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Chapter 5

Interaction between *Mycobacterium tuberculosis* and Human Host: Role of Cytokines in Pathogenesis and Treatment Monitoring

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http://dx.doi.org/10.5772/intechopen.76543

Abstract

Tuberculosis is one of the most prevalent infections of human beings. According to WHO Global tuberculosis report 2016, there were 10.4 million new incidents of TB cases worldwide, and 580,000 new cases of multidrug resistant (MDR) tuberculosis. Monitoring the effectiveness of tuberculosis treatment and timely diagnosis of latent tuberculosis is an important problem for immunological research. Interaction of M. tuberculosis and the human immune system begins with phagocytosis of mycobacteria by macrophages and activate the immune response through the cytokines and chemokines release. The balance of proinflammatory and immunoregulatory cytokines and chemokines production may reflect the level of host-parasite interaction (e.g., elimination or persistence of the microbe). The review presents current clinical trends in studies on proinflammatory (IL-12, IL-1, INF-II, TNF-α) and immunoregulatory (IL-10 and TGF-β) cytokines, as well as matrix metalloproteases and hemoxygenase 1 to characterize the success of antituberculous chemotherapy. Monitoring the effectiveness of tuberculosis treatment will require the use new combinations of cytokines, chemokines, and nonspecific inflammatory factors which combinations have not yet been determined. The most promising area is studying of immunoregulatory cytokines, (e.g., IL-10, TGF-β), cell migration factors (e.g., IP-10/CXCL-10, MIG/CXCL/9), and markers of nonspecific inflammation (e.g., HO-1, SAA and MMP-1,3,9).

Keywords: *Mycobacterium tuberculosis*, immunopathology, cytokines, chemokines

1. How the TB pathogen infects the host

According to the existing scientific data based on numerous experimental studies, the macrophages and myeloid subpopulation of the dendritic cells from the upper and lower

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respiratory tract are first contacted with inhaled foreign antigens (including mycobacteria), phagocytize them through several receptors (mannose receptor, surfactant D, DC-SIGN-specific for dendritic cells, integrated molecule, C-lectin receptor, Nod-like molecules, and Toll-like molecules). Subsequently, some of the phagocytosed mycobacteria are transferred by dendritic cells to regional lymph nodes where they are localized through the interaction of the CCR7 receptor with the ligand CCL19/21 expressed on the endothelial cells of the lymph nodes. This was shown in an experiment using mycobacteria labeled with fluorescent protein GFP [1]. These dendritic cells have on the surface of CCR7, which binds to ligands CCL19/21, expressed on endothelial cells of lymph nodes. Here, the dendritic cells produce a sufficient amount of IL-12p40, IL-12Rβ1, and CD11c and provide the initialization of the immune response through the priming of T helper 1 in the subsequent production of effector and memory cells and their migration to the sites of invasion, where the inflammatory response provides the development the specific granuloma formation. Also, mycobacteria can be lysed in phagolysosomes or die in apoptotic macrophages, or killed with cytolytic molecules of NK- or CD8+ cells.

Mycobacterium tuberculosis, found in macrophages, has genetic deterministic mechanisms of avoiding digestion inside phagocytes and persistent persistence in the host tissues [2]. In the initial stages, mycobacterial expression of the phthiocerol dimycoceroserate molecule (PDIM) limits the recognition of Mtb through Toll receptors (TLRs) and the phenolic glycolipids (PGLs). The mycobacterial cell wall lipids prevent the fusion of MTB containing phagosomes with lysosomes through a decrease recruitment of vacuolar H+/ATPase. The mycobacterial lipids lipoarabinomannan, trehalose dimycolate, and the sulfolipids also block the phagosome/lysosome fusion. In addition, bacterial phosphatase SapM and serine/threonine kinase PknG disrupt the dephosphorylation of phosphatidylinositol 3-phosphate (PI3P) and protein kinase G (PknG) and disturb the phagosome maturation [3].

MTB has genes that prevent their death in macrophages through an aphototic mechanism. Superoxide dismutase A (SodA) interferes with the caspase-dependent mechanism of macrophage apoptosis. The nuG gene, which is part of the fourteenth operon gene and codes type I NADH dehydrogenase (NDH-1), is important for neutralizing reactive oxygen species (ROS) created by macrophage phagocyte oxidase (NOX2). The importance of the operon of the 7-gene (Rv3654c-Rv3660c) for the inhibition of apoptosis of mycobacteria is also described. This mechanism is associated with inhibition of caspase-8 pre-mRNA splicing and a decrease in this protein in macrophages [4]. Virulent mycobacteria can suppress the early stages of apoptosis by cleaves the amino terminus of annexin 1 and subsequent inhibition of annexin 1 deposition on the apoptotic membrane.

The predominance of necrotic death of macrophages results in reduced mycobacterial antigen presentation by macrophages and dendritic cells [5]. On one hand, this stimulates the further accumulation of neutrophils, monocytes, macrophages, lymphocytes, and dendritic cells in the granulomas; and on the other hand, the spread of the pathological process to the surrounding tissues. Adequate TNF-α production promotes the formation of granulomas, while

excessive production of TNF-α due to stimulation with MTB tricalcium dimycolate leads to a progression of the process. Mycobacteria use their adenylate cyclase to deliver excess cAMP to the macrophage cytoplasm. This leads to suppression macrophage functions and provides a pro-granulomatous response.

Inhibition of the apoptosis process is facilitated by the region of difference 1 (RD1), and type VII secretion system (ESX) of the mycobacterium genome. This leads to activation of Calpain, and Ca^{2+}-activated protease and calcification of necrosis. The resulting tissue hypoxia contributes to the activation of the restorative regulator (DosR) of the mycobacterial genome and their transition to the dermatological state with low metabolic activity [6]. The survival of such mycobacteria occurs within the microlipid droplets that are formed when the cell membranes of the host cells are destroyed [7].

Reactivation of mycobacteria and subsequent exacerbation of the tuberculosis process usually occurs when the host's immune system is weakened or suppressed due to physiological or pathological factors. This process is provided by the Rpf system of mycobacterial genes. The production of Rpf proteins, which are structurally close to the lytic transglycosylases, leads to the hydrolysis of peptidoglycans of cell membranes and intercellular fibers, increases the permeability of tissue for blood and lymph and the inflow of nutrients. The result of these processes is activation of the mycobacteria vital activity [8].

2. Cytokines and chemokines dynamics in TB diagnosis and treatment

The pathogen and innate immunity system interaction begins with the recognition of the conservative mycobacteria antigenic structures (so-called pathogen-associated molecular patterns—PAMR). This process depends on the interaction with PPR (pathogen recognition receptors), TOL-like receptors (TOLRs), NOD-like nucleotide oligomerization domain (NOD)-like receptors, C-lectin, and mannose-binding lectin-MBL receptors. These interactions stimulate the production of pro-inflammatory cytokines and chemokines that facilitate the migration activation of cells to the sites of inflammation [9]. Microbial invasion is accompanied by host cells and tissues damage and release the parts of collagen fibers, RNA, DNA fragments, and membrane phospholipids degradation product. Such damage-associated molecular patterns (DAMP) are an additional inflammatory stimulus. Almost every cell and even platelets contains a PRR repertoire and can react to such damage factors. Interaction of microbial PAMP and endogenous DAMP causes the systemic inflammatory response (or SIRS—systemic inflammatory response syndrome) [10]. All these stimuli activate the cytokines and chemokines production and the host acquired immunity reactions. The secretion of interferons (and primarily the INF-γ) by T-helper 1 (Th1) is a key point in the control of mycobacterial infections. The strength of Th1 response does not always correlate with bacterial clearance and increased host resistance. T-helper 17 (Th17) and regulatory T cells (Tregs)

subpopulations are also important. Numerous cytokines, chemokines, and other humoral factors provide the macrophages and lymphocytes interaction. This section presents the main results of experimental and clinical studies on several major cytokine families.

3. Interleukin IL-12 family

IL-12 interleukin family is represented by four heterodimeric members: IL-12p70, IL-23, IL-27 and the newest member IL-35 consisting of each of the specific subunits. These cytokines have sufficient homology in the structure of subunits and interaction with receptors, but play a different role in the generation and maintenance of reactions of acquired immunity. Heterodimeric IL-12p70 consists of two homodimeric subunits IL-12p40 and IL-12p35, which are predominantly produced and function in various immunological compartments (IL-12p40 and IL-12p35 in inflammation foci).

Heterodimeric IL-12p70 consists of two homodimeric subunits IL-12p40 and IL-12p35, which are predominantly produced and function in various immunological compartments (IL-12p40 and IL-12p35 in inflammation foci, and IL-12p70 in lymph nodes) by binding to appropriate receptors (IL-12Rβ1 and/or IL-12Rβ2 or the IL-23R). Mutations in the subunits of IL-12 or their receptors and, accordingly, inadequate character of the produced signal causes an increased sensitivity to mycobacterial infection. The presence of adequate amounts of IL-12p70 in the lymph nodes ensures a sufficient production of Th1 and production of INF-γ [11]. IL-23 and IL-27 are released in the early stages of mycobacterial infection in the lungs and in the lymph nodes. IL-23 potentiates the production of IL-17, and IL-27 promotes the production of INF-γ. Possibilities of clinical use of IL-12, as in other and other key cytokines (INF-γ, TNF-α, IL-1β, and IL-6) for diagnosis and monitoring of tuberculosis are largely limited by the presence of allelic variants of these proteins. For example, the presence of polymorphism in the IL-12B gene influences the spontaneous and antigen-stimulated production of the IL-12p40 subunit, where the patient with the AA genotype are weak, and the SS is a strong producer of IL-12p40 [12].

4. Interleukin IL-1 family

The IL-1 cytokine family is represented by 11 members, of which IL-1α, IL-1β, IL-18, and IL-33 were studied in an experiment model and patients with pulmonary and extrapulmonary tuberculosis. The available now data point to the important role of IL-1α and IL-1β. The significance of IL-18 and IL-33 for the course of the tuberculosis process has not yet been adequately studied. Unlike IL-1α, IL-1β is produced in an inactive form. Its change into the active form occurs in the inflamasomes after the cell activation (predominantly in lung macrophages and dendritic cells, and in other organs epithelial, endothelial cells, and fibroblasts) with the corresponding signal through the NLRP3 receptor on the proforma of the caspase-1 enzyme. There is another variant of the formation of the active form of IL-1β, which proceeds outside inflamasomas, after the action of other proteolytic enzymes (chymase, cathepsin, elastase). The main function of IL-1 consist of the control pro-inflammatory reactions in case of damage

due to PAMP activation with viruses and bacteria, or and DAMP activation in host tissues by acting with the crystals of uric acid and adenosine-5, triphosphate [13].

IL-1α and IL-1β independently exert their action primarily through type I IL-1 receptor (IL-1R1). IL-1β causes differentiation of monocytes into macrophages and increases their phagocytic and antigen-presenting capacity [14]. The clinical IL-1α and IL-1β level significance for course of the tuberculosis process and the effectiveness of chemotherapy has not yet revealed a significant difference between the groups of patients with active and latent tuberculosis, unlike cytokines such as IL-2 and IFN-γ [15].

The significance of IL-18 for antituberculosis immunity, which, like IL-1β, is a pro-inflammatory factor, is currently at the stage of experimental studies. IL-18 plays the role of Th1 lymphocyte differentiating factor. IL-18 knockout mice are more susceptible to aerosol challenge with mycobacteria and produce less IFN-γ compared to wild-type mice [14].

5. Tumor necrosis factor family

To date, the family of tumor necrosis factors is represented by the main member TNF-α and its functional analogues LTα and LTβ lymphotoxins, among which the first factor is most significant for intracellular infections. The pro-inflammatory cytokine tumor necrosis factor (TNF-α) is produced predominantly by macrophages in response to stimuli activating through Toll-like receptors and also can be expressed by activated T, B and NK cells. TNF exists as a trimer in the form of transmembrane protein and in a secreted form in serum. Both forms act by binding to TNFRp55 and TNFRp75 receptors, the soluble form interacting predominantly with TNFRp55, and the membrane form interacting with TNFRp75.

TNF is a multipotent cytokine playing roles in the processes of apoptosis, activation, differentiation, and recruitment of cells into inflammatory foci. With regard to tuberculosis, TNF-α is involved in the differentiation of T cells secreting Th1 cytokines, the formation of tuberculous granulomas with activation of phagocytic macrophages and epithelioid cells, killing mycobacteria in cooperation with INF-γ, stimulation of macrophage apoptosis containing mycobacteria, stimulation production of chemokines (s CCL-2, -3, -4, -5, -8) and endothelial cell expression of adhesion molecules (CD54), which leads to accumulation of cells in inflammatory foci [16]. Variants of allelic polymorphism of the TNF-α molecule at positions -238, -308, -857, -863, related to sensitivity to tuberculosis are described. Based on the meta-analysis of published clinical and genetic studies on the polymorphism variants of the TNF-α molecule Yu et al. [17] suggested that substitutions in the positions of TNF-α-308G > A and -238G > A are significantly associated with susceptibility to pulmonary tuberculosis regardless of ethnic status and the presence of HIV infection. Substitutions in the TNF-α molecule at positions -308G > A, -863C > A, and -238G > A are associated with susceptibility to pulmonary tuberculosis in uninfected HIV. The Asian population of tuberculosis patients is characterized by the replacement of -238G > A, whereas for the African population -308G > A.

To date, several studies have been conducted on sufficiently large contingents of tuberculosis patients to determine the TNF-α content in serum and cell cultures supernatant stimulated

by various mycobacterial antigens. Its high production in supernatants of blood cultures was revealed by stimulation of 10 μg/ml of *M. tuberculosis* H37Rv sonicate in untreated patients, preserved in the effective course of chemotherapy and decreased, while the treatment course was ineffective. However, there were no significant differences between groups of patients, depending on the extent of the tuberculous process in the lungs. The authors concluded that the level of TNF-α production correlates more with the activity of the process than with its severity [18]. When comparing the production of TNF-α in a group of patients enrolled in a repeat chemotherapy course with its level determined during the initial course, its lower culture supernatants content was revealed along with other factors (IFN-γ, IP-10, MIG, and IL-2). The of blood culture of patients with active tuberculosis stimulated by monoantigens and their combination (ESAT-6, CFP-10, ESAT-6 + CFP-10, and ESAT-6 + CFP-10 + TB7.7) was shown that these combinations of antigens cause high production of TNF-α, whereas one antigen TB7.7 is not effective [19]. However, in these studies, the authors conclude that the study of TNF-α production level is not very informative for evaluating the effectiveness of treatment of patients with tuberculosis.

6. Interferon type II

Cytokines of the interferons family and especially interferon type II (IFN-γ) are the most studied factors of antituberculosis immunity due to great importance for phagocytosis and subsequent killing of mycobacterium tuberculosis. IFN-γ is produced primarily by activated CD4+ and CD8+T cells and to a lesser extent by γδ T cells, NK T cells and NK cells belonging to the innate immunity system [15]. To date, two polymorphisms of IFN-γ gene in +874 (A/T) and + 5644 (A/G) are particularly important for the output level in patients with tuberculosis, in which the former is more often associated with susceptibility to tuberculosis infection, but this fact is not absolute and varies in different geographical regions [12]. The highest level of IFN-γ gene expression is detected in Th1-activated lymphocytes which stimulate the macrophages for killing of mycobacteria, enhance the cytotoxic activity of other cells, induce apoptosis in skin and mucosal epithelial cells, regulate the expression of MHC class I and II proteins, and antigen presentation. Take into account the key role of IFN-γ in antituberculosis immunity, a large number of experimental studies have been devoted to this problem which are summarized in a number of reviews [20]. The initial clinical studies on serum IFN-γ in patients with pulmonary and extrapulmonary forms of tuberculosis was shown that the concentration of this factor was significantly higher in comparison with the same group after treatment, the group of contacts and healthy volunteers. There is also a significant individual variability in the IFN-γ concentration in all studied groups [21]. The study of the IFN-γ synthesis in lymphocyte culture stimulated with PWM (5 μg/ml), PHA (10 μg/m), and tuberculin RT (12.5 and 25 μg/ml) in patients with different clinical forms of tuberculosis and a group of contacts revealed no differences in IFN-γ production in response to mitogens and a reliable increase in production in response to stimulation with tuberculin [1]. In a similar study of patients with different clinical forms of pulmonary tuberculosis (120 patients and 144 healthy volunteers of the same place of residence), the production of IFN-γ in lymphocyte culture was determined by stimulation with *M. tuberculosis* H37Rv sonicate at a dose of 10 μg/ml before chemotherapy and up to 6 months its conduct. It was shown that the IFN-γ production in the

whole patient group before the start of treatment was low, increased during chemotherapy, and was compared to the volunteer level by 6 months. However, after subdividing the patient group according to the severity of the tuberculosis process, it was shown that in patients with small and medium severity of the process IFN-γ production before treatment was significantly higher than in severe lungs changes. During the course of chemotherapy, the IFN-γ production increased in most patients, but there was a significant variability and even a drop in the concentration of IFN-γ in some patients with small and medium process severity to the initial level [18].

Since the beginning of the 2000s, several laboratory tests (interferon gamma release assays (IGRAs)) were developed for the IFN-γ revealing in the culture medium and intracellularly. The first of these was a QFT test (QuantiFERON-TB test, Cellestis Limited, Carnegie, Victoria, Australia), which used the ELISA method to determine the amount of IFN-γ secreted by cultured blood cells in response to PPD in comparison to the control antigenic stimulus of *M. avium* and saline as a negative control. However, the specificity of QFT was less than that of the Mantou skin test. The next-generation test (QuantiFERON-TB Gold—QFT-G) appeared in 2005. It used the proteins encoded by the RD1-locus of *M. tuberculosis* (ESAT-6 and CFP-10) as an antigenic stimulus. The test effectiveness t was determined as differences in the IFN-γ concentration in response to ESAT-6 or CFP-10 stimulation minus the values of spontaneous production of the factor. The ESAT-6/CFP-10 proteins are absent in vaccine strains of mycobacteria, but are present in *M. kansasii*, *M. szulgai*, and *M. marinum*, and sensitization with these mycobacteria can cause false-positive results of IGRA.

The next modification of the IGRAs-test (QuantiFERON-TB Gold In-Tube-QFT-GIT) was developed to more accurately determine antigen-stimulated synthesis IFN-γ which largely depended on preserving the activity of patient's blood cells samples. The blood was immediately placed in test tubes with lower antigenic stimuli in order to avoid loss of viability during transport of the samples to the laboratory. A mixture of 14 peptides copying the entire amino acid sequence of ESAT-6 and CFP-10 proteins and part of the TB7.7 sequence was used as antigenic stimuli. As a negative and positive control, test tubes with heparin and PHA were used. After a 16- to 24-h-incubation, the concentration of IFN-γ c was determined by the ELISA used in the QFT-G test. Exceeding response of the tested patient by 25–50% compared to the zero control was considered a positive indicator, less than 25% was interpreted as a negative result, while at QFT-G it was estimated as an intermediate.

The fourth T-Spot test began to use in 2008. The IFN-γ-secreting cells were determined in the 96-plate test well using the ELISpot method. The proteins ESAT-6 and CFP-10 were used separately as an antigenic stimulus. The result was determined by the difference in the number of cells (spots) compared to the control (spontaneous production by IFN-γ cells without antigenic stimulus). Because QFT-G, QFT-GIT, T-Spot, and skin tests (TST) examine various aspects of the immune response, use different antigens and have different criteria for interpreting the results, the tests are not interchangeable [22].

The development in the early 2000s of a new multiplex method of simultaneous multiple studies of genes and protein factors opened new possibilities for studying cytokines, chemokines, and the inflammation acute phase proteins in various tuberculosis clinical forms [2]. The results obtained revealed many humoral immunological criteria for active and latent tuberculosis in

different age and geographical contingents of patients. Various immunodominant proteins were used as an antigenic stimulus, but mainly it was the combination of the RD1 peptides ESAT-6 and CFP-10 genomic locus of the mycobacteria. Despite some inconsistency of the results, the most informative is the combination of cytokines and chemokines (IFN-γ, IP-10, MIG, TNF-a, and IL-2), which showed the greatest differences between patients with active tuberculosis and a control group of healthy volunteers. The diagnostic significance of the combination of IFN-γ, IP-10, and MIG was the highest (96.3%). In cases of unsuccessful treatment or reactivation of the process, the concentration of this group of factors was less significant and the diagnostic sensitivity decreased by 20–25% [23]. Kellar et al. [24] identified differences in the production of IFN-γ and other cytokines and chemokines in response to stimulation of blood culture in patients with active tuberculosis with monoantigens and their combination (ESAT-6, CFP-10, ESAT-6 + CFP-10, and ESAT-6 + CFP-10 + TB7.7). The IFN-γ production was determined by ELISA, multiplex analysis, and quantitative immunomicroarray methods. The two latest methods was used to examine the production of cytokines IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, GM-CSF, GRO, MCP-1, MIP-1α, MIP-1β, MMP-9, RANTES, TNF-α, and VEGF. The ELISA method identified significant differences in the IFN-γ production in response to ESAT-6, CFP-10, TB7.7 and a combination of ESAT-6+CFP-10, and ESAT-6+CFP-10+TB7.7 compared to the control group. The differences in the production of IFN-γ in response to ESAT-6+CFP-10 versus ESAT-6+CFP-10+TB7.7 are not reliable. The response to a mixture of antigens is always greater than for individual antigens. Multiplex analysis showed that the antigens ESAT-6, CFP-10, ESAT-6+CFP-10, and ESAT-6+CFP-10+TB7.7 stimulated production of not only IFN-γ, but also IL-2, IL-8, MCP-1, and MIP-1β in patients compared with control group. The immunomicroarray method revealed that when stimulating ESAT-6+CFP-10+TB7.7, a greater number of IFN-γ, IL-2, IL-6, IL-8, IP-10, MCP-1, MIP-1β, and TNF-α, than in the control group. The authors conclude that the determination of many cytokines improves the diagnosis of tuberculosis in comparison with the IFN-γ determination only. Four cytokines are the best diagnostic markers for mycobacterial infection (sensitivity and specificity were 100% for IL-2, IL-6, IP-10, and MIP-1β.) The sensitivity of the commercial multiplex analysis test system for IFN-γ was 91.6% and specificity of 100%.

There are studies characterizing the cytokine spectrum of the antigen-stimulated blood cells in children and adolescents patients with active and latent tuberculosis in comparison with those who do not contact the tuberculosis patients. Kellar et al. [24], using multiplex analysis, studied a spectrum of 29 cytokines and blood chemokines in a group of children and adolescents (135 people, including 46 with latent tuberculosis, 11 patients with active tuberculosis and 35 uninfected persons, age 2–15 years) after stimulation with peptides QFT-G, PPD, and recombinant ESAT-6 protein. In general, the level of cytokines after stimulation with peptides QFT-G (IFN-γ, IL-2, IP-10, and IL-13) was significantly lower in children without infection than in patients with tuberculosis, the most significant in children older than 5 years of age. Children with latent TB have a high IFN-γ, IL-2, IP-10, IL-13, and IL-5 level in comparison with uninfected children. The spontaneous and antigen-stimulated (QFT-G) TGF-β1 production was significantly higher in children with tuberculosis over 5 years of age than in the similar group with latent tuberculosis. In the age group under the age of 5 years, there was no significant difference in the cytokine production in children with latent tuberculosis compared to uninfected controls. Other relationships were obtained with PPD stimulating blood cultures. Higher IL-2, IL-13, and IL-4 production were detected in comparison with recombinant ESAT-6 stimulation [24, 25]. Armand et al.

[27] stimulated blood cultures with QFT-G test system peptides from children and adolescents aged 4–15 years with latent and various forms of tuberculosis and patients without signs of disease. The multiplex analysis of the of 14 cytokines and chemokines production revealed significant differences in IL-2, INF-γ, IL-5, IP-10 level between the control group and tuberculosis patients. There were no differences in the production of TNF-α. For patients with more severe forms of tuberculosis found no differences in the production of these factors except for a significant decrease in production of IL-5 and IL-13. The increase TNF-α production, as well as the high IL-2, INF-γ level were observed in children with lymph node form tuberculosis of the 4–15 years age group with positive skin tests Mantoux and DST [26].

7. Immunoregulatory cytokines IL-10 and TGF-β

Interleukin-10 (IL-10) is a multifunctional regulatory cytokine of inflammatory responses. Numerous studies have shown that IL-10 acts as a general inhibitor of proliferative responses of both T helper (Th) 1 and Th2 cells *in vitro* and *in vivo*. IL-10 regulates inflammation through the suppression of the production of cytokines such as IL-1α, IL-1β, IL-6, IL-8, IL-12, and tumor necrosis factor-alpha in activated macrophage and interferon gamma in T cells [27].

TGF-β is the main representative of the family, which nowadays consists of 35 factors, including 5 isoforms of TGF-β, bone morphogenic protein (BMP), growth differentiation factors (GDFs), activin, and inhibin factors. TGF-β is secreted in an inactive form (L-TGF-β) and the molecule is activated after the action of plasmin, thrombospodin-1, reactive oxygen radicals, and αVβ6 integrin [28].

In the case of tuberculosis, the main producers of IL-10 and TGF-β are the regulatory antigen-specific Th1 and Th3 cells, respectively, which are activated after cooperation with dendritic cells and stipulate an optimal balance of interaction with specific effector T cells and control of tuberculosis infection for the purpose of excessive immunopathological response [29]. Nowadays, there are several studies in which the levels of IL-10 and TGF-β were determined in patients with various forms of tuberculosis and during specific chemotherapy. Jang et al. [30] found a significantly higher serum TGF-β content in patients with active pulmonary tuberculosis compare to the control group and no difference were found for serum level of IL-10. Chowdhurya et al. [31] showed a significantly higher serum production of not only TGF-β, but also IL-10 in patients with newly diagnosed tuberculosis before treatment compared to the control group of healthy volunteers. At the same time, serum TGF-β (as well as IFN-γ, TNF-α, and IL-6) level correlated with the severity of bacteremia and the radiological spread of the process. The study of TGF-β and IL-10 levels at 2, 4, and 6 months of treatment showed that within 2 months the level remained high and then gradually decreased to the fourth and sixth months, approaching the control group. Interesting results are also found for IL-6 dynamics. This factor decreased from second to fourth treatment and then did not change. The authors suggested that IL-6 can serve as a marker of the effectiveness of chemotherapy and tested the dynamics of cytokine in patients with different levels of initial bacterial release. The obtained individual curves of this cytokine content accurately reflected the effectiveness of the course of chemotherapy. Decrease in TGF-β serum production and insignificant IL-10 level at the end of the 6-month course of chemotherapy was revealed in the

study by Ameglio et al. [32]. Also, there was a difference in the production of these cytokines was observed depending on the severity of fibrotic changes in the lung tissue.

8. Chemokines. CXCL-10 (IP-10)

Chemokines play a central role in the recruitment of cells into the mycobacteria infected lung, which contributes to the delimitation of the focus of inflammation from the surrounding healthy tissues. However, under specific conditions, chemokines can also cause the disproportionate inflammation and subsequent progression of the process with formation of foci of destruction of lung tissue. In humans, the balance between protective and damaging inflammation, as well as the levels of inflammation can depend on many factors, including host genotype, bacterial strain, co-morbidities, and nutritional status. Understanding the nature and the effect of these interactions between the host, mycobacteria and the environment can serve as a basis for effective therapeutic and vaccination strategies. With tuberculosis as well as other infections, the inflammatory process is regulated by a cascade of reactions, including action adhesion molecules and chemoattractant. Chemokines are a family of small proteins, which, upon binding to membrane G protein-coupled receptors, guide the gradient-driven migration of leukocytes. Chemokines are classified into the CXC-, CC-, C-, and CX3C-subfamilies according to the arrangement of four conserved cysteine residues, which are important for maintenance of their tridimensional structure. Monin [33], summarizing the available literature data, identify the positive, negative, and terrible role of chemokines in tuberculosis, which probably exists with all heavy infections. To date, the dynamics of change and the diagnostic significance of only one chemokine IP-10 have been studied most fully in tuberculosis.

Interferon gamma inducible protein 10 (IP-10), or CXCL10, is a member of the CXC family of α-chemokines that stimulate the migration and adhesion of activated Th 1 cells through binding to the CXCR3 receptor [30]. IP-10 is secreted directly by macrophage cells infected with viruses and bacteria, and even more so after T cells recognize specific peptides on the surface of antigen-presenting cells. The secretion of IP-10 is enhanced by T cell secreted INF-γ and the numerous pro-inflammatory cytokines IL-2, IFN-α, IFN-β, IL-27, IL-17, IL-23, TNF-α, and IL -1β which are secreted by antigen-presenting cells [34]. Ruhwald et al. [35] determined various sets of cytokines and chemokines in blood supernatants of patients with tuberculosis obtained by QFT-IT test using the multiplex method and revealed a high concentration of IP-10 compared to the control group. In subsequent studies in the adult contingent of tuberculosis patients, groups contacting with patients and age-matched control, it was confirmed that IP-10 is statistically higher produced in patients with tuberculosis and gives comparable results with the QFT-IT test [34].

Several studies have examined the diagnostic value of IP-10 in children aged 1–17 years in countries with high and low incidence of tuberculosis. To determine the antigen of stimulated chemokine production, the multiplex analysis method was used in comparison with the determination of the INF-γ in the QFT-IT test. It was shown that in children and adolescents, as well as in adults, spontaneous production of IP-10 was higher in children with latent and active tuberculosis, compared to the group of healthy and antigen-stimulated synthesis, gave

more stable results compared with the production of INF-γ. Also, there are no age differences in the production of IP-10 [24, 36]. In the study, Ruhwald et al. [37], a cut-off point 673 pg/ml was determined for the antigen-stimulated IP-10 synthesis for the adult contingent, above which the reaction was considered diagnostically positive. Lighter et al. [19] identified a similar indicator for children and adolescents, which was 732 pg/ml. The authors showed that the IP-10 level in plasma was higher in patients with active tuberculosis than in the group of latent tuberculosis. Antigen-stimulated synthesis, on the contrary, was higher in the group of latent tuberculosis.

Studies on the dynamics changes of IP-10 in the serum and supernatant of blood cultures are single. Hong et al. [35] studied the chemokine values in 32 adult patients with pulmonary tuberculosis before and after 2 months of specific treatment. The authors showed that the initial serum level of IP-10 correlated with the degree of detection of MBT in the smear. At 0–1°, the average score was 132.5 pg/ml, and at grade 2–299.2 pg/ml. The patients studied on the basis of the severity of clinical signs were divided into groups of low and medium/severe risk of the course of the disease. The initial serum level of IP-10 groups did not differ, but in the low-risk group it significantly decreased during treatment, and remained unchanged in the middle/severe risk group. Similar trends in the change in the chemokine content during treatment are shown in the study of the antigen of stimulated synthesis in a 24-h blood culture, where the QFT-IT system was used as antigens [38].

In our studies, statistically significant increases in spontaneous and antigen-stimulated (20 μg/ml PPD and 1 μg/ml ESAT 6/CFP 10) production of IP-10 in children and adolescents with different forms of pulmonary tuberculosis were found compared with the group of latent tuberculosis. Decreased production of IP-10 revealed patients with lymph nodes form and focal tuberculosis by the third and especially by the sixth month of follow-up. In the group of patients with destructive tuberculosis, IP-10 production decreased after 3 months of treatment in patients with positive dynamics of the disease (disappearance of signs of tuberculous intoxication and cessation of bacterial release). The torpid and progressive nature of the tuberculosis process (the preservation of bacterial release for more than 6 months and the spread of the pulmonary tissue lesion) was not accompanied by a decrease in the production of chemokine IP-10 [39].

9. Matrix metalloproteases

Matrix metalloproteinases (MMPs) are a multigenic family of structurally and functionally similar Zn- and Ca-dependent endopeptidases that are able to modify all known components of the extracellular matrix, as well as many non-matrix molecules. The MMPs family consists of more than 60 enzymes, of which 20 are found in human tissues. A common feature of MMPs is the ability to hydrolyze the main components of the extracellular matrix containing Zn^{2+} ions in the active site of molecules using Ca^{2+} ions for molecular stabilization. They are secreted from the cells in an inactive form and their catalytic activity is suppressed by specific tissue inhibitors of metalloproteinases (TIMPs). Based on the MMP's primary structure, substrate specificity and cellular localization and they are classified into three groups, including six subfamilies.

10. Family I

MMPs secretory type (classical, free, soluble):

- collagenase (MMP-1, MMP-8, MMP-13, MMP-18);
- gelatinases (MMP-2, MMP-9);
- stromelysins (MMP-3, MMP-10, MMP-15);
- matrilizines (MMP-7, MMP-26).

11. Family II

MMPs associated with cell membranes (membrane type MT-MMP-14, -15, -16, -17, -24, -25).

12. Family III

Unclassified MMPs, with no relation to known subfamilies (MMP-11, -12, -19, -20).

Synthesis and secretion of metalloproteinases is realized by various blood and tissue cells (neutrophils, monocytes, macrophages, fibroblasts, osteoclasts, chondrocytes, keratinocytes, endothelial, and epithelial cells) but also by oncogene-transformed cells. MMPs induced by tissue growth factors such as epidermal growth factor, fibroblast growth factor, cytokine-TNFα, TNFβ, IL-1, IL-6, melatonin, hormones and neuropeptides, and oxidative stress. The importance of MMR for the pathogenesis and nature course of the disease is shown to arthritis, psoriasis, atopic dermatitis, atherosclerosis, neurodegenerative diseases, strokes, ischemic myocardial damage, periodontitis, primary open-angle glaucoma, thyroid gland diseases, chronic infections, and much more [40, 41]. Chang et al. [28] using the myeloid line THP-1 showed that *M. tuberculosis* and the main cell wall component LAM stimulate gene expression of MMP-1 and MMP-9 and the MMP-9 release in culture media suggesting their involvement in the digestion of collagen I to IV types, and indirect stimulation of IL-1, TNF-α. Subsequent studies showed that stimulation with virulent *M. tuberculosis* strain causes gene expression of MMP-1, -3, -7, and -10, and the expression of MMP-1 is stimulated more than with vaccine strain *M. bovis* (BCG) [7]. Similar results were obtained on the culture of blood mononuclear cells. MMP-1, -7, and -9 were found immunohistochemically in tuberculosis granuloma epithelioid and Pirogov-Langhans cells [42].

There are single clinical studies to identify MMP-1, -2, -8, and -9 in pleural fluid in tuberculous pleurisy and cerebrospinal fluid in patients with tuberculous meningitis. Parks et al. [7] conducted a comparative study of MMP-1, -2, -3, -7, -8, -9, and TIMP-1 and TIMP-2 in sputum by multiplex analysis in patients with tuberculosis during 24 weeks of antituberculous chemotherapy. The authors showed that the concentration of these factors was significantly higher in patients than in the control group of healthy volunteers except MMP-7, while the level of MMP-3 and -9 increased 15.2-fold and 14.4 times. The MMP concentration decreased during

the treatment and for MMP-1. MMP-3 and MMP-8 concentration significantly different at the second, eighth and twenty-fourth weeks, while the TIMP-1 and-2 concentration increased at 2 and 8 weeks and TIMP-2 significantly by the twenty-fourth week of follow-up. Comparison of this data with microbiological indicators revealed that the concentration of MMP-2, MMP-8, MMP-9, and TIMP-2 was significantly higher in MTB positive patients before treatment. MMP-3, MMP-8, and TIMP-1 were increased significantly at 2 weeks of treatment in MBT positive patients in comparison with MBT negative. Unfortunately, the authors do not give data on the correlation of MMP and TIMP concentrations with the presence of cavernous lung lesions. The availability of such information can be useful for predicting the growth of destructive processes in lung tissue.

13. Markers of oxidative stress. Hemoxygenase 1

Hemoxygenase is a microsomal enzyme, which consists of inducible and constitutive isozymes (HO-1, HO-2) and catalyzes the decomposition of heme to CO, Fe+2, and biliverdin with conversion to bilirubin by biliverdin reductase. HO-1 is a major intracellular source of iron and carbon monoxide (CO). The investigated isoforms of HO are the products of various hmox-1 and hmox-2 genes. Hemoxygenase-1 is an inducible isoform of the enzyme, the synthesis of which is enhanced by the influence of temperature, heme components, heavy metal ions, cytokines, and reactive oxygen radicals.

HO-1 is an important part of the biological mechanism for protecting against oxidative stress and tissue damage and from excessive inflammation. The end-products of heme breakdown are potently antioxidant and anti-inflammatory, and in addition, modulation, cell proliferation and cell death, either positively or negatively, but in a manner that seems to relate to the re-establishment of a homeostasis in many diseases pathogenic mechanisms. The protective value of hemoxygenase-1 is inhibition of the synthesis of inflammatory factors (IL-1, IL-6, IL-8, TNF-α), anti-inflammatory cytokines (IL-10), and heme degradation products and their metabolic derivatives. Carbon monoxide CO reduces the production of inducible NO-synthase (iNOS), cyclooxygenase-2, the corresponding inflammatory mediators—NO and prostaglandins. More detailed information on the structure and function of HO-1 can be found in the reviews of Ryter et al. [43] and Soares and Bach [12].

Diagnosis of tuberculosis is not particularly difficult in the presence of clinical, radiological, and especially microbiological data. The presence of negative microbiological results and latent forms of infection, even with positive results of tuberculin skin (TST) or IFN-γ tests, stimulates the search for additional laboratory markers. Studies in patients with active tuberculosis demonstrated a decrease in systemic concentrations of antioxidants and increased spontaneous generation of free radicals compared to those without tuberculosis, reflecting the excessive oxidative stress associated with this disease. Increased expression of HO-1 was observed in the plasma of people with various pulmonary pathologies, including acute respiratory distress syndrome, chronic obstructive pulmonary disease, and asthma. Elevations of HO-1 levels are identified in malaria, leishmaniasis and sepsis, and plasma levels of HO-1 are often associated with the severity of many other diseases [44]. However, clinical studies of the significance of NO-1 to confirm the diagnosis of tuberculosis and evaluate the effectiveness of treatment are few.

To date, there is a single clinical study conducted on a cohort of infected *M. tuberculosis* and infected individuals from South India with a high incidence of tuberculosis. Plasma samples were collected in 97 patients with active pulmonary tuberculosis (PTB), 35 patients with extrapulmonary tuberculosis, (EPTB), 39 people with LTBI and 40 healthy donors under the cohort study program on tuberculosis [44]. Levels of HO-1, IL-10, TNF-α, IFN-γ, and IL-17 in plasma were measured by ELISA. Levels of C-reactive protein (CRP) and Serum Amyloid Protein-A (SAA) were determined using the Bioplex multiplex ELISA system.

The authors found significantly higher systemic levels of HO-1 in patients with active pulmonary or extrapulmonary tuberculosis (medians [IQR]: 5.8 [3.2–11.6] and 3.45 [2.0–4, 5] ng/ml, respectively, $P \leq 0.01$) than persons with latent tuberculosis or healthy donors (1.3 [0.78–1.5] and 1.4 [1.0–1.9] ng/ml, respectively, $P = 0.49$). Among patients with active pulmonary tuberculosis, those with bilateral lung lesions had higher systemic NO-1 levels compared to patients with unilateral lesions detected by the chest x-ray, indicating a possible association between HO-1 and the anatomical prevalence of the disease. The systemic levels of HO-1 were higher in those with positive AFB staining in sputum than in patients with negative smears. With an effective course of chemotherapy, the elevated NO-1 concentration in the plasma returned to background levels. This was not observed in patients with a negative result of treatment, as determined by positive sputum cultures at the end of the course of drug therapy.

The authors evaluated the ability of HO-1 to distinguish between active tuberculosis and latent TB infection in combination with two other markers of inflammation, CRP and SAA, whose significance is increased with active TB [45]. Elevated levels of HO-1, CRP, and SAA have been detected in plasma in patients with active tuberculosis (PTB and/or EPTB) compared to individuals with LTBI. In addition, the authors compared the significance of HO-1, CRP, and SAA for establishing the diagnosis of PTB or EPTB. Systemic levels of HO-1 and CRP were significantly higher in PTB cases, while SAA concentrations were slightly reduced. Further analysis showed that HO-1 was the most informative parameter for recognizing pulmonary from extrapulmonary tuberculosis. It was also found that in individuals with active pulmonary tuberculosis, the levels of HO-1 positively correlated with IL-10 levels ($r = 0.59$, $P = 0.001$) and negatively correlated with TNF-a levels ($r = -0.31$, $P = 0.002$). There were no significant correlations between the levels of HO-1 and IFN-γ or HO-1 and IL-17. The correlation coefficient of HO-1 and IL-10 was higher in patients with a higher bacillary index.

14. Concluding remarks

Diagnosis of tuberculosis is not particularly difficult in the presence of clinical, radiological, and especially microbiological data. The difficulty arises from negative results of microbiological examination and minor clinical manifestations of tuberculosis. In addition, a significant problem is infected persons without clinical manifestations with a negative result of tuberculin skin (TST) or IFN-γ test results. They make up a huge reservoir of latent tuberculosis infection (LTBI). In some of these latently infected individuals, the infection becomes active and seriously affects the epidemiological situation. These two problems will stimulate the search for additional and new markers of the disease.

The scientific studies carried out to date show a significant increase in the effectiveness of IGRAs-test in combination with the determination of chemokine CXCL/IP-10 for the diagnosis of latent forms of tuberculosis. Monitoring the effectiveness of treatment of tuberculosis will require the use of other combinations of cytokines, chemokines, and nonspecific inflammatory factors. This will depend on the sensitivity and resistance (MDR-TB) of mycobacteria to antituberculosis drugs and the nature of the course of tuberculous inflammation (positive, torpid or progressive dynamics of the disease) associated with genetically determined parasite-host interactions. The optimal combination of such factors has not yet been determined, but probable search vectors indicate on the definition of dynamics immunoregulatory cytokines (e.g., IL-10, TGF-β), chemokines characterizing cell migration (e.g., IP-10/CXCL-10, MIG/CXCL/9) and factors nonspecific inflammation (e.g., HO-1, SAA and MMP-1,3,9). Comparability of the results of these studies will depend on the proximity of the methodological platforms.

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