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Original Article

Evaluation of neutrophil function and activation markers in response to inactive mycobacterium tuberculosis and IL-17 stimulation in tuberculosis patients

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Abstract

Aim: Neutrophils are thought to play a role in the pathogenesis of tuberculosis (Tb). This study aimed to evaluate the function and activation markers of peripheral neutrophils in Tb patients to clarify their involvement in disease progression.
Materials and Methods: The study included three groups: seven untreated Tb patients, seven cystic fibrosis (CF) patients, and seven healthy controls. Neutrophils were isolated from peripheral blood using Ficoll-Histopaque gradient centrifugation and cultured in RPMI 1640 medium with L-glutamine. Inactivated Mycobacterium tuberculosis was added both in the presence and absence of recombinant interleukin (IL)-17. Neutrophil functions—including phagocytosis, chemotaxis, and oxidative burst—as well as surface activation markers, such as CD11b, CD63, and CD66b, were analyzed via flow cytometry.
Results: Neutrophil functions and activation markers in response to IL-17 and bacilli stimulation in Tb patients were similar to those in healthy controls, but were significantly reduced in CF patients. Surface CD11b and CD66b expression, as well as the oxidative burst index of neutrophils from patients with Tb and CF, were inhibited by IL-17.
Conclusion: Neutrophil functions and activation markers in patients with mycobacterial infections were comparable to those in healthy subjects. In contrast, neutrophil functions were reduced in CF patients compared to both healthy subjects and Tb patients, though this difference lacked statistical significance. Notably, IL-17 was found to suppress the expression of CD11b, CD66b, and the oxidative burst index on neutrophil surfaces in both Tb and CF patients. These findings highlight the need for further research with larger cohorts to investigate neutrophil apoptosis and cytokine receptor expression in cell culture supernatants, offering deeper insights into neutrophil roles in Tb infection.

Keywords: Neutrophils, interleukin-17, tuberculosis, cystic fibrosis, flow cytometry

INTRODUCTION

Tuberculosis (Tb) is a chronic infectious disease with a high global morbidity and mortality rate. According to the World Health Organization (WHO), approximately one-quarter of the world's population is infected with Mycobacterium tuberculosis (1). Primary Tb infection typically occurs through the inhalation of M. tuberculosis bacilli, which initiates an immune response. Neutrophils, macrophages, and natural killer (NK) cells—key components of the innate immune system—interact through cytokines such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-12, and interferon (IFN)-γ, forming the first line of defense against Tb.

In cases where the innate immune response is insufficient, the adaptive immune system is activated through IL-12 and IFN-γ, released by macrophages . T helper 1 (Th1) and cytotoxic T cells maintain the defense by producing IFN-γ, which helps control

the infection (2). Importantly, not every Tb infection progresses to active disease; however, among those who do develop the disease and remain untreated, the mortality rate is approximately 50% (1).

Recent studies have focused on the role of T lymphocytes and cytokine functions in Tb to inform vaccine development, leading to a wealth of publications on the subject. However, the involvement of neutrophils in Tb infection has not been as extensively explored. Research on neutrophil extracellular traps (NETs) has highlighted the contribution of neutrophils to antimycobacterial defense (3). NETs are extracellular structures composed of nuclear DNA, histones, and granule enzymes—such as neutrophil elastase—that function by trapping and killing bacteria and viruses (4,5).

Findings on neutrophil functions at the molecular level offer valuable insights into how neutrophils combat Tb infection. While several studies have investigated neutrophils activated by heat-inactivated Mycobacterium tuberculosis, the cytokine interleukin-17 (IL-17) represents a relatively new area of interest due to its role in innate immunity. IL-17 is a proinflammatory cytokine secreted by various immune cells, including CD4+T cells, γδ T cells, innate lymphoid cells, and neutrophils (6).

In a study by McAllister et al., patients with cystic fibrosis (CF) were found to have elevated levels of IL-17 and IL-17F in their sputum and bronchoalveolar lavage fluid (7). Building on these findings, Dubin et al. proposed that CF is a Th17-driven disease (8). In the present study, we aimed to evaluate the functions and activation markers of peripheral neutrophils in patients with Tb to better understand the role of neutrophils in the pathogenesis of the disease.

MATERIALS AND METHODS

The study protocol and procedures were approved by the Ethics Committee of Istanbul University (Decision date: February 10, 2009, No: 4358). All participants were fully informed about the objectives of the study and provided written informed consent prior to participation.

HIV-negative Tb patients admitted to the Chest Diseases Department of Cerrahpasa Medical Faculty, İstanbul University, and Yedikule Chest Diseases and Thoracic Surgery Research and Training Hospital were included in the study. These patients had been diagnosed with pulmonary Tb based on radiological imaging and microbiological examination of sputum. All patients were recently diagnosed, and no treatment had been initiated at the time of inclusion in the study.

CF patients with bacterial colonization in the respiratory tract flora—Haemophilus influenzae, Staphylococcus aureus, or Pseudomonas aeruginosa—who were not receiving antibiotic or corticosteroid treatment were included as the patient control group. These patients were being followed up for CF at the Pediatrics Department of Cerrahpasa Medical Faculty, İstanbul University.

Healthy volunteers with no history of respiratory tract infections or autoimmune diseases were included as the healthy control group.

Neutrophils isolated from both patient and control groups were stimulated with heat-inactivated Mycobacterium tuberculosis bacilli and recombinant IL-17 and were evaluated for function and activation markers.

Heat-Inactivation of Mycobacterium tuberculosis

The Mycobacterium tuberculosis H37Rv (ATCC 27294) strain was cultured in Löwenstein-Jensen medium for six weeks, reaching a concentration of 6×10^8 CFU/mL. The strain was then inactivated by heating in a water bath at 80°C for 1 hour. After confirming that the strain could no longer be cultured, it was stored in a -80°C deep freezer until use in neutrophil experiments.

Dilutions of the Chemicals Used

- ***Recombinant IL-17A:***

Twenty-five µg of lyophilized cytokine was dissolved in 25 µL of distilled water. This solution was then diluted 1:100 by adding 2475 µL of PBS. For the study, stock solutions were prepared by further diluting 500 µL of this cytokine solution with 4500 µL of PBS, resulting in a final dilution of 1:10.

- ***Dihydrorhodamine 123 (DHR-123):***

Ten mg of lyophilized DHR-123 was dissolved in 5 mL of DMSO and aliquoted into 500 µL centrifuge tubes.

- ***Phorbol Myristate Acetate (PMA):***

Five mg of PMA was dissolved in 5 mL of DMSO and aliquoted into 500 µL centrifuge tubes.

- ***Formyl-Methionyl-Leucyl-Phenylalanine (FMLP):***

Fifty mg of FMLP was dissolved in 12.5 mL of DMSO and aliquoted into 500 µL centrifuge tubes.

Neutrophil Isolation from Peripheral Blood

Ten to fifteen milliliters of blood from patients and healthy volunteers were diluted with PBS at a 1:1 ratio and layered onto Ficoll at a 1:2 ratio. The samples were centrifuged at 800G for 20 minutes at 20°C, after which the plasma, mononuclear cell interphase, and Ficoll layers were carefully removed.

A red blood cell lysis solution, containing 8.25 g of NH_4Cl, 1 g of $KHCO_3$, and 37 mg of EDTA per liter, was added to the remaining erythrocyte and granulocyte-rich pellet, which was then kept on ice for 10 minutes. The solution was centrifuged at 4°C at 1400 rpm for 7 minutes, and the lysis solution was reapplied for an additional 5 minutes.

The resulting pellet, now free of erythrocytes, was transferred to a clean centrifuge tube and washed twice with PBS by

centrifugation at 4°C for 7 minutes at 1400 rpm. After washing, five milliliters of RPMI 1640 cell culture medium were added, and the cells were counted using a counting chamber.

The purity of the neutrophil preparation was assessed by flow cytometry, ensuring that 5000 cells were present in the granulocyte gate, achieving a final purity of 90-95%.

Cell Culture Conditions

Isolated neutrophils were resuspended in RPMI 1640 cell culture medium and plated in 6- or 24-well cell culture plates, depending on the number of cells obtained. The plates were incubated for 20 hours in a 5% CO_2 incubator at 37°C under the specified culture conditions. Data regarding cell culture conditions is presented in Table 1.

Table 1. Cell culture conditions

Well type	Control well	IL-17 well	Bacilli well	Bacilli + IL-17 well
Neutrophil concentration	1x10^6 cells/mL	1x10^6 cells/mL	1x10^6 cells/mL	1x10^6 cells/mL
IL-17 concentration	-	25 ng/mL IL-17	-	25 ng/mL IL-17
M. tuberculosis concentration	-	-	6x10^5/mL heat- nactivated *M. tuberculosis*	6x10^5/mL heat-inactivated *M. tuberculosis*

Flow Cytometric Analysis of the Cells

After the 20-hour incubation, cells from the culture wells were collected into 15 mL centrifuge tubes. The cells were centrifuged at 2000 rpm for 10 minutes, after which the supernatant was discarded. The cell pellets were resuspended in 1 mL of PBS for subsequent flow cytometric analysis.

Flow Cytometric Analysis of Neutrophil Functions

For phagocytosis, oxidative burst, and chemotaxis assays, 20 µL of cell suspension, 5 µL of DHR-123, and 1 mL of PBS were added to designated flow cytometry tubes. The tubes were incubated in a 37°C water bath for 5 minutes. Then, the following reagents were added to their respective tubes: 10 µL of Escherichia coli (1×10^6 CFU/mL) to the phagocytosis tube, 2.5 µL of PMA to the oxidative burst tube, and 2.5 µL of FMLP to the chemotaxis tube.

An initial measurement was taken using a flow cytometer, counting 5000 cells in the granulocyte gate. After 20 minutes of incubation in a water bath, a second measurement was performed. Flow cytometric index values were calculated by dividing the fluorescence intensity at the 20th minute by the initial values. Index values ≥1.5 were considered normal.

Additionally, for surface marker analysis, 100 µL of cell suspension and 5 µL of FITC-labeled monoclonal antibody were added to each tube. Following 15 minutes of incubation in the dark, 500 µL of PBS was added to each tube. A flow cytometer was used to count 5000 cells in the granulocyte gate, and the expression levels of CD11b, CD63, and CD66b were assessed.

Flow Cytometric Analysis of Activation Markers

Flow cytometer tubes were prepared for the measurement of activation markers CD11b, CD63, and CD66b.

Statistical Analysis

Intragroup comparisons were performed using the Wilcoxon test, while comparisons between groups were made using appropriate statistical methods. A p-value of <0.05 was considered statistically significant.

RESULTS

Patient demographics regarding Tb patients (n=7, mean age=36.29±12.94 years), CF patients (n=7, mean age=17±3.53 years) and healthy subjects (n=7, mean age=29.14±9.96 years) are presented in Table 2.

Table 2. Patient demographics

	Tuberculosis		Cystic fibrosis		Healthy control	
No.	**Age**	**Gender**	**Age**	**Gender**	**Age**	**Gender**
1	21	F	12	F	33	M
2	25	F	23	M	26	M
3	25	F	19	M	25	M
4	35	M	20	M	25	F
5	49	M	14	F	25	F
6	48	M	17	F	50	M
7	51	M	15	F	20	F

F: female, M: male

The mean and standard deviation values of phagocytic activation measurements in healthy controls were as follows: 1.47±0.78 for the control well, 1.61±0.86 for the IL-17 well, 1.32±0.63 for the bacilli well, and 1.09±0.35 for the bacilli+IL-17 well combination. The IL-17-stimulated value was significantly higher than the value obtained after incubation with bacilli alone (p=0.043).

Flow cytometric display of neutrophil population is shown in Figure 1, whereas phagocytosis measurement printouts are shown in Figures 2 and 3.

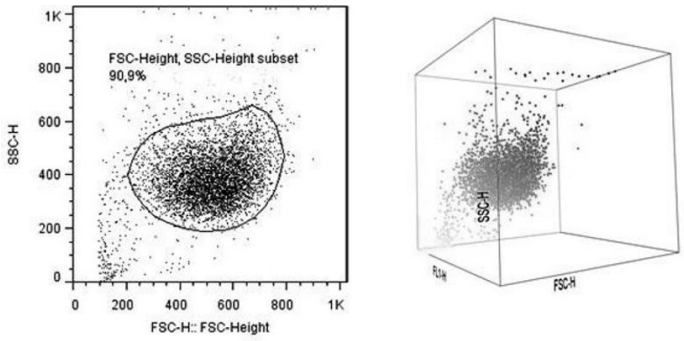


Figure 1. Flow cytometric display of neutrophil population after isolation

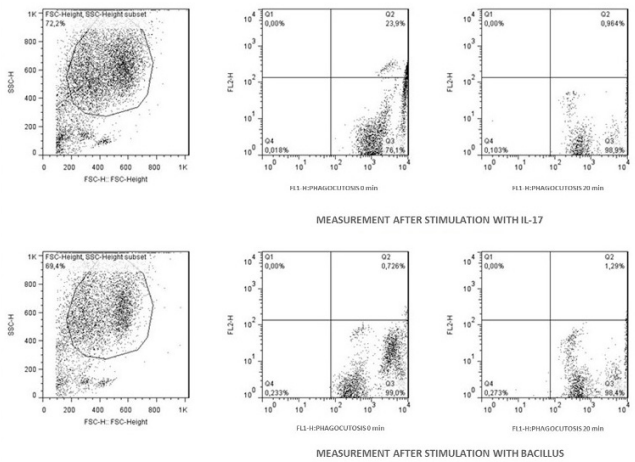
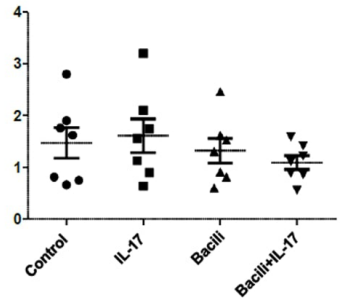


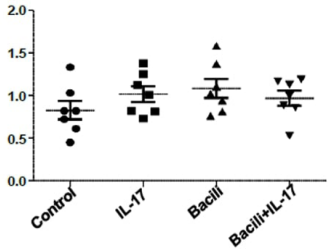
Figure 2. Phagocytic activity in healthy controls



	p
CONTROL IL-17	0,398
CONTROL BACILLI	0,128
CONTROL BACILLI+IL-17	0,128
BACILLI IL-17	0,043
IL-17 BACILLI+IL-17	0,063
BACILLI BACILLI+IL-17	0,176

Figure 3. Changes in phagocytic activity in healthy subjects

For CF patients, the mean and standard deviation values of phagocytic activation measurements were: 0.83±0.29 for the control well, 1.02±0.25 for the IL-17 well, 1.08±0.30 for the bacilli well, and 0.97±0.23 for the bacilli+IL-17 well combination. No statistically significant differences were observed in these results (Figure 4).



	p
CONTROL IL-17	0,176
CONTROL BACILLI	0,063
CONTROL BACILLI+IL-17	0,398
BACILLI IL-17	0,398
IL-17 BACILLI+IL-17	0,499
BACILLI BACILLI+IL-17	1,00

Figure 4. Phagocytic activity in cystic fibrosis patients

DISCUSSION

In this study, we investigated the functions of neutrophils in the innate immune response in newly diagnosed Tb patients who had not yet received treatment. We compared their neutrophil function to that of CF patients with neutrophilic lung inflammation and healthy subjects. Neutrophil activation markers, adhesion molecules, and changes in neutrophil functions—chemotaxis, phagocytosis, and oxidative burst—were evaluated in response to stimulation by inactivated Mycobacterium tuberculosis bacillus, IL-17, and their combination using flow cytometry.

Although neutrophil accumulation at the site of mycobacterial infection during the early stages of Tb is believed to play a role in disease pathogenesis, most research has focused on the host's Th1-type immune response to Mycobacterium tuberculosis, an intracellular pathogen. The role of neutrophils, which are typically involved in defense against extracellular infections, has been largely overlooked in the context of TB defense.

In recent years, with the discovery of NETs, there has been a growing focus on the role of neutrophils in antimycobacterial defense in both murine and human studies. NETs are composed of DNA and antimicrobial proteins, and their formation in response to mycobacterial infections occurs in a time-dependent manner (3).

In one study involving both murine and human models, neutrophils were shown to assist dendritic cells in the cross-presentation of Mycobacterium bovis BCG antigens to T cells, highlighting their role in adaptive immunity (9).

IL-17, a cytokine secreted by Th17 and γδ T cells, plays a key role in neutrophil accumulation at sites of inflammation by stimulating the production of chemokines such as IL-8. Additionally, IL-17 enhances neutrophil production in the bone marrow by inducing growth factors like granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor

(GM-CSF) (10). It also promotes the production of the ICAM-1 adhesion molecule in fibroblasts and smooth muscle cells (11). Therefore, this study also investigated the effect of IL-17 on the stimulation of neutrophils isolated from peripheral blood.

Neutrophils play a crucial role in the immune response by eliminating pathogenic microorganisms and interacting with various immune system cells. These functions are carried out through several mechanisms, including migration to the infection site in response to chemical stimuli, adhesion to pathogens via specific molecules, phagocytosis, and ultimately the destruction of microorganisms through both oxygen-dependent and oxygen-independent pathways (12). These processes can be evaluated by examining adhesion molecules and activation markers. CD11b, the α subunit of the Mac-1 integrin, forms non-covalent bonds with CD18 and is involved in the adhesion and recognition of opsonized particles. CD63 is expressed on the cell membrane during the release of azurophilic granule contents, while CD66b is involved in cell adhesion and appears on the cell surface during neutrophil activation (13).

Although macrophages are the primary targets of Mycobacterium tuberculosis, neutrophils can also be infected by the bacilli. These neutrophils, which rapidly migrate to the infection site, are capable of killing the bacilli they phagocytose both intracellularly and through extracellular mechanisms such as NETs (14). However, the precise role of neutrophils in antimycobacterial defense remains controversial.

Pedrosa et al. observed that mouse neutrophils were present at the infection site early in the infection and also a few days after recovery. They suggested that neutrophils may have a protective role by halting bacterial proliferation through non-phagocytic killing mechanisms (15). In our study of untreated Tb cases, although neutrophil function values were higher compared to healthy subjects, no significant differences were observed.

When examining chemotaxis and phagocytic activity measurements within TB patients, no significant changes were detected following antigen or cytokine stimulation. However, in the assessment of oxidative burst, neutrophils exposed solely to bacilli exhibited significantly higher values compared to those exposed to the combination of IL-17 and bacilli ($p=0.043$).

These results suggest that while bacilli ingested by neutrophils during Tb infection enhance intracellular bacillus death by increasing the production of reactive oxygen species, IL-17 does not appear to influence this intracellular killing process. Dlugovitzky et al. reported that stimulating polymorphonuclear leukocytes from Tb patients with heat-inactivated Mycobacterium vaccae led to an increased oxidative index and elevated CD11b expression on the cell surface (16). In our study, no significant change was observed in the percentage of cells expressing CD11b, although the mean fluorescence intensity (MFI) was higher ($p=0.018$) after IL-17 stimulation compared to the bacilli+IL-17 combination. This finding suggests that bacilli may inhibit the surface expression of adhesion molecules.

Similarly, there was no significant difference in the percentage of cells expressing CD66b, but the MFI following IL-17 stimulation was significantly lower than the control value ($p=0.018$). This indicates that the antigen may have had no effect on CD66b levels in Tb cases, and that IL-17 may exert a suppressive effect on CD66b expression.

This finding also suggests that neutrophil function is impaired during the course of mycobacterial infection. Fiorenza et al. demonstrated that oxidative burst values and CD11b surface expression of neutrophils isolated from Tb patients were significantly lower than those of healthy controls, both before and after stimulation with bacilli (17). The observed increase in neutrophil counts in Tb, alongside a decrease in bacillus counts, supports this view (18). However, the effectiveness of neutrophils in killing Mycobacterium tuberculosis remains controversial. While some studies have shown that neutrophils can effectively kill bacilli (19), others have reported that they are not successful in this regard (20).

Despite these inconsistencies, neutrophils have been shown to play a role in controlling infection by producing chemokines (21), inducing granuloma formation (22), and transferring microbicidal molecules to infected macrophages (23).

In addition to controlling infection, neutrophils have been implicated in the pathogenesis of Tb. More intense neutrophil accumulation has been observed in Tb lesions of genetically susceptible mice compared to resistant mice, suggesting a role for neutrophils in disease progression (24).

CF is a chronic disease caused by mutations in the CFTR gene, characterized by multi-organ involvement, particularly affecting the respiratory and gastrointestinal systems. Recurrent lung infections and chronic bronchopulmonary damage are the leading causes of morbidity and mortality in CF patients. A key pathological feature of CF is chronic neutrophilic inflammation of the airways (25). In CF, although the migration of peripheral blood neutrophils to the airways is increased, these cells are ineffective at preventing bacterial colonization, particularly by Pseudomonas aeruginosa. These neutrophils rapidly undergo apoptosis and necrosis, releasing toxic products such as neutrophil elastase, reactive oxygen species, myeloperoxidase, DNA, and actin into the respiratory tract (26).

Pyocyanin, a toxin secreted by Pseudomonas colonizing the respiratory tract, has been shown to increase neutrophil apoptosis and the production of reactive oxygen species (27). In our study, neutrophils from CF patients were found to be unresponsive to cytokine and antigenic stimuli, with no significant differences compared to healthy subjects. While there was no significant change in the percentage of cells expressing CD11b in CF patients, the MFI values obtained after IL-17 stimulation were lower compared to other stimuli. This suggests that IL-17 suppresses the expression of adhesion molecules on the surface of neutrophils in CF patients, a finding not previously reported in the literature.

The percentage of CD63-expressing neutrophils showed no significant difference, but the MFI of CD63 after IL-17 stimulation was significantly lower compared to both bacilli (p=0.018) and the bacilli+IL-17 combination (p=0.018). Similarly, there was no significant difference in the percentage of neutrophils expressing CD66b. However, the MFI of CD66b was significantly lower with cytokine stimulation compared to bacillus+cytokine stimulation (p=0.018).

It has been reported that IL-17 plays a role in neutrophil homeostasis, as neutrophils are one of its target cells (28). While IL-17 directs neutrophils to sites of inflammation, it may also impair their function (29). Moreover, IL-17 has been shown to negatively impact neutrophil survival by suppressing the anti-apoptotic effects of GM-CSF (30).

Elevated levels of IL-17A and IL-17F have been detected in the sputum and bronchoalveolar lavage fluids of CF patients (31). According to our data, IL-17 promotes neutrophilic phagocytosis in healthy individuals but does not cause a significant change in Tb and CF patients. In terms of oxidative burst values, IL-17 stimulation in neutrophils from Tb patients was lower compared to stimulation with Mycobacterium tuberculosis.

When comparing neutrophil functions among Tb patients, CF patients, and healthy subjects, Tb patients had higher values than both healthy subjects and CF patients, although these differences were not statistically significant. CD63 expression on the cell surface significantly decreased (p=0.046) only after neutrophils from healthy subjects were stimulated with the bacillus+IL-17 combination. No significant changes were observed in other markers or in CD63 percentages for the other groups.

In terms of mean fluorescence intensities, bacillus stimulation significantly increased CD11b levels in neutrophils from healthy subjects (p=0.046). Similarly, bacillus stimulation significantly increased CD66b levels in healthy individuals (p=0.046). In Tb patients, the mean fluorescence intensity of CD11b was significantly higher with bacillus stimulation compared to bacillus+IL-17 stimulation (p=0.018). Additionally, IL-17 stimulation significantly decreased the mean fluorescence intensity of CD66b (p=0.018).

CONCLUSION

Neutrophil functions and activation markers in patients with mycobacterial infection were comparable to those of healthy subjects. However, neutrophil functions were found to be reduced in CF patients compared to both healthy subjects and Tb patients, although this difference was not statistically significant. Our findings suggest that IL-17 suppresses CD11b and CD66b expression, as well as the oxidative burst index, on the surface of neutrophils in both Tb and CF patients. To further elucidate the role of neutrophils in Tb infection, future studies with larger patient cohorts, focusing on apoptosis levels and cytokine receptor expression in cell culture supernatants, are recommended.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
This work was supported by the Research Fund of Istanbul University (Project number: 3161) and was conducted as part of a master's thesis in Immunology.

Ethical Approval
The study protocol and procedures were approved by the Ethics Committee of İstanbul University (Decision date: February 10, 2009, No: 4358). All participants were fully informed about the objectives of the study and provided written informed consent prior to participation.

REFERENCES

1. WHO: World Health Organization: Global Tuberculosis report 2022. ISBN 978-92-4-006172-9 https://www.who.int/publications/i/item/9789240061729 access date 26.02.2023.
2. Cooper AM, Cell-mediated immune responses in tuberculosis. Annu Rev Immunol. 2009;27:393-422.
3. Ramos-Kichik V, Mondragón-Flores R, Mondragón-Castelán M, et al. Neutrophil extracellular traps are induced by Mycobacterium tuberculosis. Tuberculosis (Edinb). 2009;89:29-37.
4. Brinkmann V, Zychlinsky A. Beneficial suicide: why neutrophils die to make NETs. Nat Rev Microbiol. 2007;5:577-82.
5. Fisher KL, Rajkumar-Bhugeloo K, Moodley D, et al. Investigating neutrophil cell death in TB pathogenesis. Gates Open Res. 2022;5:175.
6. Hu S, He W, Du X, et al. IL-17 production of neutrophils enhances antibacteria ability but promotes arthritis development during Mycobacterium tuberculosis infection. EBioMedicine. 2017;23:88-99.
7. McAllister F, Henry A, Kreindler JL, et al. Role of IL-17A, IL-17F, and the IL-17 receptor in regulating growth-related oncogene-alpha and granulocyte colony-stimulating factor in bronchial epithelium: implications for airway inflammation in cystic fibrosis. J Immunol. 2005;175:404-12.
8. Dubin PJ, McAllister F, Kolls JK. Is cystic fibrosis a TH17 disease?. Inflamm Res. 2007;56:221-7.
9. Morel C, Badell E, Abadie V, et al. Mycobacterium bovis BCG-infected neutrophils and dendritic cells cooperate to induce specific T cell responses in humans and mice. Eur J Immunol. 2008;38:437-47.
10. Aggarwal S, Gurney AL, IL-17: prototype member of an emerging cytokine family, J Leukoc Biol. 2002;71:1-8.
11. Ivanov S, Linden A, Interleukin-17 as a drug target in human disease. Trends Pharmacol Sci. 2009;30:95-103
12. Cruse JM, Lewis RE. Illustrated Dictionary of Immunology. 3rd edition. CRC Press, Florida, 2009;530-1.
13. Stiem ER, Ochs DH, Winklestein JA. Immunologic Disorders in Infants and Children. 5th edition. Saunders, Philadelphia, 2004;111-3.

14. Urban CF, Lourido S, Zychlinsky A. How do microbes evade neutrophil killing?. Cell Microbiol. 2006;8:1687-96.

15. Pedrosa J, Saunders BM, Appelberg R, et al. Neutrophils play a protective nonphagocytic role in systemic Mycobacterium tuberculosis infection of mice. Infect Immun. 2000;68:577-83.

16. Dlugovitzky D, Fiorenza G, Farroni MA, et al. Immunological consequences of three doses of heat-killed Mycobacterium vaccae in the immunotherapy of tuberculosis. Respir Med. 2006;100:1079-87.

17. Fiorenza G, Farroni MA, Bogué C, et al. Functional characteristics of neutrophils and mononuclear cells from tuberculosis patients stimulated in-vitro with heat killed M. tuberculosis. Arch Med Res. 2007;38:526-33.

18. Fulton SA, Reba SM, Martin TD, Boom WH. Neutrophil-mediated mycobacteriocidal immunity in the lung during Mycobacterium bovis BCG infection in C57BL/6 mice. Infect Immun. 2002;70:5322-7.

19. Jones GS, Amirault HJ, Andersen BR. Killing of Mycobacterium tuberculosis by neutrophils:a nonoxidative process. J Infect Dis. 1990;162:700-4.

20. Denis M. Human neutrophils, activated with cytokines or not, do not kill virulent Mycobacterium tuberculosis. J Infect Dis. 1991;163:919-20.

21. Riedel DD, Kaufmann SH. Chemokine secretion by human polymorphonuclear granulocytes after stimulation with Mycobacterium tuberculosis and lipoarabinomannan. Infect Immun. 1997;65:4620-3.

22. Sugawara I, Udagawa T, Yamada H. Rat neutrophils prevent the development of tuberculosis. Infect Immun. 2004;72:1804-6.

23. Tan BH, Meinken C, Bastian M. Macrophages acquire neutrophil granules for antimicrobial activity against intracellular pathogens. J Immunol. 2006;177:1864-71.

24. Eruslanov EB, Lyadova IV, Kondratieva TK. Neutrophil responses to Mycobacterium tuberculosis infection in genetically susceptible and resistant mice. Infect Immun. 2005;73:1744-53.

25. Goldberg JB, Pier GB. The role of the CFTR in susceptibility to Pseudomonas aeruginosa infections in cystic fibrosis. Trends Microbiol. 2000;8:514-20

26. Tirouvanziam R, Gernez Y, Conrad CK, et al. Profound functional and signaling changes in viable inflammatory neutrophils homing to cystic fibrosis airways. Proc Natl Acad Sci USA. 2008;105:4335-9.

27. Usher LR, Lawson RA, Geary I, et al. Induction of neutrophil apoptosis by the Pseudomonas aeruginosa exotoxin pyocyanin: a potential mechanism of persistent infection. J Immunol. 2002;168:1861-8.

28. Stark M, Huo Y, Burcin T, et al. Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17. Immunity. 2005;22:285-94.

29. Zelante T, De Luca A, Bonifazi P, et al. The IL-23/IL-17 pathway promotes inflammation and impairs antifungal immune resistance. Eur. J. Immunol. 2007;37:2695-706.

30. Dragon S, Saffar AS, Shan L, Gounni AS. IL-17 attenuates the anti-apoptotic effects of GM-CSF in human neutrophils. Mol Immunol. 2008;45:160-8.

31. McAllister F, Henry A, Kreindler JL, et al. Role of IL-17A, IL-17F, and the IL-17 receptor in regulating growth-related oncogene-alpha and granulocyte colony-stimulating factor in bronchial epithelium: implications for airway inflammation in cystic fibrosis. J Immunol. 2005;175:404-12.