1% ($p_{A/a} < 0.001$), respectively). In patients with disseminated PTB, however, an opposite pattern was observed, since the secretion level of IL-12p70 in carriers of the aa genotype and a allele was significantly decreased as compared to carriers of the Aa genotype and A allele (32.2% ($p_{Aa/aa} < 0.001$; $p_{A/a} < 0.05$)) (Fig. 3).

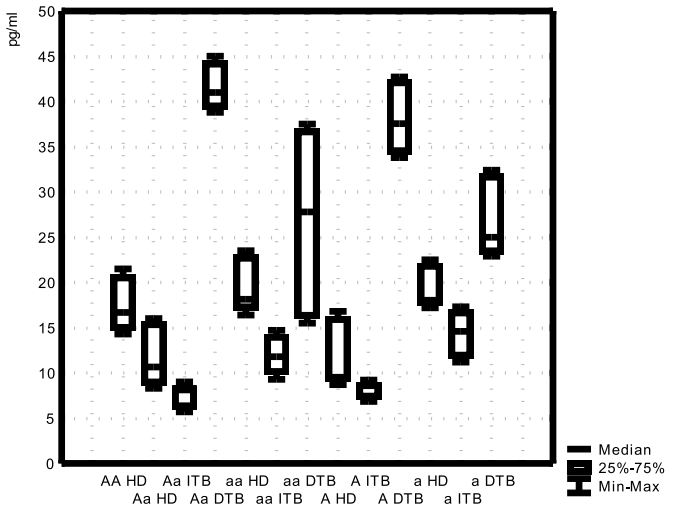


Fig. 3. Association between IL-12 secretion by mDCs and IL12B gene polymorphism IL12B/insertion in healthy donors and patients with infiltrative and disseminated PTB. Note: HD – healthy donors, ITB – infiltrative tuberculosis, DTB – disseminated tuberculosis. Results are presented as median, lower and upper quartiles. In legend: Aa, aa. – genotypes of IL12B/insertion polymorphism; A, a. – alleles of IL12B/insertion polymorphism.

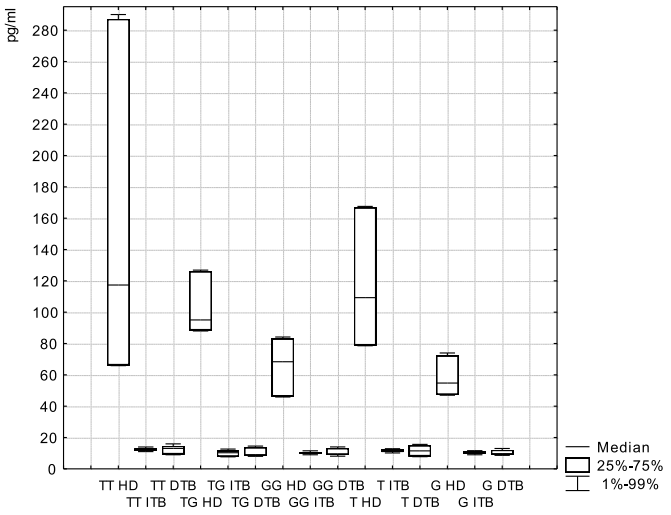


Fig. 4. Association between IL-27 secretion by mDCs and IL27 gene polymorphism 2905T/G in healthy donors and patients with infiltrative and disseminated PTB. Note: HD – healthy donors, ITB – infiltrative tuberculosis, DTB – disseminated tuberculosis. Results are presented as median, lower and upper quartiles. In legend: TT, TG, GG – genotypes of 2905T/G polymorphism; T, G – alleles of 2905T/G polymorphism.

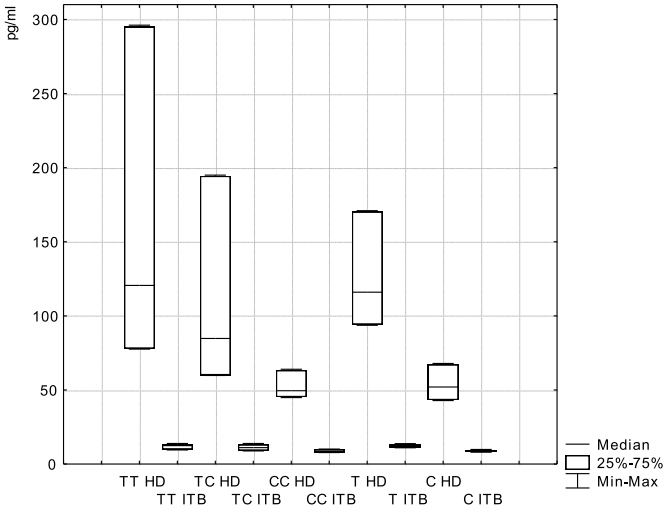


Fig. 5. Association between IL-27 secretion by mDCs and IL27 gene polymorphism 4730T/C in healthy donors and patients with infiltrative PTB. Note: HD – healthy donors, ITB – infiltrative tuberculosis, DTB – disseminated tuberculosis. Results are presented as median, lower and upper quartiles. In legend: TT, TC, CC – genotypes of 4730T/C polymorphism; T, C – alleles of 4730T/C polymorphism.

3.3. Association of IL27 2905 T/G, 4730T/C and −964A/G polymorphisms with IL-27 secretion by mDCs in TB

Analysis of the allele frequency distribution of *IL27* 2905 T/G polymorphism revealed a high incidence of carriers of the homozygous TT genotype among patients with infiltrative and disseminated PTB ($\chi^2 = 165.55$ and $\chi^2 = 188.76$ ($p < 0.001$), respectively). The TT genotype and the T allele of the polymorphism were associated with the incidence of both infiltrative (OR = 27.14 (15.47–47.62) and OR = 11.25 (7.53–16.81)) and disseminated PTB (OR = 52.09 (26.55–102.22) and OR = 18.72 (11.38–30.79)) (Suppl. 3, Table 3-3).

IL-27 2905T/G polymorphism was associated with a decrease in mDCs-derived IL-27 secretion in comparison with control group in both infiltrative and disseminated PTB patients, regardless of alleles and genotypes ($p_1 < 0.001$). At the same time, no statistically significant difference was observed between infiltrative and disseminated PTB patients with different alleles and genotypes of the 2905T/G polymorphism. (Fig. 4).

The frequency spectrum of the genotypes of the *IL27* 4730 T/C polymorphism in patients with infiltrative PTB had statistically significant differences both compared to the control group ($\chi^2 = 39.06$ ($p < 0.001$)) and to patients with disseminated PTB ($\chi^2 = 28.59$ ($p < 0.001$)). Carriers of the CC genotype were significantly more frequent among patients with infiltrative PTB, and this genotype was reliably associated with the infiltrative form of PTB (OR = 3.48 (2.19–5.55)) while no association with disseminated PTB was identified (Suppl. 3, Table 3-3). The analysis of genotypes and alleles frequencies of the *IL27* gene polymorphism −964A/G revealed no significant difference between patients with infiltrative and disseminated PTB. However, in patients with infiltrative PTB, the distribution of the genotypes of the polymorphism was different from that of healthy donors ($\chi^2 = 9.53$ ($p = 0.009$)). Additionally, the association of the GG genotype of −964A/G polymorphism with the development of infiltrative PTB (OR = 1.65 (1.05–2.59)) was identified (Suppl. 3, Table 3-3).

The level of mDC-derived IL-27 secretion in patients with infiltrative PTB, regardless of the genotypes and alleles of the *IL27* gene polymorphisms 4730T/C and −964A/G, was lower ($p_1 < (0.05–0.001)$) than in the control group (Figs. 5 and 6). At the same time, secretion of IL-27 by mDCs in carriers of the CC genotype and the C allele of 4730T/C polymorphism, as well as the *GG* genotype of −964A/G polymorphism was significantly decreased (*4730T/C*: $p_{TT/CC} < 0,05$ and $p_{T/C} < 0,05$; -964A/G: $p_{AA/GG} < 0,05$) as compared to other alleles and genotypes (Figs. 5 and 6).

4. Discussion

One of the key mechanisms underlying an immunoregulatory function of DCs is their ability to produce pro-inflammatory cytokines, thereby creating a particular cytokine environment in an inflammatory site and significantly influencing the pathway of an immune response. Thus, the differentiation of naïve T cells into Th1 lymphocytes, which is particularly important for a successful anti-tuberculosis immune response, requires the presence of the IL-12 family cytokines IL-12, IL-23, and IL-27, which are produced mainly by DCs. Another pro-inflammatory cytokine, IL-18, works in synergy with IL-12 by enhancing

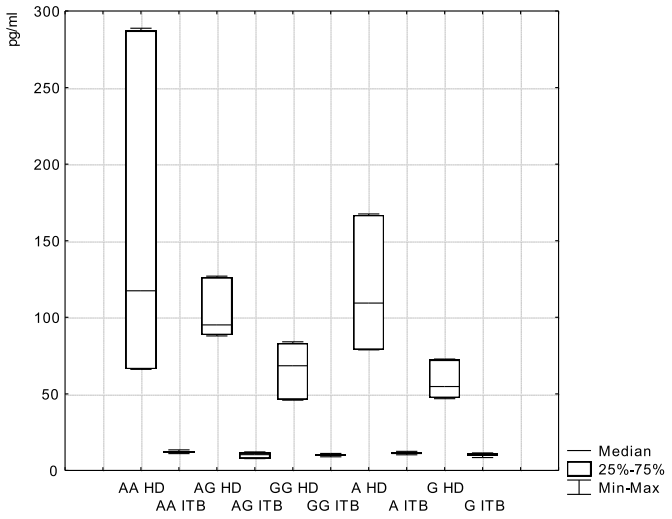


Fig. 6. Association between IL-27 secretion by mDCs and IL27 gene polymorphism −964A/G in healthy donors and patients with infiltrative PTB. Note: HD – healthy donors, ITB – infiltrative tuberculosis, DTB – disseminated tuberculosis. Results are presented as median, lower and upper quartiles. In legend: AA, AG, GG – genotypes of −964A/G polymorphism; A, G – alleles of −964A/G polymorphism.

its promoting effect on the IFNγ secretion by innate immune cells and T lymphocytes [17]. At the same time, it was shown that in the absence of IL-12, IL-18 induces the production of IFNγ-antagonist IL-4, thereby favoring the development of Th2 immune response [17]. In our study, the C allele and CC genotype of the *IL18* 105 A/C polymorphism was associated with considerable reduction of IL-18 secretion by mDCs in patients with disseminated PTB compared to control individuals (Fig. 2), suggesting a predisposing role of this polymorphism in the development of the disseminated form of PTB.

Similar to IL-18, IL-12 is predominantly produced by antigen-presenting cells, namely dendritic cells and macrophages. A number of studies investigated an association between *IL-12B* gene polymorphisms and secretion of IL-12 by immune cells, as well as an overall susceptibility to TB [18,19]. P. Selvaraj and colleagues showed that among healthy individuals, the level of IL-12p40 was significantly lower in carriers of the AA genotype of the *IL12B* gene polymorphism (+1188) as compared to the carriers of the AC genotype. Moreover, in TB patients the CC genotype of the *IL12B* gene polymorphism (+1188) was associated with an increase in IL-12p40 levels in PBMC culture supernatants compared to the carriers of other alleles and genotypes [20], which further indicates an important role of hereditary factors in the dysregulation of adaptive immunity against *Mtb*.

IL-12 has a heterodimeric structure, and its functional activity is associated primarily with the p40 subunit [21], which hyperproduction (2–3 times higher compared to IL-12p70 production) is considered to be a sign of an active immune response. At the same time, an excessive level of IL-12p40 secretion may result in the formation of inactive p40-p40 homodimers, which act as active antagonists of heterodimeric IL-12p70 by blocking IL-12 cell receptors [9,21]. In the present study we mainly focused on studying IL-12p70 production, since, compared IL-12p40, IL-12 secretion has a more inducible nature and is activated in response to antigen stimulation. Moreover, in contrast to other available studies, we evaluated the secretion of IL-12 by differentiated DCs (as opposed to PBMC cultures) in different clinical forms of PTB.

The changes in the secretion of IL-12p70 by mDCs in TB patients were multidirectional. It was found that in patients with infiltrative PTB, both genotypes (Aa and aa) and alleles (A and a) of the *IL-12B* gene polymorphism (IL12B/insertion) were associated with decreased level of IL-12p70 compared to control group. In contrast, in disseminated PTB patients carrying the same alleles and genotypes of the polymorphism, IL-12p70 secretion level was higher in comparison with that of healthy donors (Fig. 3). On one hand, this indicates the association of the *IL12B* gene polymorphism (IL12B/insertion) with the IL-12p70 secretion variability in PTB and supports its predisposing role in the development of both infiltrative (in case of the Aa genotype) and disseminated (in case of the aa genotype) PTB. On the other, this data suggests a connection between the pattern of mDC-derived IL-12p70 secretion and the development of a particular clinical form of PTB. Furthermore, it is evident that in patients with infiltrative TB, a decrease in the production of IL-12p70 by mDCs combined with persistent secretion of IL-18 might lead to an unfavorable outcome of the disease in the case of predominance of Th2 immune response [4].

Mtb is capable of inhibiting the cytokine-secretory activity of dendritic cells, thereby promoting the suppression of the antigen-specific immune response of $CD4^+$ T cells and shifting the balance towards the Th2 and Treg pathways [22–24]. Apparently, an increase in the mDC-derived IL-12 secretion in association with a genetically determined reduction of IL-18 secretion in patients with disseminated TB is either defined solely by the genetic predisposition, or has a compensatory epigenetic nature, for instance, due to the massive bacteremia that often accompanies the course of this clinical form of PTB. Madan-Lala et al. (2014) showed that the mutant strain hip 1 of *Mtb* induced increased levels of secretion of the key Th1-inducing cytokine IL-12 as well as other pro-inflammatory cytokines (IL-23, IL-6, TNFα, and IL-1β) in DC culture via an activation of MyD88-and TLR2/9-dependent signaling pathways [25].

The results of ELISA showed that in patients with infiltrative PTB, the secretion of IL-27, a known synergist of IL-12 and a member of the IL-12 cytokine family, was lower than in healthy donors, regardless of genotypes and alleles of the *IL27* 4730 T/C and 964A/G polymorphisms. Minimal levels of IL-27 secretion were associated with homozygous CC genotype of 4730T/C polymorphism and GG genotype of −964A/G polymorphism, which allows us to speculate on the possible predisposing role of these polymorphisms in the development of infiltrative PTB (Figs. 5 and 6). In addition, a reduced secretion of IL-27 was identified in carriers of the *IL27* 2905 T/G polymorphism with both infiltrative and disseminated PTB. At the same time, there was no association between secretion of IL-27 and allele and genotype frequency spectrum of 2905T/G polymorphism, which, however, does not entirely disprove the predisposing role the T allele and the TT genotype of this polymorphism in the development of both infiltrative and disseminated TB (Fig. 4).

The primary effect of IL-27 revolves around promotion of $CD4^+$ T cells differentiation into Th1 cells. However, IL-27 was also shown to act as a suppressive factor, since its structural subunit Ebi 3 is a target for the key transcription factor of regulatory T cells, Foxp3 [17,26]. In addition, IL-27, in contrast to IL-12, can enhance proliferation of not only activated, but also naive $CD4^+$ T cells [17,27,28]. It is possible that the observed imbalance of cytokine secretion by mDCs in PTB patients is due to the ability of cytokines from the same family to replicate each other's function. Apparently, in the deficit of IL-27, its biological functions are substituted by IL-12. Moreover, we can speculate that a decrease in the secretion of IL-18 in disseminated PTB constitutes a negative factor in the pathogenesis of this clinical form of tuberculosis, because, as indicated above, IL-18 is able to synergize with IL-12 to promote the secretion of IFNγ, a key mediator of anti-tuberculous immunity. Thus, the polymorphism IL12B/insertion is associated with an intact secretion of IL-12 by mDCs and therefore represents a favorable factor in the pathogenesis of disseminated PTB.

Despite a substantial amount of research, the role of cytokine gene polymorphisms in the pathogenesis of pulmonary tuberculosis still remains to be fully elucidated and therefore represents a relevant issue in the context of anti-tuberculosis immunity studies. Thus, E. Peresi et al. (2013) investigated and influence of *IFNG, IL12B, TNF, IL17A, IL10* and *TGFB1* gene polymorphisms on the immune response in TB patients receiving anti-tuberculosis treatment. The authors showed that the

polymorphisms IFNG +874T/A, IFNG +2109A/G, IL12B + 1188 A/C, IL10 -819C/T and TGFB1 +21C/T were associated with the changes in the secretion of corresponding cytokines and therefore might be substantially involved in the development of immune response against *Mtb* by modulating cytokine secretion, especially against the background of tuberculosis treatment [29]. In contrast, in the present study the cytokine-secretory capacity of DCs was investigated prior to administration of anti-tuberculosis drugs, since they are known to exert a prominent immunosuppressive effect. In another study, Y. Hu and colleagues demonstrated a positive association between the *IFNG* gene polymorphism (−874) and the active form of tuberculosis infection, whilst the IL-10 gene polymorphism was associated with latent infection [30]. Together, these data, along with the results of the present study, further substantiate an important role of cytokine gene polymorphisms in the etiology and pathogenesis of tuberculosis.

5. Conclusions

In patients with PTB, the deficit of IL-18 secretion by mDCs was associated with the *IL18* 105 A/C polymorphism in disseminated clinical form of the disease. IL-27 secretion deficit was in turn associated with polymorphisms of the *IL27* gene: 4730T/C and −964A/G in infiltrative PTB and 2905T/G in both clinical forms of the disease. At the same time, secretion level of IL-12p70 in carriers of both genotypes (Aa and aa) and alleles (A and a) of the polymorphism IL12B/insertion with infiltrative PTB was lower than in the control group, while in patients with disseminated PTB it was higher in comparison with that of healthy donors. This indicates not only the modulating effect of the *IL12B* gene polymorphism towards the mDC-derived secretion of IL-12p70, but also its association with the development of a particular clinical form of PTB. The Aa genotype of the polymorphism IL12B/insertion of the *IL12B* gene; CC genotype of the 4730T/C polymorphism and GG genotype of 964A/G polymorphism of the *IL27* gene, predispose to the development of infiltrative PTB.

In turn, predisposition to the development of disseminated TB is associated with the C allele and CC genotype of the *IL18* 105 A/C polymorphism, as well as a allele and aa genotype of the polymorphism IL12B/insertion. The T allele and TT genotype of the *IL27* 2905 T/G polymorphism predisposes to the development of both clinical forms of the disease in association with the pronounced reduction of IL-27 secretion by mDCs.

Conflicts of interest

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

Conformity with ethics

The clinical study was carried out in accordance with the Helsinki Declaration of the World Medical Association “Ethical Principles of Scientific Medical Research with Human Participation” as amended in 2000 and the “Rules of Clinical Practice in the Russian Federation” approved by Order No. 266 of the Ministry of Health of the Russian Federation, 19.06.2003. All patients signed informed consent prior to participation in the study in accordance with Protocol No. 1698 dated November 15, 2010, approved by the local ethics committee at the Siberian State Medical University.

Acknowledgments

The research was carried out with the financial support of the Grant Council of the President of the Russian Federation for state support of the leading scientific schools of the Russian Federation under the grants SS-7906.2016.7 “The role of galectins in the pathogenesis of malignant tumors of blood and other localizations” (Head, Academician RAS V.V. Novitskiy) and SS-2690.2018.7 “Molecular factors of dysregulation of the homeostasis of immunocompetent blood cells in socially significant diseases” (Head, Corresponding Member of RAS O.I. Urazova). This research was supported by the Program of Increasing the Competitiveness of TSU.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tube.2019.02.003.

References

[1] Prabhu Anand S, Selvaraj P, Jawahar MS, Adhilakshmi AR, Narayanan PR. Interleukin-12B & interleukin-10 gene polymorphisms in pulmonary tuberculosis. Indian J Med Res 2007;126(2):135–8.
[2] Henao MI, Montes C, Paris SC, Garcia LF. Cytokine gene polymorphisms in Colombian patients with different clinical presentations of tuberculosis. Tuberculosis (Edinburgh) 2006;86(1):11–9.
[3] Morahan G, Kaur G, Singh M, Rapthap CC, Kumar N, Katoch K, et al. Association of variants in the IL12B gene with leprosy and tuberculosis. Tissue Antigens 2007;69(Suppl. 1):234–6.
[4] Sallakci N, Coskun M, Berber Z, Gurkan F, Kocamaz H, Uysal G, et al. Interferon gamma gene +874T-A polymorphism is associated with tuberculosis and gamma interferon response. Tuberculosis (Edinburgh) 2007;87(3):225–30.
[5] Gonzalo X, Ambroggi M, Cordova E, Brown T, Poggi S, Drobniewski F. Molecular epidemiology of Mycobacterium tuberculosis, Buenos Aires, Argentina. Emerg Infect Dis 2011 Mar;17(3):528–31.
[6] Blischak JD, Tailleux L, Myrthil M, et al. Predicting susceptibility to tuberculosis based on gene expression profiling in dendritic cells. Sci Rep 2017 Jul;7(1):5702.
[7] Abramov DD, KofiadiI A, Utkin KV, Trofimov DYu, Haitov PM, Alekseev LP. Polymorphism of single nucleotides in the genes of cytokines and their receptors: the biological effect and identification methods. Immunology 2011;5:275–80.
[8] Churina EG, Urazova OI, Novitski VV, Esimova IE. Secondary immunological deficiency in patients with pulmonary tuberculosis. Immunodiagnostics and immunotherapy. Tomsk: Pechatnaya manufaktura; 2013. p. 84.
[9] Espinosa-Cueto P, Magallanes-Puebla A, Castellanos C, Mancilla R. Dendritic cells that phagocytose apoptotic macrophages loaded with mycobacterial antigens activate CD8 T cells via cross-presentation. PLoS One 2017 Aug;12(8):e0182126.
[10] Griffiths KL, Ahmed M, Das S, et al. Targeting dendritic cells to accelerate T-cell activation overcomes a bottleneck in tuberculosis vaccine efficacy. Nat Commun 2016 Dec;7:13894.
[11] Satake Y, Nakamura Y, Kono M, et al. Type-1 polarised dendritic cells are a potent immunogen against Mycobacterium tuberculosis. Int J Tuberc Lung Dis 2017 May;21(5):523–30.
[12] Chkadua GZ, Zabotina TN, Burkova AA, et al. Adaptation of the method of human dendritic cell cultivation from the peripheral blood monocytes for clinical practice. Russ Biother J 2002;1(3):55–61.
[13] Maver A, Medica I, Salobir B, et al. Polymorphisms in genes coding for mediators in the interleukin cascade and their effect on susceptibility to sarcoidosis in the Slovenian population. Int J Mol Med 2007;20:385–90.
[14] Kim H-L, Cho SO, Kim S-Y. Association of *interleukin-18* gene polymorphism with body mass index in women. Reprod Biol Endocrinol 2012:10–31.
[15] Tsai Yi-Yu, Lin Jane-Ming, Wan Lei, et al. Interleukin gene polymorphisms in age-related macular degeneration. Invest Ophthalmol Vis Sci 2008; February;49:693–8.
[16] Huang Na, Liu Lin, Wang Xiao-Zhen, et al. Association of interleukin -12 and IL-27 gene polymorphisms with chronic obstructive pulmonary disease in a Chinese population. DNA Cell Biol 2008;27(9):527–31.
[17] Ketlinski SA, Simbircev AS. Cytokines. SPb. Foliant" publisher; 2008. p. 552.
[18] Tso HW, et al. Associations between IL12B polymorphisms and tuberculosis in the Hong Kong Chinese population. J Infect Dis 2004 Sep;190(5):913–9.
[19] Morris GA, et al. Interleukin 12B (IL12B) Genetic Variation and Pulmonary Tuberculosis: a Study of Cohorts from The Gambia, Guinea-Bissau, United States and Argentina. PLoS One 2011 Feb;6(2):e16656.
[20] Selvaraj P, Alagarasu K, Harishankar M, et al. Cytokine gene polymorphisms and cytokine levels in pulmonary tuberculosis. Cytokine 2008;41(1):26–33.
[21] Jones LL, Vignali DA. Molecular interactions within the IL-6/IL-12 cytokine/receptor superfamily. Immunol Res 2011 Oct;51(1):5–14.
[22] Sia JK, Bizzell E, Madan-Lala R, Rengarajan J. Engaging the CD40-CD40L pathway augments T-helper cell responses and improves control of Mycobacterium tuberculosis infection. PLoS Pathog 2017 Aug;13(8):e1006530.
[23] Sakai S, Kauffman KD, Sallin MA, et al. CD4 T cell-derived IFN-γ plays a minimal role in control of pulmonary Mycobacterium tuberculosis infection and must Be actively repressed by PD-1 to prevent lethal disease. PLoS Pathog 2016 May;12(5):1005667.
[24] Churina EG, Urazova OI, Novitski VV. Regulatory T cells and antituberculosis immunity. Tomsk: Pechatnaya manufaktura; 2014. p. 156.
[25] Madan-Lala R, Sia JK, King R, et al. Mycobacterium tuberculosis impairs dendritic cell functions through the serine hydrolase Hip1. J Immunol 2014 May;192(9):4263–72.
[26] Yoshida H, Hunter CA. The immunobiology of interleukin-27. Annu Rev Immunol 2015;33:417–43.

[27] Xia H, Ye ZJ, Zhou Q, et al. IL-27 and IL-27-producing CD4$^+$ T cells in human tuberculous pleural effusion. Tuberculosis (Edinb) 2014 Dec;94(6):579–88.
[28] Iwasaki Y, Fujio K, Okamura T, Yamamoto K. Interleukin-27 in T cell immunity. Int J Mol Sci 2015 Jan;16(2):2851–63.
[29] Peresi E, Cardoso Oliveira L, Laurentino da Silva W, et al. Cytokine polymorphisms, their influence and levels in Brazilian patients with pulmonary tuberculosis during antituberculosis treatment. Tuberc Res Treat 2013;2013:285094.
[30] HuY Wu L, Li D, et al. Association between cytokine gene polymorphisms and tuberculosis in a Chinese population in Shanghai: a case-control study. BMC Immunol 2015;16(8).

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