

MECHANISMS

Levels of TNF, TNF autoantibodies and soluble TNF receptors in patients with bronchial asthmaElena Alexeevna Golikova¹, Julia Anatolievna Lopatnikova¹, Tatiana Vladimirovna Kovalevskaya-Kucheryavenko², Vera Makarovna Nepomnyashih², and Sergey Vitalievich Sennikov¹¹Laboratory of Molecular Immunology, Federal State Budgetary Institution "Research Institute of Clinical Immunology", Russian Academy of Medical Sciences Siberian Branch, Novosibirsk, Russia and ²Department of Allergology, Clinic of immunopathology, Federal State Budgetary Institution "Research Institute of Clinical Immunology", Russian Academy of Medical Sciences Siberian Branch, Novosibirsk, Russia**Abstract**

Objectives: The aim of the study was to evaluate the potential contribution made by tumor necrosis factor (TNF) autoantibodies to the pathogenesis of bronchial asthma (BA). **Methods:** We used affinity chromatography methods and a magnetic separation procedure to purify human autoantibodies specific to TNF. The autoantibodies were used as a calibration material to determine the absolute content of autoantibodies to TNF using enzyme-linked immunosorbent assay (ELISA). TNF content and levels of soluble receptors to TNF were determined using the ELISA commercial test kits. **Results:** We demonstrated significant increases in the levels of TNF and soluble TNF receptors in the sera of patients with uncontrolled and controlled BA, as compared with healthy donors. Levels of autoantibodies of the IgG2 and IgG4 subclasses were significantly higher in sera from patients with uncontrolled BA than in healthy donors. Levels of IgG2 autoantibodies were significantly higher in sera from patients with uncontrolled BA than in patients with controlled BA. **Conclusions:** BA is associated with changes in the levels of not only TNF and soluble receptors for TNF, but also autoantibodies to TNF. Given the magnitude of the changes in the levels of different subclasses of autoantibodies to TNF, we propose that these autoantibodies might contribute to the pathogenesis of BA.

Keywords

Autoantibodies, bronchial asthma, chromatography, tumor necrosis factor

History

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Introduction

Tumor necrosis factor (TNF) is one of the most important immunoregulatory cytokines and possesses a wide spectrum of actions. TNF is found in the serum of healthy individuals at the minimum detectable levels, but its level increases in various diseases [1] and this increase might be connected with disease severity. For example, TNF plays an important part in determining the progress of bronchial asthma (BA) [2].

The mechanism of action of TNF in BA remains unclear, but it is thought to act directly on the smooth muscle of the respiratory tract [3] or to affect the release of the cysteinyl leukotrienes LTC₄ and LTD₄ [4]. TNF directly induces the release of histamine from human mast cells [5] and participates in positive autocrine regulation by increasing the secretion of cytokines from human mast cells [6]. In consequence, it is possible that TNF participates in the interaction of mast cells with smooth muscles and, more importantly, in the development of hypersensitivity of the respiratory tract.

TNF exerts several other effects that might be involved in the pathogenesis of asthma. TNF is a chemoattractant for neutrophils and eosinophils; it can enhance the cytotoxic effect of eosinophils on endothelial cells. It is also involved in T-cell activation and induces T cells to produce cytokines. TNF augments the epithelial expression of adhesion molecules, such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, which play an important role in the migration of T cells to the lungs and the subsequent development of hypersensitivity of the respiratory tract [2].

It has been shown that inhalation of recombinant TNF by healthy persons result in the development of hypersensitivity and neutrophilia of the respiratory tract [7]. Treatment of asthma patients with TNF also enhances respiratory tract hypersensitivity [8].

Owing to the significant role of TNF, its biological activity is subject to complex regulation. The control involves membrane-bound TNF receptors (TNFRs), which transduce the cytokine signal to the cell, and soluble receptors (sTNFRs), which restrict the effects of TNF in the circulation [9,10]. In BA, it has been shown that the serum level of sTNFRs increases [11], which might be evidence for the participation of these receptors in the pathogenesis of BA as factors that restrict the biological activity of TNF.

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Autoantibodies to TNF might also play an important role in the regulation of TNF activity [12]. Currently, autoantibodies to immunoregulatory cytokines are recognized by researchers as important effector molecules that can regulate the immune response. In recent years, autoantibodies that are specific to interferons (INF- α , β and γ), interleukins (IL-1 α –6, 8–10 and 12), growth factors (granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, leukaemia inhibitory factor, oncostatin M and vascular endothelial growth factor) and TNF have been discovered [13–15]. It is thought that autoantibodies fulfil several essential biological functions. These include the neutralization of cytokines secreted from tissues, the regulation of the biological activities of cytokines to prevent systemic reactions and cytokine deposition through the formation of immune complexes, and prolonging the persistence of cytokines in the systemic circulation in a similar manner to that attributed to soluble receptors [16]. Despite the fact that the biological roles of autoantibodies have not yet been fully identified, an increasing amount of data have indicated their participation in the pathogenesis of various diseases.

To determine the possible contribution of autoantibodies to TNF in the pathogenesis of BA, we determined the level of TNF autoantibodies in patients with BA and healthy donors simultaneously, and estimated the levels of the mediator itself and its soluble receptors.

Materials and methods

Donors

The group of patients with BA comprised 15 persons who had been hospitalised in the clinic of Novosibirsk Research Institute of Clinical Immunology, Siberian Branch of the Russian Academy of Medical Sciences. The diagnosis of BA was verified in accordance with the 2008 the Global Initiative for Asthma GINA criteria. Blood samples were taken from each patient before treatment of BA and from 11 patients in whom the disease had been controlled by symptomatic treatment. Patients were referred to uncontrolled BA group based on the listed symptoms. BA became controlled for all patients after the in-hospital recrudescence cure. The following factors confirmed controlled course of BA: the absence of day and night asthma symptoms and restrictions in daily activities, normal or near-normal lung function and the absence of BA exacerbations. According to spirometry, values of FEV1 measured in the group of patients with acute BA after course of therapy were >80% higher than predicted values [17]. Based on these parameters, patients were added to the group of controlled BA and were sampled the peripheral blood.

Human peripheral blood from 24 healthy donors was obtained from the Novosibirsk Center of Blood. The status of the donors was determined through a questionnaire and the latex test for CRP (LLC Olvex diagnosticum, Russia). The individuals with serum CRP concentration <6 mg/mL were included as donors.

The control group was staffed with focus on sex and mean age to best correlate with the experimental group. The experimental group has an average age of 36.2 years and is split 40% men to 60% women. The control group has an average age of 34 years and is split 45% men and 55% women.

Reagents and materials

Tris(hydroxymethyl)aminomethane, 2-mercaptoethanol, sodium azide and cellulose dialysis membranes were purchased from Sigma-Aldrich (Sigma-Aldrich, St.Louis, MO, USA). Glycine was obtained from Merck (Merck, Whitehouse Station, NJ, USA). Triton X-100 was supplied by GERBU (Germany). Sodium dodecyl sulfate, ammonium persulfate, N,N,N',N'-tetramethylethylenediamine and Coomassie brilliant blue R 250 were purchased from Serva (Serva, Heidelberg, Germany). Acrylamide and guanidine hydrochloride were supplied by Fluka (Fluka, St.Louis, MO, USA). Ethanol, glacial acetic acid, hydrochloric acid, sodium chloride, potassium dihydrogen phosphate, sodium hydrogen phosphate dodecahydrate and bromophenol blue were obtained from Reahim (Reahim, Moscow, Russia). The molecular weight standard proteins for electrophoresis were Broad Range SDS-PAGE Molecular Standards from Bio-Rad (Bio-Rad, Hercules, CA, USA).

We used the following chromatographic sorbents: Bio-Gel P-6 DG (Bio-Rad, USA), Protein G Sepharose 4 Fast Flow (GE Healthcare, Pittsburgh, PA, USA) and Affi-Gel 15 (Bio-Rad, USA).

Recombinant human TNF was provided by the Center for Immunological Engineering (Russia). Polyclonal rabbit antibodies to TNF were provided by FBUN State Scientific Center for Virology and Biotechnology Vector (Russia).

Isolation of Immunoglobulin G (IgG) autoantibodies from human serum

Peripheral blood was sampled using the Vacuette system with serum clot activator (Greiner, Austria), in compliance with all necessary antiseptic procedures. Serum was obtained by centrifugation (3000 rpm or 855 g) of whole blood within 15 min of sampling. The serum was transferred into the buffer that was required for affinity purification of antibodies using a Bio-Gel P6 DG column (Bio-Rad). A 3-mL aliquot of the sample was applied to the column and then the proteins were eluted in the working buffer (20 mM PBS, pH 7.4); sorbent volume in the column was 10 mL; the flow rate was 0.2–0.6 mL/min. The fraction corresponding to the first peak was collected. The fractions from the Bio-Gel P6 DG column were applied to a protein G sepharose fast flow affinity column (GE Healthcare). The sample (3 mL) was applied to the column after it had been equilibrated with the working buffer (20 mM PBS, pH 7.4); sorbent volume in the column was 1.5 mL; the flow rate was 0.1–0.5 mL/min. And after the optical density had fallen to zero, the working buffer has changed for the elution buffer (0.1 M Gly-HCl, pH 2.5) and the proteins were eluted. The eluted fractions were neutralized to physiological pH with 1 M Tris-HCl (pH 9.0). After the target fractions had been collected from the column, non-specifically associated components were removed by washing with 20 mM PBS that contained 0.1% Triton X100 (pH 7.4) for 1 min, and the column was regenerated according to the manufacturer's recommendations. Selected antibody fractions were pooled and dialyzed for 12 h at 4 °C against ≥ 100 volumes of 20 mM PBS (pH 7.4).

Isolation of antibodies to TNF from a general pool of IgG autoantibodies

The dialyzed antibody fractions from the protein G sepharose fast flow column were applied to a TNF-bound Affi-Gel 15 column (Bio-Rad, USA); sorbent volume in the column was 1.5 mL. TNF was bound to the affinity sorbent according to the manufacturer's instructions. The sample (3 mL) was applied to the column after it had been equilibrated with working buffer (20 mM PBS, pH 7.4). The flow rate was 0.4 mL/min. And after the optical density had fallen to zero the working buffer has changed for 1 M guanidine-HCl (pH = 7.4) to remove non-specifically associated components. The column was then washed with the working buffer and the proteins eluted with the same elution buffer as used above. The eluted fractions were neutralized to physiological pH with 1 M Tris-HCl (pH = 9.0). After that the column was washed again with working buffer. Collected using the elution buffer antibody fractions were pooled and dialyzed for 12 h at +4 °C against ≥ 100 volumes of 20 mM PBS (pH = 7.4). The dialyzed antibodies were concentrated to 100–200 mL using a concentrator (Labconco, USA) at +4 °C and dialyzed for 12 h at +4 °C against ≥ 100 volumes of 20 mM PBS (pH = 7.4). They were then aliquoted and stored at +4 °C for later use.

Magnetic separation

Magnetic particles (Dynabeads M-280, Dynal Biotech, Norway) coated with streptavidin were used for magnetic separation. Rabbit polyclonal antibodies to TNF were labelled with biotin (ORIGEN, USA) as follows: 2 mg of biotin were dissolved in 1 mL of distilled water, 40 mL of the resulting solution were taken and added to 250 mL of rabbit polyclonal antibodies (5 mg/mL) and allowed to stand for half an hour with constant stirring. To stop the reaction, a crystal of tris(hydroxymethyl)aminomethane was added. After that the resulting solution was applied to the PD-10 Desalting column (GE Healthcare, USA) prewashed with five volumes of 20 mM PBS (pH = 7.4). The column was connected to a prearranged chromatograph and the fraction corresponding to the first peak was collected (working buffer – 20 mM PBS, (pH = 7.4), flow rate – 1 mL/min). Then the collected fraction of rabbit polyclonal antibodies to TNF labelled with biotin (30 µg) was co-incubated with magnetic beads (150 µL). After washing with 20 mM PBS (pH = 7.4) using a magnet, the resulting complex was incubated with the antibody fractions obtained from the affinity chromatography procedure for 3.5 h. The supernatant containing the purified autoantibody fractions was then collected using a magnet to separate the beads. Protein concentrations before and after magnetic separation were measured using the NanoDrop 2000c system (Thermo Fisher Scientific Inc., USA) according to the manufacturer's protocol.

Enzyme immunoassay

IgG subclasses and TNF contents were determined using solid-phase enzyme-linked immunosorbent assay (ELISA). The levels of the different IgG subclasses were determined by using an ELISA test kit for the different subclasses (IgG-ELISA-BEST, Vector-Best, Novosibirsk, Russia). TNF

content was determined using the commercial test kit TNF-alpha – IFA – BEST (Vector-Best, Novosibirsk, Russia). The levels of soluble receptors to TNF types I and II were determined using Human soluble TNFRI ELISA Kit and Human soluble TNFRII ELISA Kit (RayBioTech, Norcross, GA, USA). The immunosorbent assays were conducted using the commercial test systems according to the manufacturers' instructions.

The relative contents of antibody subclasses IgG1, IgG2, IgG3 and IgG4 to TNF were determined using an ELISA kit, including recombinant human TNF (Center for Immunological Engineering, Russia), plates with highly-adsorptive surfaces (Costar, Tewksbury, MA, USA), reagents from Immunotech (Russia), phosphate-buffered saline, TMB 1-component solution and a stop- solution, as well as monoclonal antibodies against the classes and subclasses of human immunoglobulins labelled with horseradish peroxidase (Polignost, Saint-Petersburg, Russia). The immunoassay protocol was as follows: TNF recombinant protein (40 µg/mL) was adsorbed on microtiter ELISA plates to 100 µL volume and incubated for 2 h at 37 °C, then for 16–18 h at 4 °C. Non-specific binding sites were blocked by incubation with 1% BSA for 1 h at 37 °C, with a preliminary washing of the plates with PBS containing 0.05% Tween 20. Serum (100 µL) was introduced at 1:5 dilution (after each incubation, the plate was washed 5× with PBS). The plate was incubated for 2 h at 37 °C, 100 µL conjugates were introduced at 1:100 dilution, and after a further incubation for 1 h at 37 °C, 100 µL TMB solution was added followed by incubation for 30 min at 37 °C. Stop solution (100 µL) was added and the absorbance was read on a microtiter plate spectrophotometer at 450 nm with a 620 nm reference. The fraction of antibodies to TNF obtained by affinity chromatography from the sera of apparently healthy donors was used for calibration to determine the autoantibody content. Previously, the content of IgG subclasses in the purified fraction had been measured by commercial ELISA test kit.

Statistics

Statistical analysis was performed using the program Statistica 7.0 (StatSoft, Tulsa, OK, USA). Results are presented as medians and interquartile ranges. Statistical significance of differences was determined using Kruskal–Wallis ANOVA and multiple comparisons test. For all tests, $p < 0.05$ was considered significant.

Results

Levels of TNF and sTNFR-I and II

Table 1 shows the levels of TNF and sTNFR-I and II in serum from patients with uncontrolled and controlled BA and in healthy donors.

The level of TNF was significantly higher in the serum of patients with uncontrolled BA than in healthy donors ($p < 0.0005$). The highest level of TNF was in the serum of patients with uncontrolled BA (1.04 ± 0.20 pg/mL). In patients with controlled BA, the level of TNF was lower (0.58 ± 0.15 pg/mL), but the difference was not significant.

Table 1. Levels of TNF, sTNFR-I and sTNFR-II in sera from patients with uncontrolled and controlled BA, and healthy donors. Data are presented as the mean $\pm$ SEM.

Group	TNF (pg/mL)	sTNFR-I (pg/mL)	sTNFR-II (pg/mL)
Patients with uncontrolled BA	1.04 $\pm$ 0.20 ^a (n = 15)	1185.72 $\pm$ 189.16 ^a (n = 14)	2825.82 $\pm$ 89.03 ^{a,b} (n = 15)
Patients with controlled BA	0.58 $\pm$ 0.15 (n = 11)	1106.00 $\pm$ 103.80 ^a (n = 11)	2839.95 $\pm$ 99.38 ^{a,b} (n = 11)
Healthy donors	0.22 $\pm$ 0.13 (n = 24)	132.55 $\pm$ 26.43 (n = 24)	1620.89 $\pm$ 169.24 ^b (n = 24)

^aSignificant ($p < 0.005$) in comparison with healthy donors;

^bSignificant ($p < 0.0005$) in comparison with level of TNFR-I (pg/mL).

Table 2. Levels of TNF autoantibodies by IgG subclass in sera from patients with uncontrolled and controlled BA, and healthy donors. The results are shown in ng/mL. Data are presented as the median with the interquartile range.

Group	IgG1	IgG2	IgG3	IgG4
Patients with uncontrolled BA	162.000 (143.200–185.500) (n = 12)	85.470 ^a (79.190–160.820) (n = 11)	173.810 (127.970–238.610) (n = 13)	64.635 ^a (53.820–89.450) (n = 10)
Patients with controlled BA	232.500 (199.600–340.600) (n = 8)	62.450 ^b (43.620–93.850) (n = 10)	131.130 (126.390–137.450) (n = 9)	48.730 (43.640–53.820) (n = 9)
Healthy donors	176.100 (133.800–204.300) (n = 18)	62.450 (42.570–69.780) (n = 14)	121.650 (94.770–154.840) (n = 15)	36.635 (29.000–63.360) (n = 16)

^aSignificant ($p < 0.05$) in comparison with healthy donors;

^bSignificant ($p < 0.05$) in comparison with patients with uncontrolled BA.

In all the groups, the serum level of sTNFR-II was significantly higher than that of sTNFR-I ($p < 0.000005$ in healthy donors; $p < 0.00005$ in patients with uncontrolled BA and $p < 0.0005$ in patients with controlled BA). We also found that the levels of sTNFR-I and II were significantly higher in the serum of patients with uncontrolled BA than in the serum of healthy donors ($p < 0.000005$ and $p < 0.00005$, respectively). In patients with controlled BA, the levels of sTNFR-I and II were also significantly higher than those in healthy donors ($p < 0.000005$ and $p < 0.0005$, respectively).

Serum levels of TNF autoantibodies

The absolute values of autoantibodies to TNF in the blood serum of patients with BA and healthy donors were determined by the method of immune-enzyme analysis (IEA) using fractions of autoantibodies to TNF as a calibration material. These fractions were obtained from the blood serum of the same healthy donors through affinity chromatography and magnetic separation. First, the levels of the different IgG subclasses in the purified fraction of autoantibodies to TNF were determined using a commercial IEA kit: 46 ng/mL for IgG1, 102 ng/mL for IgG2, 24 ng/mL for IgG3 and 48 ng/mL for IgG4. These values could then be used to calculate the levels of the different subclasses in the serum of the participants.

Table 2 shows the levels of IgG1, IgG2, IgG3 and IgG4 autoantibodies to TNF in the serum of patients with uncontrolled and controlled BA and in healthy donors. All subclasses of IgG were detected in the serum of all groups. In the serum of patients with uncontrolled BA, there were significantly higher levels of IgG2 and IgG4 than in healthy

donors ($p < 0.005$ for IgG2, $p < 0.05$ for IgG4). The levels of IgG2 were significantly higher in the serum of patients with uncontrolled BA than in patients with controlled BA ($p < 0.05$).

In patients with uncontrolled BA, the serum level of IgG1 autoantibodies to TNF was significantly higher than those of IgG4 ($p < 0.0005$). In addition, the level of IgG3 autoantibodies to TNF was significantly higher than that of and IgG4 ($p < 0.0005$). Similar results were obtained for patients with controlled BA, namely, the level of IgG1 autoantibodies to TNF was significantly higher than those of IgG2 and IgG4 ($p < 0.005$ and $p < 0.00005$). Furthermore, the level of IgG3 autoantibodies to TNF was significantly higher than that of IgG4 ($p < 0.05$) (Figure 1).

In healthy donors, the serum level of IgG1 autoantibodies to TNF was significantly higher than those of IgG2 and IgG4 ($p < 0.005$ and $p < 0.00005$, respectively). In addition, the level of IgG3 autoantibodies to TNF was significantly higher than the level of IgG4 ($p < 0.005$) (Figure 2).

Positive correlations were identified between the levels of IgG1 autoantibodies in the serum of patients with uncontrolled and controlled BA ($R^2 = 0.91$; $p < 0.05$); between the levels of IgG1 and IgG3 autoantibodies to TNF in serum of healthy donors ($R^2 = 0.83$; $p < 0.05$); and between the levels of IgG2 TNF autoantibodies and the cytokine itself in the serum of patients with controlled BA ($R^2 = 0.90$; $p < 0.05$).

Discussion

To determine the possible contribution of TNF autoantibodies to the pathogenesis of BA, we determined the level of TNF autoantibodies in patients with BA and healthy donors, and estimated the serum levels of the cytokine itself and its

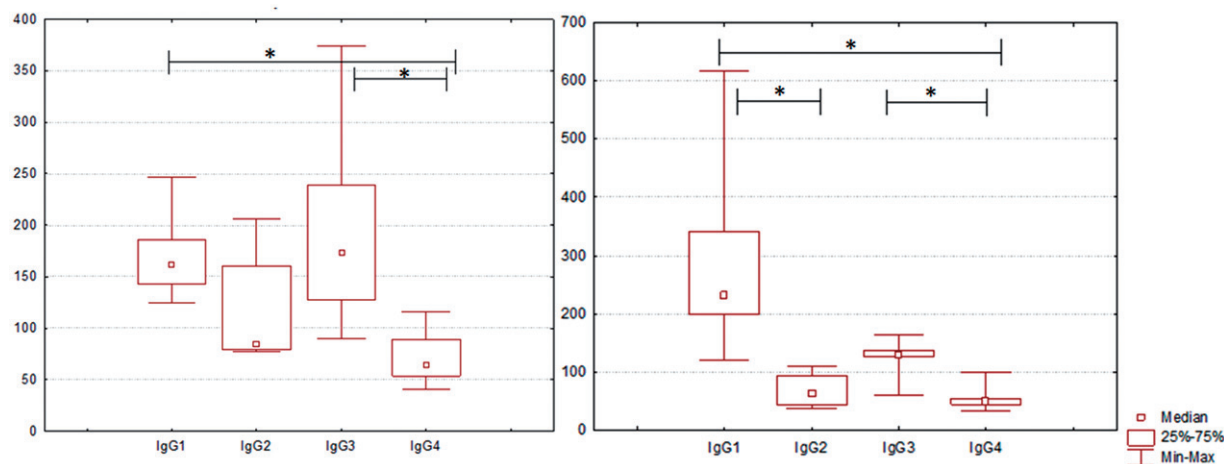


Figure 1. Median levels of TNF autoantibodies by subclass in the sera of patients with uncontrolled BA (A) and patients with controlled BA (B). Results are shown in ng/mL. *Significant ($p < 0.05$).

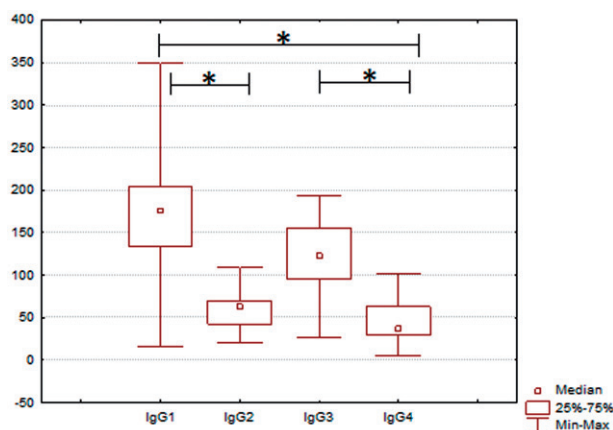


Figure 2. Levels of TNF autoantibodies by subclass in sera from healthy donors. Results are shown in ng/mL. *Significant ($p < 0.05$).

soluble receptors. It has been reported previously that the production of TNF by macrophages and monocytes is increased in peripheral blood upon the administration of antigen to patients with BA [18,19], and that the level of TNF mRNA is increased in bronchoalveolar lavage from patients with asthma [20]. In the study reported herein, we showed that the level of TNF in the serum of patients with uncontrolled BA was significantly higher than that in healthy controls, which confirmed that the cytokine is involved in the pathogenesis of BA. We also showed a nonsignificant decrease in the level of TNF after the disease had been controlled. Certainly the main pathogenetic changes in bronchial asthma take place in airways, including with participation of immunocompetent cells. In such a way more pronounced changes of the mediators content occur locally but the use of bronchoalveolar lavage for content measurements of TNF, soluble receptors and autoantibodies to TNF is much more traumatic comparing with peripheral blood puncture. Such a procedure is not applicable for healthy donors. Nevertheless it was shown activated immune cells get into the bloodstream, so the determination of immunomodulating molecules albeit indirectly reflects local pathogenetic processes in bronchial asthma.

There are two known types of sTNFR, which are produced not only by local synovial cells, but also by circulating peripheral blood cells, and this production is intensified under the influence of TNF [21]. Soluble receptors participate in the regulation of TNF functions *in vivo*. On the one hand, they can neutralise TNF within the systemic circulation and inhibit its biological activity by blocking cytokine action and competing with TNFRs on the cell surface, as well as strengthening its paracrine effects [22]. On the other hand, complexes of the receptors with TNF can be considered as a pool of bound receptors, from which they can be released gradually [23].

In all groups in our study, there was a predominance of sTNFR-I over sTNFR-II. We also found that the levels of both receptor types were significantly higher in the serum of patients with uncontrolled and controlled BA than in healthy donors, which might be evidence for the active involvement of sTNFRs in the pathogenesis of BA [11].

At present, most studies that investigate the role of cytokine autoantibodies express the levels of these antibodies in relative units. For example, when levels are analysed using tagged antibodies, the results are obtained in optical density units, which depend on the particular equipment and the activity of each enzyme used in a given study [24–26]. However, this approach makes it difficult to compare data obtained by different research groups and impossible to compare the levels of different classes and subclasses of autoantibodies within each donor group. To resolve these problems, we estimated the absolute quantities of the different IgG subclasses of TNF autoantibodies using an autoantibody fraction that had been obtained from serum of healthy donors with the aid of affinity chromatography and supplementary purification with magnetic separation [27]. The purity of the fractions obtained and IgG identity by molecular weight were confirmed by electrochemiluminescence, immune enzyme analysis and Laemmli protein electrophoresis [28].

The results showed that, in patients with uncontrolled BA, IgG1 and IgG3 were the predominant subclasses of autoantibodies to TNF. At the same time, in the serum of patients with controlled BA, and in healthy donors, the level of IgG1 autoantibodies significantly exceeded those of the other subclasses, whereas the level of IgG3 autoantibodies

was significantly higher than those of IgG4. We also found a positive correlation between the levels of IgG1 and IgG3 autoantibodies to TNF in the serum of healthy donors. Both IgG1 and IgG3 are complement-fixing subclasses of autoantibodies.

Previously, several research groups have shown that IgG1 and IgG3 autoantibodies are predominant in patients with autoimmune diseases [29–31], which differs from the typical distribution of antibodies to foreign antigens (IgG1 > IgG2 > IgG3 > IgG4) [32]. In the present study, we also found that IgG1 and IgG3 autoantibodies to TNF predominated over the IgG2 and IgG4 subclasses in the serum of patients with BA and healthy donors. The predominance of the IgG1 and IgG3 subclasses among TNF autoantibodies points to the possible active participation of the autoantibodies in activation of the complement system, because it is known that IgG1 and IgG3 actively bind C1q complement component, whereas IgG2 is capable of only weak binding and IgG4 does not bind at all [33].

The levels of IgG2 and IgG4 autoantibodies to TNF in patients with uncontrolled BA were significantly higher than those in healthy donors, and during the transition from uncontrolled to controlled BA, the levels of IgG2 decreased significantly. As mentioned above, IgG2 is only capable of weak complement activation. We did identify a positive correlation between the levels of IgG2 autoantibodies to TNF and TNF itself in patients with controlled BA. Therefore, it is possible that IgG2 autoantibodies to TNF regulate immune reactions in patients with BA by promoting the deposition of TNF through the formation of immune complexes and increasing the duration of TNF within the systemic circulation, similarly to the role of soluble TNF receptors, and perhaps heightening the effect of the cytokine.

In the present study, the significant differences in the levels of IgG2 and IgG4 autoantibodies in patients with uncontrolled BA in comparison with healthy donors provide evidence for the involvement of these autoantibodies in the pathogenesis of BA.

Conclusion

We demonstrated an increase in the levels of TNF and sTNFR-I and II in the serum of patients with uncontrolled and controlled BA as compared with healthy donors. In relation to different IgG subclasses of autoantibody, there was a significant increase in the levels of IgG2 and IgG4 autoantibodies in the serum of patients with uncontrolled BA in comparison with healthy donors. In addition, the levels of IgG2 autoantibodies decreased significantly when patients transitioned from uncontrolled to controlled BA. Thus, it is likely that autoantibodies to TNF are involved in the pathology of BA, in addition to TNF itself and soluble TNF receptors.

Declaration of Interest

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