Applications of humanized mice in *Mycobacterium tuberculosis* infections (Review)

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Received March 20, 2025; Accepted October 9, 2025

DOI: 10.3892/mmr.2026.13898

Abstract. Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (*M.tb*), which poses a notable threat to human health. The present review aims to explore the application of humanized mice in the study of *M.tb* infections. Due to differences in immune responses between mice and humans, humanized mice with human immune systems have been developed as models to characterize human immune responses to *M.tb*. The present review searched for research on humanized mice and tuberculosis in Web of Science and PubMed using the keywords 'humanized', 'mice' or 'mouse' and 'tuberculosis', and summarized the findings. Humanized non-obese diabetic (NOD).Cg-Rag1^{tm1MoM}Il2rg^{tm1Wjl} and NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl} mice have the potential to accelerate the screening of vaccine candidates, therapeutic regimens and the 'bench to bedside' translation process. New therapies, such as IgG1 P1AM25 in humanized Fcγ receptor mice and phage DS6A in humanized NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl} Tg(cytomegalovirus-interleukin-3, granulocyte-macrophage colony-stimulating factor and KIT ligand)1Eav/MloySzJ mice, may have potential for treating tuberculosis. The humanized bone marrow-liver-thymus and human leukocyte antigens transgenic mouse models are effective tools for studying the co-infection of *M.tb* and human immunodeficiency virus (HIV). The present review highlights the key role of humanized mouse models in advancing the understanding of *M.tb* infection, including host-pathogen interactions, immune evasion mechanisms, vaccine development, therapeutic interventions and co-infection with HIV. In conclusion, humanized mice provide a powerful platform for bridging the gap between preclinical research and clinical tuberculosis therapeutics.

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Key words: Mycobacterium tuberculosis, tuberculosis, humanized mouse model, immune response, therapy, vaccine

Contents

1. Introduction

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (*M.tb*), primarily affecting lung cells and characterized by granuloma formation (1,2). It has historically been the leading cause of mortality from a single pathogen (3). The World Health Organization (WHO) reported 10.80 million cases and 1.25 million mortalities from tuberculosis in 2023 (4). A total of 30 countries with a high tuberculosis burden account for 87% of the global cases in 2023, including India, Indonesia and China, accounting for 26.0, 10.0 and 6.8% respectively (4,5). Furthermore, the 2019 coronavirus disease pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, makes the global tuberculosis mortality toll in 2020 exceed that observed in 2015 and even 2012 (6-8). The End Tuberculosis Strategy composed by the WHO aims to reduce the incidence of tuberculosis by 90%, to <10 cases per 100,000 individuals within the global population annually by 2035 (9). This would require an annual decline in tuberculosis incidence to accelerate from the current decline of 2% per year to 20% per year (10,11). A total of three key areas of tuberculosis research are important to reducing incidence: i) Vaccine development; ii) improved diagnostic tools and iii) improved treatment options (11,12).

Traditional mice, as valuable experimental models, are widely used in *M.tb* research, including studies on pathogenesis, drug development and vaccine assessment (13-15). Although traditional mice share some genomic and physiological traits with humans (2), marked differences in immune responses and pathogenesis following *M.tb* infections, such as

collagen destruction, limit the utility of these models (16,17), hindering progress in *M.tb* research (18). A key difference in pathogenesis between mice and humans is that human tuberculosis forms organized, caseous necrotizing granulomas with a macrophage core and a peripheral rim of lymphocytes (19), whereas *M.tb*-infected mice form loose granulomata-like structures without giant cells (20). These differences highlight the notable need for a novel animal model that accurately mimics the pathogenesis of *M.tb* infections in humans.

Humanized mice, which are immunodeficient recipients engrafted with human cells or tissues or that express human gene products, emphasize the evolutionary specificity and diversity of human genotype and phenotype (21,22). Currently, common humanized mouse models of *M.tb* infection are created by grafting human hematopoietic cells and bone marrow-liver-thymus (BLT) tissues, and by transferring human leukocyte antigen (HLA) genes and receptor genes, such as T cell receptor (TCR), toll-like receptor (TLR) and Fcγ receptor (FcγR), to immunodeficient mice (Figs. S1-S11). The humanized mouse model has become an appealing alternative for studying human infectious diseases, including tuberculosis, as it closely mimics the human immune system (23,24) and is increasingly used to study host responses and immunopathology (17,25-27). These models are also being used with increasing frequency as preclinical tools to assess the efficacy of novel drugs and vaccines and to investigate underlying mechanisms in *M.tb* infections (2,28). The present review systematically searched for studies on humanized mice and tuberculosis in the Web of Science (https://www.webofscience.com) and PubMed (https://pubmed.ncbi.nlm.nih.gov/) databases, using the keywords 'humanized', 'mice' or 'mouse' and 'tuberculosis'. The present review summarizes advances in the application of humanized mice for studying immune responses, therapy development and vaccine assessment in *M.tb* infections and *M.tb*-human immunodeficiency virus (HIV) co-infections.

2. Application of humanized mice in *M.tb*-induced immune response and pathology

An incomplete understanding of the human immune response to *M.tb* infections and its associated protections has hindered the development of tuberculosis vaccines and therapies. Further exploration of the immune response and pathogenesis induced by *M.tb* infections is important (Table I and Fig. 1) (17,24-27,29-34). Several humanized mouse models have been developed to investigate this issue (24,29,30).

A total of five research groups have utilized humanized mice to analyze the immune response induced by *M.tb* and its antigens (25,26,29,31-33). A research group successfully developed humanized non-obese diabetic (NOD). Cg-Prkdc^{scid}Il2rg^{tm1sug}/JicTac (NOG) mice by injecting human CD34$^+$ hematopoietic progenitor cells (HPCs) into mice, resulting in the generation of human CD45$^+$ cells (Fig. S1) (31). The researchers observed a multi-subpopulation human T-cell response (CD4$^+$ CD45RA$^-$ CD45RO$^+$ subpopulation) and the expression of cytokines and chemokines, such as interleukin (IL)-2, tumor necrosis factor-α (TNF-α), interferon-γ, perforin and granulysin, following *M.tb* infection (31). In HLA transgenic mice injected with the class III human genes, HLA DQ and/or DR genes (Fig. S1), the variable T-helper (Th) response to the *M.tb* antigen early secreted antigenic target (ESAT)-6-31-45 depended on the HLA haplotype of transgenic mice rather than a single DR or DQ HLA molecule (25).

A team created human CD1 transgenic mice and *M.tb* antigen mycolic acid (MA)-specific TCR (DN1)/CD1 transgenic mice by transferring human CD1 and CD1b-restricted MA-specific TCR genes into mouse models, resulting in successful expression of human CD1 and DN1 TCRs in transgenic mice (Fig. S2) (26,29). The authors found that intranasal inoculation of MA-loaded micellar nanocarriers activated and proliferated adoptively transferred DN1 T cells, eliciting MA-specific T cell responses in humanized mice infected with the *M.tb* antigen MA. Additionally, active DN1 T cells congregated in pulmonary granulomas and provided protection against *M.tb* infection (26,29). A group built humanized NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl} Tg [cytomegalovirus-IL-3, granulocyte-macrophage colony-stimulating factor, KIT ligand (KITLG)]1Eav/MloySzJ (NSG-SGM3) mice by engrafting human CD34$^+$ hematopoietic stem cells (HSCs), which differentiate into human CD45$^+$ cells (Fig. S3) (32). *M.tb* infection increases colony-forming units (CFUs) and the CD4$^+$/CD8$^+$ T cell ratio, leading to immune cell infiltration around a necrotic nucleus. The immune response to tuberculosis is complex and requires further investigation.

Several researchers have investigated the role of TNF in humanized mice infected with *M.tb* (Fig. S3). Humanized TNF knock-in mice can survive and control *M.tb* load during infection, while TNF knock-out mice die rapidly. Administration of TNF blockers, such as infliximab, etanercept or adalimumab, subsequently increases *M.tb* levels and hyperinflammation in *M.tb*-infected humanized TNF knock-in mice (33). Therefore, TNF may carry out an important role in controlling *M.tb* infection.

Some researchers have examined *M.tb*-induced pathology and clarified that damage to the pulmonary extracellular matrix via collagen destruction may initiate caseous necrosis, as seen in humans, rather than be a result of necrosis in humanized C57BL6 mice. This mouse model generates human matrix metalloproteinase 1 (MMP-1) and forms human giant cells following *M.tb* infection (Fig. S4) (17).

Organized granulomas are a characteristic pathological feature of human tuberculosis, preventing the spread of *M.tb (*24). A total of four research teams have developed humanized mouse models for granuloma formation. The first team developed a humanized BLT mouse model by grafting human fetal liver-thymus tissues and CD34$^+$ fetal liver cells. The human immune system was well-reconstructed, as evidenced by the generation of human CD45$^+$ cells (Fig. S4). Organized granulomatous lesions with an acellular center, *M.tb* periphery, caseous necrosis and cholesterol crystal similar to human tuberculosis granulomas were observed in the humanized mice infected with *M.tb* but not in traditional mice (24). The second team developed humanized NOD.Cg-Rag1^{tm1MoM}Il2rg^{tm1Wjl} (NRG-A2) mice with transgenes for human HLA-A2.1/A*02:01 (A2) and humanized HLA I/II-transgenic mice with transgenes for human HLA-DR4/DRB1*04:01 (DRAG) and A2. Both mouse models expressed human CD45$^+$ leukocytes (Fig. S5). These two mouse models showed similar *bacillus* loads and dissemination

Table I. Summary of humanized mouse models used in *M.tb*-induced immune response and pathology studies.

First author/s, year	*M.tb* strain	Mouse strain	Method of humanization	Human cell engraftment (%)	CFUs	Lesions/ granulomas	Humoral immunity	Cellular immunity	Application of the humanized mice	(Refs.)
Grover *et al*, 2017	*M.tb* H37Rv (TMCC #102)	Non-obese diabetic Cg-Prkdc^{scid} Il2rg^{tm1sug}/ JicTac (NOG) mice	Graft with human CD34^{+} HPCs	>30	-	-	-	CD4^{+} and CD8^{+} T cell response	To evaluate human T-cell immune responses in vaccine development.	(31)
Smart *et al*, (2014)	ESAT-6	HLA transgenic mice	Transfer of human DR and DQ HLA genes	-	-	-	ESAT-6 immunodo-minant epitope, IFN-γ and IL-10/12/17	-	To determine the pathogenicity or therapeutic nature of a peptide in the context of HLA alleles.	(25)
Shang *et al*, (2018)	MA-Mc	CD1 and DN1 trans-genic mice	Transfer of human CD1 and CD1b-restricted MA-specific TCR genes	-	-	-	IFN-γ, TNF-α and IL-2	MA-specific T cells	To evaluate the protective efficacy of vaccines.	(26)
Zhao *et al*, (2015)	MA-Mc and *M.tb* H37Rv	CD1 and DN1 trans-genic mice	Transfer of human CD1 and CD1b-restricted MA-specific TCR genes	-	-	-	-	The activation of DN1 and *M.tb* antigen Ag85B-specific CD4^{+} T cells	To evaluate the protective efficacy of vaccines.	(29)
Bohorquez *et al*, 2024	*M.tb* H37Rv	NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl} Tg1Eav/ MloySzJ (NSG-SGM3) mice	Graft with human CD34^{+} stem cells	>1	Increased CFUs in lung and spleen	Immune cell infiltration around a necrotic nucleus	Decreased MCP-1 and PDGF and increased IL-1Rα and IL-13	Increased CD4^{+}/CD8^{+} T cell ratio	A reproducible animal model for *M.tb* infection.	(32)

Table I. Continued.

First author/s, year	*M.tb* strain	Mouse strain	Method of humanization	Human cell engraftment (%)	CFUs	Lesions/ granulomas	Humoral immunity	Cellular immunity	Application of the humanized mice	(Refs.)
Olleros *et al*, 2015	*M.tb* H37Rv	TNF mice	Human TNF knock-in	-	A few scattered *M.tb*	Well-organized pulmonary granulomas	-	-	To evaluate whether human-specific TNF-neutralizing drugs compromise host defense functions.	(33)
Al Shammari *et al*, 2015	*M.tb* H37Rv	C57BL/6 mice	Transfer of human MMP-1 and MMP-9 genes	-	Similar to wide-type mice	Typical caseous necrosis	No difference in TNF-α, IL-1β/12, IFN-γ, MCP-1 and IP-10 expression between humanized mice and wild-type mice	-	Investigate the impact of extracellular matrix on granuloma.	(17)
Calderon *et al*, 2013	*M.tb* td Tomato H37Rv	BLT mice	Graft with human fetal liver, thymus tissue and $CD34^+$ HSCs	27	Occurs in the lung and spreads to the liver and spleen	Organized granulomatous lesions, caseous necrosis, bronchial obstruction and crystallization of cholesterol deposits	-	Human $CD3^+$ T cells are recruited to and organized at sites of inflammation	To understand the human immune response to *M.tb*.	(24)
Ledpard *et al*, 2022	*M.tb* H37Rv	NOD.Cg-$Rag1^{tm1MoM}$ $Il2rg^{tm1Wjl}$ (NRG)-A2 and DRAG-A2 mice	Transfer of human A2 and DRAG genes	20-35	Both models have similar CFUs	Both models form granulomatous tissues .	-	Higher $CD3^+$ T cell levels in NRG mice than in DRAG-A2 mice	To investigate the pathology, disease course and immune responses of co-infection.	(34)

Table I. Continued.

First author/s, year	*M.tb* strain	Mouse strain	Method of humanization	Human cell engraftment (%)	CFUs	Lesions/ granulomas	Humoral immunity	Cellular immunity	Application of the humanized mice	(Refs.)
Arrey *et al*, 2019	*M.tb* H37Rv	NSG mice	Graft with $CD34^+$ human HSCs and HPCs	55	Lung CFU increases with time	Develop caseous necrotic granulomas similar to human tuberculosis	IL-8 increases with time	$CD4^+$ T cells and macrophages increase with time	To mimic pediatric and immunocompromised individuals.	(30)
Heuts *et al*, 2013	*M.tb* Harlingen strain	NSG mice	Graft with $CD34^+$ HSCs	-	Higher bacterial titers in humanized mice than non-humanized mice	Small and large macro scopic lesions occasionally surrounded by a collagen layer, extensive necrosis and granulomas	Higher levels of IFN-γ, CXCL9 and CXCL10 mRNA are observed in infected mice than in uninfected mice	Giant cells and $CD3^+$ cells	Formation and maintenance of human granuloma in tuberculosis.	(27)

M.tb, *Mycobacterium tuberculosis*; ESAT-6, a *M.tb* antigen; CFU, colony-forming units; HPCs, hematopoietic progenitor cells; HSCs, hematopoietic stem cells; HLA: human leukocyte antigen; IFN-γ, interferon-γ; MA-Mc, mycolic acid-loaded micellar nanocarriers; MMP, matrix metalloproteinase; IL, interleukin; TNF, tumor necrosis factor; MCP-1, monocyte chemotactic protein 1; TCR, T-cell receptor; DN1, MA-specific TCR; PDGF, platelet-derived growth factor; IP-10, IFN-γ-inducible protein 10; CXCL, chemokines; BLT, bone marrow-liver-thymus; DRAG, HLA-DR4/DRB1*04:01; A2, HLA-A2.1/A*02:01; NSG, NOD.Cg- $Prkdc^{scid}Il2rg^{tm1Wjl}$.

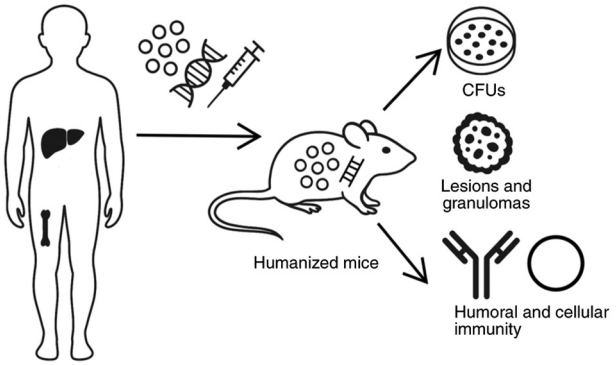

Figure 1. Construction and application of a humanized mouse model infected with *Mycobacterium tuberculosis*. CFUs, colony-forming units.

potential. However, the DRAG-A2 mice developed more classical, well-organized granulomas following *M.tb* infection than the NRG-A2 mice (34).

The third team created humanized human immune system (HIS)-NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl} (HIS-NSG) mice by injecting human CD34$^+$ HSCs and HPCs, which resulted in the generation of human CD45$^+$ cells (Fig. S6). After *M.tb* infection, the mice developed caseous necrotic granulomas resembling human tuberculosis, with a core of necrotic debris and a peripheral cell layer (30). The fourth team generated humanized NSG mice by injecting human CD34$^+$ HSCs into mice, resulting in the production of human CD45$^+$ cells (Fig. S6). Compared with non-humanized mice, the humanized mice developed irregular or circular granulomas, characterized by numerous human giant cells and bacilli in the core, surrounded by CD3$^+$ T cells or a collagen layer and increased necrosis following *M.tb* infection (27). Among these models, the humanized BLT, DRAG-A2 and HIS-NSG mice exhibited characteristics of human tuberculosis granulomas, making them suitable for further research. Overall, the observations made in these humanized mouse models provide valuable insights for developing protective biomarkers of tuberculosis and identifying precise host-pathogen interactions.

Humanized mice have markedly enhanced the current understanding of the immune responses and pathological processes induced by *M.tb* (28). The aforementioned models have enabled detailed investigations of human-specific immune cell interactions, cytokine profiles and granuloma formation. However, current models still have limitations in fully recapitulating the complexity and heterogeneity of human immune responses. Furthermore, several questions remain to be addressed. This includes: i) The proportion of human immune cells in the peripheral blood of humanized mice that is suitable for use in further research; and ii) any changes that have occurred in the body weight, survival rate, *M.tb* burdens, lesions and the expressions of antibodies, CD4$^+$ T cells, CD8$^+$ T cells, cytokines and chemokines in humanized mice compared with humans, traditional mice and negative controls (24). The degree to which these indices increase or decrease may indicate whether the humanized mice are a suitable *M.tb* infection model. A 'gold standard' humanized mouse model could then be generated based on these indices and possibly utilized for future research on vaccines and therapies.

3. Application of humanized mice in tuberculosis vaccine research

Vaccination generates long-lasting host immunity, an important component in global tuberculosis control and eventual eradication. The *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) vaccine has been used for >100 years (35). BCG is the only licensed tuberculosis vaccine, but its efficacy against pulmonary tuberculosis is limited, providing protection for only 10-15 years (35-38). Therefore, new tuberculosis vaccines are needed to create a protective environment in the lungs (37,39). Previous research has employed humanized mouse models to develop more effective vaccines (Table II) (3,31,40-43). The protective efficacy of four new vaccines against *M.tb* infection was tested via subcutaneous injection, similar to BCG administration, using transgenic mice. The peptide-based vaccine ACP, containing Th1 the immunodominant peptides antigen Ag85B$_{12\text{-}26}$, CFP21$_{12\text{-}26}$ and PPE18$_{149\text{-}163}$ derived from *M.tb* antigens, has the following effects: i) It reduces lung pathological lesions; ii) increases levels of interferon (IFN)-γ$^+$ T lymphocytes, Th1-type cytokines, such as IFN-γ and TNF-α, and antibodies, resulting in an immunoglobulin ratio of IgG2a/IgG1>1; and iii) stimulates a stronger cellular immune response. However, the ACP vaccine does not enhance the protective efficacy of BCG following *M.tb* infection in humanized C57BL/6 (HLA-A11$^{+/+}$ downregulator of transcription 1$^{+/+}$H-2-β2 microglobulin$^{-/-}$/ intracellular amyloid β$^{-/-}$) mice expressing human HLA genes (Fig. S7) (3).

The MP3RT peptide-based vaccine, containing the mycobacterial antigens Mpt51, Mpt63, Mpt64, Mtb8.4, PPE18, PPE44, PPE68, resuscitation promoting factor RpfA, RpfB, RpfE and TB10.4, reduces *M.tb* load, pulmonary lesions and inflammatory cell numbers, and increases IFN-γ$^+$ and CD3$^+$IFN-γ$^+$ T lymphocytes, IFN-γ cytokine and MP3RT-specific IgG antibodies. However, the MP3RT vaccine does not restore animal weight as with BCG in humanized C57BL/6 mice expressing human HLA genes after *M.tb* infection (Fig. S3) (40). Similarly, the Ag85A/B chimeric DNA vaccine also fails to provide improved protection compared with BCG in restoring animal weight, reducing *M.tb* load and alleviating lung lesions and inflammatory cell infiltration in humanized C57BL/6 mice challenged with *M.tb* (40). The CL075:antigen 85B peptide 25 (Ag85Bp25)-PS vaccine, a combination of a polymer nanocarrier encapsulating the TLR agonist CL075-PS with *M.tb* Ag85Bp25, primes Ag85B-specific CD4$^+$ adaptive immune responses similar to BCG in humanized TLR8 neonatal mice expressing human TLR genes (Fig. S7) (41).

Unlike traditional subcutaneous BCG inoculation, some researchers have explored the efficacy of the respiratory mucosal route for vaccine delivery, mimicking the *M.tb* infection pathway. In humanized NRG mice, engrafted with human CD34$^+$ HSCs and expressing human CD45$^+$ cells (Fig. S8), BCG is injected subcutaneously to protect against *M.tb* infection, similar to its effect in humans. The respiratory mucosal vaccination pathway using virus-vectored *M.tb* Ag85A (AdHu5Ag85A) induces a more robust CD4$^+$ and CD8$^+$ T cell response, reduces *M.tb* burdens and decreases granulomatous lesions in humanized NRG mice compared with control mice after *M.tb* infection (42). Intranasal inoculation with a trivalent

Table II. Summary of humanized mouse models used in tuberculosis vaccine studies.

First author/s, year	Vaccine (administration method)	*M.tb* strain	Mouse strain	Method of humanization	Human cell engraftment level	Body weight	CFUs	Lesions/ granulomas	Humoral immunity	Cellular immunity	Application of the humanized mice	(Refs.)
Gong *et al*, 2022	ACP and BCG (subcutaneous)	*M.tb* H37Rv	C57BL/6 and HLA A11/DR1 mice	Transfer of human HLA-$A11^{+/+}DR1^{+/+}$ H2-$\beta2m^{-/-}$/ $Ia\beta^{-/-}$ [9]	-	-	Lower CFUs were observed in the ACP and PBS group than those in BCG and BCG + ACP group	Lower granuloma numbers were observed in BCG, ACP and BCG + ACP groups than in the PBS group	IL-2, IFN-γ, IgG1, IgG2a and IgG2a/ IgG1>1	IFN-γ T lympho-cytes	To evaluate the protective efficacy of the vaccines.	(3)
Gong *et al*, 2017	BCG, MP3RT and ag85A/B DNA vaccine (subcutaneous)	*M.tb* H37Rv	C57BL/6 mice	Transfer of human HLA-$A11^{+/+}DR1^{+/+}$ H2-β2m-/-/ Iaβ-/-	-	MP3RT and DNA vaccines could not restore weight of mice as BCG could	BCG, MP3RT and DNA vaccines decreased the CFUs of mouse liver	BCG, MP3RT and DNA vaccines decreased the lesion area of mouse lung	MP3RT induced the expre-ssion of IgG	MP3RT induced the generation of $CD3^+$ $CD4^+$ T cells and $CD3^+$ $IFN\text{-}\gamma^+$ Th1 cells	To evaluate the protective efficacy of the vaccines.	(40)
Dowling *et al*, 2017	BCG or Ag85B/p25 (subcutaneous)	*M.tb* Ag85B/ p25	TLR8 mice	Transfer of human TLR8 gene	-	-	-	-	-	Ag85B/ p25 induced Ag85B-specific $CD4^+$ T cells	To evaluate the immunogenicity of vaccines.	(41)

Table II. Continued.

First author/s, year	Vaccine (administration method)	*M.tb* strain	Mouse strain	Method of humanization	Human cell engraftment level	Body weight	CFUs	Lesions/ granulomas	Humoral immunity	Cellular immunity	Application of the humanized mice	(Refs.)
Yao *et al*, 2017	Human serotype 5 adenovirus vaccine (intranasal) and BCG (subcutaneous)	*M.tb* H37Rv	NRG mice	Graft with human CD34$^+$ HSCs	>1%	-	AdHu5Ag 85A reduced observed CFUs	AdHu5Ag 85A reduced granulomatous lesions	IFN-γ, TNF-α and IL-2	AdHu5 Ag85A induced *M.tb*-specific and secondary human T cell responses	To predict the protective efficacy of novel tuberculosis vaccines and therapeutic strategies.	(42)
Afkhami *et al*, 2023	Tri:ChAd:TB (intranasal)	M.tb H37Rv (ATCC 27294)	NRG mice	Graft with human CD34$^+$ HSCs	10%	Tri:ChAd:TB restored the weights of mice	Tri:ChAd:TB reduced observed CFUs	Tri:ChAd:TB reduced the granulomatous lesions	-	-	To evaluate the protective efficacy of the vaccines.	(43)
Grover *et al*, 2017	BCG (subcutaneous) or ESAT-6 (intranasal)	*M.tb* H37Rv (TMCC #102)	NOG mice	Graft with human CD34$^+$ hematopoietic progenitor cells	>30%	-	ESAT-6 and BCG did not reduce observed CFUs	ESAT-6 and BCG led to the accumulation of cellular aggregates	ESAT-6 increased the expression of IL-2 and granzyme B	ESAT-6 could not reduce CD8$^+$ granzyme perforin+$^+$ T cells as BCG could	To evaluate human T-cell immune responses in vaccine development.	(31)

CFUs, colony-forming units; BCG, *Mycobacterium bovis* bacillus Calmette-Guerin; IgG, immunoglobulin-γ; ESAT, early secreted antigenic target; IAβ, intracellular amyloid β; β2m, β2 microglobulin; TLR8, Toll-like receptor 8; DR1, down-regulator of transcription 1.

adenoviral-vectored (Tri:ChAd:TB) vaccine, containing the *M.tb* antigens, Ag85A, TB10.4 and RpfB, targeting antigens expressed during the acute, chronic and dormant phases of *M.tb* provides robust protection against pulmonary *M.tb* challenge in humanized NRG mice, engrafted with human HSCs and reconstituting human $CD45^+$ leukocytes (Fig. S8). The treated mice maintain stable weight, with reduced *M.tb* burden, fewer pathological changes and less granulomatous lesions compared with unvaccinated mice (43). In humanized NOG mice, the *M.tb* antigen ESAT-6 vaccine, administered via intranasal immunization, fails to effectively stimulate granzyme$^+$ perforin$^+$ $CD8^+$ T cells or control *bacillus* CFUs, unlike BCG after *M.tb* infection (31).

Humanized mouse models hold potential as tools for preclinical evaluation of tuberculosis vaccines (31). These models enable the testing of human-specific immune responses, such as T-cell priming and memory formation, following vaccination (31). These models bridge the gap between basic research and clinical application (44), however, challenges persist. To the best of our knowledge, the humanized mice used to evaluate these vaccines have not undergone comprehensive immune and pathological assessments. Additionally, the similarity between mice and humans in terms of immunity and pathology remains to be fully elucidated, which may raise questions regarding the validity of vaccine evaluation data generated using these models. Furthermore, the ACP, MP3RT, Ag85B/p25, ESAT-6 and Ag85A/B DNA vaccines did not demonstrate superior efficacy compared with the traditional BCG vaccine and the efficacy of the AdHu5Ag85A and Tri:ChAd:TB vaccines was not directly compared with BCG (3,31,40-43). To the best of our knowledge, the method of administering vaccines via the respiratory mucosa has not identified a vaccine superior to BCG. Therefore, further consideration is needed for the development and clinical application of subsequent vaccines.

4. Application of humanized mice in tuberculosis therapy research

Researchers have tested and developed several therapeutic strategies for tuberculosis using humanized mice models (Table III) (30,45-48). To eliminate species differences in drug metabolism, researchers developed the humanized 8HUM mouse model by replacing 33 mouse cytochrome P450 (CYP) genes, pregnane X receptor (PXR) and constitutive androstane receptor (CAR) with 8 human genes, including human CYP genes (CYP1A1, CYP1A2, CYP2C9, CYP2D6, CYP3A4 and CYP3A7), as well as human PXR and CAR (Fig. S9), which are the CYPs responsible for the majority of drug metabolism in humans (49). Both the humanized 8HUM mice and C57BL/6J mice exhibit similar CFU counts and maintain comparable body weight following *M.tb* infection. In humanized 8HUM mice, the efficacy of three anti-tuberculosis drugs was tested, revealing that moxifloxacin reduces *M.tb* CFU counts compared with the untreated group. However, pretomanid and bedaquiline do not eliminate the *bacillus* load when compared with C57BL/6J mice. Efavirenz, a CYP3A4 inducer, has no effect on the pharmacokinetics or efficacy of bedaquiline. However, its combination with bedaquiline reduces *M.tb* load in humanized 8HUM mice compared with C57BL/6J mice (45). In addition, the drug disposition pathways and drug-drug interactions of several substances, such as rifampicin, the herbal medicine St. John's Wort and S-acenocoumarol, in humanized 8HUM mice are similar to those in humans (50). This suggests that the 8HUM model can facilitate the clinical development of drugs and serve as an appropriate tool for drug development. Notably, the researchers observed changes in *bacillus* load and weight in humanized 8HUM mice after *M.tb* infection (45). However, they did not compare the reduction in *M.tb* CFU by pretomanid and bedaquiline with the untreated group or investigate immune responses and pathological changes (45). Therefore, the viability of humanized 8HUM mice for studying *M.tb* infection requires further investigation.

Moxifloxacin has been proposed as an addition to the standard tuberculosis chemotherapy regimen, which comprises rifampicin, isoniazid and pyrazinamide, to enhance therapeutic efficacy. Some studies support this approach (51-53), while others contradict it (54,55). Researchers tested this hypothesis using humanized HIS-NSG mice (Fig. S6) and found that adding moxifloxacin did not reduce *M.tb* CFUs in organs compared with mice denied the addition (30). Therefore, further investigation is needed to determine whether moxifloxacin should be added to the standard chemotherapy regimen.

Due to the inevitable drug resistance associated with traditional antibiotics, novel therapies, such as necrosis inhibitors, monoclonal antibodies and phage therapy, must be developed to address this issue. Previous report indicate that macrophage apoptosis promotes microbial escape and propagation (56). The effect of necrosis inhibition on the dissemination of *M.tb* in humanized NSG mice was examined. *M.tb* loads in mice treated with the necrosis inhibitor necrostatin-1s were comparable with those in control mice, as observed in humanized NSG mice, which produce human $CD45^+$ cells and are engrafted with human $CD34^+$ cord blood stem cells (Fig. S9) (46). Therefore, necroptosis inhibition does not reduce *M.tb* dissemination, contradicting a previous finding (56).

A research team evaluated the protective efficacy of human monoclonal antibodies targeting *M.tb* surface glycans, which FcγR mediates (57). In humanized FcγR mice expressing human FcγR I/II/IIB/IIIA/IIIB genes (Fig. S10), IgG1 P1AM25, a monoclonal antibody with a high affinity for the *M.tb* surface glycan arabinomannan, reduces pulmonary *M.tb* CFUs following infection (47).

In addition to the aforementioned therapies, researchers have identified the potential benefits of phage therapy. Humanized NSG-SGM3 mice, engineered with HSCs and the human cytokine/chemokine genes *IL-3*, *granulocyte-macrophage colony-stimulating factor* and *KITLG*, generate human immune cells (Fig. S10). Following aerosolized *M.tb* infection, phage DS6A-treated mice gain weight, and display improved lung function, reduced lung inflammation and clearance of *M.tb* from the spleen compared with untreated mice (48).

Humanized mice have proven valuable in evaluating the efficacy of anti-tuberculosis drugs and host-directed therapies (48). These models provide insight into the effects of drugs on human immune cells and facilitate the assessment of potential immune-modulatory treatments. New therapies for tuberculosis, such as IgG1 P1AM25 and phage DS6A, have

Table III. Summary of humanized mouse models used in tuberculosis therapy studies.

First author/s, year	*M.tb* strain	Therapy	Mouse strain	Method of humanization	Human cell engraftment level	Body weight	CFUs	Lesions/ granulomas	Humoral immunity	Application of the humanized mice	(Refs.)
Macleod *et al*, 2024	*M.tb* H37Rv	Moxifloxacin, pretomanid and bedaquiline	8HUM mice	Transfer of human CYP1A1, CYP1A2, CYP2C9, CYP2D6, CYP3A4 and CYP3A7 genes, as well as PXR and CAR	-	-	Moxifloxacin reduced observed CFUs	-	-	Drug metabolism model.	(45)
Arrey *et al*, 2019	*M.t*b H37Rv	Moxifloxacin, rifampicin, isoniazid and pyrazinamide	NSG mice	Graft with $CD34^+$ human hematopoietic stem cells; and hematopoietic progenitor cells	55%	-	Addition moxifloxacin to isoniazid, rifampicin and pyrazinamide did not reduce observed CFUs	-	-	To mimic pediatric and immunocompromised individuals.	(30)
Stutz *et al*, 2018	*M.tb* H37Rv	Necrostatin-1s necrosis inhibitor	NSG mice	Graft with $CD34^+$ cord blood stem cells	50%	-	Necrostatin-1s did not reduce observed CFUs	-	-	To understand the pathophysiology of *M.tb* infection.	(46)
Liu *et al*, 2023	*M.tb* Erdman	IgG1 P1AM25	FcγR mice	Transfer of human FcγR genes	-		Human IgG1 P1AM25 reduced observed lung CFUs	-	-	Investigation of the role of human IgG against tuberculosis.	(47)

Table III. Continued.

First author/s, year	*M.tb* strain	Therapy	Mouse strain	Method of humanization	Human cell engraftment level	Body weight	CFUs	Lesions/ granulomas	Humoral immunity	Application of the humanized mice	(Refs.)
Yang *et al*, 2024	*M.tb* H37Rv	Phage DS6A	NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl} Tg1Eav/MloySzJ (NSG-SGM3) mice	Transfer of human IL-3, GM-CSF and KITLG genes and graft with human HSCs	-	DS6A increased mouse body weight	DS6A reduced observed splenic CFUs	DS6A reduced inflammation and caused other pathological changes	DS6A increased IgM levels	Evaluation of the therapeutic efficacy of phage.	(48)

CFUs, colony-forming units; CYP, cytochrome; PXR, pregnane X receptor; CAR, constitutive androstane receptor; 8HUM, human CYP1A1, CYP1A2, CYP2C9, CYP2D6, CYP3A4 and CYP3A7 genes, as well as PXR and CAR; FcγR, Fcγ receptor; GM-CSF, granulocyte-macrophage colony stimulating factor; KITLG, KIT ligand; NSG, NOD.Cg- Prkdc^{scid}Il2rg^{tm1Wjl}.

shown promising efficacy in humanized mice and warrant further investigation (47,48). However, current humanized mouse models often fail to accurately replicate the pharmacokinetics and pharmacodynamics of drugs in humans, limiting their predictive value (50,51). Additionally, as the aforementioned studies have shown, variability in immune cell reconstitution across different humanized mouse strains leads to inconsistencies in therapeutic outcomes. To address these challenges, future research should focus on enhancing the physiological relevance of drug metabolism and improving the consistency of human immune cell engraftment.

5. Application of humanized mice in *M.tb* and HIV co-infections

Since the mid-1980s, the HIV epidemic has contributed to an increase in tuberculosis cases (58). Co-infection with *M.tb* and HIV has become a notable obstacle in the treatment of tuberculosis (38). HIV infection leads to a decline in CD4$^+$ T cells, creating an opportunity for latent tuberculosis infection (LTBI) to reactivate into active tuberculosis (38,59). HIV-infected individuals are 16-27 times more likely to develop tuberculosis compared with healthy individuals. Co-infection with HIV and LTBI increases the risk of progression to active tuberculosis from 10% over a lifetime to 10% per year (60). The public health impact of HIV and *M.tb* co-infection underscores the need to investigate the interaction between these pathogens and develop novel prophylactics and therapeutics. This requires reliable animal models for evaluation (Table IV) (32,34,61-63).

The BLT, HLA transgenic and NSG-SGM3 mouse models were developed to study the immune responses triggered by *M.tb* and HIV co-infection. In BLT humanized mice, transplanted with human fetal liver-thymus tissues and CD34$^+$ HSCs expressing human CD45$^+$ leukocytes (Fig. S4), HIV infection increases production of the pro-inflammatory cytokines, IL-1β and IL-6, resulting in poorly organized granulomas, worse pulmonary lesions, increased neutrophils and higher *M.tb* load and propagation. These effects are more severe following intravenous HIV infection followed by intranasal *M.tb* infection compared with *M.tb* infection alone. Furthermore, pulmonary pneumonia and vascular occlusions with endothelialitis develop in HIV/*M.tb* co-infected mice (61). Following co-infection with HIV and *M.tb*, humanized NRG-A2 mice with human A2 transgenes express human CD45$^+$ leukocytes (Fig. S5) and develop granulomatous lesions similar to those in mice infected with *M.tb* alone (34), which is inconsistent with previous finding (61). After *M.tb* and HIV co-infection, humanized NSG-SGM3 mice (Figs. S3 and S11) exhibit a similar infection pattern to humans, with a decrease in CD4$^+$ T cells and an increase in HIV burden compared with uninfected mice (32,62).

Additionally, humanized NSG-SGM3 mice have been used to evaluate the role of sirtinol in controlling *M.tb* following HIV and *M.tb* co-infection. The authors of the study found that sirtinol reduces CFU counts in the lung and spleen and decreases lung lesions (Fig. S11) (62).

Additionally, a humanized mouse model of *M.tb* relapse was created in the context of HIV. In humanized BLT mice, injected with human BLT tissues and generating human

Table IV. Summary of humanized mouse models used in *M.tb* and HIV co-infections studies.

First author/s, year	*M.tb* strain	Mouse strain	Method of humanization	Human cell engraftment level	CFUs	Lesions/ granulomas	Humoral immunity	Cellular immunity	Application of the humanized mice	(Refs.)
Nusbaum *et al*, 2016	HIV-1 JR-CSF and *M.tb* H37Rv	BLT mice	Graft with human fetal liver, thymus tissue and $CD34^+$ hematopoietic stem cells	27%	HIV/*M.tb* co-infection increased observed CFUs	HIV/*M.tb* co-infection increased the inflammatory cell influx and granulomatous area. HIV p24 + cells localized to *M.tb* lesions in the lung. Pulmonary pneumonia and vascular occlusions with endothelialitis developed in HIV/*M.tb* co-infected mice.	HIV/*M.tb* co-infection increased the expression of IL-1β, IL-6 and IP-10	HIV/*M.tb* co-infection promoted neutrophil accumulation	Pre-clinical model to identify mechanisms of co-infection pathobiology and test therapeutic interventions prior to use in NHPs or human subjects.	(61)
Lepard *et al*, 2022	HIV-1 R5 and *M.tb* H37Rv	NRG mice	Transfer of human leukocyte antigen (HLA)-A2.1 A*02:01 genes	20-35%	-	HIV/*M.tb* co-infection did not increase the granulomatous area in the NRG mice.	-	-	To investigate the pathology, disease course and immune responses of co-infection.	(34)
Bohorquez *et al*, 2024	*M.tb* H37Rv and HIV-1 BaL	NSG-SGM3 mice	Engraft human $CD34^+$ stem cells	>1%	HIV/*M.tb* co-infection did not increase observed CFUs compared with *M.tb* infection	-	Increased IP-10, granulocyte-macrophage colony stimulating factor and IFN-γ	Decreased $CD4^+/CD8^+$ T cell ratio	A reproducible animal model for *M.tb* and HIV co-infection.	(32)

Table IV. Continued.

First author/s, year	*M.tb* strain	Mouse strain	Method of humanization	Human cell engraftment level	CFUs	Lesions/ granulomas	Humoral immunity	Cellular immunity	Application of the humanized mice	(Refs.)
Singh *et al*, 2024	HIV-1 BaL and *M.tb*^{1} H37Rv	NSG-SGM3 mice	Engraft human CD34^{+} stem cells	-	Sirtinol decreased observed CFUs	Sirtinol decreased observed lesions	-	-	A new therapy for HIV-1 and *M.tb* co-infections.	(62)
Huante *et al*, 2020	*M.tb* H37Rv and HIV-1 JR-CSF	BLT mice	Graft with human bone marrow, liver and thymus	-	HIV increased observed CFUs in mice after tuberculosis chemo-therapy	HIV increased the granuloma-tous inflamma-tion in mice after tuberculo-sis chemotherapy	HIV/*M.tb* co-infection increased the expression of tumor necrosis factor-α, IL-17, IL-6, CCL2 and CCL10 in the lung	-	Post-chemotherapy tuberculosis relapse model.	(63)

CFUs, colony-forming units; HIV, human immunodeficiency virus; IL, interleukin; IFN-γ, interferon-γ; IP, IFN-γ-inducible protein; NHPs, non-human primate; BLT, bone marrow-liver-thymus; NRG, NOD.Cg-Rag1tm1MoMIl2rgtm1Wjl; NSG-SGM3, NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl} Tg1Eav/MloySzJ.

$CD45^+$ cells (Fig. S4), rifampin and isoniazid treatment reduces *M.tb* loads and facilitates granuloma resolution following *M.tb* infection. However, subsequent HIV infection increases *M.tb* CFU counts (63). Studies on *M.tb* and HIV co-infection in humanized mice are limited, making it difficult to draw definitive conclusions. However, they provide valuable insights for future research.

Analysis of the data of patients with pulmonary tuberculosis in China from 2005 to 2021 revealed that the total recurrence rate was 0.47 per 100 person-years (64). Thus, it is important to establish latent *M.tb* infection and control reactivation under immunocompromised conditions, particularly in the context of HIV co-infection, as HIV markedly increases the risk of tuberculosis reactivation (65). A strategy to achieve this is to use low-dose *M.tb* inoculation in humanized mice, which can induce a dormant state similar to latent tuberculosis. The depletion of $CD4^+$ T cells can be used to create an immunocompromised environment, facilitating latency (66). In HIV co-infection models, where HIV-induced immune dysfunction is present, *M.tb* can establish latency and reactivation can be triggered by immune reconstitution or withdrawal of immunosuppressive treatments (65). The aforementioned immunocompromised-latent *M.tb* models will allow for the study of tuberculosis relapse and provide valuable insights into therapeutic strategies for preventing reactivation in immunocompromised individuals.

Humanized mice can model *M.tb* and HIV co-infection, a complex clinical scenario with important public health implications. These models enable the investigation of the mutual impact of both pathogens on immune function and allow for testing combined therapeutic strategies. Despite these advantages, current models have limitations in fully capturing the dynamic interplay between HIV and *M.tb* in humans. Limitations include incomplete immune cell development, poor lymphoid tissue organization and the short lifespan of some models (67). Advancing humanized mouse models to support long-term studies and more comprehensive immune reconstruction is a notable priority.

6. Limitations and prospects

Since the discovery of *M.tb* in 1882, numerous vaccines and treatments against *M.tb* infection have been developed; however, tuberculosis incidence and mortality have not decreased as expected (68). The humanized mouse model holds promise for studying tuberculosis, but several aspects need improvement.

Compared with traditional mice, humanized mice generate immune responses that resemble those in humans during *M.tb* infection more closely (31). NSG or NRG humanized mice, transplanted with human HSCs, are primarily used for short-term studies of tuberculosis immune responses (27,30,32,34). By contrast, humanized BLT mice, transplanted with human BLT, are used for long-term studies, such as co-infection with *M.tb* and HIV (61,63). For specific purposes, researchers have developed unique humanized mouse models. For instance, humanized TNF knock-in mice were developed to study the role of TNF in tuberculosis (33).

Of the humanized mice infected with *M.tb* discussed in 23 studies, 11 were generated by injecting human $CD34^+$ cells. The frequency of human $CD45^+$ cells in the peripheral blood of humanized mice ranged from 1 to 55%. Typically, human $CD45^+$ T cells make up >25% of the peripheral blood in successfully constructed humanized mice (69). This variability in frequency limits comparisons between humanized mouse models. Incomplete replication of the human immune system and variability in human immune cell frequencies across humanized mice led to inconsistent results, limiting the interpretation of human immune responses to *M.tb*. Furthermore, treating *M.tb* requires the collaboration of multiple systems, not just the immune system (70). Therefore, no animal model can fully replicate human *M.tb* infection. The results of *M.tb* infection and treatment in humanized mice suggest the need for caution in their interpretation.

Of the 23 humanized mouse models for tuberculosis, three were engrafted with human BLT tissues and four were transgenic for human HLA. These differences in transplantation methods highlight the need to select the optimal mouse model for experiments based on specific research requirements (2). Additionally, these studies of *M.tb* infection using humanized mice are varied, emphasizing the need for relevant institutions to establish protocols that standardize the use of humanized mice. This will enable the comparison of results across laboratories and facilitate the development of effective vaccines and therapeutic regimens.

Mice remain the primary animal model for tuberculosis research, accounting for 61% of all tuberculosis-infected models, due to their ease of use, low cost and ability to rapidly evaluate vaccine and drug efficacy (71,72). Non-human primates (NHPs) are considered ideal models for studying tuberculosis due to their similar pathogenesis to humans. However, NHP models make up only 1% of all tuberculosis animal models and are limited by ethical and economic constraints (72).

There remain ambiguities in *M.tb* research using humanized mice, including inadequate replication of the human lung microenvironment and strain-specific differences. Future research should focus on developing advanced models with improved engraftment protocols, enhanced lung-specific humanization and greater diversity in *M.tb* strains to address these issues.

The use of humanized mouse models in *M.tb* infection research has substantial practical and medical value. Compared with traditional mice, humanized models provide a more accurate representation of human immune responses, improving current understanding of tuberculosis pathogenesis in humans and aiding in the development of effective interventions (27,31,48).

The present review summarizes the following two key contributions to medical progress: i) The investigation of co-infections. Humanized mouse models are important for studying co-infections, such as HIV and *M.tb*, which are common in human populations (61). These models replicate the immunopathological effects of co-infection, including $CD4^+$ T cell depletion and granulomatous lesion formation, providing insights into disease progression and potential therapeutic targets (61); and ii) preclinical evaluations of therapeutics. Humanized mouse models serve as valuable platforms for

testing new anti-tuberculosis drugs and vaccines (3,48). Their ability to replicate human immune responses allows for the evaluation of therapeutic efficacy and safety prior to clinical trials, potentially speeding up the development of effective treatments (48).

The utilization of humanized mouse models in tuberculosis research is of notable practical and medical importance. These models bridge the gap between animal studies and human clinical applications, facilitating a deeper understanding of disease mechanisms and the development of effective interventions. Their role in advancing tuberculosis research is notable, particularly in the face of challenges such as drug resistance and co-infections.

7. Conclusion

Co-infection with other pathogens, such as HIV and SARS-CoV-2, continues to exacerbate the challenges of treating tuberculosis, with limited strategies available (32,34,61-63). Although several vaccines and therapeutic agents are undergoing clinical trials, there is a substantial need to identify safe and effective vaccines and drugs to prevent and treat *M.tb* infection (3,30,31,40-43,45-48), especially in cases of HIV-*M.tb* co-infection. To address this, a number of humanized mouse models have been developed to study the immune response and pathogenesis of tuberculosis, as well as the interactions between HIV and *M.tb*, to accelerate the screening process. However, greater efforts are required to implement the End Tuberculosis Strategy.

Acknowledgements

Not applicable.

Funding

The present study was supported by grants from the National Natural Science Foundation of China (grant nos. 82273696 and 81973105).

Availability of data and materials

Not applicable.

Authors' contributions

BH drafted the manuscript, with substantial input from the other authors. HY was responsible for funding acquisition. FL, JL, JW, YC and HY all contributed to reviewing and editing the manuscript. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Use of artificial intelligence tools

During the preparation of this work, artificial intelligence tools were used to improve the readability and language of the manuscript, and subsequently, the authors revised and edited the content produced by the artificial intelligence tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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