Review

Mycobacterium tuberculosis in a Trap: The Role of Neutrophil Extracellular Traps in Tuberculosis

Luiz Henrique Agra Cavalcante-Silva , Fernanda Silva Almeida , Arthur Gomes de Andrade , Fernando Cézar Comberlang , Leonardo Lima Cardoso , Shayenne Eduarda Ramos Vanderley , Tatjana S. L. Keesen *

Posted Date: 23 May 2023

doi: 10.20944/preprints202305.1582.v1

Keywords: NETs; innate immunity; mycobacteria.

Review

Mycobacterium tuberculosis in a Trap: The Role of Neutrophil Extracellular Traps in Tuberculosis

Luiz Henrique Agra Cavalcante-Silva, Fernanda Silva Almeida, Arthur Gomes de Andrade, Fernando Cézar Comberlang, Leonardo Lima Cardoso, Shayenne Eduarda Ramos Vanderley, and Tatjana S. L. Keesen *

Immunology of Infectious Diseases Laboratory, Department of Cellular and Molecular Biology, Federal University of Paraiba, João Pessoa 58051-900, PB, Brazil; luiz0710@gmail.com (L.H.A.C.-S.); fernandaalmeida.ufpb@gmail.com (F.S.A.); arthurg.hit@gmail.com (A.G.d.A.); fcezar14@gmail.com (F.C.C.); leonardo15lima16@gmail.com (L.L.C.); shayenne.erv@gmail.com (S.E.R.V.)

* Correspondence: tat.keesen@cbiotec.ufpb.br; Tel.: +55-83-32167371

Abstract: *Mycobacterium tuberculosis* complex causes tuberculosis (TB), a disease that causes pulmonary inflammation but can also affect other tissues. Despite macrophages having a defined role in TB immunopathogenesis, other innate immune cells, such as neutrophils, are involved in this process. These cells have high phagocytic ability and a microbial-killing machine comprised of enzymes, antimicrobial peptides, and reactive oxygen species. In the last two decades, a new neutrophil immune response, the neutrophil extracellular traps (NETs), has been intensely researched. NETs comprise DNA associated with histones, enzymes, and antimicrobial peptides. These structures are related to antimicrobial immune response and some immuno-pathogenesis mechanisms. This mini review highlights the role of NETs in tuberculosis and how they can be helpful as a diagnostic tool and/or therapeutic target.

Keywords: NETs; innate immunity; mycobacteria

1. Introduction

Tuberculosis (TB) is an infectious-contagious disease mainly affecting the lungs and other tissues [1]. Several factors have limited TB control, including timely diagnosis and treatment, imperfect diagnostic testing, multi-drug resistance, and treatment abandonment [2–5]. High TB-burden countries use attenuated *Mycobacterium bovis* strain-based vaccine (Bacille Calmette-Guerin, BCG) to prevent severe TB at birth or among school-age children. However, this vaccine has limited effects on adult pulmonary TB [6]. In 2020, approximately 10 million people had tuberculosis, but 40% of these cases were undiagnosed and reported [7]. This was followed by a rate of 1.5 million death from TB worldwide. WHO's End TB Strategy proposes targets of a 95% reduction in the absolute number of TB deaths and a 90% reduction in the TB incidence rate by 2035 [8].

The pathogenesis of TB is complex and involves many immune system cells, such as macrophages, lymphocytes, and neutrophils [9,10]. These last cells may have a beneficial and a detrimental role in TB development [11]. Neutrophil functions comprise various antimicrobial peptides besides crosstalk with different immune cells. Additionally, neutrophils can release extracellular traps (i.e., DNA, histones, and various granular and cytoplasmic proteins) that mediate many immune responses [12,13]. This mini review discusses the role of neutrophil extracellular traps (NETs) in tuberculosis and how it could be helpful to understand this disease, bringing insight into developing new treatments/diagnostic tools.

2. Tuberculosis

Tuberculosis (TB) is one of the oldest infectious diseases in the history [14]. It is caused by the *Mycobacterium tuberculosis* complex, comprising species including *M. bovis*, BCG strain of *M. bovis*, *M.*

 doi:10.20944/preprints202305.1582.v1

africanum, *M. caprae*, *M. microti*, *M. canettii*, and *M. pinnipedii*. Most TB cases are attributed to *M. tuberculosis (Mtb)*, which accounts for 98% of human cases [15,16].

Patients with TB can be classified into two distinct clinical forms: latent TB infection (LTBI) and active TB disease [17]. Those with latent TB infection are asymptomatic and non-transmissible [18]. At the same time, those with active TB disease show general symptoms such as fever, fatigue, lack of appetite, weight loss, back pain, and night sweat. In addition, those with lung disease may have a persistent cough and hemoptysis (coughing up blood) in its advanced form, which includes pulmonary disease that can result in further transmission. Some patients in the active form of the disease may be asymptomatic and are better described as having subclinical tuberculosis. It is estimated that 5 to 10% of people exposed to *Mtb* develop its active form [16,19–21].

Extra-pulmonary TB (EPTB) form is also an important clinical issue, as it can occur in any part of the body following the initial spreading of *Mtb* to the pulmonary draining lymphatic system [22]. *Mtb* is taken up by lymphatic vessel non-professional phagocytes such as endothelial cells and fibroblasts, which then spread via lymph to other organ lymphatic systems. Symptomatic EPTB occurs in nearly 10% of infected people. However, pulmonary TB prevails since alveoli and airways are the main gateway of *Mtb* air-borne infection and its transmission. EPTB can also undergo latency and active disease independent of pulmonary TB. The lesions of TB include the peritoneum, kidneys, bone marrow, spleen, pleural, meningeal, peritoneal membranes, skin, and genito-urinary tract [19,23,24].

At the start of infection, alveolar macrophages internalize *Mtb*, which provides a cellular environment for the bacteria to replicate intracellularly [25,26]. This safeguards the host from the mycobacteria and permits the infection to establish itself and persist in the latent phase. These bacteria-filled immune cells can be transported through the alveolar barrier to cause widespread dissemination [19]. Before adaptive immune responses are established, the intracellular replication and spread of *Mtb* to neighboring pulmonary lymph nodes and other extrapulmonary tissues occur through lymphatics and blood circulation. The failure to restrict bacterial replication may lead to the release of *Mtb* into the extracellular environment; after that, it actively replicates and releases pathogenic proteins and lipids to interact with hosts. In this way, complex host-pathogen interactions determine the course of *Mtb* infection [19,27].

At the onset of *Mtb* infection, the secretion of cytokines and chemokines such as interferons, tumor necrosis factor-α (TNF-α), IL-6, IL-12, IL-17, IL-23, CCL2, CCL3, CCL5, CXCL8, and CXCL10, attracts additional phagocytes to the site of infection, which puts immune pressure on the bacteria [28,29]. In addition, they stimulate adaptive immune responses but also cause inflammation and destruction of lung tissue that can be triggered by M1 macrophages responding to *Mtb* via iNOS-RNS, ROS, TNF-α, and IL-6, associated with TLR 2/4 pathways, including MAPK and Jun activation. The arrival of neutrophils is an initial defense against infection but can also limit immunity against the bacteria and offer a conducive environment for its replication. Innate immunity plays a crucial role in the long-term control of *Mtb* infection, controlling inflammation and directing adaptive immune responses [15,19,30,31].

Monocytes and neutrophils are attracted to the site of *Mtb* infection [10,31]. Still, monocyte recruitment may inadvertently promote bacterial dissemination through polarization from M1 to an anti-inflammatory M2 phenotype expressing IL-10, TGF-β1, IL-10, CCL-24, and arginase-1, while inhibiting soluble antibacterial factors with TNF-α, IL-12, IL-6, and NO, favoring bacterial replication. This M2 phenotype is associated with TB, leprosy, and other chronic inflammatory diseases such as asthma and parasitic infections, but also with the resolution of non-infectious inflammatory lesions. Moreover, monocytes can transport *Mtb* to pulmonary lymph nodes and coordinate with dendritic cells to stimulate $CD4^+$ T cell production after infection. Neutrophil and monocyte recruitment is a hosting strategy to contain bacterial replication, but it is coopted by the bacterium to facilitate its growth and dissemination [9,19,31].

During *Mtb* infection, dendritic cells (DCs) are essential in priming adaptive immune responses [32]. Professional antigen-presenting cells initiate adaptive immunity by presenting *Mtb* antigens in the context of major histocompatibility complex (MHC), costimulatory molecules, and cytokines.

 doi:10.20944/preprints202305.1582.v1

Depletion of CD11c-expressing cells, a pan-DC marker, after *Mtb* infection impairs bacterial control and delays the onset of adaptive immunity, highlighting the importance of DCs in mobilizing responses capable of controlling bacterial replication. After infection, DCs migrate to the lung-draining lymph nodes and mature to initiate antigen-specific T cell responses. IL-12 secreted by myeloid cells and essential for IFN-γ induction is required for DC migration during *Mtb* infection. Antigen-specific T cells can be primed by infected migratory DCs and uninfected resident lymph node DCs. *Mtb* infection impairs antigen presentation by macrophages and dendritic cells, affecting antigen-specific T cell responses [15,31,32].

The active disease develops in weeks or more extended periods due to *Mtb* slow replication within macrophages and variation in strain virulence. This triggers macrophage activation and the induction of adaptive immune responses, resulting in the fusion of phagosomes and lysosomes and the secretion of cytokines such as IFN-α, TNF-α, IFN-β, IL-1β, IL-6, and IL-12 [33,34]. While these cytokines aid in the host's bactericidal response, they may also cause severe lung inflammation and tissue destruction, a sign of active tuberculosis. HIV infection is the main risk factor linked to the progression from latent TB infection to active TB. HIV weakens the immune system by decreasing the numbers of $CD4^+$ T cells and impairing the function of both $CD4^+$ T cells and $CD8^+$ T cells, essential for protection against active TB [9,35,36]. Diabetes Mellitus (DM) patients are more susceptible to TB due to reduced *Mtb* phagocytosis, altered cytokines, and impaired glucose tolerance. Furthermore, drug–drug interactions between TB and DM treatments can reduce the effectiveness of both treatments and the potential worsening of drug side effects. Consequently, it is essential to establish early diagnosis and treatment for both TB and DM to prevent the increased risk of cardiovascular complications and death [37]. Additional risk factors for reactivation TB include aging, malnutrition, and medical conditions that impede immunity, such as renal failure, cancer, and immunosuppressive therapy [9,35,36].

The development of adaptive immunity against *Mtb* begins right after the bacteria spreads into the lymph nodes, where granulomas form. This immune response is mediated by T lymphocytes ($CD4^+$ and $CD8^+$) that release cytotoxins and recruit B lymphocytes and plasma cells [38]. $CD4^+$ T cells, especially Th1 cells that produce interferon-γ (IFN-γ), are critical for developing a successful adaptive immunity. MHC class II and I molecules expressed by APCs also play a vital role in the presentation of bacterial antigens to T lymphocytes, allowing them to produce IFN-γ and other cytokines, like interleukin-2 (IL-2) and tumor necrosis factor-alpha (TNF-α). Studies have suggested that reducing $CD4^+$ T cells in HIV-1 infection is connected to a higher bacterial load and a broader spread of tuberculosis during TB-HIV co-infection, emphasizing the importance of T-cell-mediated adaptive immunity in the defense against *Mtb* [38–40].

$CD8^+$ T lymphocytes are critical in the cellular response against mycobacteria, as they can eliminate infected cells or bacilli. Cytotoxic serine proteases, held in cytolytic granules, are a part of the granzyme (Gzms) family and have a significant role in the operations of $CD8^+$ T cells. $CD4^+$ cytotoxic T cells, which secrete GzmB and perforin, have also been identified. Granzyme K, discharged by activated $CD8^+$ T cells, encourages reactive oxygen species (ROS) production in target cells and leads to fast cell death by causing single-stranded nicks of chromosomal DNA and cell membrane damage. In TB patients, $CD8^+$ T cells with GzmK are more abundant and clonally expanded in tuberculous pleural effusion than in peripheral blood. GzmK is associated with inflammation, as it is upregulated during acute and chronic inflammation [41].

One of the hallmarks of TB is granuloma formation [26,42]. Granulomas represent a complex immune response of the host against *Mtb* infection responsible for tuberculosis. They aim to confine the bacteria to a specific location and prevent dissemination to other body parts. In pulmonary tuberculosis, granulomas comprise macrophages, dendritic cells, neutrophils, fibroblasts, T lymphocytes, and B lymphocytes, which organize into a compact and organized structure. Plasma cells, $CD4^+$, $CD8^+$, and B T lymphocytes are arranged at the periphery, forming a peripheral lymphatic rim in the granuloma that limits mycobacterial replication [9,15,19,31,43]. Adaptive immunity is crucial in *Mtb* containment in granulomatous latency [19].

 doi:10.20944/preprints202305.1582.v1

3. Neutrophils

Neutrophils comprise a significant portion of the circulating leukocytes in humans, accounting for approximately 50-70%. These cells have a characteristic segmented nucleus, a roughly 7-10 micrometers diameter, and play a critical role, especially in antimicrobial response [44–49]. The clinical significance of their abundance lies in predicting severe infections that may follow congenital or acquired conditions causing a decrease in neutrophil count in the bloodstream [50]. These cells were thought to be transcriptionally inactive. However, recent findings led to a paradigm shift, revealing subpopulation heterogeneity and crucial inflammatory and repair functions [51,52].

During development in the bone marrow, myeloid progenitors undergo sequential maturation to generate mature neutrophils that are released to the circulation in a process that gauges the extramedullary pool size. Although the kinetics of circulating neutrophils and their precursors remains somewhat uncertain, there has been increasing evidence about the remarkable plasticity that can be molded by the different tissues where they can be redistributed throughout your lifetime [53,54]. That can be explained by the transcriptional diversity of neutrophils during maturation, known as “neutrotime”, a transcriptional continuum for neutrophils in the bone marrow, blood, and spleen, suggesting a dominant developmental spectrum that underlies neutrophil heterogeneity under normal conditions [55]. Such diversity also includes low-density neutrophils (LDNs), which may manifest immunosuppressive or pro-inflammatory properties. Proinflammatory LDNs are known as low-density granulocytes (LDGs), and immunosuppressive LDNs are known as granulocytic myeloid-derived suppressor cells (G-MDSCs). Additionally, specific tumor-associated neutrophils (TANs) can be further categorized into N1 and N2 subsets, representing functions that are either anti-tumorigenic or pro-tumorigenic, respectively [56,57].

Neutrophil cytoplasm contains granules and secretory vesicles that play a crucial role in their function. As the first line of defense, neutrophils are the initial responders against a wide range of pathogens. These effector functions include phagocytosis with phagolysosome activity, degranulation of antimicrobial molecules and various enzymes involved in inflammation regulation, formation of neutrophil extracellular traps (NETs), and playing a role in tissue repair and wound healing by releasing growth factors [12,58,59].

The development of inflammation can occur in response to both infectious and sterile injuries, with PAMPs and DAMPs triggering similar pro-inflammatory responses through the activation of PRRs [60–62]. Neutrophils are recruited to both types of inflammation, but their recruitment and subsequent pro-inflammatory responses can be detrimental in sterile injuries such as obesity-associated inflammation [63]. The ability of neutrophils to distinguish between infectious and sterile inflammation is currently unknown, as both types of inflammation can activate similar receptors and intracellular pathways, leading to similar outcomes. However, different neutrophil types (pro-inflammatory versus pro-resolving) may be recruited depending on the type of inflammation and the specific ligands and cells involved [56].

Neutrophil recruitment to inflamed tissues is a multi-step process involving upregulating adhesion molecules on endothelial cells, allowing for neutrophil tethering, rolling, and eventually firm adhesion [12,64]. The interaction between adhesion molecules and chemokine receptors on neutrophils and their ligands on endothelial cells mediates this process. Neutrophil activation and chemotaxis toward the site of inflammation are also critical steps in this process [12,63]. Once adhered, neutrophils may display probing behavior before crawling toward endothelial cell junctions to transmigrate across the endothelial layer. The transmigration process can occur either paracellularly or transcellularly, with the latter being mediated by specialized structures called transmigratory cups. While the mechanisms of neutrophil migration through the endothelial basement membrane are not fully understood, it is suggested that neutrophils preferentially migrate through regions with low levels of extracellular matrix molecules and gaps between pericyte regions [65,66].

Neutrophils, traditionally viewed as pro-inflammatory cells, have been shown to exhibit anti-inflammatory and healing characteristics [63]. In addition, they aid in removing dead cells and bacteria, perform wound debridement, and express proteases that benefit tissue repair. Neutrophils also facilitate monocyte recruitment and promote their removal from the tissue, which contributes to

 doi:10.20944/preprints202305.1582.v1

the resolution of inflammation and promotes healing and tissue repair. Additionally, it is seen that neutrophils release growth factors that promote angiogenesis, which can be exploited by cancer cells to promote their growth and spread [67].

In TB immunopathogenesis, neutrophils have a dual role [11,68,69]. High levels of these cells can be found in patients with active TB in the bloodstream and bronchoalveolar fluid [70–72]. Additionally, these cells are involved in central nervous system tuberculosis development [73]. Furthermore, neutrophils can phagocyte mycobacteria, and their antimicrobial machinery can display some antimycobacterial activities [44]. Further, it has been demonstrated that alveolar mucosa increases the capacity of the neutrophil to recognize and kill *Mtb* [74]. However, the dysregulated granulocytic influx is related to disease progression [75], and massive neutrophilic infiltration has been found in mouse models of TB [76]. Thus, neutrophil is also implicated in the exacerbation of lung tissue damage. In the following section, we describe the role of neutrophil extracellular traps in tuberculosis.

4. Neutrophil extracellular trap in tuberculosis

Neutrophil extracellular traps (NETs) are web-like structures composed of chromatin and antimicrobial proteins released by neutrophils. The primary function of NETs is to capture and kill microorganisms [77]. The DNA strands within NETs act as a physical barrier, immobilizing bacteria and fungi. At the same time, the antimicrobial proteins associated with the NETs, such as histones, neutrophil elastase, and myeloperoxidase, possess potent microbicidal properties that can directly kill pathogens [78]. Moreover, NETs also play a role in modulating immune responses. They can activate other immune cells, such as macrophages and dendritic cells, to enhance their antimicrobial activities. Additionally, NETs can interact with immune system components, including antibodies and complement proteins, facilitating the recognition and clearance of pathogens [79]. While NETs are crucial for host defense, their dysregulation has been associated with various inflammatory and autoimmune diseases. Excessive or prolonged NET formation can damage tissue, as NETs contain toxic components that can harm healthy cells and contribute to chronic inflammation [78–81].

Although NETs have infection control actions [82], the role of these neutrophil traps is still controversial and being investigated in diseases caused by mycobacteria [83]. Initially, it was only known that NETs could capture microorganisms. However, it is now recognized that such structures exert a direct microbicidal action against infectious agents [84]. Studies have already demonstrated the microbicidal activity of NETs against various pathogens, including bacteria, fungi, protozoa, and viruses [77,85–88]. However, other studies reveal that these extracellular structures only can capture but not kill *Mtb*, something already observed in vitro and *in vivo*, which may be related to the complex structure of its cell wall and its multiple detoxification mechanisms [89–93].

A current and relevant question regarding the role of NETs in tuberculosis is whether their release can be more beneficial or harmful to the host. This is because *Mtb* and BCG can mediate the release of NETs stimulation, and excessive formation of these structures can cause tissue damage [89,92,94]. Different mechanisms of 'NETosis' induced by *Mtb* and associated tissue damage have already been reported. Dang et al. [83] observed that the extracellular sphingomyelinase Rv0888 of *Mtb* in recombinant *M. smegmatis* could act as a virulence factor, inducing the release of NETs with associated myeloperoxidase (MPO), which leads to the aggravation of lung lesions through apoptosis via caspase-3. Additionally, extracellular traps induced by *Mtb* stimulate macrophages to release IL-6, TNF-α, and IL-1β, inflammatory cytokines that can recruit more neutrophils to the site of infection, exacerbating the inflammatory response and lung injury [95]. In addition, NETs associated with the NF-kB-dependent matrix metalloproteinase-8 (MMP-8) enzyme leading to collagen degradation in the lung tissue of humans with TB, and increased levels of NETs in patients' sputum have been observed [96]. Teixeira et al. [97] also observed that NETosis could be induced by type 1 IFN through the blocking of GM-CSF, which leads to the exacerbated formation of NETs and consequent growth of mycobacteria and worsening of pulmonary pathology, reinforcing the evidence that neutrophil traps when not controlled, contribute to the maintenance of pulmonary immunopathology in tuberculosis. Moreover, Su et al. [98] demonstrated that the induction of low-density granulocyte

 doi:10.20944/preprints202305.1582.v1

generation occurs during mycobacterium tuberculosis infection, facilitated by the promotion of neutrophil extracellular trap formation through the ROS pathway.

Another mechanism capable of inducing NETosis is the release of ESAT-6 by the mycobacterial ESX-1 system [99]. Francis et al. [99] demonstrated that the ESAT-6 protein acts as a leukocidin, causing neutrophil necrosis through a high influx of Ca^{2+} that leads to NET release, correlating the ESX-1 system as one of the virulence factors of *Mtb*. Moreover, the release of these NETs not only removes the threat of neutrophils and creates a necrotic environment rich in nutrients and conducive to extracellular bacillary growth. Therefore, in addition to aggravating tissue damage, NETs, when released excessively, also contribute in other ways to the progression of the pathology, and *Mtb* seems to know how to exploit this well.

On the other hand, one of the characteristics of active pulmonary tuberculosis is the presence of necrotic cores containing leukocytes and extracellular bacteria, which can undergo cavitation. Therefore, these lesions are associated with the potential for airborne transmission of the disease [99,100]. In addition, these granulomas are hypoxic, leading to decreased formation of NETs, probably caused by the decrease of reactive oxygen species (ROS) [101]. However, the consequences of this decrease are still unclear.

Furthermore, studying markers that help indicate tuberculosis's stages is paramount for its treatment. In this regard, Schechter et al. [102] observed that the release of NETs in TB could be identified through blood plasma by detecting extracellular DNA, human neutrophil elastase (HNE), and MPO. In addition, treatment with antibiotics decreased plasma levels of NETs. Similarly, De Melo et al. [103] reported higher levels of citrullinated histone H3, a marker of NETs, in the peripheral blood of patients with pulmonary tuberculosis and lung tissue damage. Additionally, Meier et al. [104] also observed increased levels of NETs in the total blood of tuberculosis progressors up to 6 months before the diagnosis of the disease, as well as their additional activation in tuberculosis patients. Such evidence suggests the detection of NETs could be an essential biomarkers in tuberculosis [72]. Furthermore, it again brings to light the formation of NETs associated with the immunopathology of the disease.

As previously mentioned, *Mtb* can induce the release of NETs but is not killed by these traps, which is interesting considering that NETs can lead to the death of other microorganisms. However, the mechanisms by which the mycobacterium evades extracellular traps have yet to be fully elucidated. Nevertheless, it is known that many bacterial species have developed ways to escape death by NETs. For example, *Streptococcus pyogenes*, *Staphylococcus aureus*, and *S. pneumoniae* produce DNases, which are seen as virulence factors for impairing NETosis [105–107]. Additionally, *S. pneumoniae* has a polysaccharide capsule that reduces the binding of NETs and modifies the lipoteichoic acids of its cell wall, altering its charge and disrupting its affinity with antimicrobial factors[107]. With the same goal, Group A *Streptococcus* (GAS) produces the M1 protein, inhibiting the action of the broad-spectrum antibacterial cathelicidin LL-37. Thus, inadequate LL-37 activity helps the survival of GAS within NETs [108]. Furthermore, it was demonstrated that LL37 complexed with DNA are internalized by macrophages after neutrophils release and then inhibit mycobacteria growth intracellularly [94]. Thus, a evade this mechanism seems a plausible way to mycobacteria escape NETs antimicrobial activity.

Furthermore, bacteria can escape from NETs through other mechanisms, such as releasing peptidases, forming biofilm, and inhibiting ROS production [106]. Concerning *Mtb*, to what extent this mycobacterium exploits these escape mechanisms has yet to be fully clear. Still, it is known that mycobacteria have a complex cell wall structure with a high lipid content, which hinders the penetration of antibacterial agents [109]. Additionally, *Mtb* is also sensitive to the action of LL-37, and here, the inhibition of this peptide by the pathogen is also something that can be investigated. Likewise, its detoxifying mechanisms should be better studied to bring to light, in greater completeness, the factors that contribute to the survival of *Mtb* in NETs, which would aid in discovering new therapeutic targets for tuberculosis.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 May 2023 doi:10.20944/preprints202305.1582.v1

5. Conclusions

The role of neutrophil extracellular traps (NETs) in diseases caused by mycobacteria, particularly tuberculosis, is still controversial and under investigation. While studies have shown the microbicidal activity of NETs against various pathogens, including bacteria, fungi, protozoa, and viruses, some research suggests that NETs can capture but not kill *M. tuberculosis*, possibly due to the complex structure of its cell wall and evasion mechanisms. Also, excessive formation of NETs in TB can lead to tissue damage and exacerbate the inflammatory response and lung injury (Figure 1). However, NETs levels in the serum plasm of TB patients could be explored more to find a new way to detect the disease progression. Understanding the interaction between *M. tuberculosis* and NETs could lead to identifying new therapeutic targets for TB.

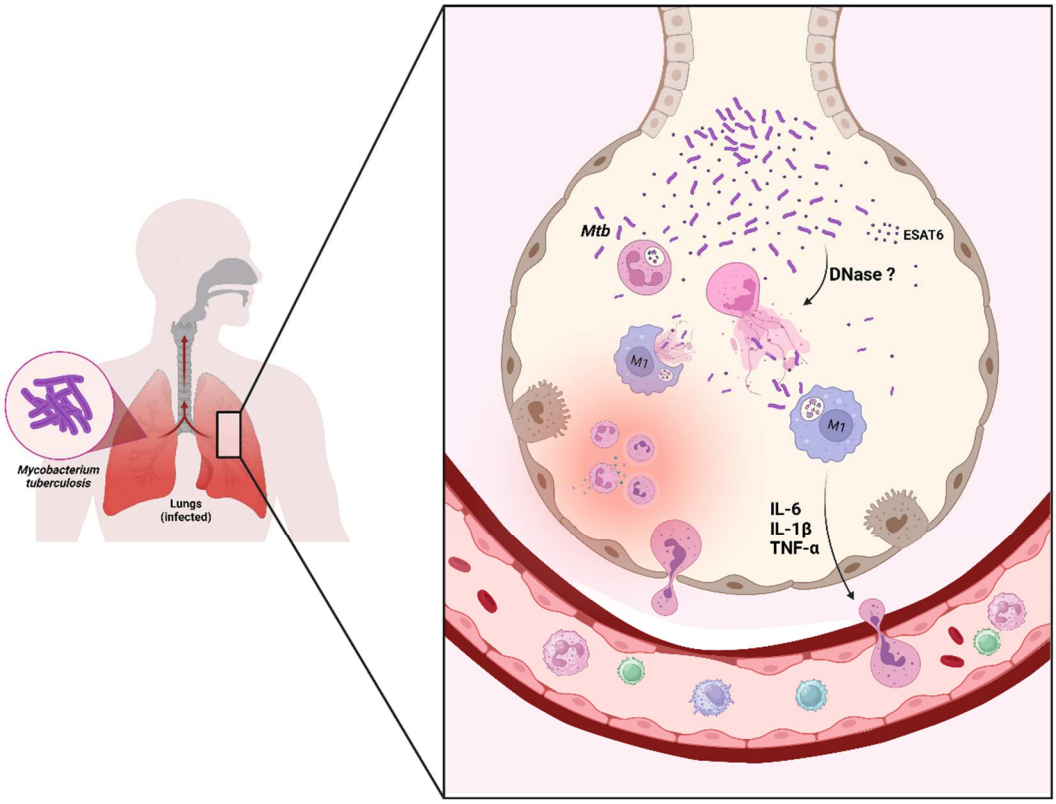

Figure 1. *Mycobacterium tuberculosis* and its antigens (e.g., ESAT-6) induce neutrophil extracellular traps release (NETs). This mechanism can capture but not kill *M. tuberculosis*. Some resistance mechanisms may be associated (e.g., perhaps DNase release). The NETs can induce macrophages to release cytokines (e.g., IL-6, IL-1β, and TNF-α) that favor neutrophil influx. Macrophages can also internalize NETs; in macrophage lysosomes, NETs peptides could kill *M. tuberculosis*. Created with Biorender.com.

Author Contributions: Conceptualization, L.H.A.C.-S.; writing—original draft preparation, L.H.A.C.-S., F.S.A., A.G.A., F.C.C. and S.E.R.V.; writing—review and editing, T.S.L.K.; and supervision, T.S.L.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by fellowships "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Number: 88887.704195/2022-00; 88887.717944/2022-00)" and "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Number: 440939/2020-8)".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Garrido-Cardenas, J.A.; de Lamo-Sevilla, C.; Cabezas-Fernández, M.T.; Manzano-Agugliaro, F.; Martínez-Lirola, M. Global tuberculosis research and its future prospects. *Tuberculosis (Edinb).* 2020, *121*, doi:10.1016/J.TUBE.2020.101917.
2. Nogueira, B.M.F.; Krishnan, S.; Barreto-Duarte, B.; Araújo-Pereira, M.; Queiroz, A.T.L.; Ellner, J.J.; Salgame, P.; Scriba, T.J.; Sterling, T.R.; Gupta, A.; et al. Diagnostic biomarkers for active tuberculosis: progress and challenges. *EMBO Mol. Med.* **2022**, *14*, doi:10.15252/EMMM.202114088.
3. Dicks, K. V.; Stout, J.E. Molecular Diagnostics for Mycobacterium tuberculosis Infection. *Annu. Rev. Med.* **2019**, *70*, 77–90, doi:10.1146/ANNUREV-MED-040717-051502.
4. Lucena, L.A. de; Dantas, G.B. da S.; Carneiro, T.V.; Lacerda, H.G. Factors Associated with the Abandonment of Tuberculosis Treatment in Brazil: A Systematic Review. *Rev. Soc. Bras. Med. Trop.* **2023**, *56*, doi:10.1590/0037-8682-0155-2022.
5. Yadav, P. Challenges & Solutions for Recent Advancements in Multi-Drugs Resistance Tuberculosis: A Review. *Microbiol. insights* **2023**, *16*, 117863612311524, doi:10.1177/11786361231152438.
6. Singh, S.; Saavedra-Avila, N.A.; Tiwari, S.; Porcelli, S.A. A century of BCG vaccination: Immune mechanisms, animal models, non-traditional routes and implications for COVID-19. *Front. Immunol.* **2022**, *13*, doi:10.3389/FIMMU.2022.959656.
7. Pai, M.; Kasaeva, T.; Swaminathan, S. Covid-19's Devastating Effect on Tuberculosis Care - A Path to Recovery. *N. Engl. J. Med.* **2022**, *386*, 1490–1493, doi:10.1056/NEJMP2118145.
8. World Health Organization (WHO) Global Tuberculosis Report 2022. *Geneva World Heal. Organ.* **2022**.
9. Kanabalan, R.D.; Lee, L.J.; Lee, T.Y.; Chong, P.P.; Hassan, L.; Ismail, R.; Chin, V.K. Human tuberculosis and Mycobacterium tuberculosis complex: A review on genetic diversity, pathogenesis and omics approaches in host biomarkers discovery. *Microbiol. Res.* **2021**, *246*, doi:10.1016/J.MICRES.2020.126674.
10. Scriba, T.J.; Coussens, A.K.; Fletcher, H.A. Human Immunology of Tuberculosis. *Microbiol. Spectr.* **2016**, *4*, doi:10.1128/microbiolspec.TBTB2-0016-2016.
11. Borkute, R.R.; Woelke, S.; Pei, G.; Dorhoi, A. Neutrophils in Tuberculosis: Cell Biology, Cellular Networking and Multitasking in Host Defense. *Int. J. Mol. Sci.* **2021**, *22*, doi:10.3390/IJMS22094801.
12. Liew, P.X.; Kubes, P. The Neutrophil's Role During Health and Disease. *Physiol. Rev.* **2019**, *99*, 1223–1248, doi:10.1152/physrev.00012.2018.
13. Burn, G.L.; Foti, A.; Marsman, G.; Patel, D.F.; Zychlinsky, A. The Neutrophil. *Immunity* **2021**, *54*, 1377–1391, doi:10.1016/J.IMMUNI.2021.06.006.
14. Loddenkemper, R.; Murray, J.F. History of Tuberculosis. In *Essential Tuberculosis*; Springer, Cham: Cham, 2021; pp. 3–9.
15. Guerra-De-Blas, P.D.C.; Torres-González, P.; Bobadilla-Del-Valle, M.; Sada-Ovalle, I.; Ponce-De-León-Garduño, A.; Sifuentes-Osornio, J. Potential Effect of Statins on Mycobacterium tuberculosis Infection. *J. Immunol. Res.* **2018**, *2018*, doi:10.1155/2018/7617023.
16. Pai, M.; Behr, M.A.; Dowdy, D.; Dheda, K.; Divangahi, M.; Boehme, C.C.; Ginsberg, A.; Swaminathan, S.; Spigelman, M.; Getahun, H.; et al. Tuberculosis. *Nat. Rev. Dis. Prim.* **2016**, *2*, doi:10.1038/NRDP.2016.76.
17. Dowdy, D.W.; Raviglione, M.C. Basic and Descriptive Epidemiology of Tuberculosis. In *Essential Tuberculosis*; Springer, Cham, 2021; pp. 29–36.
18. Campaniço, A.; Harjivan, S.G.; Warner, D.F.; Moreira, R.; Lopes, F. Addressing Latent Tuberculosis: New Advances in Mimicking the Disease, Discovering Key Targets, and Designing Hit Compounds. *Int. J. Mol. Sci. 2020, Vol. 21, Page 8854* **2020**, *21*, 8854, doi:10.3390/IJMS21228854.
19. Ferluga, J.; Yasmin, H.; Al-Ahdal, M.N.; Bhakta, S.; Kishore, U. Natural and trained innate immunity against Mycobacterium tuberculosis. *Immunobiology* **2020**, *225*, doi:10.1016/J.IMBIO.2020.151951.
20. Simmons, J.D.; Stein, C.M.; Seshadri, C.; Campo, M.; Alter, G.; Fortune, S.; Schurr, E.; Wallis, R.S.; Churchyard, G.; Mayanja-Kizza, H.; et al. Immunological mechanisms of human resistance to persistent Mycobacterium tuberculosis infection. *Nat. Rev. Immunol.* **2018**, *18*, 575–589, doi:10.1038/S41577-018-0025-3.
21. Warner, D.F.; Koch, A.; Mizrahi, V. Diversity and disease pathogenesis in Mycobacterium tuberculosis. *Trends Microbiol.* **2015**, *23*, 14–21, doi:10.1016/J.TIM.2014.10.005.
22. Bruchfeld, J.; Forsman, L.D.; Fröberg, G.; Niward, K. Extrapulmonary Tuberculosis. In *Essential Tuberculosis*; Springer, Cham: Cham, 2021; pp. 259–266.
23. Rodriguez-Takeuchi, S.Y.; Renjifo, M.E.; Medina, F.J. Extrapulmonary Tuberculosis: Pathophysiology and Imaging Findings. *Radiographics* **2019**, *39*, 2023–2037, doi:10.1148/RG.2019190109.
24. Wilkinson, R.J.; Rohlwink, U.; Misra, U.K.; Van Crevel, R.; Mai, N.T.H.; Dooley, K.E.; Caws, M.; Figaji, A.; Savic, R.; Solomons, R.; et al. Tuberculous meningitis. *Nat. Rev. Neurol. 2017 1310* **2017**, *13*, 581–598, doi:10.1038/nrneurol.2017.120.
25. Ahmad, S. Pathogenesis, immunology, and diagnosis of latent Mycobacterium tuberculosis infection. *Clin. Dev. Immunol.* **2011**, *2011*, doi:10.1155/2011/814943.

26. Jellinger, K.A.; Bustin, S.; Alsayed, S.S.R.; Gunosewoyo, H. Tuberculosis: Pathogenesis, Current Treatment Regimens and New Drug Targets. *Int. J. Mol. Sci. 2023, Vol. 24, Page 5202* **2023**, *24*, 5202, doi:10.3390/IJMS24065202.
27. Palucci, I.; Delogu, G. Host Directed Therapies for Tuberculosis: Futures Strategies for an Ancient Disease. *Chemotherapy* **2018**, *63*, 172–180, doi:10.1159/000490478.
28. Domingo-Gonzalez, R.; Prince, O.; Cooper, A.; Khader, S.A. Cytokines and Chemokines in Mycobacterium tuberculosis Infection. *Microbiol. Spectr.* **2016**, doi:10.1128/MICROBIOLSPEC.TBTB2-0018-2016.
29. Mundra, A.; Yegiazaryan, A.; Karsian, H.; Alsaigh, D.; Bonavida, V.; Frame, M.; May, N.; Gargaloyan, A.; Abnousian, A.; Venketaraman, V. Pathogenicity of Type I Interferons in Mycobacterium tuberculosis. *Int. J. Mol. Sci.* **2023**, *24*, doi:10.3390/IJMS24043919.
30. Ernst, J.D. Mechanisms of M. tuberculosis Immune Evasion as Challenges to TB Vaccine Design. *Cell Host Microbe* **2018**, *24*, 34–42, doi:10.1016/J.CHOM.2018.06.004.
31. Sia, J.K.; Rengarajan, J. Immunology of Mycobacterium tuberculosis Infections. *Microbiol. Spectr.* **2019**, *7*, doi:10.1128/MICROBIOLSPEC.GPP3-0022-2018.
32. Kim, H.; Shin, S.J. Pathological and protective roles of dendritic cells in Mycobacterium tuberculosis infection: Interaction between host immune responses and pathogen evasion. *Front. Cell. Infect. Microbiol.* **2022**, *12*, 891878, doi:10.3389/FCIMB.2022.891878.
33. Zumla, A.; Rao, M.; Parida, S.K.; Keshavjee, S.; Cassell, G.; Wallis, R.; Axelsson-Robertsson, R.; Doherty, M.; Andersson, J.; Maeurer, M.J. Inflammation and tuberculosis: host-directed therapies. *J. Intern. Med.* **2015**, *277*, 373–387, doi:10.1111/JOIM.12256.
34. Domingo-Gonzalez, R.; Prince, O.; Cooper, A.; Khader, S.A. Cytokines and Chemokines in Mycobacterium tuberculosis Infection. *Microbiol. Spectr.* **2016**, *4*, doi:10.1128/MICROBIOLSPEC.TBTB2-0018-2016.
35. Kumar, P. Adult pulmonary tuberculosis as a pathological manifestation of hyperactive antimycobacterial immune response. *Clin. Transl. Med.* **2016**, *5*, doi:10.1186/S40169-016-0119-0.
36. Bell, L.C.K.; Noursadeghi, M. Pathogenesis of HIV-1 and Mycobacterium tuberculosis co-infection. *Nat. Rev. Microbiol.* **2018**, *16*, 80–90, doi:10.1038/NRMICRO.2017.128.
37. Ssekamatte, P.; Sande, O.J.; van Crevel, R.; Biraro, I.A. Immunologic, metabolic and genetic impact of diabetes on tuberculosis susceptibility. *Front. Immunol.* **2023**, *14*, doi:10.3389/FIMMU.2023.1122255.
38. Jasenosky, L.D.; Scriba, T.J.; Hanekom, W.A.; Goldfeld, A.E. T cells and adaptive immunity to Mycobacterium tuberculosis in humans. *Immunol. Rev.* **2015**, *264*, 74–87, doi:10.1111/IMR.12274.
39. Chai, Q.; Lu, Z.; Liu, C.H. Host defense mechanisms against Mycobacterium tuberculosis. *Cell. Mol. Life Sci.* **2020**, *77*, 1859–1878, doi:10.1007/S00018-019-03353-5.
40. Urdahl, K.B. Understanding and overcoming the barriers to T cell-mediated immunity against tuberculosis. *Semin. Immunol.* **2014**, *26*, 578–587, doi:10.1016/J.SMIM.2014.10.003.
41. Jiang, J.; Cao, Z.; Xiao, L.; Su, J.; Wang, J.; Liang, J.; Yang, B.; Liu, Y.; Zhai, F.; Wang, R.; et al. Single-cell profiling identifies T cell subsets associated with control of tuberculosis dissemination. *Clin. Immunol.* **2023**, *248*, doi:10.1016/J.CLIM.2023.109266.
42. Ehlers, S.; Schaible, U.E. The granuloma in tuberculosis: Dynamics of a host-pathogen collusion. *Front. Immunol.* **2012**, *3*, 411, doi:10.3389/FIMMU.2012.00411/BIBTEX.
43. Gideon, H.P.; Hughes, T.K.; Tzouanas, C.N.; Wadsworth, M.H.; Tu, A.A.; Gierahn, T.M.; Peters, J.M.; Hopkins, F.F.; Wei, J.R.; Kummerlowe, C.; et al. Multimodal profiling of lung granulomas in macaques reveals cellular correlates of tuberculosis control. *Immunity* **2022**, *55*, 827-846.e10, doi:10.1016/J.IMMUNI.2022.04.004.
44. Parker, H.A.; Forrester, L.; Kaldor, C.D.; Dickerhof, N.; Hampton, M.B. Antimicrobial Activity of Neutrophils Against Mycobacteria. *Front. Immunol.* **2021**, *12*, doi:10.3389/FIMMU.2021.782495.
45. Naumenko, V.; Turk, M.; Jenne, C.N.; Kim, S.J. Neutrophils in viral infection. *Cell Tissue Res.* **2018**, *371*, 505–516, doi:10.1007/s00441-017-2763-0.
46. Cavalcante-Silva, L.H.A.; Carvalho, D.C.M.; Lima, É. de A.; Galvão, J.G.F.M.; da Silva, J.S. d. F.; Sales-Neto, J.M. de; Rodrigues-Mascarenhas, S. Neutrophils and COVID-19: The road so far. *Int. Immunopharmacol.* **2021**, *90*, doi:10.1016/j.intimp.2020.107233.
47. Rosales, C. Neutrophil: A Cell with Many Roles in Inflammation or Several Cell Types? *Front. Physiol.* **2018**, *9*, 113, doi:10.3389/fphys.2018.00113.
48. Galani, I.E.; Andreakos, E. Neutrophils in viral infections: Current concepts and caveats. *J. Leukoc. Biol.* **2015**, *98*, 557–564, doi:10.1189/JLB.4VMR1114-555R.
49. Urban, C.F.; Backman, E. Eradicating, retaining, balancing, swarming, shuttling and dumping: a myriad of tasks for neutrophils during fungal infection. *Curr. Opin. Microbiol.* **2020**, *58*, 106–115, doi:10.1016/J.MIB.2020.09.011.
50. Newburger, P.E. Autoimmune and other acquired neutropenias. *Hematol. Am. Soc. Hematol. Educ. Progr.* **2016**, *2016*, 38–42, doi:10.1182/ASHEDUCATION-2016.1.38.
51. Kumar, S.; Dikshit, M. Metabolic Insight of Neutrophils in Health and Disease. *Front. Immunol.* **2019**, *10*, doi:10.3389/FIMMU.2019.02099.

52. Shim, H.B.; Deniset, J.F.; Kubes, P. Neutrophils in homeostasis and tissue repair. *Int. Immunol.* **2022**, *34*, 399–407, doi:10.1093/INTIMM/DXAC029.
53. Palomino-Segura, M.; Sicilia, J.; Ballesteros, I.; Hidalgo, A. Strategies of neutrophil diversification. *Nat. Immunol.* **2023**, *24*, 575–584, doi:10.1038/S41590-023-01452-X.
54. Hidalgo, A.; Chilvers, E.R.; Summers, C.; Koenderman, L. The Neutrophil Life Cycle. *Trends Immunol.* **2019**, *40*, 584–597, doi:10.1016/j.it.2019.04.013.
55. Grieshaber-Bouyer, R.; Radtke, F.A.; Cunin, P.; Stifano, G.; Levescot, A.; Vijaykumar, B.; Nelson-Maney, N.; Blaustein, R.B.; Monach, P.A.; Nigrovic, P.A.; et al. The neutrotime transcriptional signature defines a single continuum of neutrophils across biological compartments. *Nat. Commun.* **2021**, *12*, doi:10.1038/S41467-021-22973-9.
56. Ley, K.; Hoffman, H.M.; Kubes, P.; Cassatella, M.A.; Zychlinsky, A.; Hedrick, C.C.; Catz, S.D. Neutrophils: New insights and open questions. *Sci. Immunol.* **2018**, *3*, eaat4579, doi:10.1126/sciimmunol.aat4579.
57. Deniset, J.F.; Kubes, P. Neutrophil heterogeneity: Bona fide subsets or polarization states? *J. Leukoc. Biol.* **2018**, *103*, 829–838, doi:10.1002/JLB.3RI0917-361R.
58. Cassatella, M.A.; Östberg, N.K.; Tamassia, N.; Soehnlein, O. Biological Roles of Neutrophil-Derived Granule Proteins and Cytokines. *Trends Immunol.* **2019**, *40*, 648–664, doi:10.1016/J.IT.2019.05.003.
59. Phillipson, M.; Kubes, P. The Healing Power of Neutrophils. *Trends Immunol.* **2019**, *40*, 635–647, doi:10.1016/j.it.2019.05.001.
60. Kawai, T.; Akira, S. The role of pattern-recognition receptors in innate immunity: Update on toll-like receptors. *Nat. Immunol.* **2010**, *11*, 373–384, doi:10.1038/ni.1863.
61. Netea, M.G.; Balkwill, F.; Chonchol, M.; Cominelli, F.; Donath, M.Y.; Giamarellos-Bourboulis, E.J.; Golenbock, D.; Gresnigt, M.S.; Heneka, M.T.; Hoffman, H.M.; et al. A guiding map for inflammation. *Nat. Immunol.* **2017**, *18*, 826–831, doi:10.1038/NI.3790.
62. Medzhitov, R. Inflammation 2010: New Adventures of an Old Flame. *Cell* **2010**, *140*, 771–776, doi:10.1016/j.cell.2010.03.006.
63. Kolaczkowska, E.; Kubes, P. Neutrophil recruitment and function in health and inflammation. *Nat. Rev. Immunol.* **2013**, *13*, 159–175, doi:10.1038/nri3399.
64. Chatfield, S.M.; Thieblemont, N.; Witko-Sarsat, V. Expanding Neutrophil Horizons: New Concepts in Inflammation. *J. Innate Immun.* 2018, *10*, 422–431.
65. Muller, W.A. Mechanisms of Leukocyte Transendothelial Migration. *Annu. Rev. Pathol. Mech. Dis.* **2011**, *6*, 323–344, doi:10.1146/annurev-pathol-011110-130224.
66. Hyun, Y.; Hong, C. Deep insight into neutrophil trafficking in various organs. *J. Leukoc. Biol.* **2017**, *102*, 617–629, doi:10.1189/jlb.1ru1216-521r.
67. Powell, D.R.; Huttenlocher, A. Neutrophils in the Tumor Microenvironment. *Trends Immunol.* **2016**, *37*, 41–52, doi:10.1016/j.it.2015.11.008.
68. Wu, F.; Zhao, S.; Yu, B.; Chen, Y.M.; Wang, W.; Song, Z.G.; Hu, Y.; Tao, Z.W.; Tian, J.H.; Pei, Y.Y.; et al. A new coronavirus associated with human respiratory disease in China. *Nature* **2020**, *579*, 265–269, doi:10.1038/s41586-020-2008-3.
69. Hilda, J.N.; Das, S.; Tripathy, S.P.; Hanna, L.E. Role of neutrophils in tuberculosis: A bird's eye view. *Innate Immun.* **2020**, *26*, 240–247, doi:10.1177/1753425919881176.
70. Panteleev, A. V.; Nikitina, I.Y.; Burmistrova, I.A.; Kosmiadi, G.A.; Radaeva, T. V.; Amansahedov, R.B.; Sadikov, P. V.; Serdyuk, Y. V.; Larionova, E.E.; Bagdasarian, T.R.; et al. severe tuberculosis in humans correlates best with neutrophil abundance and lymphocyte deficiency and does not correlate with antigen-specific CD4 T-cell response. *Front. Immunol.* **2017**, *8*, 963, doi:10.3389/FIMMU.2017.00963/BIBTEX.
71. Lowe, D.M.; Bandara, A.K.; Packe, G.E.; Barker, R.D.; Wilkinson, R.J.; Griffiths, C.J.; Martineau, A.R. Neutrophilia independently predicts death in tuberculosis. *Eur. Respir. J.* **2013**, *42*, 1752–1757, doi:10.1183/09031936.00140913.
72. van der Meer, A.J.; Zeerleder, S.; Blok, D.C.; Kager, L.M.; Lede, I.O.; Rahman, W.; Afroz, R.; Ghose, A.; Visser, C.E.; Zahed, A.S.M.; et al. Neutrophil extracellular traps in patients with pulmonary tuberculosis. *Respir. Res.* **2017**, *18*, doi:10.1186/S12931-017-0663-1.
73. Poh, X.Y.; Loh, F.K.; Friedland, J.S.; Ong, C.W.M. Neutrophil-Mediated Immunopathology and Matrix Metalloproteinases in Central Nervous System - Tuberculosis. *Front. Immunol.* **2022**, *12*, doi:10.3389/FIMMU.2021.788976.
74. Arcos, J.; Diangelo, L.E.; Scordo, J.M.; Sasindran, S.J.; Moliva, J.I.; Turner, J.; Torrelles, J.B. Lung Mucosa Lining Fluid Modification of Mycobacterium tuberculosis to Reprogram Human Neutrophil Killing Mechanisms. *J. Infect. Dis.* **2015**, *212*, 948–958, doi:10.1093/INFDIS/JIV146.
75. Gopal, R.; Monin, L.; Torres, D.; Slight, S.; Mehra, S.; McKenna, K.C.; Junecko, B.A.F.; Reinhart, T.A.; Kolls, J.; Báez-Saldańa, R.; et al. S100A8/A9 proteins mediate neutrophilic inflammation and lung pathology during tuberculosis. *Am. J. Respir. Crit. Care Med.* **2013**, *188*, 1137–1146, doi:10.1164/RCCM.201304-0803OC/SUPPL_FILE/DISCLOSURES.PDF.

76. Lovewell, R.R.; Baer, C.E.; Mishra, B.B.; Smith, C.M.; Sassetti, C.M. Granulocytes act as a niche for Mycobacterium tuberculosis growth. *Mucosal Immunol. 2020 141* **2020**, *14*, 229–241, doi:10.1038/s41385-020-0300-z.
77. Brinkmann, V.; Reichard, U.; Goosmann, C.; Fauler, B.; Uhlemann, Y.; Weiss, D.S.; Weinrauch, Y.; Zychlinsky, A. Neutrophil Extracellular Traps Kill Bacteria. *Science (80-.).* **2004**, *303*, 1532–1535, doi:10.1126/science.1092385.
78. Papayannopoulos, V. Neutrophil extracellular traps in immunity and disease. *Nat. Rev. Immunol.* 2018, *18*, 134–147.
79. Brinkmann, V. Neutrophil Extracellular Traps in the Second Decade. *J. Innate Immun.* 2018, *10*, 414–421.
80. Narasaraju, T.; Tang, B.M.; Herrmann, M.; Muller, S.; Chow, V.T.K.; Radic, M. Neutrophilia and NETopathy as Key Pathologic Drivers of Progressive Lung Impairment in Patients With COVID-19. *Front. Pharmacol.* **2020**, *11*, doi:10.3389/fphar.2020.00870.
81. Hahn, J.; Knopf, J.; Maueröder, C.; Kienhöfer, D.; Leppkes, M.; Herrmann, M. Neutrophils and neutrophil extracellular traps orchestrate initiation and resolution of inflammation. *Clin. Exp. Rheumatol.* **2016**, *34*, 6–8.
82. Porto, B.N.; Stein, R.T. Neutrophil Extracellular Traps in Pulmonary Diseases: Too Much of a Good Thing? *Front. Immunol.* **2016**, *7*, doi:10.3389/FIMMU.2016.00311.
83. Dang, G.; Cui, Y.; Wang, L.; Li, T.; Cui, Z.; Song, N.; Chen, L.; Pang, H.; Liu, S. Extracellular Sphingomyelinase Rv0888 of Mycobacterium tuberculosis Contributes to Pathological Lung Injury of Mycobacterium smegmatis in Mice via Inducing Formation of Neutrophil Extracellular Traps. *Front. Immunol.* **2018**, *9*, doi:10.3389/FIMMU.2018.00677.
84. Yipp, B.G.; Kubes, P. NETosis: how vital is it? *Blood* **2013**, *122*, 2784–2794, doi:10.1182/BLOOD-2013-04-457671.
85. Delgado-Rizo, V.; Martínez-Guzmán, M.A.; Iñiguez-Gutierrez, L.; García-Orozco, A.; Alvarado-Navarro, A.; Fafutis-Morris, M. Neutrophil Extracellular Traps and Its Implications in Inflammation: An Overview. *Front. Immunol.* **2017**, *8*, doi:10.3389/FIMMU.2017.00081.
86. Hiroki, C.H.; Toller-Kawahisa, J.E.; Fumagalli, M.J.; Colon, D.F.; Figueiredo, L.T.M.; Fonseca, B.A.L.D.; Franca, R.F.O.; Cunha, F.Q. Neutrophil Extracellular Traps Effectively Control Acute Chikungunya Virus Infection. *Front. Immunol.* **2020**, *10*, doi:10.3389/FIMMU.2019.03108.
87. Urban, C.F.; Nett, J.E. Neutrophil extracellular traps in fungal infection. *Semin. Cell Dev. Biol.* **2019**, *89*, 47–57, doi:10.1016/J.SEMCDB.2018.03.020.
88. Guimarães-Costa, A.B.; Nascimento, M.T.C.; Froment, G.S.; Soares, R.P.P.; Morgado, F.N.; Conceição-Silva, F.; Saraiva, E.M. Leishmania amazonensis promastigotes induce and are killed by neutrophil extracellular traps. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 6748–6753, doi:10.1073/PNAS.0900226106.
89. Ramos-Kichik, V.; Mondragón-Flores, R.; Mondragón-Castelán, M.; Gonzalez-Pozos, S.; Muñiz-Hernandez, S.; Rojas-Espinosa, O.; Chacón-Salinas, R.; Estrada-Parra, S.; Estrada-García, I. Neutrophil extracellular traps are induced by Mycobacterium tuberculosis. *Tuberculosis (Edinb).* **2009**, *89*, 29–37, doi:10.1016/J.TUBE.2008.09.009.
90. Filio-Rodríguez, G.; Estrada-García, I.; Arce-Paredes, P.; Moreno-Altamirano, M.M.; Islas-Trujillo, S.; Ponce-Regalado, M.D.; Rojas-Espinosa, O. In vivo induction of neutrophil extracellular traps by Mycobacterium tuberculosis in a guinea pig model. *Innate Immun.* **2017**, *23*, 625–637, doi:10.1177/1753425917732406.
91. Hahn, S.; Giaglis, S.; Chowdury, C.S.; Hösli, I.; Hasler, P. Modulation of neutrophil NETosis: interplay between infectious agents and underlying host physiology. *Semin. Immunopathol.* **2013**, *35*, 439–453, doi:10.1007/S00281-013-0380-X.
92. Nakamura, K.; Nakayama, H.; Sasaki, S.; Takahashi, K.; Iwabuchi, K. Mycobacterium avium-intracellulare complex promote release of pro-inflammatory enzymes matrix metalloproteinases by inducing neutrophil extracellular trap formation. *Sci. Rep.* **2022**, *12*, doi:10.1038/S41598-022-09017-Y.
93. Mendoza-Aguilar, M.D.; Arce-Paredes, P.; Aquino-Vega, M.; Rodríguez-Martínez, S.; Rojas-Espinosa, O. Fate of Mycobacterium tuberculosis in peroxidase-loaded resting murine macrophages. *Int. J. mycobacteriology* **2013**, *2*, 3–13, doi:10.1016/J.IJMYCO.2012.11.002.
94. Stephan, A.; Batinica, M.; Steiger, J.; Hartmann, P.; Zaucke, F.; Bloch, W.; Fabri, M. LL37:DNA complexes provide antimicrobial activity against intracellular bacteria in human macrophages. *Immunology* **2016**, *148*, 420–432, doi:10.1111/IMM.12620.
95. Braian, C.; Hogea, V.; Stendahl, O. Mycobacterium tuberculosis- induced neutrophil extracellular traps activate human macrophages. *J. Innate Immun.* **2013**, *5*, 591–602, doi:10.1159/000348676.
96. Schechter, M.C.; Buac, K.; Adekambi, T.; Cagle, S.; Celli, J.; Ray, S.M.; Mehta, C.C.; Rada, B.; Rengarajan, J.; Delgado-Rizo, V.; et al. Neutrophil-Derived MMP-8 Drives AMPK-Dependent Matrix Destruction in Human Pulmonary Tuberculosis. *Front. Immunol.* **2013**, *7*, 1–13, doi:10.1182/blood-2013-04-457671.

97. Moreira-Teixeira, L.; Stimpson, P.J.; Stavropoulos, E.; Hadebe, S.; Chakravarty, P.; Ioannou, M.; Aramburu, I.V.; Herbert, E.; Priestnall, S.L.; Suarez-Bonnet, A.; et al. Type I IFN exacerbates disease in tuberculosis-susceptible mice by inducing neutrophil-mediated lung inflammation and NETosis. *Nat. Commun.* **2020**, *11*, doi:10.1038/S41467-020-19412-6.
98. Su, R.; Peng, Y.P.; Deng, Z.; Deng, Y.T.; Ye, J.Q.; Guo, Y.; Huang, Z.K.; Luo, Q.; Jiang, H.; Li, J.M. Mycobacterium tuberculosis Infection Induces Low-Density Granulocyte Generation by Promoting Neutrophil Extracellular Trap Formation via ROS Pathway. *Front. Microbiol.* **2019**, *10*, doi:10.3389/FMICB.2019.01468.
99. Francis, R.J.; Butler, R.E.; Stewart, G.R. Mycobacterium tuberculosis ESAT-6 is a leukocidin causing Ca2+ influx, necrosis and neutrophil extracellular trap formation. *Cell Death Dis.* **2014**, *5*, doi:10.1038/CDDIS.2014.394.
100. Hunter, R.L. The Pathogenesis of Tuberculosis-The Koch Phenomenon Reinstated. *Pathog. (Basel, Switzerland)* **2020**, *9*, 1–25, doi:10.3390/PATHOGENS9100813.
101. Ong, C.W.M.; Fox, K.; Ettorre, A.; Elkington, P.T.; Friedland, J.S. Hypoxia increases neutrophil-driven matrix destruction after exposure to Mycobacterium tuberculosis. *Sci. Rep.* **2018**, *8*, doi:10.1038/S41598-018-29659-1.
102. Schechter, M.C.; Buac, K.; Adekambi, T.; Cagle, S.; Celli, J.; Ray, S.M.; Mehta, C.C.; Rada, B.; Rengarajan, J. Neutrophil extracellular trap (NET) levels in human plasma are associated with active TB. *PLoS One* **2017**, *12*, doi:10.1371/JOURNAL.PONE.0182587.
103. De Melo, M.G.M.; Mesquita, E.D.D.; Oliveira, M.M.; Da Silva-Monteiro, C.; Silveira, A.K.A.; Malaquias, T.S.; Dutra, T.C.P.; Galliez, R.M.; Kritski, A.L.; Silva, E.C. Imbalance of NET and Alpha-1-Antitrypsin in Tuberculosis Patients Is Related With Hyper Inflammation and Severe Lung Tissue Damage. *Front. Immunol.* **2019**, *9*, doi:10.3389/FIMMU.2018.03147.
104. Meier, S.; Seddon, J.A.; Maasdorp, E.; Kleynhans, L.; du Plessis, N.; Loxton, A.G.; Malherbe, S.T.; Zak, D.E.; Thompson, E.; Duffy, F.J.; et al. Neutrophil degranulation, NETosis and platelet degranulation pathway genes are co-induced in whole blood up to six months before tuberculosis diagnosis. *PLoS One* **2022**, *17*, doi:10.1371/JOURNAL.PONE.0278295.
105. Ríos-López, A.L.; González, G.M.; Hernández-Bello, R.; Sánchez-González, A. Avoiding the trap: Mechanisms developed by pathogens to escape neutrophil extracellular traps. *Microbiol. Res.* **2021**, *243*, 126644, doi:10.1016/J.MICRES.2020.126644.
106. Arazna, M.; Pruchniak, M.P.; Demkow, U. Neutrophil extracellular traps in bacterial infections: strategies for escaping from killing. *Respir. Physiol. Neurobiol.* **2013**, *187*, 74–77, doi:10.1016/J.RESP.2013.03.002.
107. Manda, A.; Pruchniak, M.P.; Araźna, M.; Demkow, U.A. Neutrophil extracellular traps in physiology and pathology. *Cent. J. Immunol.* **2014**, *39*, 116–121, doi:10.5114/CEJI.2014.42136.
108. Lauth, X.; Von Köckritz-Blickwede, M.; McNamara, C.W.; Myskowski, S.; Zinkernagel, A.S.; Beall, B.; Ghosh, P.; Gallo, R.L.; Nizet, V. M1 Protein Allows Group A Streptococcal Survival in Phagocyte Extracellular Traps through Cathelicidin Inhibition. *J. Innate Immun.* **2009**, *1*, 202, doi:10.1159/000203645.
109. Brennan, P.J. Structure, function, and biogenesis of the cell wall of Mycobacterium tuberculosis. *Tuberculosis* **2003**, *83*, 91–97, doi:10.1016/S1472-9792(02)00089-6.