Herald of Science of S.Seifullin Kazakh Agrotechnical Research University: Veterinary Sciences. – Astana: S. Seifullin Kazakh Agrotechnical Research University, 2025. – № 4 (012). – P.34-54. - ISSN 2958-5430, ISSN 2958-5449

doi.org/ 10.51452/kazatuvc.2025.4(012).2103
UDC 616-002.5 **Research article**

Immunoactive Proteins of *Mycobacterium bovis*: From Molecular Mechanisms to Biomarkers and Vaccines

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Received: 11.12.2025 **Accepted:** 25.12. 2025 **Published:** 30.12.2025

Abstract

Background and Aim. *Mycobacterium bovis* is a zoonotic member of the *Mycobacterium tuberculosis* complex and the causative agent of bovine tuberculosis, posing significant challenges to veterinary health, wildlife management and public health. Despite high genomic similarity to *M. tuberculosis*, M. bovis exhibits distinct host–pathogen interaction strategies that influence immune recognition, persistence and disease progression.

This review aims to systematize and critically synthesize current knowledge on immunoactive proteins of *M. bovis* and to elucidate their roles in modulation of innate and adaptive immune responses, with particular emphasis on mechanisms of immune evasion, diagnostic relevance and vaccine potential.

Materials and Methods. This narrative review synthesizes peer-reviewed literature retrieved from international scientific databases. Studies addressing molecular genetics, proteomics, tran-scriptomics, host immune signaling pathways, and experimental infection models of *M. bovis* were critically evaluated. Special attention was given to proteins involved in phagocytosis, autophagy, Toll-like receptor (TLR) signaling and cytokine regulation, as well as to comparative studies with M. tuberculosis.

Results. The analysis indicates that *M. bovis* actively modulates macrophage defense mechanisms through multiple molecular axes, including inhibition of phagosome–lysosome fusion, selective activation of PINK1–Parkin-dependent mitophagy and suppression of xenophagy. Key immunoactive proteins, such as ESAT-6, CFP-10, MPB70/80/83, PE/PPE proteins and lipoproteins, play central roles in shaping both inflammatory and regulatory immune responses. Activation of cytosolic DNA sensors, particularly Interferon-Inducible Protein 204 (IFI204), and downstream IFN-β signaling is more pronounced in *M. bovis* infection and contributes to species-specific immune responses. Differential expression and secretion of these proteins underpin their value as diagnostic biomarkers and potential vaccine antigens.

Conclusion. Immunoactive proteins of *M. bovis* form a complex molecular network that enables immune modulation, intracellular persistence and host adaptation. Their functional significance extends beyond virulence, positioning them as promising targets for Differentiating Infected from Vaccinated

Animals (DIVA)-compatible diagnostics and second-generation vaccines. An integrated understanding of these mechanisms is essential for improving control strategies for bovine tubercu-losis and reducing the risk of zoonotic transmission.

Keywords: Ag85; diagnostics; ESX-1; immunomodulation; MPB70/80/83; PE/PPE.

Introduction

M. bovis causes tuberculosis in domestic and wild animals and can also infect humans, making it a dangerous zoonotic infection. It is a member of a group of bacteria grouped under the *M. tuberculosis* complex and is well adapted to infect different animals. This poses significant challenges to both agriculture and public health [1, 2]. Its genetic material is almost identical to that *of M. tuberculosis* DNA (more than 99.95%). However, the *M. bovis* genome lacks certain regions and contains single nucleotide substitutions, as well as additional genes absent from the *M. tuberculosis* core genome, contributing to functional variability and immune adaptation. [3, 4, 5]. The most notable differences between *M. bovis* and other bacteria in the complex concern the genes that are responsible for the structure of the cell wall and for the proteins secreted to the outside. It is at these sites that the greatest diversity is found [6, 7, 8, 9].

Immunoactive proteins play a key role in how M. bovis interacts with the immune system and causes disease. Particularly important are those proteins that the bacterium secretes through the specialized ESX-1 system, such as ESAT-6 and CFP-10. Also important are lipoproteins and antigens from the MPB70, MPB80 and MPB83 families. These proteins help the bacteria to survive inside the body and influence the development of infection [10, 11, 12].

Due to the work of ESX-1 system proteins, when the immune system cells such as macrophages and dendritic cells are infected, the body reacts more strongly by activating bacterial DNA recognition, increasing the production of the protective agent IFN-β and triggering autophagy [13, 14, 15]. However, *M. bovis* is able to defend itself against these reactions by inducing mitophagy (destruction of mitochondria), which helps it to avoid other cellular defense mechanisms such as xe-nophagy. This allows the bacterium to persist longer inside cells and develop new, more dangerous forms [16, 17, 18, 19]. It has also been found that differences between strains (e.g., amino acid sub-stitution in the ESAT-6 protein) can affect the strength of the innate immune response and even the outcome of the disease [20].

Because of its molecular features, *M. bovis* poses a serious problem: the infection is difficult to detect, treat and develop an effective vaccine against it. MPB70, MPB80 and MPB83 are strongly immunogenic and serve as valuable biomarkers for detecting infection and distinguishing infected from vaccinated hosts. [21, 22, 23, 24]. On the other hand, even among different strains of this bacterium, large differences are found, making it difficult to find stable and accurate targets for diagnosis and treatment. Therefore, to develop effective solutions, comprehensive methods are needed, including the study of genes, proteins, metabolites, and other biological components simultaneously [25, 26, 27, 28].

Against the backdrop of globalization and increasing bacterial resistance to drugs, the im-portance of *M. bovis* in veterinary medicine continues to grow. In some regions, zoonotic tuberculo-sis is still widespread. This creates serious economic problems for farmers, hinders infection control in wildlife and increases the risk of human infection. To effectively manage this infection, a deeper understanding of how *M. bovis* immunoactive proteins influence disease progression, how they interact with the immune system, and how they can be used to identify infected and immunized animals is needed.

This review discusses the major *M. bovis* proteins, their role in protecting the bacterium from immune responses, in disease development, and their application in diagnosis, infection control, and the development of new vaccines [29, 30].

Materials and Methods

Literature search strategy. The present study was conducted as a structured narrative review of published data focusing on immunoactive proteins of *M. bovis* and their role in host–pathogen interactions. A comprehensive literature search was performed using international scientific databases, including PubMed, Scopus, Web of Science, and Google Scholar. The literature review covered publications from 1998 to 2025.

Search queries included combinations of the following keywords and their derivatives: *M. bovis,* immunoactive proteins, ESX-1, ESAT-6, CFP-10, MPB70, MPB83, Ag85 complex, PE/PPE proteins,

lipoproteins, autophagy, mitophagy, xenophagy, IFI204, TLR signaling, biomarkers, vaccines, and zoonotic tuberculosis.

Inclusion and exclusion criteria. Peer-reviewed original research articles, reviews, and experimental studies written in English were included if they addressed:

(i) molecular and cellular mechanisms of *M. bovis* interaction with the host immune system;

(ii) functional characterization of immunoactive proteins;

(iii) comparative analyses between *M. bovis* and *M. tuberculosis*;

(iv) diagnostic, prognostic or vaccine-related applications of *M. bovis* antigens.

Publications focusing exclusively on non-tuberculous mycobacteria or lacking immunological or molecular relevance were excluded.

Data extraction and analysis. Relevant data were extracted manually and organized according to protein families and functional pathways, including ESX-1–associated secreted proteins, Ag85 complex, MPB70/80/83 antigens, PE/PPE proteins, and lipoproteins. Particular attention was paid to experimental evidence describing modulation of phagocytosis, autophagy, mitophagy, Toll-like receptor signaling, and cytokine responses.

Comparative transcriptomic, proteomic and secretomic studies were analyzed to identify species-specific expression patterns and regulatory mechanisms distinguishing *M. bovis* from other members of the *M. tuberculosis* complex. Emphasis was placed on studies using macrophage infection models, animal models, and multi-omics approaches.

Synthesis and interpretation. The collected data were qualitatively synthesized to identify recurring molecular mechanisms, convergent immune evasion strategies, and consistent diagnostic or vaccine-related targets. No meta-analysis or quantitative statistical evaluation was performed, due to the heterogeneity of experimental designs across the included studies. The final interpretation integrates molecular, immunological and applied aspects to provide a comprehensive framework for understanding the role of immunoactive proteins in *M. bovis* pathogenesis, diagnosis and control.

Results and Discussion

The results of the literature analysis are presented below in a thematic manner, integrating molecular, cellular and immunological data to elucidate the biological basis of *M. bovis* pathogenicity and its interaction with the host immune system. The discussion begins with fundamental aspects of *M. bovis* biology and pathogenesis as a framework for understanding subsequent immune evasion mechanisms.

Biology and pathogenesis of M. bovis

M. bovis is one of the major members of the *M. tuberculosis* complex (MTBC). Although its genetic material is almost identical to the genome of *M. tuberculosis* (more than 99.95% similarity), these bacteria differ in which hosts they infect, how they develop the disease, and how they cope with the body's immune defenses [1]. The main difference between *M. bovis* is not the presence of unique genes, but rather how exactly the genes responsible for virulence and interaction with the immune system work. Whole genome studies show that the biggest differences between the bacteria relate to the structure of the cell wall and the proteins that the bacterium secretes outward. It is these proteins that determine how the bacterium will interact with the animal's immune system [7].

Pangenomic analysis (the study of all genes of different strains) showed that *M. bovis* has both a common set (approximately 2700 common genes) and a huge additional set of over 3800 genes that are only found in individual strains. This suggests that the *M. bovis* genome remains “open”, meaning it can change, but it does not have a strictly unique set of proteins common to all strains. When scientists grouped these genes by function, it turned out that many of them are partic-ularly associated with the regulation of other genes, fat metabolism, and secretory systems, especial-ly the so-called ESX (or Type VII) systems [2]. In *M. bovis*, for example, the ESX-1 system works more actively during infection of bovine cells than in *M. tuberculosis*, causing a stronger innate im-munity response [31]. Comparison of gene and protein activities in *M. bovis* and *M. tuberculosis* showed that *M. bovis* induces a stronger cellular defense response, in particular, pathways recogniz-ing foreign DNA are activated and IFN-β protein production is increased [32]. These differences are not due to the emergence of completely new proteins, but rather to the way in which already known antigens, such as ESAT-6, CFP-10, TB27.4, and

MPB70/80/83, are regulated. For example, *M. bovis* has a mutation in the Rv0444c gene, which affects the regulation of another gene (SigK), and this explains why it produces some proteins more strongly [33, 34].

M. bovis is able to subtly influence the host cells' defense mechanisms, especially the work of macrophages, the immune cells that normally engulf and destroy bacteria. One such mechanism is autophagy, which is the process by which a cell "digests" unwanted or dangerous elements. *M. bovis* has learned to control this process so that it does not die inside the cell. Specifically, the bacterium triggers mitophagy the destruction of mitochondria – thus interfering with another defense response, xenophagy, which is designed to kill invading bacteria. It alters the PINK1-PRKN pathway to avoid being seen and destroyed [19]. Also, during infection, IFI204 protein activity is enhanced, which triggers another form of autophagy accompanied by the release of the defense signaling protein IFN-β. These processes are part of the bacterium's strategy that allows it to hide from innate immunity [13].

M. bovis most often affects farm animals, especially cows, and this is due to both the characteristics of the bacterium itself and the way the immune system of these animals responds to infection [35]. However, *M. bovis* can infect not only cattle but also a wide variety of animal species, including domestic and wild animals, making it difficult to control the infection and eliminate it completely. In addition, although *M. bovis* is less likely to cause tuberculosis in humans compared to *M. tuberculosis*, such cases pose a serious risk. Especially because this pathogen is resistant to one of the main drugs, pyrazinamide, which complicates treatment [36].

The peculiarities of how *M. bovis* causes disease are not related to the appearance of some new, unique proteins, but to the way the work of already known genes is regulated. This allows the bacterium to change its properties, adapt to different conditions, cause different immune reactions, and infect many species, including humans.

Immune response and evasion mechanisms

Following *M. bovis* infection, both innate and adaptive immune responses are activated; however, the pathogen has evolved effective mechanisms to evade these defenses and establish persistent intracellular infection. To understand how it all starts, we need to look at exactly how the bacterium interacts with the innate immune system, specifically how it is engulfed by the macrophage (this is the cell that captures and destroys foreign microorganisms). Below is a diagram showing the basic steps of how a macrophage "eats" *M. bovi*s, as well as how the bacterium itself interferes with this process and escapes destruction.

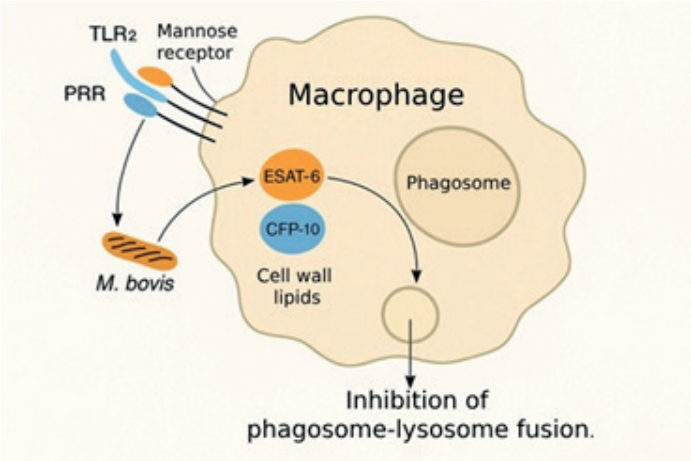

Figure 1 – Intracellular pathway of *M. bovis* phagocytosis in the macrophage, showing points of pathogen intervention, including inhibition of phagosome-lysosome fusion, suppression of oxygen burst, and DNA release into the cytosol

Recent studies have increasingly demonstrated how *M. bovis* differs from other closely related bacteria in the M. tuberculosis complex and what molecular tricks it uses to survive in the body. One interesting example is how this bacterium "tricks" immune cells, especially macrophages. One of these three important mechanisms of how *M. bovis* controls the cellular processes of mitophagy and xenophagy is explained below.

How *M. bovis* switches the macrophage to mitophagy and prevents it from destroying bacteria. *M. bovis* is adept at interfering with the defense systems of the macrophage, a cell that normally should engulf and digest foreign invaders. As a result of mitophagy activation, the cell begins to spend resources not on fighting the bacteria, but on "cleaning up the broken parts". As a result, the xenophagy system is weakened and the bacterium remains alive inside the macrophage. This deception is obtained through the involvement of one important signaling protein, p-TANK-binding kinase 1 (TBK1), which shifts the cell's attention from microbes to mitochondria. Scientists have proven that if mitophagy is blocked (e.g., by drugs or genetic interventions that inhibit the PINK1 protein), the macrophage begins to actively destroy *M. bovis* again. [16].

Figure 2 shows how exactly *M. bovis* interferes with the cell's defense processes, forcing it to focus on mitophagy rather than on destroying the bacterium itself.

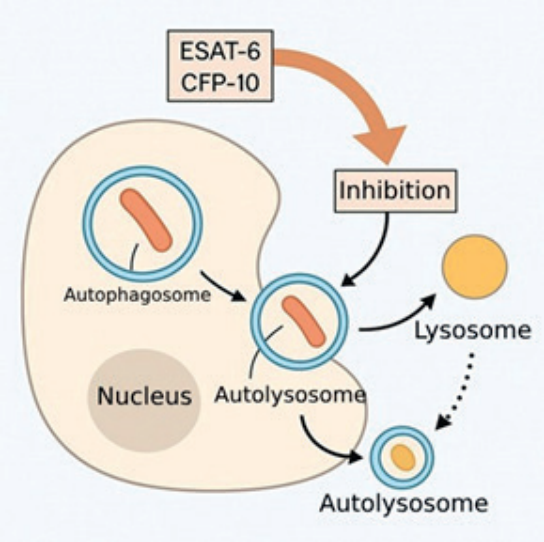

Figure 2 – Selective activation of mitophagy and suppression of *M. bovis* xenophagy through the PINK1-Parkin pathway and p-TBK1 regulation. This "cheating" of autophagy ensures the survival of the mycobacterium in the macrophage

Role of IFI204 in IFN-B activation and autophagy. One of the important elements of macrophage defense against *M. bovis* infection is the IFI204 protein, which works as a "sensor" of foreign DNA inside the cell. When the bacterium enters a macrophage, it is trapped in a special capsule called a phagosome. But if this capsule is destroyed, the bacterial DNA is released into the cell fluid (cytosol), where it is detected by IFI204. This triggers two defense processes at once. The first pathway: IFI204 activates the proteins stimulator of interferon genes (STING) and TBK1, which in turn trigger another protein IRF3. This leads to the production of interferon IFN-β, a signaling molecule that helps the cell fight infection. The second pathway: it starts the activation of autophagy, a process in which the cell "packs" unwanted or harmful elements (including bacteria) into bubbles and recycles them. At this point, the level of special proteins, such as LC3, increases and autophagosomes are formed, sort of like "garbage cans" inside the cell [13]. If the IFI204 gene is turned off in the macrophage (e.g., in a knockout experiment), IFN-β production drops severely and autophagy becomes weaker. As a result, the bacterium can more easily multiply inside the cell. In addition, *M. bovis* itself is adept at interfering with these defense pathways. It produces special microRNAs, such as miR-199a, that block the TBK1 protein. This impairs both IFN-β production and autophagy, which helps the bacterium to survive inside the macrophage even longer [37].

Differences in pathogen recognition between *M. bovis* and other MTBC members. Although *M. bovis* and *M. tuberculosis* have almost the same genetic information, the immune system recognizes them differently and this affects how the body responds to infection. *M. bovis* has been found to elicit a stronger immune response at the cellular level. In particular, macrophages, which engulf and destroy pathogens, react more actively to foreign DNA in the cytoplasm when infected with *M. bovis,* which triggers enhanced production of type I interferon (IFN-β) [7, 38]. Toll-like receptors, especially TLR2,

located on the surface of macrophages, also play an important role in the primary recognition of mycobacteria. These receptors are the first to come into contact with the pathogen and trigger a chain of signals aimed at activating the innate immune response. All these differences emphasize that, despite genetic similarities, *M. bovis* and *M. tuberculosis* interact differently with the immune system, which has implications for diagnosis, treatment and vaccine development (Figure 3).

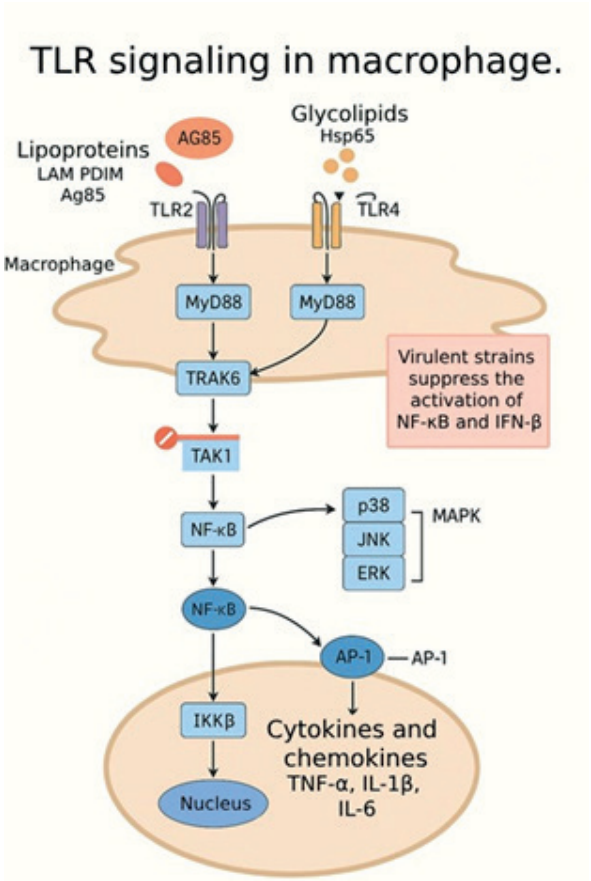

Figure 3 – Signaling pathway of innate immune response activation via TLR2 during *M.bovis* infection. Participation of adaptor proteins MyD88, NF-κB and production of proinflammatory cytokines is shown

One of the key features of *M. bovis* is the enhanced operation of the ESX-1 system, which secretes special proteins such as ESAT-6 and CFP-10. These proteins help bacterial molecules penetrate the host cell faster, which in turn activates the immune defense, especially the pathway associated with the IFI204, STING and interferon IFN-β proteins. This response is stronger in *M. bovis* than in *M. tuberculosis* infection. With regard to adaptive immunity, *M. bovis* induces active production of defense substances such as IFN-γ and IL-22, which are involved in the fight against infection. However, the strength and specificity of this response can vary greatly depending on the particular strain of the bacterium and which proteins it secretes [10, 39]. In addition, even small changes in the structure of key proteins (e.g., replacement of one amino acid residue in the ESAT-6 protein) can either enhance the immune system response or, on the contrary, make the pathogen less visible to the organism [15].

Classification of immunoactive proteins. ESX-1 secreted proteins (ESAT-6, CFP-10)

The secreted proteins of the ESX-1 system, primarily ESAT-6 and CFP-10, are important molecules by which *M. bovis* interacts with the host immune system and induces disease development. These proteins are encoded in a region of the genome known as difference region 1 (RD1), which is present in virulent strains of *M. bovis* but absent in the attenuated BCG vaccine strain. These proteins are secreted through a specialized type VII secretion system.

Increased expression of esx-1 in *M. bovis* and implications for the immune response. Comparison of gene and protein activities showed that *M. bovis* produces more components of the ESX-1 system, and

thus more ESAT-6 and CFP-10 proteins, than *M. tuberculosis*, especially in bovine macrophages. This leads to more severe damage to the phagosome membrane and active release of bacterial DNA into the cell cytosol. As a result, the mechanisms of innate immune response are activated, the IFI204 sensor is activated, the STING-TBK1 signaling pathway is triggered, and the synthesis of type I interferon (IFN-β) begins. Thus, *M. bovis* causes a stronger launch of defense mechanisms related to DNA recognition in the cytosol, stimulates the production of inflammatory molecules and forms an immune response with pronounced antiviral characteristics in cattle macrophages. This, in turn, affects the development and course of infection compared to infection caused by *M. tuberculosis* [38, 40].

Genetic differences in ESAT-6 and their influence on the immune response. It is significant that the ESAT-6 protein, which is part of the ESX-1 system, may differ slightly in its structure in different strains of M. bovis. For example, in some virulent strains, a single amino acid substitution, the socalled T63A mutation, which is located at the end of the ESAT-6 molecule, has been found. Studies have shown that this substitution enhances the ability of the bacterium to disrupt the phagosome membrane, activate the production of interferon IFN-β and trigger an inflammatory response in the macrophage. As a result, the immune system begins to produce inflammatory signaling agents more actively, making the response stronger compared to strains lacking this mutation [41, 42]. These differences between the protein variants are important because they may explain why different animals or humans experience infection differently, and why not all tests and vaccines work equally well.

The importance of ESAT-6 and CFP-10 in diagnosis. The ESAT-6 and CFP-10 proteins elicit a particularly strong immune system response, and it is this property that is utilized in modern TB tests such as IGRA (interferon release assays) and skin tests [43]. These tests are used to detect M. bovis infection in cows, deer and other animals. Importantly, these proteins are not present in the BCG vaccine strain, so that infected animals can be distinguished from simply vaccinated animals – this is called the DIVA-strategy. Tests containing ESAT-6 and CFP-10 peptides (e.g., Bovigam) show high accuracy because they induce a specific response only in infected individuals [44, 45]. In addition, these proteins are considered as possible components of future vaccines because they induce the desired type of immune response, a cellular response involving Th1-lymphocytes, which is essential to combat mycobacterial infections [46].

Ag85 complex (Ag85A, Ag85B, Ag85C)

The Ag85 complex, comprising three proteins, Ag85A, Ag85B, and Ag85C, is one of the most studied in mycobacteria such as *M. bovis* and *M. tuberculosis*. These proteins play an important role both in the development of infection and in the activation of immune defenses.

Role in cell wall construction. All three proteins of the Ag85 complex are enzymes involved in the synthesis of a special part of the mycobacterial cell envelope, mycolic acids. They work as transporters of these acids, combining them with other components to form a stable and dense cell wall. One important product of this process is the cord factor (TDM), which helps the bacterium survive in the host. If the work of Ag85C protein is blocked, the bacterium will not be able to build its shell normally, it will start to accumulate intermediates, and this will prevent its growth and reproduction [47].

During infection, especially in conditions of oxygen deficiency or increased acidity (which occurs in the body during chronic inflammation), it is Ag85B that begins to be actively produced, which indicates its important role in the survival of the bacterium during prolonged infection [48].

Impact on immune response and prospects for vaccines. The proteins of the Ag85 complex elicit a strong immune system response. They are particularly well recognized by the body's defense cells and trigger the so-called Th1-type immune response – with the production of substances such as IFN-γ, IL-2 and TNF-α, which help to control and suppress mycobacteria. In experiments with mice and cattle, it has been shown that a particularly active protective immunity is formed in response to Ag85B [49, 50]. Due to this fact, the proteins of the Ag85 complex began to be used in the creation of new vaccines. For example, vaccines based on modified virus (MVA85A) or BCG vaccines with increased Ag85B production were developed. These vaccines provided stronger protection compared to conventional BCG, as evidenced by both less damage in animal tissues and higher levels of protective substances such as IL-17 [51]. In addition, the combination of Ag85 with other proteins, such as ESAT-6, yields an even broader and stronger immune response. Therefore, Ag85 is now considered not only as a basis for new vaccines, but also as an important marker in diagnostics and can be used in tests that measure the level of protective cytokines (e.g., IFN-γ) to detect infection [27].

The proteins MPB70, MPB80 and MPB83 are among the most important antigens of the bacterium *M. bovis* for the immune system. These proteins are not only actively produced in the body, but also well recognized by the immune system and are of great importance in the diagnosis of tuberculosis in animals. MPB70 and MPB80 are soluble proteins that are secreted into the external environment and are almost not found in other mycobacteria such as *M. tuberculosis*. In contrast, MPB83 is a protein attached to the surface of the mycobacterium, making it particularly accessible to immune cells [52, 12]. These three proteins are produced in high amounts in M. bovis due to mutations in a regulatory gene (anti-SigK), which enhances the function of the SigK protein responsible for turning on these antigens. The BCG vaccine strain and *M. tuberculosis* lack such mutations, so their levels of these proteins are much lower [53, 11].

In terms of immune response, MPB70 and MPB80 enter the external environment and activate immunity at a distance, whereas MPB83 acts on the surface of the bacterium itself, directly contacting the body's defense cells. This difference is important in the development of tests and vaccines [54, 55].

These proteins are particularly valuable for diagnostic purposes because vaccinated animals (e.g., those that have received BCG) do not have a false reaction to them. Therefore, MPB70, MPB80 and MPB83 are used in various diagnostic tests, such as ELISA and interferongamma tests, allowing infected animals to be accurately distinguished from vaccinated animals. This makes them an excellent choice for implementing the socalled DIVA strategy. Interestingly, even though these proteins are quite similar, they differ in their structure at the sites where immune cells attach to them (called epitopes). For example, MPB70 and MPB83 have sites that differ even in individual amino acids. This is important in the development of accurate tests and vaccines: these proteins can be combined to improve the recognition of infection and reduce the risk of false results [54]. MPB83 is considered to be a particularly promising marker; antibodies to it appear at the earliest stages of infection, so it is convenient to use for early diagnosis. Also, the level of MPB70 protein can be useful for assessing the stage of the disease. In general, the high activity of these proteins and their immune uniqueness make them essential components in the control of tuberculosis in ani-mals [55].

PE/PPE proteins, components of phagocytosis and autophagy suppression

PE/PPE proteins and other special proteins secreted by M. bovis play an important role in how this bacterium avoids destruction by the immune system and continues to survive within the body.

PE/PPE proteins are a large group of proteins (there are over 160 in *M. tuberculosis*, and almost as many in *M. bovis*), many of which are found on the surface of the bacterium or secreted outward through the specialized ESX-5 system. It used to be thought that these proteins were simply different in structure and therefore difficult for the immune system to recognize. But it is now known that they do much more than that. They interfere with the immune system cells' ability to recognize the bacterium, affect the operation of key signaling pathways (e.g. TLR2, NF-κB and MAPK), can both enhance and suppress inflammation, and are involved in maintaining cell wall structure and nutrient transfer [56, 57, 58].

Some PE/PPE proteins (e.g., PE_PGRS20 and PE_PGRS47) are particularly important for bacterial survival within macrophages. These proteins interfere with the initiation of autophagy and block Rab1A, a protein that triggers autophagy. If these proteins are removed, the bacterium becomes vulnerable, as cells begin to destroy it more efficiently [59].

In addition to PE/PPE proteins, *M. bovis* has other defense tools. One of them is the PknG protein. It prevents the macrophage from connecting the phagosome to the lysosome, i.e. it actually blocks the “digestion” of the bacterium. Without this protein, the bacterium cannot survive inside the cell. Another protein, Zmp1, inhibits the inflammatory response by reducing the production of IL-1β signaling protein and simultaneously interferes with the formation of the phagolysosome as a key element for killing the bacterium [56, 57].

Thus, M. bovis utilizes a complex system of PE/PPE proteins and effectors such as PknG and Zmp1 to “fool” the immune system. It camouflages itself, interferes with its own processing inside cells, and regulates inflammation so that it doesn't cause too strong a response. All of this allows it to persist in the body for a long time. Disrupting these proteins makes the bacterium less dangerous, which makes them interesting targets for creating new drugs or vaccines aimed at disrupting immune evasion mechanisms [60].

Lipoproteins (LprG, LpqH, etc.), TLR2 stimulators

Mycobacterial lipoproteins such as LprG, LpqH, LprA, LppX, et al. – are special proteins found on the surface of microbes and are linked to fatty molecules. These proteins play an important role in how the body recognizes infection. They interact with special "sensors" on immune cells such as macrophages and dendritic cells. These sensors are called TLR2 receptors and allow the immune system to quickly detect mycobacteria and initiate a defense response [61, 62].

1.Immunomodulation and TLR2 signaling. Mycobacterial lipoproteins are recognized by special receptors on the surface of immune cells, TLR2 together with TLR1 or TLR6. This triggers a chain of signals inside the cell, including important molecules such as NF-κB and AP-1, resulting in the release of inflammatory substances such as TNF-α, IL-6, IL-12 and IL-1β. One of the most active proteins, LpqH, also promotes the production of interferon-γ and helps the cell to better present antigens to other elements of the immune system [63]. In order for lipoproteins to trigger such a reaction, they need a special fatty "tag" at their origin. If the enzymes responsible for such a tag do not work, lipoproteins will not be able to turn on the immune response effectively. In different strains o*f M. bovis* and *M. tuberculosis*, these "tags" may differ slightly, which affects the strength of the immune response [64].

2.Involvement in intracellular signaling, inflammation and antibacterial mechanisms. Lipoproteins not only trigger inflammation but also activate defense mechanisms in cells. For example, the same LpqH turns on a signaling chain that, through calcium and other proteins, activates the production of catheplicidin, a substance that destroys mycobacteria. This process is also linked to vitamin D and autophagy, a mechanism by which the cell "eats" harmful microbes [65].

Some lipoproteins, such as LprG, help mycobacteria to survive. They are involved in fat transport and strengthen the cell wall of the mycobacterium, which prevents the fusion of the phagosome with the lysosome and thus prevents the bacterium from dying. If LprG is removed, the mycobacterium is more easily destroyed inside the cell and the immune system recognizes it better [66].

Interestingly, if there are too many lipoproteins on the surface of the mycobacterium (as in some particularly dangerous forms, such as *M. abscessus*), this can cause too much inflammation, which itself can damage tissue. That is, lipoproteins can both help to fight infection and aggravate the course of the disease [67].

3.Diagnostic and vaccine potential. Mycobacterial lipoproteins, especially the LpqH protein, are active in inducing a strong immune response, in particular promoting the production of interferon-γ and triggering autophagy, the process by which a cell destroys harmful bacteria. Due to these properties, lipoproteins have attracted the attention of scientists as potential candidates for the creation of new vaccines against tuberculosis, as well as for the development of more accurate diagnostic methods [64]. These proteins play an important role in linking the two levels of immune defense: innate and adaptive. This helps the body to not just react to infection, but to turn on more effective control mechanisms that can prevent the disease from becoming chronic.

Lipoproteins produced by mycobacteria such as *M. bovis* and other closely related species serve a dual function. On the one hand, they trigger the innate immune response through TLR2 receptors, induce inflammation and activate defense mechanisms in cells, such as autophagy and the production of specific antimicrobial substances. On the other hand, these proteins can specifically regulate these same processes at different stages to "tune in" to the host immune system. Mycobacteria are able to change the amount and structure of their lipoproteins to achieve the right balance not to irritate the immune system too much to avoid its complete destruction, but also not to remain completely undetected. This fine-tuning helps them survive in the body for a long time, which is important for their ability to cause chronic forms of disease [67].

Comparative and integrative 'omics' data

Analysis and comparison of complex 'omics' data (transcriptomics, proteomics and secretomics) between *M. bovis* and *M. tuberculosis* has helped to reveal why these closely related bacteria cause tuberculosis with different features in animals and humans. Studies have shown how they interact differently with the body and the immune system.

Transcriptomics and proteomics: unique expression profiles. Although the genomes of *M. bovis* and *M. tuberculosis* are nearly identical, differences in gene activity (i.e., how strongly certain genes work) lead to marked differences in their behavior [5]. In *M. bovis*, genes responsible for virulence – especially

those that control the ESX-1 secretion system – are more actively turned on in macrophages (the body's defense cells) in cows. This includes the ESAT-6, CFP-10 proteins, and the espR and phoP regulatory genes. ESX-1 proteins are secreted in higher amounts as shown by mass spectrometry techniques [68].

Also, the production of two important lipoproteins, MPB70 and MPB83, is strongly increased in *M. bovis*. This is due to mutations in the rskA regulator, which controls the sigma factor SigK. Because of this, overproduction of these proteins is activated. Analysis of proteomes (set of all proteins) by electrophoresis and mass spectrometry confirms that these antigens are more abundant in *M. bovis* than in *M. tuberculosis*, both inside the cells and among those secreted outside [69, 12]. Interestingly, the Ag85 protein complex (Ag85A/B/C) in both species is almost identical in structure, but the level of its production may differ depending on the infection conditions and the type of infected cells. This requires further study [70].

The secretome: a comparative analysis of pathogenic proteins. The secretome is a set of proteins that mycobacteria secrete into the external environment. It plays a key role in how the bacterium "communicates" with host cells and how it resists their defenses. Comparative analysis has shown that *M. bovis* has many more ESX-1 system proteins (including ESAT-6, CFP-10, EspA, EspC, EspD) in the secretome than *M. tuberculosis*. This helps *M. bovis* to degrade phagosome membranes, penetrate the cytoplasm, and trigger a strong inflammatory response through the cGAS-STING pathway with the production of type I interferon more actively. All this explains the peculiarities of zoonotic tuberculosis, i.e., tuberculosis transmitted from animals to humans [69]. In addition, the proteins MPB70/80 and MPB83, which strongly excite the immune system and can increase inflammation, are actively secreted by *M. bovis* and are considered good markers for the diagnosis of this species. In *M. tuberculosis*, these proteins are almost not produced due to the peculiarities of its regulatory systems [12]. *M. tuberculosis* secretome was also found to be more saturated with proteins associated with latent (hidden) state and stress, such as α-crystallin Rv2031c or Rv2623 protein. In contrast, in M. bovis, secreted proteins are directed at triggering inflammation and interacting with TLR2 receptors. Even among the common proteins, there are differences not only in quantity but also in chemical modifications, such as the composition of lipid "anchors" in lipoproteins. All this affects how the immune system recognizes and responds to bacteria [63, 64].

The significance of differences in 'omics'-profiles for pathogenesis and diagnosis. All the above shows that *M. bovis* induces a more active immune response by secreting many proteins that stimulate innate immunity. This can help the body to respond more quickly to infection, but at the same time creates a risk of chronicity, as the bacterium is able to "evade" the immune attack by rearranging the work of macrophages. *M. tuberculosis*, although close in genetics, acts differently. It prefers to trigger those genes that allow it to "hide" in the body – it activates proteins that work under conditions of stress and contribute to the transition to the latent phase. This makes human tuberculosis prone to a long, latent course [69].

Diagnostic and applied significance

How *M. bovis* produces key proteins such as MPB70, MPB83, ESAT-6 and CFP-10 plays a critical role in current diagnostic and prognostic methods for bovine tuberculosis as well as zoonotic tuberculosis in humans. Comparative studies that analyze gene activity and protein synthesis (transcriptomics and proteomics) show that the main differences between *M. bovis* and M. *tuberculosis* are not related to the genes themselves, but to how active they are. This explains the differences in bacterial behavior in different host species and influences which antigens are used in modern diagnostic tests [7, 1].

Protein expression and secretion as a basis for multicomponent diagnostics and vaccines. In *M. bovis*, the proteins MPB70, MPB80 and MPB83 are consistently produced in large quantities much higher than in *M. tuberculosis*. This is due to changes in the gene regulator RskA, which normally suppresses the activity of another gene, sigma factor K. In the case of *M. bovis*, this regulation is disrupted, and the sigma factor works without restrictions, which causes active production of these proteins [12]. These proteins are hardly produced in BCG vaccine strains, while they are very scarce in *M. tuberculosis*. Therefore, MPB70/80/83 are considered excellent markers to distinguish infected from vaccinated animals (so-called DIVA-diagnostics – Differentiating Infected from Vaccinated Animals). This is crucial for tracking the epidemic, understanding its magni-tude, and assessing how well the vaccine is working [69]. On the other hand, ESAT-6 and CFP-10 proteins, which are encoded in the

RD1 genetic region, are absent in most BCG vaccine strains but are actively produced in both *M. bovis* and *M. tuberculosis*. These proteins are the basis of modern IGRA tests (cellular immune response tests), as they induce a strong T-cell response. Current ap-proaches combine multiple antigens, such as MPB70/83 and ESAT-6/CFP-10, to improve diagnostic accuracy. These combined ELISA and IGRA tests not only allow for better detection of TB, but also for judging the stage of infection. This is possible because the immune system responds differently to these proteins at different times: first, a rapid T-cell response to ESAT-6 and CFP-10, and later the production of antibodies to MPB70 and MPB83 [71, 72].

Detection of circulating peptides and integration with host markers. Thanks to modern protein analysis methods such as LC-MS/MS and MALDI-TOF and the development of very sensitive antibodies, it is now possible to directly detect individual mycobacterial peptides (e.g. MPB70 and Ag85B) in the blood even when the number of bacteria in the body is still very low. This makes it possible to detect tuberculosis at the earliest stages, including latent and asymptomatic forms, which is beyond the scope of standard immunologic tests [72]. However, detection of such peptides alone does not always give an accurate result due to the fact that the immune system may react differently in different animals. To improve accuracy, scientists have begun to combine data on mycobacterial antigens with information on proteins and signaling molecules of the organism itself, so-called inflammatory markers, such as SAA (serum amyloid A), HP (haptoglobin), IFN-γ (interferon-gamma), IP-10, and TNF-α. This approach allows for a more comprehensive diagnostic panel that works better in real-world settings, both in laboratories and in veterinary field examinations [73]. These complex biomarkers are characterized by their ability to detect infection at stages when conventional tests (antibody or T-cell tests) have not yet been successful. Combinations such as the presence of MPB83 peptide in the blood along with high levels of IFN-γ and low levels of IL-10 are associated with a higher risk of developing active tuberculosis. This makes it possible to use such markers not only to detect the disease, but also to monitor its development, prevent it, and assess the effectiveness of treatment or vaccination [71].

Cytokine profile: role in diagnosis and prognosis. Today, scientists are actively studying how the levels of various cytokines – proteins that regulate the immune response – change in the body. Especially important are such molecules as IFN-γ, IL-22, TNF-α, IL-10, IP-10 and others. They are measured in multi-analyses used both for diagnosis of tuberculosis (e.g. IGRA tests) and to assess the risk of progression from latent to active infection. IFN-γ remains one of the main indicators of the body's early response to mycobacteria – especially to ESAT-6 and CFP-10 antigens. By monitoring IL-22 and TNF-α, it is possible to judge the current phase of the disease, the level of inflammation and the formation of granulomas (areas of inflammation characteristic of tuberculosis). By combining data on “inflammatory” cytokines (IFN-γ, TNF-α, IL-22) and “inflammation-suppressing” cytokines (IL-10, TGF-β), it is possible not only to diagnose infection, but also to divide infected individuals into groups according to their risk level – for example, those who may remain latent and those who are more likely to develop active disease. This is especially important when it comes to timely intervention, treatment or vaccination [71].

In general, these approaches, from analyzing the expression of mycobacterial proteins (MPB70/80/83, ESAT-6, CFP-10), through their direct detection in blood, to analyzing body proteins and cytokines, make it possible to move towards more accurate and “smart” diagnostics of TB, especially *M. bovis*. This helps to detect even latent cases, to apply DIVA testing (to distinguish infected from vaccinated), to monitor the effectiveness of vaccination and to predict how the infection will progress in both animals and humans.

Conclusion

This review highlights that *M. bovis* represents a unique member of the *M. tuberculosis* complex with distinct molecular features of interaction with the host immune system. This review revealed that immunoactive proteins of *M. bovis*, including components of the ESX-1 secretion system, lipoproteins, PE/PPE proteins, and MPB70/80/83 antigens, play a key role in modulating the immune response by providing intracellular persistence and evasion by phagocytic and autophagic mechanisms. Active interference in innate immunity pathways (phagocytosis, autophagy, TLR sig-naling) makes these proteins not only virulence factors but also promising targets for diagnosis, monitoring, and vaccine development. Molecular and multiomic studies support the need for a comprehensive approach to

understanding the biology of *M. bovis*, especially in the face of increasing zoonotic threat and resistance to therapy. In this context, indepth studies of immunoactive proteins offer opportunities to develop highly specific biomarkers and second-generation vaccines that can effectively control bovine TB and prevent its transmission to humans. Integration of immunoactive M. bovis proteins into multicomponent diagnostic panels and next-generation vaccine platforms rep-resents a promising strategy to enhance sensitivity, specificity and translational applicability of bovine tuberculosis control tools.

Authors' Contributions

NG: conducted a comprehensive literature search, analyzed the gathered data and drafted the manuscript. AG: analyzed the gathered data and drafted the manuscript. FZh: analyzed the gathered data and performed final revision and proofreading of the manuscript. APS and OA: designed and supervised the study, conducted a comprehensive literature search, analyzed the gathered data and drafted the manuscript. All authors have read, reviewed, and approved the final manuscript.

Acknowledgements

The authors express their sincere gratitude to Kairat A. Turgenbayev and Asiya M. Borsynbayeva for their valuable consultations, methodological assistance, and continuous support through out the study and data analysis process. The authors also gratefully acknowledge the Engineering Center for Organic Agrotechnologies of S. Seifullin University for providing the necessary facilities and technical support for the experimental work.

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