Vitamin D, Cathelicidin, Prolactin, Autoantibodies, and Cytokines in Different Forms of Pulmonary Tuberculosis versus Sarcoidosis

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ABSTRACT: **Background:** Vitamin D insufficiency is associated with autoimmune and chronic inflammatory diseases such as tuberculosis and sarcoidosis.

Objectives: To evaluate the vitamin D-dependent mechanisms of immunity and autoimmunity in different forms of pulmonary tuberculosis and sarcoidosis.

Methods: We measured the serum levels of 25(OH)D and $1,25(OH)_2D$, individual autoimmune profiles, plasma concentrations of cathelicidin, several hormones, and production of nine cytokines in patients with short- and long-duration tuberculosis and sarcoidosis.

Results: The level of 25(OH)D was significantly decreased in all patients. Concentration of $1,25(OH)_2D$ was elevated only in sarcoidosis, prolactin content was augmented only in tuberculosis. We saw no expected increase of cathelicidin levels in tuberculosis and sarcoidosis. The individual mean immune reactivity levels of autoantibodies to 24 antigens were significantly lower in tuberculosis and sarcoidosis patients compared to healthy controls. Pronounced deviations from individual mean immune reactivity levels were found for several autoantigens in all patients. The induced production of interferon gamma-γ, interleukin (IL) 2, 17, and 8 by peripheral blood mononuclear cells was significantly increased in patients of both tuberculosis groups, but spontaneous production of tumor necrosis factor-α, IL-2, and IL-6 was lower in the tuberculosis patients than in healthy controls. We registered marked differences in the groups of tuberculosis patients.

Conclusions: We demonstrated the role of vitamin D deficiency in poor cathelicidin response in tuberculosis and sarcoidosis. Both diseases are accompanied by significant changes in the autoimmune profile, probably related to the status of vitamin D and cytokine regulation.

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Pulmonary tuberculosis and pulmonary sarcoidosis both belong to the category of chronic granulomatous inflammation and often pose difficulties for differential diagnosis.

Tuberculosis is still a major public health hazard. The balance between the *Mycobacterium tuberculosis* (Mtb) and the host innate and adaptive immunity mechanisms determines the disease course. The infected alveolar macrophages activate signaling pathways to combat bacterial replication and attract other immune cells to the site of infection. The Mtb alters macrophage signaling pathways, thus inhibiting phagolysosomal maturation [1].

Recent studies on vitamin D, besides its metabolic effects, confirmed its role in pathogenesis of autoimmune and chronic inflammatory diseases including tuberculosis and sarcoidosis. Its insufficiency is widely spread and associated with increased tuberculosis risk in different populations [2,3]. Conversion of 25-hydroxyvitamin D to active 1,25-dihydroxyvitamin D occurs in the immune cells and is essential for their antibacterial activity due to induction of cathelicidin (LL-37), a potent antimicrobial peptide and universal chemokine [4]. Cathelicidin possesses anti-infection properties; it plays a particular part in defense against intracellular Mtb inducing autophagy in monocytes/macrophages [5].

Vitamin D deficiency is common in patients with autoimmune diseases as well as among tuberculosis patients [3,6]. High titers of various autoantibodies present in pulmonary tuberculosis patients with vitamin D deficiency [7]. Vitamin D supplementation restores the immune functions, inducing an immature tolerogenic state of dendritic cells. It also enhances the function of T regulators, inhibits the Type 1 T helper (Th1) functions, and affects the human B cell differentiation [6]. However, with vitamin D deficiency, persistent germs can break self-tolerance and induce autoimmune diseases through activation of the antigen-presenting cells [8]. Autoimmunity also occurs in sarcoidosis [9].

The host resistance to pathogens such as Mtb depends both on the adaptive and innate immune mechanisms. T1, Th2, and Th17 cells as well as regulatory T cells are involved in the response to Mtb, but Th1 and their cytokines are recognized as the main players associated with microbicidal mechanisms [10]. The T-cell cytokines are able to regulate the vitamin D metabolism [11].

The majority of investigations of the vitamin D impact on tuberculosis did not take into account the variability of disease forms. This study partially fills that gap. Distinction was made between tuberculosis patients with short-time and long-time duration of disease. In addition, we compared the levels of vitamin D and several hormones, as well as immunological data in pulmonary forms of tuberculosis and sarcoidosis.

PATIENTS AND METHODS

A total of 112 participants were enrolled in the study. Blood samples were obtained from April until June 2015 from patients at the Saint Petersburg Research Institute of Phthisiopulmonology (SPbRIP) and those at the tuberculosis sanatorium near Saint Petersburg (59° north latitude). The institutional review board of the SPbRIP approved all studies involving humans. All processing of information obtained from the participants was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

The participants were divided into four groups:

- Patients with infiltrative tuberculosis (n=33)
- Patients with fibrous cavernous tuberculosis (n=22)
- Patients with sarcoidosis (n=16)
- Healthy donors (n=41)

The mean age of patients was 35.4 years (range 17–65), 73% were male. The mean age of healthy donors was 31.5 years (range 17–56), 44% were male. The chest X-ray findings, direct smear microscopy, mycobacterial culture results, and clinical data were abstracted from the medical records. Tuberculosis manifests in many forms. In our study, two groups of tuberculosis patients had different clinical and X-ray characteristics, but all tuberculosis patients were in active phases of the disease.

Infiltrative tuberculosis (IT) is the most common type of the secondary tuberculosis. In our investigation the IT patients (mean age 22.9 years, range 17–33) had 1 to 3 segments of the lung affected (81.4%), and a more benign course of disease compared to the fibrous cavernous tuberculosis (FCT) group. Of the IT patients, 38% excreted Mtb. Physical signs of tuberculosis were absent in 49.1% of patients.

The FCT patients had progressive disease lasting from 4 to 19 years, with an average duration of 11.2 years. The multiple involvement of lung segments and signs of lung destruction were observed in all of them. Mtb excretion was found in 91%. Of the FCT patients, 89% had manifestations of tuberculosis on physical examination. All FCT patients had multi-drug Mtb resistance and some degree of respiratory insufficiency. The mean age of FCT patients group was 47.9 (range 29–65).

The overwhelming majority of the population in Russia has been bacillus Calmette-Guerin-vaccinated, which was the case for all donors involved.

The concentration of 25-hydroxyvitamin D (calcifediol) was measured by enzyme-linked immunosorbent assay (ELISA) (Immunodiagnostic Systems Ltd, UK) with assay sensitivity of 5 nmol/L.

The level of 1,25-dihydroxyvitamin D (calcitriol) was measured by ELISA (Immunodiagnostic Systems Ltd, UK). The kit was a complete assay system intended for purification of calcitriol in the human serum by immunoextraction followed by quantitation by the enzyme immunoassay. The sensitivity of the assay was 2.5 pg/ml.

Cathelicidin (human LL-37) levels in plasma was measured by ELISA (Hycult Biotech, the Netherlands). The detection limit was 0.1 ng/ml.

The prolactin level was measured by the PRL AccuBind ELISA test system (Monobind Inc., USA), which had a detection limit 0.15 ng/ml. The prolactin, procalcitonin, 3-iodotironin, thyroxin, thyroid-stimulating hormone (TSH), and cortisol levels were measured by ELISA (Vector Best Baltica, Russia).

The cytokine production by peripheral blood mononuclear cells (PBMC) was measured using the whole-blood ELISA (Vector Best Baltica, Russia). We determined cytokine concentrations in the supernatants of purified protein derivative (PPD)-stimulated PBMCs (induced) and in the unstimulated (spontaneous) duplicates from patients and healthy donors. The sensitivity and range of the cytokine detection was different for various cytokines as reported by the manufacturer, namely, IFN-γ, IL-18: sensitivity 2 pg/ml, range 0–1000 pg/ml; IL-2, IL-10: 1 pg/ml, 0–500 pg/ml; IL-1β, IL-6 0.5 pg/ml, 0–300 pg/ml; IL-8: 2 pg/ml, 0–250 pg/ml; TNF-α: 1 pg/ml, 0–250.

The individual serum profiles of multiple autoantibodies (AAB) and the integral immune reactivity was performed by the method of ELI-viscero-test, (Immunculus, Russia) which is based on the ELISA technology [12]. The method evaluates the relative serum content of AAB to 24 major antigens simultaneously. For every person, the test measures the average overall antigens characterizing the individual mean immune reactivity (MIR). The results of the ELI-viscero-test do not characterize the absolute AAB concentrations but rather their deviations in percentage from the individual MIR. Deviation ranges for each antigen in healthy population were established in to be between -20 and + 10 percent from MIR [12].

STATISTICAL ANALYSIS

Statistical analysis was performed using STATISTICA (data analysis software system), version 7 (StatSoft, Inc.USA). Mean

values were compared using the *t*-test for the data that were normally distributed and the Mann–Whitney test for the data that were not normally distributed. Statistical significance was set at $P < 0.05$.

RESULTS

The peripheral blood characteristics of the two tuberculosis groups were different. Both groups displayed monocytosis, thrombocytosis, and increased erythrocyte sedimentation rate (ESR), but changes in the FCT group were much more significant. Monocytosis was present in 76.5% of FCT patients, thrombocytosis in 85.7%, and increased ESR in 82.4%.

VITAMIN D IN TUBERCULOSIS AND SARCOIDOSIS

The serum level of 25(OH)D was low even in healthy adult donors constituting 19.3 ± 1.4 ng/ml (mean ± standard error); but in all patients it was significantly lower, namely 13.2 ± 1.7 in sarcoidosis ($P < 0.05$), 11.7 ± 1.8 ($P < 0.001$) in IT and 8.2 ± 1.4 ng/ml ($P < 0.001$) in FCT [Table 1]. The serum concentration of the $1,25(OH)_2D$ was increased in sarcoidosis patients only (50.4 ± 16.6 vs. 35.5 ± 12.8 pg/ml in healthy subjects, $P < 0.05$) [Table 1].

CATHELICIDIN AND HORMONE LEVELS

There was no increase of the cathelicidin level in tuberculosis or sarcoidosis. The cathelicidin content was 39.8 ± 9.7 ng/ml in healthy donors, 45.1 ± 18.3 ng/ml in IT, 50.1 ± 32.8 ng/ml in FCT, and 39.8 ± 8.3 ng/ml in sarcoidosis patients [Table 1].

The prolactin content in sarcoidosis patients did not differ from that of controls, but it was significantly higher in the IT group (22.3 ± 3.4 vs. 7.7 ± 0.9 ng/ml, $P < 0.01$) and FCT (19.4 ± 3.3, $P < 0.05$ [Table 1]. The levels of procalcitonin, 3-iodotironin, thyroxin, and TSH were normal in all groups. In a few cases of tuberculosis (5 out of 17), the blood cortisol level was elevated.

Table 1. Vitamin D, cathelicidin, prolactin levels (the mean ± standart error, M±m; n is the number of patients in the groups)

	Healthy subjects	Sarcoidosis	Infiltrative tuberculosis	Fibrous-cavernous tuberculosis
25 (OH)D, ng/ml	19.3 ± 1.4	13.2 ± 1.7 $P < 0.05$	11.7 ± 1.8 $P < 0.001$	8.2 ± 1.4 $P < 0.001$
(n)	(8)	(8)	(13)	(8)
$1,25(OH)_2D$, pg/ml	35.5 ± 12.8	50.4 ± 16.6 $P < 0.05$	32.1 ± 14.7	40.7 ± 19.2
(n)	(10)	(10)	(10)	(11)
Cathelicidin, ng/ml (n)	39.8 ± 9.7 (20)	39.8 ± 8.3 (8)	45.1 ± 18.3 (24)	50.1 ± 32.8 (12)
Prolactin, ng/ml	7.7 ± 0.9	9.1 ± 1.1	22.3 ± 3.4 $P < 0.01$	19.4 ± 3.3 $P < 0.05$
(n)	(9)	(9)	(21)	(10)

INDIVIDUAL SERUM AAB PROFILES

Deviation of MIR from zero reflects the individual immune system activity. Normal range of MIR deviations from the population average is between -25% and +5% [12]. MIR was significantly decreased both in tuberculosis and sarcoidosis compared to healthy controls. It was decreased in 100% patients with IT, in 67% of patients with FCT, and in 91% of sarcoidosis patients.

We found pronounced deviations of AAB toward several auto-antigens from MIR levels in all patients. The IT patients most often had increased levels of AAB to the dsDNA (4/9 patients). The predominance of autoantibodies to the kidney antigens, to insulin, and to the TSH-receptor was found in five of nine patients.

Significant deviations of AAB from normal ranges occurred in FCT patients predominantly to kidney antigens (7/9 patients) and to TSH receptor (6/9 patients). According to Poletaev [12], negative deviations indicate binding of AAB by the surplus of autoantigens occurring under intensive tissue decay. Compared to IT patients, each particular patient with FCT had elevated levels of AAB toward a larger number of different antigens. Compared to tuberculosis, the sarcoidosis patients demonstrated fewer deviations from normal MIR ranges. Increased levels of AAB occurred predominantly toward dsDNA (8/12) and GaM-02 (4/12).

CYTOKINES

The production of IFN-γ by the cultured PBMCs stimulated by PPD was significantly greater for IT patients than in healthy donors; the mean was 125 pg/ml vs. 47.7 pg/ml; according to Mann–Whitney U test (U = 35, $P = 0.013$) [Figure 1A]. There was no difference between IT patients and healthy donors in the spontaneous production of IFN-γ.

The stimulated production of TNF-α in the IT patients did not differ from that of healthy donors, whereas the production of TNF-α by the non-stimulated PBMC was lower in IT patients (mean 0.6 pg/ml) than in healthy individuals (mean 11.2 pg/ml), U = 19, $P = 0.0003$ [Figure 1B].

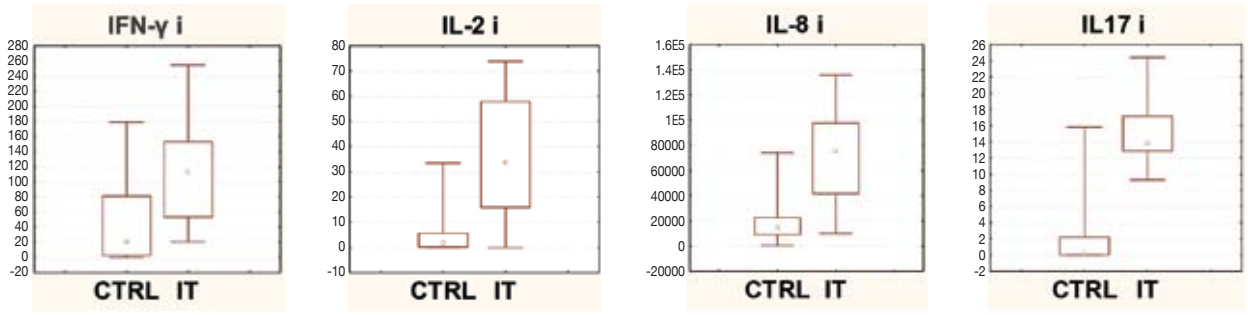
The stimulated production of IL-2 was significantly increased in IT patients (mean 37.8 pg/ml) compared to healthy donors (mean 4.8 pg/ml), U = 21, $P = 0.0014$ [Figure 1A].

The induced production of IL-17 was much higher in IT patients (14.9 pg/ml) than in controls (2.7 pg/ml), U = 10, $P = 0.00004$ [Figure 1A].

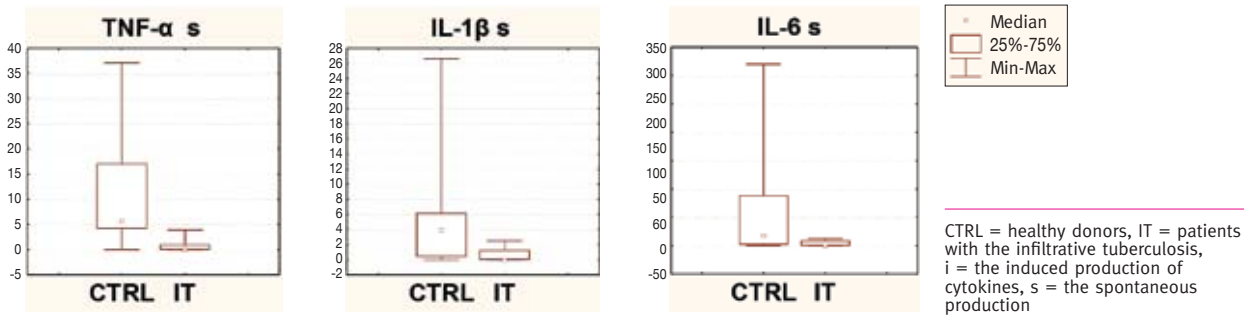
The stimulated production of IL-8 was significantly enhanced in IT patients (mean 72000 pg/ml) compared to healthy donors (mean 19300 pg/ml), U = 31, $P = 0.002$ [Figure 1A]. The spontaneous production of IL-1β was significantly lower in IT patients (0.55 pg/ml) than in controls (5.86 pg/ml), U = 34, $P = 0.004$ [Figure 1]. In addition, the spontaneous production of IL-6 was lower in IT patients (3.7 pg/ml) than in the healthy individuals (80.2 pg/ml), U = 32, $P = 0.004$ [Figure 1B].

Figure 1. Cytokine production by peripheral blood mononuclear cell in the infiltrative tuberculosis patients (pg/ml)

[A] The induced production of cytokines



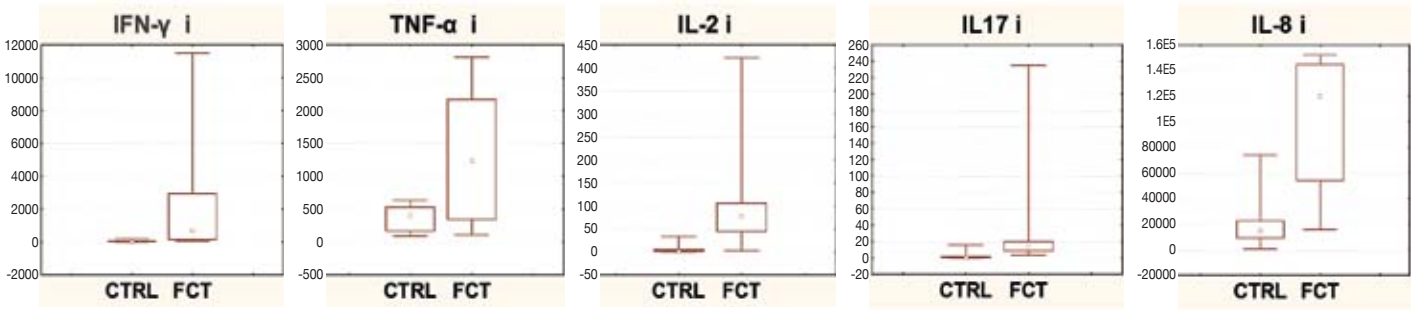
[B] The spontaneous production of cytokines



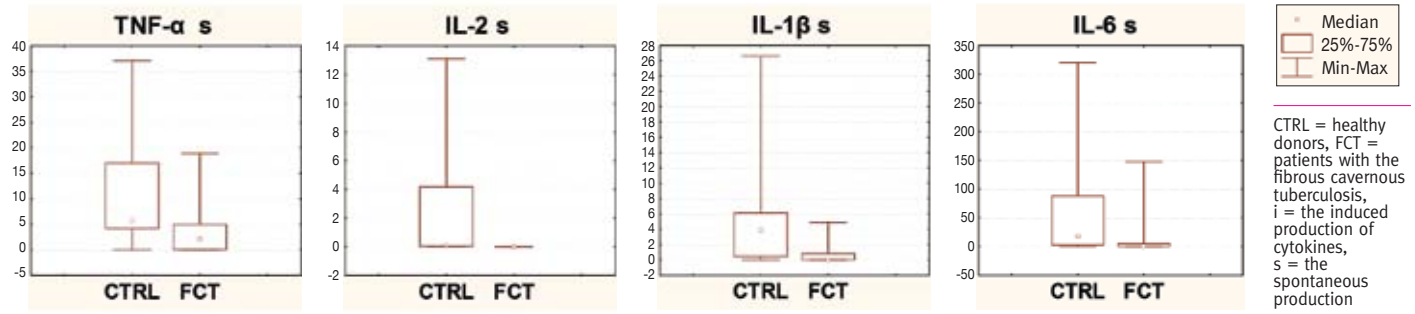
CTRL = healthy donors, IT = patients with the infiltrative tuberculosis, i = the induced production of cytokines, s = the spontaneous production

Figure 2. Cytokine production by peripheral blood mononuclear cell in the fibrous cavernous tuberculosis patients (pg/ml)

[A] The induced production of cytokines



[B] The spontaneous production of cytokines



CTRL = healthy donors, FCT = patients with the fibrous cavernous tuberculosis, i = the induced production of cytokines, s = the spontaneous production

The mean IFN-γ level in stimulated cultures was significantly higher compared to both healthy donors (mean 2212 pg/ml vs. 48 pg/ml, U = 16, *P* = 0.0003) [Figure 2A] and to patients with IT (125 pg/ml, U = 20, *P* = 0.045) [Figure 3A]. Half of the FCT patients had a many-fold increased level compared to any other patients studied.

The concentration of TNF-α stimulated by PPD in FCT patients was significantly increased compared to healthy donors (1359 pg/ml vs. 371 pg/ml, U = 35, *P* = 0.018) [Figure 2A] and was insignificantly higher than in the IT patients. The spontaneous production of TNF-α was significantly lower in FCT than in controls (4.4 vs. 11.2, U = 54, *P* = 0.045) [Figure 2B].

The induced production levels (in pg/mL) of IL-2 (107.0 vs. 4.8, U = 10, *P* = 0.0001), IL-17 (35.5 vs. 2.7, U = 14 *P* = 0.0001), and IL-8 (102500 vs. 19300, U = 14, *P* = 0.0002) were also increased in FCT, and more significantly than in IT [Figure 2A].

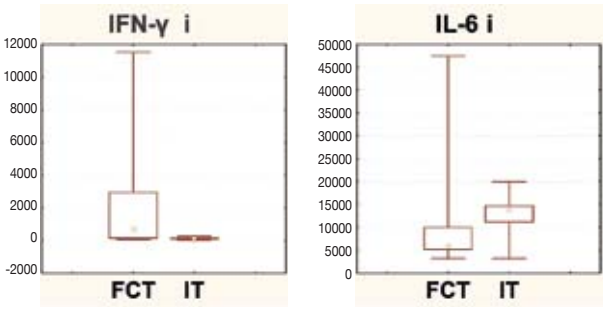
The spontaneous production of IL-2, IL-1β, and IL-6 (pg/ml) was decreased in FCT patients compared to healthy donors: IL-2 (0.0 vs. 2.2, U = 50, *P* = 0.007), IL-1β (0.7 vs. 5.7, U = 41, *P* = 0.012), IL-6 (16.6 vs. 80.2, U = 35, *P* = 0.0052) [Figure 2B].

No difference was revealed between patients of both groups and healthy donors regarding the levels of IL-10 and IL-18, both in stimulated and not stimulated cultures. We also saw no difference in the spontaneous production of IFN-γ, and IL-8 in the FCT patients against that of controls (not shown).

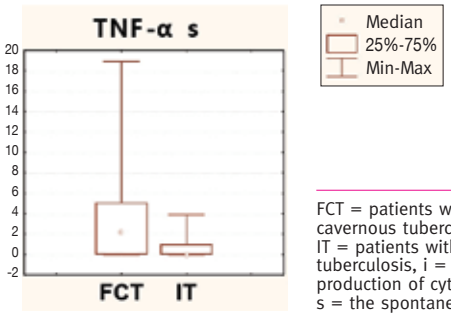
The two study groups differed in the production of cytokines. Significantly higher stimulated levels of IFN-γ occurred in FCT patients (Mann–Whitney U Test: U = 20.5, *P* = 0.045). The same was recorded for stimulated IL-6 level (U = 21.5, *P* = 0.031) [Figure 3A]. The spontaneous production of TNF-α was lower in IT than in FCT (U = 22.5, *P* = 0.038).

Figure 3. Comparison of the cytokine production by peripheral blood mononuclear cell in the fibrous cavernous and infiltrative tuberculosis patients

[A] The induced production of cytokines



[B] The spontaneous production of cytokines



FCT = patients with the fibrous cavernous tuberculosis,
IT = patients with the infiltrative tuberculosis, i = the induced production of cytokines,
s = the spontaneous production

DISCUSSION

VITAMIN D

Pulmonary forms of tuberculosis and sarcoidosis are granulomatous disorders, the latter remains a disease of unknown etiology with immunopathological mechanisms. Both have similar X-ray, pathomorphological, and genetic characteristics [9].

It was suggested that mycobacterial antigens; that is, heat shock proteins (Mtb-hsp), can serve as causative factors for both diseases. Mtb-hsp, especially Mtb-hsp65, may provide a link between infection and autoimmunity [13]. Dubaniewicz [13] hypothesized that, in genetically different individuals, the same antigens (Mtb-hsp) may induce different immune responses, leading to the development of either sarcoidosis or tuberculosis.

The vitamin D status is based on the serum levels of 25(OH) D. There is no consensus on the normal ranges for vitamin D level as yet. We considered the vitamin D levels < 20ng/ml as the deficient ones and found deficiency of the vitamin D in our study in sarcoidosis patients and especially in those with tuberculosis. The FCT patients with long-progressing tuberculosis after many exacerbations and remissions, having considerable lung lesions, manifested the deepest drop of the vitamin D level. Indeed, these patients were altered by multiple factors influencing their vitamin D status such as medicines (Rifampicin©), long hospital stays, and lack of the sun exposure. It seems unlikely that the FCT patients initially had, before the tuberculosis disease onset, such a low level of vitamin D (8.2 ± 1.43 ng/ml). It may be more common for those few patients who had co-morbid chronic hepatitis. It seems that the low level of vitamin D is the result of interactions between Mtb and the host macrophages. A novel subset of genes, whose regulation was affected specifically by infection with mycobacteria, was identified [1]. This subset includes genes involved in response to vitamin D. The mean level of $1.25(OH)_2D$ was increased only in sarcoidosis patients, probably due to its excessive production by the macrophages of sarcoidosis granulomata [14].

CATHELICIDIN

There were no significant changes in concentration of cathelicidin (LL-37) among tuberculosis or sarcoidosis patients compared to healthy donors, probably due to the low concentration of 25(OH)D in both diseases. In patients with

acute septic and non-septic infection the cathelicidin level was markedly enhanced and correlated with vitamin D status, but there was no correlation in the case of tuberculosis. Yamshchikov et al. [15] found higher LL-37 concentrations in tuberculosis patients. However the level of 25(OH)D in that study was higher than in our tuberculosis patients, and the investigations were performed in serum samples and not in freshly obtained plasma. In this case during blood clot formation, neutrophils may degranulate to be the source of additional LL-37. We also found a high prevalence of thrombocytosis in tuberculosis patients but it correlated with the disease severity, monocytosis, and enhanced ESR and not with the cathelicidin level. Lambert and colleagues [16] support our data that demonstrated an association between low cathelicidin levels and a history of bacterial pneumonia in the study of 650 individuals. In the case of an intracellular pathogen like Mtb, the intracrine mechanisms of defense are especially important and depending on the vitamin D status.

The prolactin levels were raised in both groups of tuberculosis patients, but did not differ from controls in sarcoidosis patients. There are multiple examples suggesting that the enhanced prolactin level might facilitate the autoimmune processes [17].

INDIVIDUAL SERUM AAB PROFILES

Our interest in the AAB with tuberculosis patients was motivated by the growing evidence that the autoimmune processes are triggered by infections like tuberculosis because chronic presence of Mtb can be regarded as an endogenous adjuvant [3]. The important question is whether the AAB presence eventually leads to overt autoimmune disease. To clarify this issue, we studied two groups of patients with a short-time and long-time course of tuberculosis and patients with sarcoidosis, which is a disease with an autoimmune component of pathogenesis [6].

Individual mean immune reactivity (MIR) was significantly decreased in tuberculosis and sarcoidosis compared to healthy controls, which indicates polyclonal immunosuppression [12]. Immunosuppression in pulmonary tuberculosis was demonstrated previously by other methods; the immune responses in tuberculosis may increase the spontaneous and the Mtb antigen-induced apoptosis of T cells [18]. Patients with tuberculosis and sarcoidosis had increased content of AAB toward different antigens in spite of a general background of immunosuppression. It is worth mentioning that evaluation of the relative AAB level with respect to MIR demonstrates the increase of the AAB production with greater sensitivity than the standard method measuring their absolute content under significant immunosuppression peculiar to both tuberculosis and sarcoidosis.

The increased level of AAB most often occurred with respect to the dsDNA (in IT and sarcoidosis). The tuberculosis patients also demonstrated enhanced levels of AAB to different antigens, but AAB to the kidney antigens, insulin, and the TSH-receptor prevailed. Our data are consistent with the data of other researches who demonstrated the presence of different AAB in the serum of the active tuberculosis patients [7,19].

In our study the sarcoidosis patients demonstrated fewer deviations from normal MIR ranges compared to those with tuberculosis. It seems important that patterns of the deviations in the IT, FCT, and sarcoidosis groups were different. There were no corresponding clinical symptoms and signs of overt autoimmune diseases involving the organs to which AAB presented. Despite high prevalence of anti-thyroid AAB in tuberculosis patients, we found no changes in thyroid hormones and TSH concentrations. Shen and co-authors [19] demonstrated the presence of AAB to different antigens among tuberculosis patients, suggesting that AAB are reactive in tuberculosis instead of being pathognomonic, and therefore do not require immunosuppressant therapy. We tend to accept this interpretation because the most common anti-dsDNA autoantibodies were characteristic for both tuberculosis and sarcoidosis, although the FCT patients suffering from a more severe form of tuberculosis than the IT patients had higher prevalence and a broader set of AAB.

CYTOKINES

In our study, the production of IFN-γ stimulated by PPD was enhanced in patients of both groups compared to healthy donors, but the highest level was found in FCT patients. Half of our patients had a many-fold increased level compared even to other patients in this group. We saw no association of this high level with clinical data. The essential role for IFN-γ in the resistance to Mtb has been confirmed by many researchers [10]. IFN-γ, the main cytokine of the Th1 subset, enhances the macrophage defense mechanisms via a vitamin D-dependent pathway, but it also is known for enhancement of autoimmunity [10] and is considered an endogenous adjuvant.

In the current study, we found increased stimulated levels of TNF-α in FCT but not in IT patients. This observation is important because TNF-α is known to be critical in the control of Mtb infection [10]. The induced production of one more Th1 cell cytokine, namely IL-2, was greatly increased in our tuberculosis patients, while spontaneous production did not change.

In our study, induced production of IL-17 was greatly increased in patients of both tuberculosis groups, but especially in FCT. Th1 and Th17 cells are known to be crucial for control of Mtb [10]. The major cytokine of Th17 cell: IL-17 has the dual capacity, both defensive and pathogenic. It plays an important part in pathogenesis of the autoimmune inflammation [20]. It is possible that in our study IL-17 was responsible for the autoimmune components revealed in tuberculosis.

The excessive activity of TNF-α and IFN-γ can be harmful to the host. In the early stages of tuberculosis, the IL-17 production facilitates the granuloma formation and control of bacterial

growth. During the chronic phase of tuberculosis, the balance between Th1 and Th17 responses is vital because excessive IL-17 production may cause extensive neutrophil accumulation and tissue damage [20].

Our results showed a marked increase in the stimulated production of IL-8 by patients of both tuberculosis groups, maybe partially due to the capacity of TNF-α to up-regulate the IL-8 secretion [21]. IL-8 has a central role in the neutrophil chemotaxis to areas of tuberculosis granulomata formation; however, extensive accumulation of neutrophils in tuberculosis lesions is associated with a high pathogen load [22].

The more severe the course of tuberculosis in the FCT patients, the higher the levels of IFN-γ, TNF-α, IL-17, and IL-8 whose detrimental action was not restrained by IL-10, which failed to respond by increasing its production.

We found a significant difference between the two groups of tuberculosis patients in the levels of induced production of IFN-γ and IL-6 and spontaneous production of TNF-α. Spontaneous production of TNF-α, IL-2, IL-1β, and IL-6 was significantly lower in the patients of both groups compared to controls. Low and even undetectable levels of IL-2 in tuberculosis has been reported by others [23]. The cause, however, is not clear. One possible explanation is that the blood samples were taken from the tuberculosis patients after the beginning of the treatment and serum cytokine levels decreased during anti-tuberculosis therapy. Another explanation is that in tuberculosis most active cells are recruited into granulomata, so those staying in the blood may have relatively low spontaneous cytokine-producing activities, although strong stimulation reveals their potential. Katti [23] reported similar results.

We registered significant changes in the content of vitamin D, prolactin, AAB, and cytokine production in pulmonary tuberculosis and sarcoidosis, as well as some differences between the studied forms of tuberculosis and failure of the cathelicidin response to the infection.

Our data testify to the role of vitamin D deficit for poor cathelicidin response in chronic granulomatous diseases. Both tuberculosis and sarcoidosis are accompanied by significant changes of autoimmune profile, which can be related to status of vitamin D and cytokine regulation.

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