Original Research

Autoantibodies to Tumor Necrosis Factor in Patients with Active Pulmonary Tuberculosis

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Abstract

Background: Tumor necrosis factor (TNF) plays an important role in immune responses to the causative agent of tuberculosis, *Mycobacterium tuberculosis*. Additionally, TNF can also mediate many negative disease manifestations. The aim of this study was to assess the contribution of anti-TNF autoantibodies to the pathogenesis of active pulmonary tuberculosis (TB). **Methods**: The levels of anti-TNF autoantibody classes and subclasses were determined by applying enzyme-linked immunosorbent assays (ELISAs). The levels of TNF and of its soluble receptors were also evaluated using commercial ELISA kits. **Results**: The levels of both types of soluble TNF receptors were lower patients with TB than in healthy donors. Patients with TB had higher titers of immunoglobulin (Ig)G class and IgG3 subclass anti-TNF autoantibodies in comparison with healthy donors. Patients who had a disseminated TB infection had higher TNF level and IgG, IgG1 and IgG3 autoantibody titers compared with patients who had a localized TB infection. **Conclusions**: Changes in the titers of anti-TNF autoantibody classes and subclasses were noted in patients with TB, suggesting their possible contribution to the disease pathogenesis of TB.

Keywords: anti-cytokine autoantibodies; tumor necrosis factor; pulmonary tuberculosis; enzyme immunoassay

1. Introduction

The pro-inflammatory cytokine tumor necrosis factor (TNF) plays a key role in the host immune response to the tuberculosis pathogen *Mycobacterium tuberculosis (M. tuberculosis)*. TNF performs a number of important functions during infection; for example, it stimulates T-lymphocyte and monocyte chemotaxis, phagocyte activation, macrophage apoptosis, dendritic-cell maturation followed by T-cell activation, interferon (IFN)-γ secretion, and granuloma formation. The main function of TNF during human *M. tuberculosis* infection is ensuring its latency [1,2]. Anti-TNF therapy increases the risks of contracting tuberculosis or reactivating a latent tuberculosis infection [3]. However, TNF can also participate in many negative tuberculosis manifestations, as well as directly mediate *M. tuberculosis* proliferation in human monocytes and potentiate pathogen cytotoxicity. Excessive TNF can contribute to tissue damage and the necrosis of tuberculosis lesions, thus leading to organ dysfunction [1]. Thus, although TNF is responsible for necessary immune responses to *M. tuberculosis* infection, excessive TNF production can cause severe tissue damage and various disease symptoms [1]. Some prior reports have shown evidence of an elevated serum TNF level in patients with active pulmonary tuberculosis (TB) [4]. However, studies demonstrating no significant differences between patients with TB and healthy individuals have also been published [5].

Soluble type 1 (sTNFR1, p55) and 2 (sTNFR2, p75) TNF receptors are identical to the extracellular cytokine-binding domains of membrane TNF receptors; they can compete with membrane-associated TNF receptors for binding TNF and thus inhibit TNF bioactivity [6]. Human anti-cytokine autoantibodies (ACAABs) are polyclonal and belong mainly to the IgG class [7]. Their binding avidities range from 1×10^{-4} to 5×10^{-11} M, with average values of 1×10^{-6} to 5×10^{-7} M [8]. The affinity of cytokine binding to their corresponding autoantibodies [7] is comparable to that of cytokine receptors [9], indicating that autoantibodies effectively compete for ligand-binding sites, thereby inhibiting cytokine binding to receptors and subsequent signal transduction. ACAABs are present in various organs and tissues, including blood, during both healthy and pathological states [10]. In some cases, ACAABs are associated with disease pathogenesis or predisposition to particular pathologies [11–14] and cause the manifestation of disease symptoms [15–18]. In addition to inhibiting specific cytokine functions, autoantibodies can play an important role in health and disease owing to their ability to form immune complexes [11,19,20]. Thus, autoantibodies against certain cytokines can be detected in both healthy individuals and persons with various pathologies.

So, considering prominent role of tumor necrosis factor in protective immunity against *M. tuberculosis*, its involvement in negative manifestations of pulmonary tuberculosis and important role of anti-cytokine autoantibodies in modulating biological activity of corresponding mediators and in disease pathogenesis in a number of nosologies, the aim of this study was to obtain new data on the levels of various classes and subclasses of anti-TNF autoantibodies in patients with TB and healthy individuals.

2. Materials and Methods

2.1 Study Participants

The group of patients with TB included 45 individuals who were hospitalized in the First Therapy department of Novosibirsk Research Institute of Tuberculosis. The diagnosis is formed on the basis of complaints, anamnesis, physical examination, sputum culture for the presence of Mycobacterium tuberculosis in liquid and solid media, Xpert MTB/RIF test, positive skin test, X-ray studies and the dynamics of the process against the background of anti-tuberculosis therapy. HIV infection was the exclusion criterion for the study. Several patients were diagnosed with hepatitis B and C, while they had no clinical or laboratory manifestations. Most patients were receiving anti-tuberculous therapy, but instead of whether were patients receiving therapy at the time of blood collection or not, all of them had active pulmonary tuberculosis. There were no patients with miliary tuberculosis in the study. None of the patients included in the study received steroid therapy.

Blood samples were obtained from 150 healthy donors at Novosibirsk Blood Center. Blood sampling of patients with TB and healthy individuals was performed after obtaining their informed consent.

The study protocol was approved by the local ethics committee at the Research Institute of Fundamental and Clinical Immunology.

2.2 Determination of the Serum Levels of TNF and of Its Soluble Type 1 and 2 Receptors

To obtain serum samples, 4 mL of venous blood per subject was collected into vacuum tubes with Vacuette Z Serum Clot Activator (Greiner Bio-One GmbH, Kremsmunster, Austria), decanted for 1 h, and centrifuged at 2.500 rpm for 20 min. Serum was then isolated and stored at –20 °C until use in subsequent experiments. The serum levels of TNF and of its soluble type 1 and 2 receptors were determined by conducting solid-phase enzyme-linked immunosorbent assays (ELISAs) using the following respective commercially available kits in accordance with each manufacturers' instructions: Alpha-TNF-ELISA-BEST (Vector-Best, Novosibirsk, Russia), Human TNF RI ELISA Kit, and Human TNF RII ELISA Kit (Ray-Biotech Inc., Peachtree Corners, GA, USA). The optical density of stained solutions in the plate wells was measured with an Anthos 2020 microplate reader (Anthos Labtec Instruments GmbH, Salzburg, Austria) at a wavelength of 450 nm, using a 620 nm reference wavelength.

2.3 Assessment of the Levels of Anti-TNF Autoantibody Classes and Subclasses

Serum titers of anti-TNF autoantibodies were evaluated by conducting solid-phase ELISAs using recombinant human TNF (Institute of Medical Biotechnology, The State Research Center of Virology and Biotechnology VECTOR, Berdsk, Russia) as described in our previous study [16]. Antibodies against immunoglobulin classes and subclasses, each conjugated with horseradish peroxidase, (Bialexa, Moscow, Russia) were diluted as follows: 1:499 for anti-IgM (clone 1H9c); 1:1.599 for anti-IgG (clone 2M9c); 1:499 for anti-IgA (clone 1A9c); 1:99 for anti-IgG1 (clone 2C11c); and 1:24 for anti-IgG2 (clone 52G1), anti-IgG3 (clone 5G12c), and anti-IgG4 (clone 5C7c).

2.4 Statistical Data Analysis

Statistical data analysis was performed using Graph-Pad Prism 6 software (GraphPad Software, San Diego, CA, USA). Independent samples were tested for statistical significance using non-parametric Mann-Whitney test. Data are expressed as medians and interquartile ranges. We evaluated correlation among parameters with the Spearman test. Differences with $p < 0.05$ were considered statistically significant.

3. Results

3.1 Levels of TNF and of Its Soluble Type 1 and 2 Receptors

The groups of patients with TB and healthy donors had comparable compositions, in terms of age and sex. The study group characteristics are presented in Table 1.

Table 1. The groups of patients with active pulmonary tuberculosis and healthy donors.

Characteristics of the patients with TB and the healthy individuals groups	Patients with TB (n = 45), n (%)	Healthy donors (n = 150), n (%)	*p*-value
Sex, M/F	27/18 (60/40)	83/67 (55.3/44.7)	0.611
Age, median [IQR]	31 [25–44]	30.5 [23–45]	0.512
Disseminated infection	26 (57.8)	-	-
MBT+	9 (34.6)	-	-
MBT−	17 (65.4)	-	-
Localized infection	19 (42.2)	-	-
MBT+	6 (31.6)	-	-
MBT−	13 (68.4)	-	-

MBT+: patients with TB, from whom *M. tuberculosis* was isolated; MBT−: patients with TB, from whom *M. tuberculosis* was not isolated; Localized infection: patients with TB with infection occupying one or two segments of lungs; Disseminated infection: patients with TB with infection occupying more than two segments of lungs.

The serum levels of TNF and of its soluble type 1 and 2 receptors in patients with TB and healthy individuals were assessed by ELISA. There was no difference in the TNF level between the patients with TB and the healthy individuals (Fig. 1A). A comparison of the serum level of TNF among patients with TB revealed higher TNF level in patients with disseminated infection (infection occupying more than two segments of lungs) than in patients who had a localized infection (infection occupying one or two segments of lungs) [21] (Fig. 1B) and in patients with disseminated infection from whom pathogen was isolated on bronchoscopy (the group of patients with most severe disease) than in other patients (Fig. 1C).
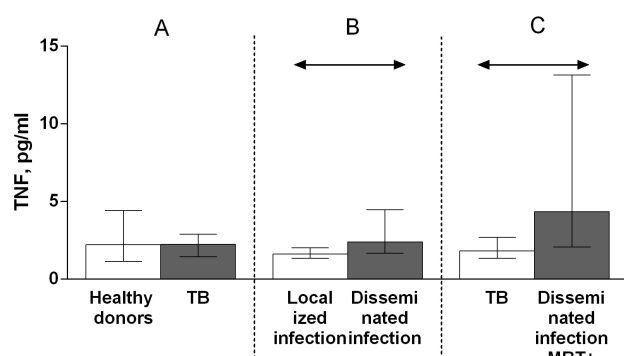

Fig. 1. Serum levels of TNF in patients with TB and healthy donors (A), patients with TB who had localized or disseminated infection (B) and in MBT+ on bronchoscopy patients with disseminated tuberculosis and other patients with TB (C). Data are presented as the median (interquartile range). Double-arrow bar indicate statistical significance. Mann–Whitney test, $p < 0.05$. MBT+ : patients with TB, from whom *M. tuberculosis* was isolated; MBT–: patients with TB, from whom *M. tuberculosis* was not isolated; Localized infection: patients with TB with infection occupying one or two segments of lungs; Disseminated infection: patients with TB with infection occupying more than two segments of lungs.

The serum levels of both types of soluble TNF receptors were lower in patients with TB than in healthy donors (Fig. 2A). There were no differences in sTNFR levels between patients with TB with disseminated and localized infection (Fig. 2B), at the same time sTNFR1 levels in patients with TB, from whom *M. tuberculosis* was isolated (MBT+) were higher in comparison with patients with TB, from whom *M. tuberculosis* was not isolated (MBT–) (Fig. 2C), sTNFR1 and sTNFR2 serum levels were higher in MBT+ on bronchoscopy patients with disseminated infection than in other patients with TB (Fig. 2D).

3.2 Titers of Anti-TNF Autoantibody Classes and Subclasses in Patients with TB and Healthy Donors

Serum titers of anti-TNF autoantibodies belonging to classes IgA, IgG, and IgM and subclasses IgG1, IgG2,

IgG3, and IgG4 in patients with TB and healthy donors were assessed by performing ELISAs. The measured titers are expressed here in optical density international units.

The IgG autoantibody titer was significantly higher in patients with TB than in healthy donors (Fig. 3A). There were no significant differences in the IgM (Fig. 4A) and IgA (Fig. 5A) autoantibody titers between patients with TB and healthy individuals.

Regarding anti-TNF autoantibody subclass, the IgG3 autoantibody titer was higher in patients with TB compared with that in healthy donors (Fig. 6A), whereas there were no significant differences in the titers of IgG1 (Fig. 7A), IgG2 (Fig. 8A), and IgG4 (Fig. 9A) autoantibodies between patients with TB and healthy individuals.

A comparison of the serum titers of anti-TNF autoantibody classes and subclasses among patients with TB revealed higher IgG (Fig. 4B), IgG1 (Fig. 6B) and IgG3 (Fig. 8B) autoantibody titers in patients with disseminated infection than in patients who had a localized infection, and higher IgG (Fig. 4C), IgA (Fig. 5C) and IgG1 (Fig. 6C) in patients with disseminated infection from whom M. tuberculosis was isolated on bronchoscopy than in other patients.

In patients with TB, positive correlations were observed between the titers of anti-TNF autoantibodies of classes IgG and IgA and between those of subclasses IgG1 and IgG3, as well as between the titers of these class and subclass groups. Among healthy individuals, positive correlations were noted between the titers of anti-TNF autoantibodies of classes IgG and IgA, those of classes IgG or IgA and the subclass IgG3, and between the groups of subclasses IgG1 and IgG4, and IgG2 and IgG4.

4. Discussion

TNF plays a key role in host defense against *M. tuberculosis*. However, it can also enhance the pathogenic effect of this disease and is associated with a number of negative manifestations of TB infection. In the literature, there are conflicting reports regarding differences in serum TNF levels between patients with TB and healthy donors; some studies have found an elevated serum TNF level in patients with TB [4], whereas others have observed no significant difference in the serum TNF titer between patients with TB and healthy donors [5]. Notably, the TNF level can decrease during anti-tuberculosis therapy [4]. One previous study evaluated the TNF production of peripheral blood mononuclear cells from patients with TB or healthy donors; mononuclear cells from patients with infiltrative and fibrous cavernous TB were shown to produce less TNF than those from healthy donors. Furthermore, although the levels of purified protein derivate-stimulated TNF production by these cells from patients with infiltrative TB did not differ from that of cells from healthy individuals, these levels were higher in cells from patients with fibrous cavernous TB than in those from healthy donors [22]. There is no generally accepted classification of the severity of pulmonary
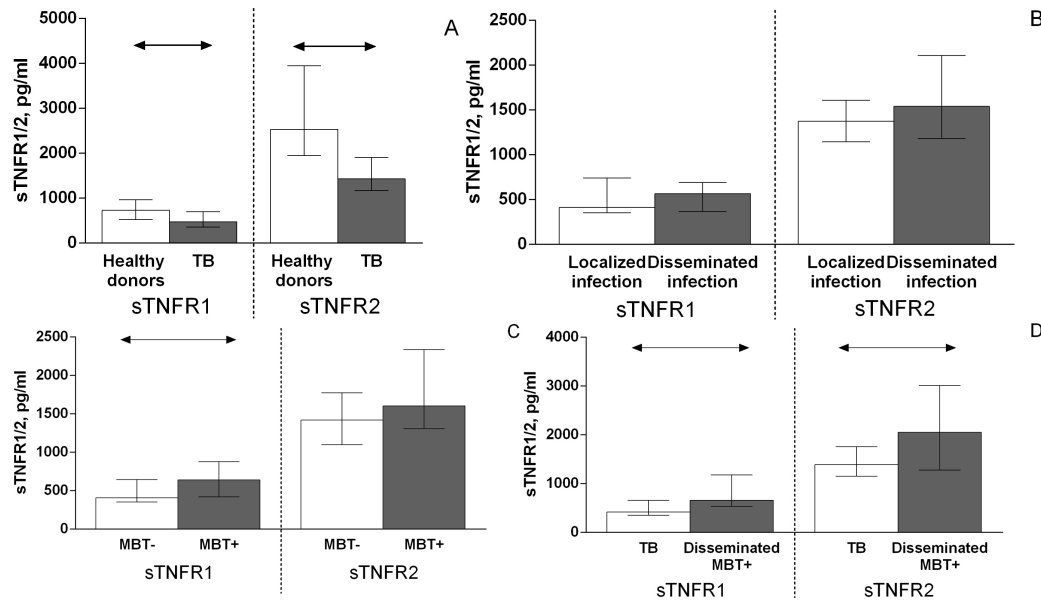
Fig. 2. Serum levels of TNF soluble type 1 and 2 receptors in patients with TB and healthy donors (A), patients with TB who had localized or disseminated infection (B), in patients with TB from whom Mycobacterium tuberculosis was (MBT+) or was not (MBT–) isolated (C) and in MBT+ on bronchoscopy patients with disseminated tuberculosis and other patients with TB (D). Double-arrow bar indicate statistical significance. Data are presented as the median (interquartile range). Mann–Whitney test, $p < 0.05$. MBT+: patients with TB, from whom *M. tuberculosis* was isolated; MBT–: patients with TB, from whom *M. tuberculosis* was not isolated; Localized infection: patients with TB with infection occupying one or two segments of lungs; Disseminated infection: patients with TB with infection occupying more than two segments of lungs.
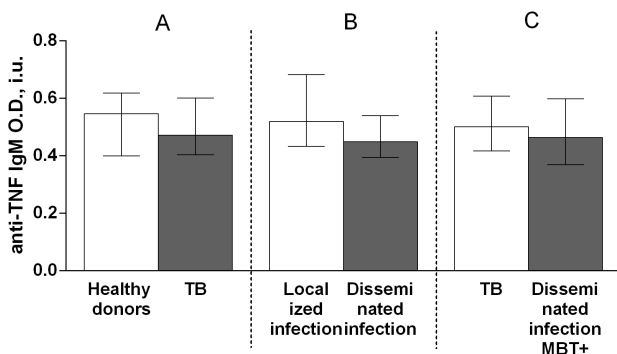

Fig. 3. Serum levels of anti-TNF IgG autoantibodies in patients with TB and healthy donors (A), patients with TB who had localized or disseminated infection (B) and in MBT+ on bronchoscopy patients with disseminated tuberculosis and other patients with TB (C). Levels anti-TNF IgG autoantibodies are expressed in international units of optical density (O.D., i.u.). Double-arrow bar indicate statistical significance. Data are presented as the median (interquartile range). Mann–Whitney test, $p < 0.05$. MBT+: patients with TB, from whom *M. tuberculosis* was isolated; MBT–: patients with TB, from whom *M. tuberculosis* was not isolated; Localized infection: patients with TB with infection occupying one or two segments of lungs; Disseminated infection: patients with TB with infection occupying more than two segments of lungs.
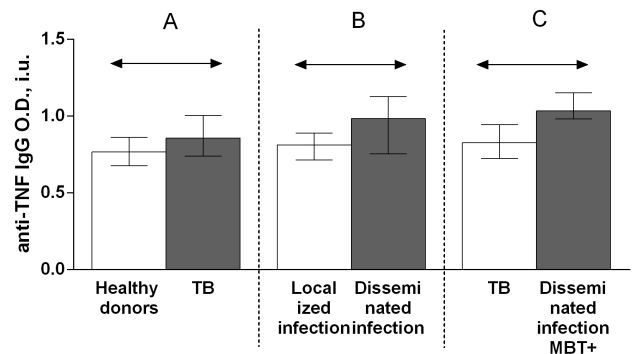

Fig. 4. Serum levels of anti-TNF IgM autoantibodies in patients with TB and healthy donors (A), patients with TB who had localized or disseminated infection (B) and in MBT+ on bronchoscopy patients with disseminated tuberculosis and other patients with TB (C). Levels anti-TNF IgM autoantibodies are expressed in international units of optical density (O.D., i.u.). Double-arrow bar indicate statistical significance. Data are presented as the median (interquartile range). Mann–Whitney test, $p < 0.05$. MBT+: patients with TB, from whom *M. tuberculosis* was isolated; MBT–: patients with TB, from whom *M. tuberculosis* was not isolated; Localized infection: patients with TB with infection occupying one or two segments of lungs; Disseminated infection: patients with TB with infection occupying more than two segments of lungs.
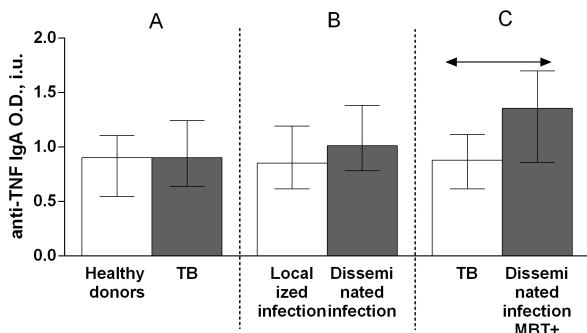
Fig. 5. Serum levels of anti-TNF IgA autoantibodies in patients with TB and healthy donors (A), patients with TB who had localized or disseminated infection (B) and in MBT+ on bronchoscopy patients with disseminated tuberculosis and other patients with TB (C). Levels anti-TNF IgA autoantibodies are expressed in international units of optical density (O.D., i.u.). Double-arrow bar indicate statistical significance. Data are presented as the median (interquartile range). Mann–Whitney test, $p < 0.05$. MBT+: patients with TB, from whom *M. tuberculosis* was isolated; MBT–: patients with TB, from whom *M. tuberculosis* was not isolated; Localized infection: patients with TB with infection occupying one or two segments of lungs; Disseminated infection: patients with TB with infection occupying more than two segments of lungs.
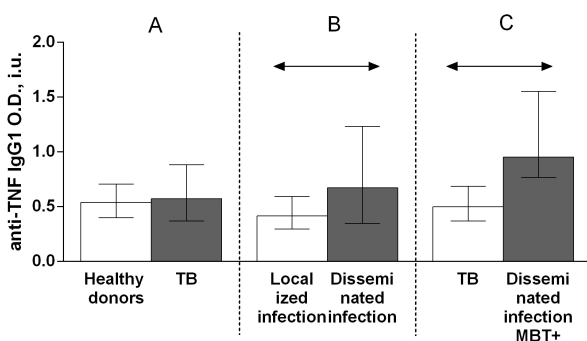

Fig. 7. Serum levels of anti-TNF IgG1 autoantibodies in patients with TB and healthy donors (A), patients with TB who had localized or disseminated infection (B) and in MBT+ on bronchoscopy patients with disseminated tuberculosis and other patients with TB (C). Levels anti-TNF IgG1 autoantibodies are expressed in international units of optical density (O.D., i.u.). Double-arrow bar indicate statistical significance. Data are presented as the median (interquartile range). Mann–Whitney test, $p < 0.05$. MBT+: patients with TB, from whom *M. tuberculosis* was isolated; MBT–: patients with TB, from whom *M. tuberculosis* was not isolated; Localized infection: patients with TB with infection occupying one or two segments of lungs; Disseminated infection: patients with TB with infection occupying more than two segments of lungs.
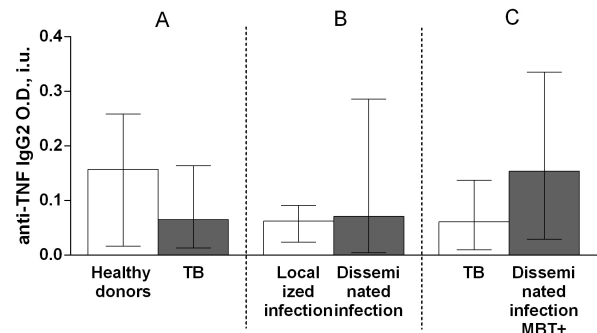

Fig. 6. Serum levels of anti-TNF IgG3 autoantibodies in patients with TB and healthy donors (A), patients with TB who had localized or disseminated infection (B) and in MBT+ on bronchoscopy patients with disseminated tuberculosis and other patients with TB (C). Levels anti-TNF IgG3 autoantibodies are expressed in international units of optical density (O.D., i.u.). Double-arrow bar indicate statistical significance. Data are presented as the median (interquartile range). Mann–Whitney test, $p < 0.05$. MBT+: patients with TB, from whom *M. tuberculosis* was isolated; MBT–: patients with TB, from whom *M. tuberculosis* was not isolated; Localized infection: patients with TB with infection occupying one or two segments of lungs; Disseminated infection: patients with TB with infection occupying more than two segments of lungs.
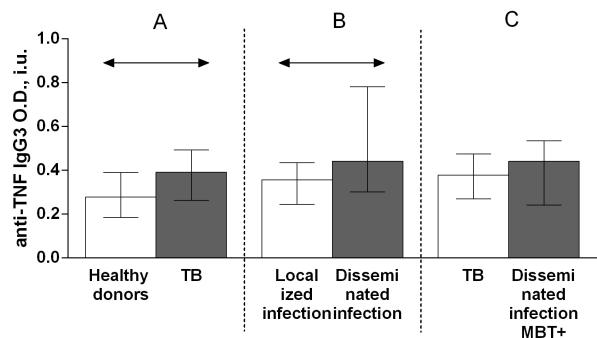

Fig. 8. Serum levels of anti-TNF IgG2 autoantibodies in patients with TB and healthy donors (A), patients with TB who had localized or disseminated infection (B) and in MBT+ on bronchoscopy patients with disseminated tuberculosis and other patients with TB (C). Levels anti-TNF IgG2 autoantibodies are expressed in international units of optical density (O.D., i.u.). Double-arrow bar indicate statistical significance. Data are presented as the median (interquartile range). Mann–Whitney test, $p < 0.05$. MBT+: patients with TB, from whom *M. tuberculosis* was isolated; MBT–: patients with TB, from whom *M. tuberculosis* was not isolated; Localized infection: patients with TB with infection occupying one or two segments of lungs; Disseminated infection: patients with TB with infection occupying more than two segments of lungs.
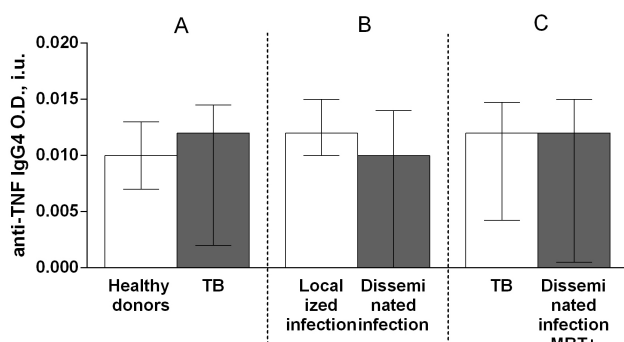
Fig. 9. Serum levels of anti-TNF IgG4 autoantibodies in patients with TB and healthy donors (A), patients with TB who had localized or disseminated infection (B) and in MBT+ on bronchoscopy patients with disseminated tuberculosis and other patients with TB (C). Levels anti-TNF IgG4 autoantibodies are expressed in international units of optical density (O.D., i.u.). Double-arrow bar indicate statistical significance. Data are presented as the median (interquartile range). Mann–Whitney test, $p < 0.05$. MBT+: patients with TB, from whom *M. tuberculosis* was isolated; MBT–: patients with TB, from whom *M. tuberculosis* was not isolated; Localized infection: patients with TB with infection occupying one or two segments of lungs; Disseminated infection: patients with TB with infection occupying more than two segments of lungs.

tuberculosis. In Russia, a classification is used according to the volume of lung damage: up to two segments inclusive — a limited process, more than two segments — a disseminated process [21]. A group of patients with disseminated infection from whom pathogen was isolated on bronchoscopy is the group with most severe disease because it is characterized by disseminated destructive process with cavitations [21]. In the present study, we did not observe significant differences in the TNF level between patients with TB and healthy individuals (Fig. 1A). However, we have found elevated level of tumor necrosis factor in patients characterized by disseminated infection compared with patients with localized infection (Fig. 1B) and in the group of patients with most severe disease with disseminated infection from whom *M. tuberculosis* was isolated on bronchoscopy than in other patients (Fig. 1C). These differences reflect the lesion volume of the lung tissue and the severity of infectious process during pulmonary tuberculosis, in which TNF plays key roles in the immune and pathological responses and mediator production is stimulated by multiple mechanisms [1,2].

Soluble receptors can compete with membrane-bound receptors for TNF binding and consequently inhibit TNF bioactivity [6]; however, the functional interactions between TNF and its soluble receptors are complex. The addition of sTNFR1 and sTNFR2 to trimeric TNF leads to the formation of complexes between them, stabilizing the active TNF form [6]. TNF can dissociate from this complex

and replace decayed TNF at the periphery, thus maintaining a constant concentration of unbound bioactive trimeric cytokine. This process seems to occur within TB lesions, as evidenced by the increased levels of TNF and of both types of its soluble receptors in bronchoalveolar fluid from patients with TB [23]. Factors sTNFR1 and sTNFR2 can act as TNF antagonists, TNF-carrier proteins, slow-release reservoirs for TNF, stabilizers of TNF bioactivity capable of increasing the cytokine half-life, and a TNF buffer by inhibiting the effect of high TNF concentrations and providing the effect of low and well-controlled cytokine levels on cells [6]. Thus, soluble TNF receptors affect local and systemic TNF bioavailability [6].

One previous study reported that patients with TB who have an elevated serum TNF level exhibit high levels of its soluble type 1 and 2 receptors [23]. However, we found that serum levels of soluble type 1 and 2 TNF receptors in patients with TB were lower compared with those in healthy donors (Fig. 2A). This difference is probably due to the lack of an increased systemic level of the cytokine itself in the serum of patients with TB. Similar to tumor necrosis factor level, sTNFR1 and sTNFR2 levels are elevated in the group of patients with disseminated infection from whom *M. tuberculosis* was isolated on bronchoscopy than compared other patients (Fig. 2D). This is, obviously, due to the regulatory mechanism, by which high levels of soluble TNF receptors are produced to neutralize cytokine excess and maintain a constant concentration of unbound bioactive trimeric cytokine and to reduce tissue damage.

The study of autoantibodies as molecules reacting with the body's own antigens is an important area of research in modern molecular immunology [24]. ACAAB induction is one of the mechanisms of regulating cytokines in health and pathology. The reason why autoantibodies lead or do not lead to the development of pathology is not completely clear. The main point of view is that if the antibody has sufficient affinity and its content is above a certain threshold concentration, the autoantibody can neutralize the biological activity of the cytokine by binding to it and preventing its interaction with its receptor on the cell. However, a number of studies have shown the ability of different antibodies to bind to the same molecule of toxins [25,26] or cytokines [27,28], which suggests that in some cases neutralization depends on the production of antibodies to several antigenic sites, which leads to the formation of immune complexes that can be effectively eliminated *in vivo* [27,28]. Moreover, monoclonal antibodies to interleukine (IL)-2, which neutralize the effects of a cytokine *in vitro*, can enhance and prolong the biological activity of IL-2 when administered *in vivo* [19]. Furthermore, there are data on different biological activity of IgG isolated from the blood serum of patients with various autoimmune diseases and healthy donors. It has been shown that IgG isolated from the blood serum of patients with different autoimmune diseases and healthy individuals has

a different effect on the biological activity of granulocyte colony-stimulating factor, in addition, in patients with one nosology, the effect of IgG obtained from individual patients may also differ [13]. In another work, incubation of mononuclear cells from healthy donors, thyroid peroxidase, and blood sera of patients with Hashimoto's thyroiditis and healthy donors was carried out. Wherein, patients had different levels of anti-thyroid peroxidase autoantibodies. It has been shown that when incubated with serum containing high levels of autoantibodies to peroxidase an increased production of TNF, IL-6 and IFN-γ by mononuclear cells is observed, and with serum with low autoantibodies content IL-10 production is detected [29]. However, anti-granulocyte-macrophage colony-stimulating factor autoantibodies purified from blood serum of patients with pulmonary alveolar proteinosis and healthy individuals posess the same biological activity [11,30]. Currently, there are only a few publications on the study of anti-TNF autoantibodies. Anti-TNF autoantibodies have been detected in the serum of healthy donors, patients with Gram-negative bacterial septicemia, and individuals with cystic fibrosis in the presence of chronic pulmonary infection, as well as in the serum of patients with various autoimmune diseases [15,31–35]. A protective effect of anti-TNF autoantibodies on the course of systemic lupus erythematosus and rheumatoid arthritis has also been described [15,34]. *In vitro* experiments demonstrated the ability of anti-TNF autoantibodies isolated from the serum of rheumatoid arthritis patients to neutralize the cytotoxic effect of TNF [32]. We have previously reported an elevated serum level of anti-TNF autoantibodies, belonging to subclasses IgG2, IgG3, and IgG4, in patients with acute rheumatoid arthritis and in individuals with an uncontrolled course of bronchial asthma, as well as an elevated serum level of subclass IgG1 autoantibodies in patients with a controlled course of bronchial asthma in comparison with healthy donors. Lower titers of anti-TNF IgG2 and IgG4 autoantibodies were reported in patients with rheumatoid arthritis in response to therapy, while lower levels of these autoantibodies, along with a significantly elevated titer of IgG1 autoantibodies, were found in patients with bronchial asthma during the transition from an uncontrolled to a controlled disease course [16–18].

The presence of high-affinity IgG autoantibodies against IFN-γ, a crucial immunoregulatory cytokine, which along with TNF is critical for protective immune responses in TB [12], has been observed in cases of *M. tuberculosis* infection. Significantly elevated titers of autoantibodies against TNF, IL-17A, and IL-31, as well as against some other autoantigens, were noted in the pleural fluid of patients with TB as compared with patients with lung cancer [36].

Herein, to determine the possible contribution of anti-TNF autoantibodies to the pathogenesis of TB, serum titers of anti-TNF autoantibodies belonging to classes IgA, IgG, or IgM and subclasses IgG1, IgG2, IgG3, or IgG4, as well

as the levels of TNF and of its soluble receptors, were assessed in both patients with TB and healthy donors. These autoantibodies were detected in serum from both patients with TB and healthy individuals. The IgG autoantibody titer was higher in patients with TB than in healthy donors (Fig. 4A), but no differences were observed in the titers of IgM (Fig. 3A) and IgA (Fig. 5A) autoantibodies between patients with TB and healthy individuals. Furthermore, the level of IgG3 autoantibodies was higher in patients with TB compared with healthy donors (Fig. 8A), whereas the titers of IgG1 (Fig. 6A), IgG2 (Fig. 7A), and IgG4 (Fig. 9A) autoantibodies in patients with TB did not differ from those in healthy individuals.

A comparison of the anti-TNF autoantibody titers among patients with TB revealed higher IgG (Fig. 4B), IgG1 (Fig. 6B) and IgG3 (Fig. 8B) titers in patients who had a disseminated infection compared with individuals who had a localized infection, as well as IgG (Fig. 4C), IgA (Fig. 5C) and IgG1 (Fig. 6C) in MBT+ on bronchoscopy patients with disseminated infection than in other patients with TB. These data point on a possible significant role for autoantibodies belonging to the IgG class, particularly those belonging to the IgG1 or IgG3 subclasses, in immune responses to TB. Various research groups have previously shown that specific autoantibodies in autoimmune disease patients are often composed mainly of antibodies belonging to the IgG1 or IgG3 subclass [37].

The observed higher levels of IgG (Fig. 4A) and IgG3 (Fig. 8A) anti-TNF autoantibodies in patients with TB compared with healthy donors, of IgG (Fig. 4B), IgG1 (Fig. 6B) and IgG3 (Fig. 8B) anti-TNF autoantibodies in patients with a disseminated infection compared with patients with a localized manifestation of TB and IgG (Fig. 4C) and IgG1 (Fig. 6C) anti-TNF autoantibodies in MBT+ on bronchoscopy patients with disseminated infection suggest an active involvement of anti-TNF autoantibodies in complement system activation. IgG1 and IgG3 immunoglobulins have the strongest ability to interact with the C1q complement component, while IgG2 is capable of weak binding, and IgG4 is unable to bind C1q [37].

Elevated IgG class (Fig. 4A) and IgG3 (Fig. 8A) subclass of anti-TNF autoantibody titers in patients with TB, as well as higher IgG (Fig. 4B), IgG1 (Fig. 6B) and IgG3 (Fig. 8B) anti-TNF autoantibody titers in individuals with a disseminated infection, and IgG (Fig. 4C) and IgG1 (Fig. 6C) anti-TNF autoantibody titers in the group of patients with most severe disease with disseminated infection from whom pathogen was isolated on bronchoscopy may indicate the damaging effect of these antibodies in pathology [20]. This effect might be caused by enhanced TNF action via the formation of a complex with TNF that increases the expression of pro-inflammatory mediators through an Fc-receptor-dependent mechanism, as is the case with anti-IL-8 autoantibodies during acute respiratory distress syndrome [20]. Moreover, some anti-cytokine antibodies can

enhance the bioactivity and increase the half-life of cytokines in vivo, acting as a reservoir of cytokines (carrier proteins) [38,39]. It has been shown in a mouse model that the administration of a cytokine with the corresponding monoclonal antibody in a low molar ratio (cytokine:mAb = 2:1) leads to an increase in the biological activity of the mediator and an increase in the half-life [38]. In a number of studies, a high level of antibodies enhances the function of the corresponding cytokines. For example, mAb to IL-3 or IL-4 increase the stimulation of proliferation of mouse mucosal mast cells [39]. However, to clarify the likely damaging role of anti-TNF autoantibodies in the pathogenesis of pulmonary tuberculosis, it is necessary to conduct tests to determine the functional activity of such antibodies and their influence on the biological effects of tumor necrosis factor.

5. Conclusions

In conclusion, our findings on the anti-TNF autoantibody titers in patients with TB and healthy donors suggest that these autoantibodies may play an important role in the immunopathogenesis of pulmonary tuberculosis.

Abbreviations

ACAAB, anti-cytokine autoantibody; ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; IFN, interferon; IL, interleukine; TB, active pulmonary tuberculosis; TNF, tumor necrosis factor.

Author Contributions

FDK, JAL, ZAL and SVS contributed to the conception, design, analysis and data interpretation, and drafting of the manuscript; FDK and JAL contributed to experimental work; ZAL performed patient recruiting; SVS contributed to revision and final approval of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The study protocol was approved by the local ethics committee at the Research Institute of Fundamental and Clinical Immunology.

Conflict of Interest

The authors declare no conflict of interest. SVS is serving as one of the Editorial Board members of this journal. We declare that SVS had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to HY.

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