TOPICS

新たな結核ワクチン

＊国立病院機構近畿中央胸部疾患センター　臨床研究センター　臨床研究センター長

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はじめに

いまだに世界の人口の1/3が結核菌の感染を受け，毎年940万人の結核患者が発生し，180万人が毎年結核で死亡している，最大の感染症の1つである[1~3]．本邦でも結核罹患率の一時的増加が認められ，1999年“結核緊急事態宣言”が旧厚生省より出された．1998年，米国CDC(疾病予防管理センター)およびACET(結核根絶諮問委員会)は結核に対し，政府・学術機関・企業が一体となってBCGに代わる新世代の結核ワクチン開発の必要性を強く主張する発表をした．BCGワクチンは成人の結核予防に無効であることがWHOより発表され，本邦でも法令改正が行われ，小・中学生・成人の定期的BCG接種が中止となった．しかしながら，BCGに代わる結核ワクチンは欧米でも臨床応用には至っていない．我々はBCGよりもはるかに強力な新しいサブユニットワクチン，DNAワクチンやリコンビナントBCGワクチンの開発に成功した(表)[4,5]．したがって，新しい結核ワクチンについて述べる[4~7]．

Ⅰ．キラーT細胞と結核免疫

結核感染に対する免疫力はキラーT，Mφ，$CD4^+$T細胞，および肉芽腫形成等の総合的な抵抗力である(図1)[1~3]．

1．キラーT細胞

結核症に対する宿主の抵抗性は細胞性免疫といって過言ではない．特に獲得免疫(キラーT細胞)が重要である．我々は$CD8^+$キラーT細胞の誘導にはヘルパーT細胞から産生されるサイトカインが必要であることを初めて示した．さらに，ヒトT細胞ハイブリドーマを世界に先駆けて作製し，IL-6がキラーT細胞分化の後期に作用することを明らかにした(図2)[8~10]．

$CD8^+$T細胞は結核感染Mφを殺して，結核菌の増殖の場をなくし結核菌を殺す役割が重要である[1~3](文献4参照)．

Ⅱ．新たな結核ワクチン(HSP65 DNA＋IL-12 DNAワクチン等)

1．新しい結核ワクチン

結核ワクチンは①サブユニットワクチン，②DNAワクチン，③リコンビナントBCGワクチン(弱毒化結核菌を含む)に大別される．

2．DNAワクチン

我々はHsp65 DNA＋IL-12 DNA(HVJ-エンベロープベクター)のワクチンはBCGワクチンよりも1万倍強力な結核予防ワクチンであることを世界に先駆けて明らかにした．

このワクチンは，結核菌に対するCD8陽性キラーT細胞の分化誘導を増強しワクチン効果とキラーT細胞活性が相関した(図3)[3,6]．

さらに，このワクチンを多剤耐性結核菌感染後にワクチン投与し，多剤耐性結核治療ワクチン効果(肺臓・肝臓・脾臓の結核菌数減少)を示した．また，超薬剤耐性結核(XDR-TB)に対しても治療効果(延命効果)をこのワクチンは発揮した(図4A)．

これらの研究が高く評価され，筆者はWHO STOP TB PartnershipおよびWHOのWGND(Working Group on New TB Drugs)のメンバーに選出された．

3．リコンビナントBCGワクチン

BCGに，種々の遺伝子を導入しリコンビナントBCGを作製した．

サブユニットワクチンMtb72f融合タンパク質のDNAを導入した72fリコンビナントBCGの作製に成功し，このワクチンはサルでも結核予防効果を示した(表)[3,11]．

4．サブユニットワクチン

Reed博士らのMtb72f融合タンパク質(Mtb39とMtb32の融合タンパク質)のサブユニットワクチンが強力な予防ワクチン効果を示した[11,12]．

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表　新しい結核ワクチンにおける動物実験モデルを用いた開発研究

ワクチン	マウス	モルモット	サル	SCID-PBL/hu	ヒト
HVJ-エンベロープ/ Hsp65 DNA＋IL-12 DNA	BCGワクチンより10,000倍強力な予防ワクチン効果	有効	有効		計画（第Ⅰ相，Ⅱ相）
	治療効果	計画	治療効果	治療効果	
	多剤耐性結核に治療効果 超薬剤耐性結核に治療効果	計画	計画		
HVJ-リポソーム/ Hsp65 DNA＋IL-12 DNA	BCGワクチンより100倍強力な予防ワクチン効果	有効	有効（100％生存）		
リコンビナント72ｆBCG	予防ワクチン効果（有効）	有効	有効		

新しい結核ワクチンの種類と動物実験モデルを用いた開発研究．SCID-PBL/hu：Severe Combined Immunodeficiency Mice〔SCIDマウス（重症複合免疫不全マウス）：Tリンパ球およびBリンパ球が欠損したマウスでヒトのT細胞が生体内に生着可能）にヒト末梢血リンパ球を$1\sim2\times10^7$個腹腔内投与し，ヒトT細胞をSCIDマウスに生着させたマウス．このマウスはヒトのT細胞よりなるので，生体内のヒト免疫応答を解析できる（文献8参照）．

我々は15K GranulysinがCD8$^+$キラーT細胞から分泌され，ヒトMφ内の結核菌を殺傷することを明らかにした[3]．さらに，granulysinワクチンは極めて有用な治療法であることを示した[3]．

5．IL-2Rγ鎖遺伝子欠損SCID-PBL/huの系で結核患者リンパ球をSCIDマウスに生着させ，ヒト結核ワクチン効果解析モデルを初めて開発した（表）[4, 8]．

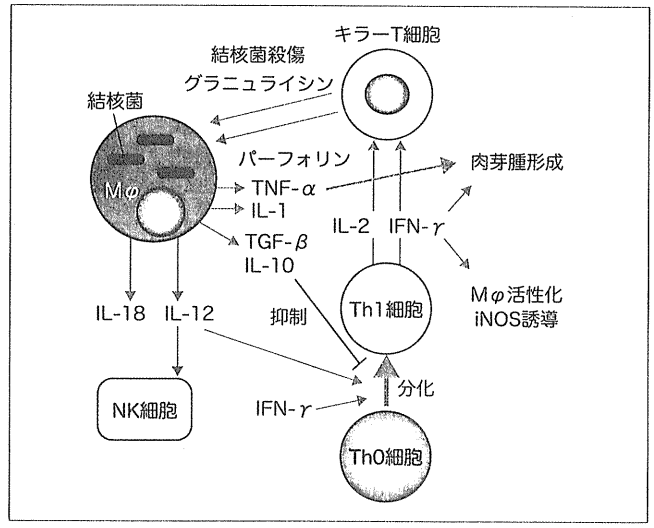

図1　抗結核免疫とマクロファージ，ヘルパーT細胞，キラーT細胞活性化
抗結核免疫におけるキラーT細胞，マクロファージ，ヘルパーT細胞，サイトカインの役割．

Ⅲ．新しい結核ワクチンの開発状況（臨床応用）

1．Stop TB Partnership（WHO）

WHOは現在進行中で，しかも臨床応用に有望な新しい結核ワクチン開発のリストを発表した．我々のHVJ/Hsp65DNA＋IL-12DNAワクチンも候補の1つとしてそのなかに推奨されている．

2．結核ワクチンの応用の可能性

①新しい結核ワクチンの臨床応用

カニクイザル（最もヒトの結核感染に近いモデル）を用いBCGより強力な予防ワクチン効果（生存率，免疫反応，赤血球沈降速度（赤沈），肺のX線像）を示すワクチン2種を開発した[3〜6, 11, 13]．すなわち，現在最も有力なものとしてHSP65 DNA＋IL-12 DNAワクチンおよび，r72f BCGワクチンがあげられる（表）．Mtb 72f融合タンパク質サブユニットワクチン[12]は第Ⅱ相となっている[7]．A. Hill博士らのワクシニアウイルス-85A DNAワクチンは，第Ⅱ相臨床試験で，Ag85Aタンパク質に対するIFN-γ産生を増強した[3, 14]．

②プライム-ブースト法

BCGをプライムし，HSP65＋

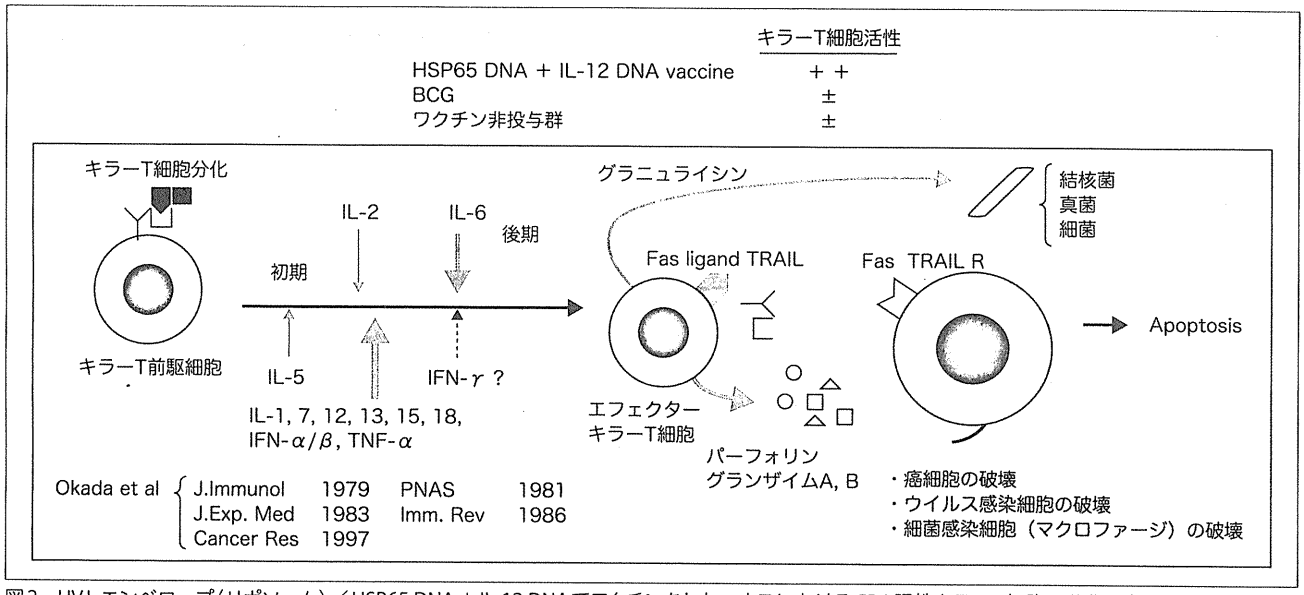

図2　HVJ-エンベロープ(リポソーム)／HSP65 DNA＋IL-12 DNAでワクチンをしたマウスにおけるCD8陽性キラーT細胞の分化過程
HVJ-エンベロープ(リポソーム)／HSP65＋IL-12 DNAワクチンによる結核菌に対するキラーT細胞の分化誘導．このワクチンは生体内で極めて強力なキラーTを誘導．一方，BCGワクチンはほとんどキラーT細胞を誘導しなかった．キラーT細胞誘導活性と予防ワクチン効果は相関した．

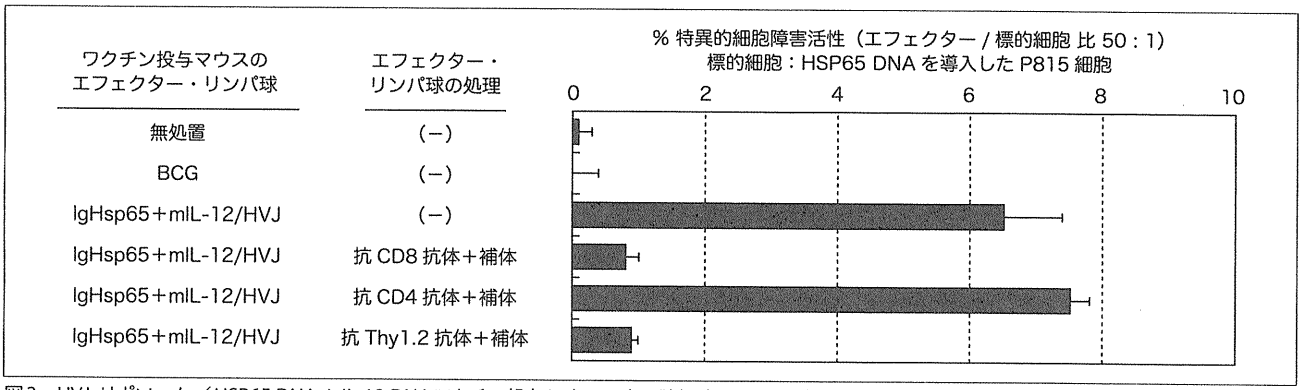

図3　HVJ-リポソーム／HSP65 DNA＋IL-12 DNAワクチン投与によるマウス脾細胞における結核菌に対するCD8陽性キラーT細胞の誘導
HSP65＋IL-12 DNAワクチンで誘導される結核菌抗原(HSP65)に対するキラーT細胞はCD8陽性，CD4陰性キラーT細胞であった．

IL-12 DNAワクチンをブーストする方法を用いた(図5A)．サルでこのプライム-ブースト法で100％の生存を示した(図5B)[3, 4, 7]．一方，BCGワクチン単独投与群は33％の生存率であった．本邦では乳幼児にBCG接種(プライム)が義務づけられていることにより，成人ワクチン(中学生，成人，老人)としてこのDNAワクチンをブーストとして用いる結核ワクチンの臨床応用案である(図5C)[3, 4, 7]．

③治療ワクチン

感染したカニクイザルの系でHVJ-エンベロープ/Hsp65 DNA＋ヒトIL-12 DNA ワクチンを投与した(図4B)．この群では100％の生存率が認められた(図4C)[3, 4, 7]．一方，コントロール群では，60％の生存率であった．このDNAワクチン投与群では，体重増加，赤沈(赤血球沈降速度)の改善，末梢血T細胞の増殖増強反応が認められた．このワクチンはカニクイザルの系において治療ワクチン効果を示し，ヒトMDR-TB，XDR-TBの治療剤として極めて有用であることが示唆された[3, 4, 7]．

おわりに

2009年WHOの委員会において我々のHSP65DNA＋IL-12DNAワクチンによる結核治療効果が高く評価された．他にMtb72fワクチンやMVA85Aワクチンが評価された．これらのワクチンが結核発症予防や治療に役立つ日が近い．

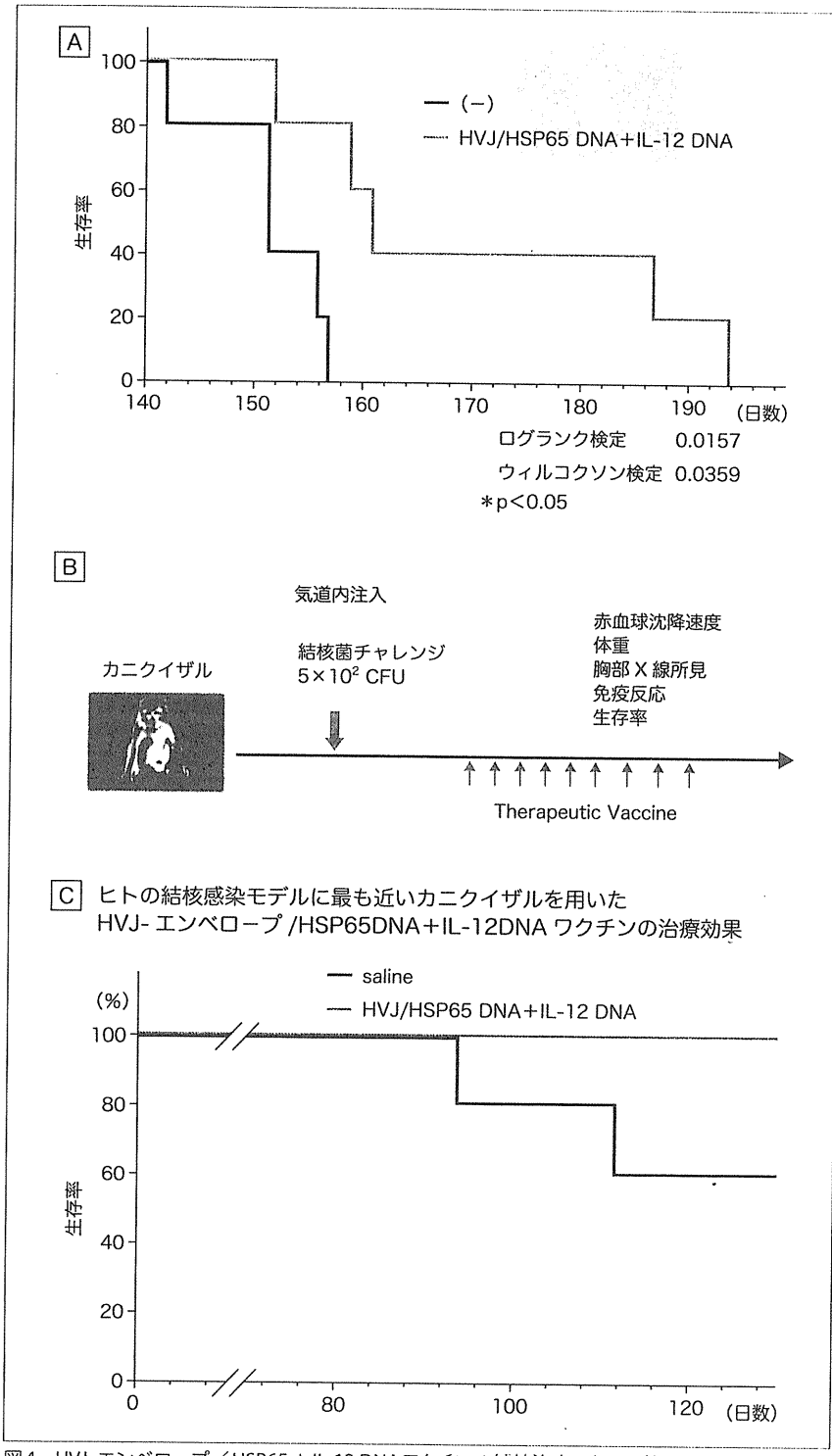

図4　HVJ-エンベロープ／HSP65＋IL-12 DNAワクチンの結核治療ワクチン効果
[A]DBA/1マウスに超薬剤耐性結核を感染させた後，HVJ-エンベロープ／HSP65＋IL-12 DNAワクチン（100 μg/マウス）で治療した．コントロール群（生食投与）との生存率を解析し，有意に延命効果を示した．[B]カニクイザルに結核菌を気道内感染させた後，HVJ-エンベロープ／HSP65＋IL-12 DNAワクチン（1つのプラスミドに2つの遺伝子を導入）で治療（i.m.）投与した．[C]このワクチン投与群では100％の生存が認められた．一方，コントロール群は60％であった．すなわち，このワクチンは結核治療効果を示した．

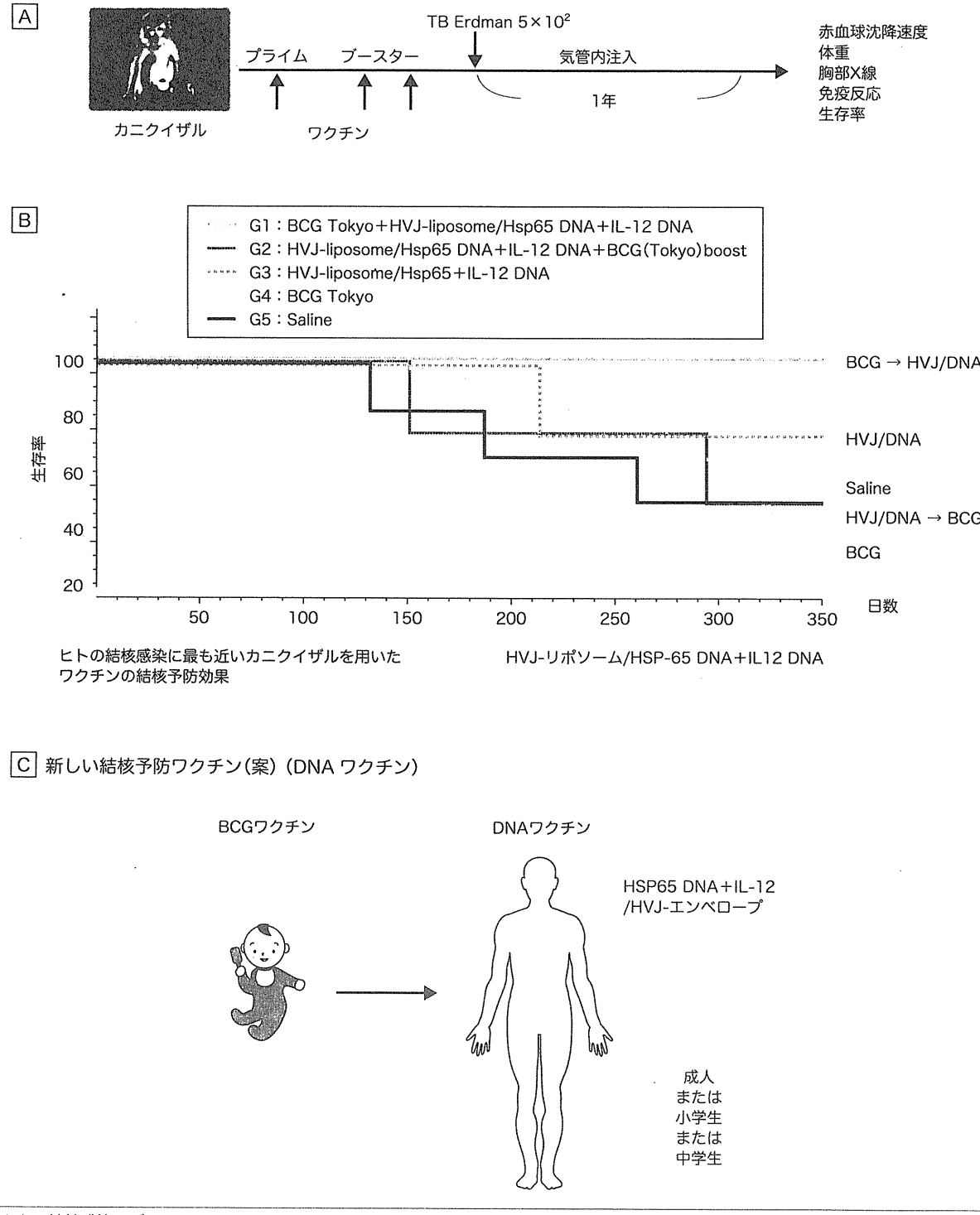

図5　ヒトの結核感染モデルに最も近い，カニクイザルを用いたHSP65＋IL-12 DNAワクチンの予防効果

Ⓐプライム-ブースト法を用い，BCGおよびHVJ-リポソーム／HSP65＋IL-12 DNAワクチンをカニクイザルに予防投与した．最終免疫後４週間後にヒト強毒結核菌を5×10^2CFU気道内注入し，１年以上生存率，免疫反応，赤血球沈降速度，体重，胸部X線所見で経過観察し，予防効果を評価した．Ⓑ BCGプライム-DNAワクチンブーストで１年間100％の生存率．一方BCGのみの群では33％の生存率．Ⓒ BCGプライム（乳幼児）-DNAワクチンブースト（成人）モデル．

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Review Article

Innate Immune Effectors in Mycobacterial Infection

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Tuberculosis, which is caused by infection with *Mycobacterium tuberculosis* (Mtb), remains one of the major bacterial infections worldwide. Host defense against Mtb is mediated by a combination of innate and adaptive immune responses. In the last 15 years, the mechanisms for activation of innate immunity have been elucidated. Toll-like receptors (TLRs) have been revealed to be critical for the recognition of pathogenic microorganisms including mycobacteria. Subsequent studies further revealed that NOD-like receptors and C-type lectin receptors are responsible for the TLR-independent recognition of mycobacteria. Several molecules, such as active vitamin D_3, secretary leukocyte protease inhibitor, and lipocalin 2, all of which are induced by TLR stimulation, have been shown to direct innate immune responses to mycobacteria. In addition, Irgm1-dependent autophagy has recently been demonstrated to eliminate intracellular mycobacteria. Thus, our understanding of the mechanisms for the innate immune response to mycobacteria is developing.

1. Introduction

In humans, tuberculosis is one of deadly infectious diseases. Indeed, approximately 2 million tuberculosis patients die every year. The risk of disease is also increased by emergence of acquired immune deficiency syndrome and development of multidrug-resistant mycobacteria [1]. Therefore, it is important to understand the host defense mechanisms against mycobacteria. Inhalation of aerosols containing *Mycobacterium tuberculosis* (Mtb) causes tuberculosis. After inhalation, Mtb invades alveolar macrophages to enter into the host and establish the infection. The host, in turn, ignites defense responses through sequential activation of immunity, a combination of innate and adaptive immune systems. In the adaptive phase of immune responses, the importance of Th1/IFN-γ-mediated responses in mycobacterial infection has been well established [2]. In contrast, although macrophages are the major target of invasion by Mtb, how the innate arm of immunity mediates host defense against mycobacteria had long remained unknown. However, the mechanisms behind innate immune responses have been revealed in the past 15 years following the identification and characterization of pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) [3]. Furthermore, it has been elucidated that TLR-dependent activation of innate immunity controls the development of adaptive immune responses [4]. The involvement of PRRs other than TLRs in the recognition of mycobacteria has also been revealed. In addition to the induction of adaptive immune responses, the PRR recognition of mycobacteria induces expression of several effector molecules participating in the innate host responses. The role of these innate effector molecules in mycobacterial infection is being elucidated. PRR-independent mechanisms for mycobacterial killing, such as autophagy, have also been revealed. In this paper, we will describe recent advances in our understanding of effectors that mediate innate immune responses against mycobacteria.

2. Toll-Like Receptors in Mycobacterial Infection

Innate immune responses after mycobacterial infection are initiated by recognition of mycobacterial components by PRRs, with mycobacterial components activating several

TLRs (Figure 1). Genomic DNA from a *Mycobacterium bovis* strain, bacillus Calmette–Guérin (BCG), have an ability to augment NK cell activity and induce type I IFNs from murine spleen cells and human peripheral blood lymphocytes. The immunostimulatory activity of mycobacterial DNA was ascribed to the presence of palindromic sequences including the 5′-CG-3′ motif, now called CpG motif [5], and now known to activate TLR9 [6]. The mycobacterial cell wall consists of several glycolipids. Among these, lipoarabinomannan (LAM) lacking mannose end capping, lipomannan (LM), and phosphatidyl-*myo*-inositol mannoside (PIM) are recognized by TLR2 [7, 8]. The 19-kDa lipoprotein of Mtb also activates macrophages via TLR2 [9, 10]. TLR4 is also presumed to recognize mycobacterial components.

The *in vivo* importance of the TLR-mediated signal in host defense to Mtb was highlighted in studies using mice lacking MyD88, a critical component of TLR signaling. MyD88-deficient mice are highly susceptible to airborne infection with Mtb [11–13]. In contrast to mice lacking MyD88, mice lacking individual TLRs are not dramatically susceptible to Mtb infection. Susceptibility of TLR2-deficient mice to Mtb infection varies between different studies [14, 15], while TLR4-deficient mice do not show high susceptibility to Mtb infection [16, 17]. A report demonstrates that TLR9-deficient mice are susceptible to Mtb infection and mice lacking both TLR2 and TLR9 are more susceptible [18]. These findings indicate that multiple TLRs might be involved in mycobacterial recognition. However, a recent report using mice lacking TLR2/TLR4/TLR9 indicated that these triple KO mice show a milder phenotype than MyD88-deficient mice [12]. Therefore, more intensive examination is required to reveal whether TLRs or molecules other than TLRs activating MyD88 mediate innate immune responses to mycobacterial infection. This study also demonstrated that Th1-like adaptive immune responses are induced even in Mtb-infected MyD88-deficient mice [12]. Therefore, the TLR/MyD88-independent component of innate immunity is involved in the induction of adaptive immune responses during mycobacterial infection. The TLR/MyD88-independent response might be induced by other PRRs described below.

3. Non-TLRs in Mycobacterial Infection

Several recent findings have indicated that PRRs other than TLRs evoke innate immune responses [19]. These include RIG-I-like receptors, NOD-like receptors (NLRs), and C-type lectin receptors. Among these PRRs, NOD-like receptors and C-type lectin receptors have been implicated in the innate recognition of mycobacteria (Figure 2).

NOD2 is a member of NLRs that recognize muramyl dipeptide (MDP), a core component of bacterial peptidoglycan, in the cytoplasmic compartment. Macrophages from NOD2-deficient mice show a defective cytokine production after Mtb infection [20]. Similarly, mononuclear cells of individuals homozygous for the *3020insC NOD2* mutation show a defective cytokine response after stimulation with Mtb [7]. Activation of the NOD2-mediated pathway is induced by stimulation with live Mtb, but not by heat-killed Mtb [8]. Live Mtb, which is localized in the phagosomal compartment within macrophages, stimulates the cytosolic NOD2 pathway by inducing phagosomal membrane damage [21]. The NOD2 ligand MDP is N-acetylated in most bacteria. However, MDP is N-glycolylated by N-acetyl muramic acid hydroxylase (NamH) in mycobacteria. Analyses using *M. smegmatis* namH mutant and NOD2-deficient mice showed that N-glycolyl MDP is recognized by NOD2. In addition, N-glycolyl MDP is the more potent NOD2 activator than N-acetyl MDP [22]. Thus, NOD2 contributed to the recognition of mycobacteria.

Several members of the NLR family, such as NLRP1, NLRP3, and IPAF, induce assembly of the inflammasome, which leads to caspase-1-dependent secretion of IL-1β and IL-18 [23]. The involvement of IL-1β and IL-18 in mycobacterial infection was demonstrated in studies using knockout mice [24–27]. A recent study demonstrated that mycobacteria inhibit the inflammasome-dependent casapase-1 activation leading to defective IL-1β production [28]. The inhibition of caspase-1 activation has further been shown to be mediated by an Mtb gene, *zmp1*, which encodes a putative Zn^{2+} metalloprotease. Thus, Mtb has a strategy that evades the inflammasome-mediated innate immune responses.

C-type lectin receptors, such as mannose receptor, were originally reported to mediate phagocytosis of mycobacteria [29]. Another C-type lectin receptor, DC-SIGN, has been shown to recognize mycobacteria, and thereby modulate the function of dendritic cells [30–32]. Recognition of mycobacteria by dectin-1 has been shown to induce gene expression such as TNF-α, IL-6, and IL-12 [33, 34]. In addition, macrophage inducible C-type lectin (Mincle) has recently been shown to recognize trehalose-6,6′-dimycolate (TDM: also called cord factor), a mycobacterial cell wall glycolipid that is the most studied immunostimulatory component of Mtb [35, 36], thereafter modulating macrophage activation. Thus, several C-type lectin receptors are involved in the recognition of mycobacteria.

CARD9 is involved in the signaling pathways of several PRRs including TLRs, NOD-like receptors, and FcRγ-associated C-type lectin receptors through association with Bcl-10 and MALT. Therefore, it is not surprising that CARD9-deficient mice are highly susceptible to Mtb infection. However, interestingly the high susceptibility of CARD9-deficient mice to the infection has been shown to be excessive inflammatory responses due to defective production of the immunosuppressive cytokine IL-10 [37]. Mincle is a member of C-type lectin receptors associated with FcRγ [38]. Accordingly, TDM-induced immune responses are mediated by the signaling pathway activating CARD9 [36, 39].

TLRs and C-type lectin receptors are expressed on the plasma membrane or the endosomal/phagosomal membrane, whereas NOD-like receptors are expressed within the cytoplasm. Indeed, distinct patterns of TLR- and NOD-like receptor-mediated gene expression profiles have been demonstrated in infection with intracellular bacteria [40]. Thus, several PRRs recognize mycobacteria in distinct sites within the host cells (macrophages) to synergistically induce effective host defense responses.

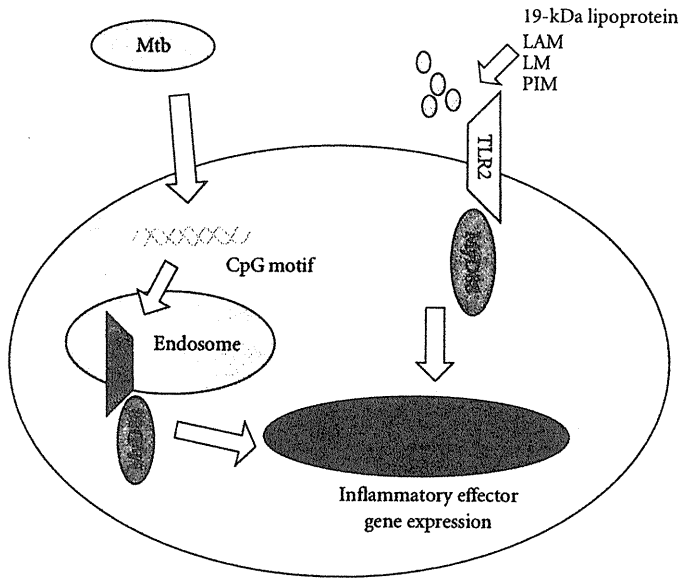

FIGURE 1: Recognition of mycobacteria by Toll-like receptors. TLR2 recognizes several mycobacterial-derived components. TLR9 recognizes mycobacterial DNA including the CpG motif within endosomal compartments. TLR-dependent recognition of mycobacteria induces activation of signaling pathways via the adaptor molecule MyD88, leading to activation of gene expression.

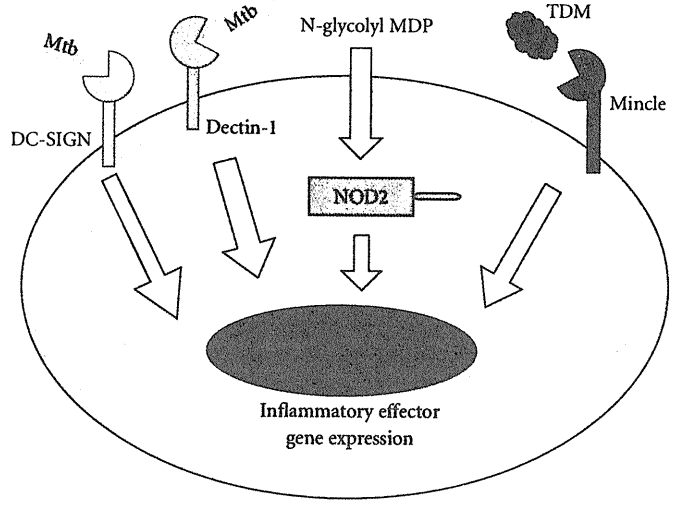

FIGURE 2: Recognition of mycobacteria by pattern recognition receptors. Several pattern recognition receptors, such as NOD-like receptors and C-type lectin receptors, mediate the TLR-independent recognition of mycobacteria. NOD2, a member of NOD-like receptors, recognizes mycobacterial N-glycolyl MDP within the cytoplasm. DC-SIGN and dectin-1 are members of C-type lectin receptors, which are implicated in the recognition of mycobacteria. In addition, Mincle has been shown to recognize TDM (a mycobacterial cell wall glycolipid).

4. Effectors for Mycobacterial Killing

The recognition of mycobacteria by several PRRs induces the expression of several genes that mediate host defense (Figure 3). Among these gene products, vitamin D receptor (VDR) and Cyp27b1, a 25-hydroxyvitamin D_3 1-α-hydroxylase that catalyzes inactive provitamin D into the bioactive form of vitamin D (1, 25 $(OH)_2D_3$), have been shown to be induced by TLR2 ligands in human macrophages [41]. Stimulation of macrophages with 1, 25 $(OH)_2D_3$ induces the expression of the antimicrobial peptide cathelicidin, and thereby enhances the antimycobacterial killing activity [42]. In addition to cathelicidin, the small cationic antimicrobial peptide defensin mediates innate immune responses to Mtb [43, 44]. Experimental infection of the lung epithelial cell line A549 with Mtb strongly induces production of human β-defensin HBD-2, which leads to Mtb killing [43]. HBD-2 expression has also been shown to be induced by TLR2 [45].

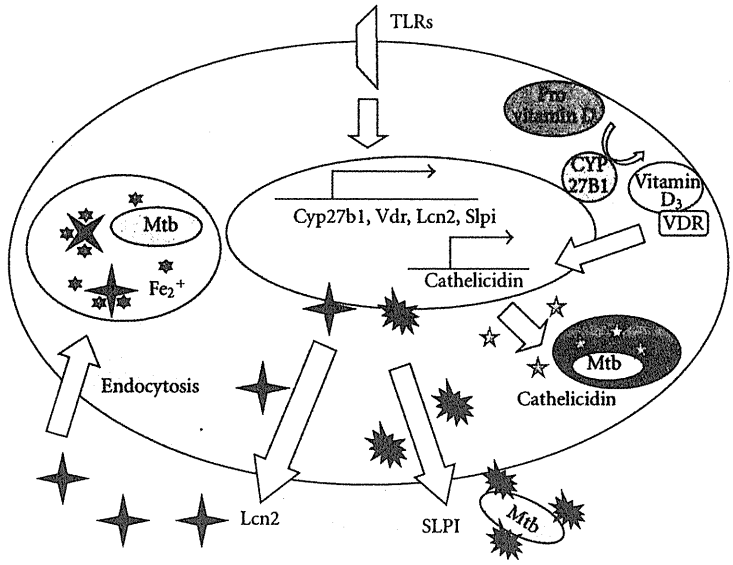

FIGURE 3: TLR-dependent innate response to mycobacteria. Several TLR-dependent gene products mediate innate immune responses to mycobacteria. Mycobacterial stimulation of TLR2 induces expression of Cyp27b1 and vitamin D receptor (VDR), both of which are involved in vitamin D_3-dependent induction of cathelicidin which directly kills mycobacteria. TLR-dependent induction of SLPI mediates disruption of the mycobacterial cell wall. Lcn2, which is also induced by TLR stimulation, is internalized into the alveolar epithelial cells and inhibits mycobacterial growth by sequestering iron uptake.

Gene expression analyses of the lung of mycobacteria-infected mice have identified several TLR-dependent genes that are involved in innate immune responses during mycobacterial infection. These genes include *Slpi*, encoding secretory leukocyte protease inhibitor (SLPI), and *Lcn2*, encoding lipocalin 2 (Lcn2). SLPI is a secreted protein composed of two cysteine-rich whey acidic protein (WAP) domains [46–48]. SLPI was named after its presence in secretions and its function as a serine protease inhibitor. SLPI was originally shown to mediate wound healing [49, 50]. SLPI is produced by bronchial and alveolar epithelial cells as well as alveolar macrophages and is secreted into the alveolar space at the early phase of mycobacterial respiratory infections. Recombinant mouse SLPI effectively inhibits the *in vitro* growth of BCG and Mtb through disruption of the mycobacterial cell wall structure. Cationic residues within the WAP domains of SLPI are essential for the disruption of mycobacterial cell walls. Moreover, SLPI-deficient mice are highly susceptible to mycobacterial infection [51]. The mechanism by which SLPI attaches to the membrane of mycobacteria has been elucidated. SLPI recognizes mannan-capped lipoarabinomannans and phosphatidylinositol mannoside, which are conserved in mycobacteria. Thus, SLPI might act as a PRR in order to bind to the mycobacterial membrane [52].

Lcn2 (also known as neutrophil gelatinase-associated lipocalin, 24p3, or siderocalin) was originally identified in the granules of human neutrophils. Lcn2 is a member of the lipocalin protein family and able to bind to small hydrophobic molecules, siderophore. It is a bacterial molecule made in iron-limited environment and facilitates iron uptake by bacteria [53–58]. The expression of Lcn2 is increased in macrophages of LPS-treated mice [59]. In addition, it is secreted into the alveolar space by alveolar macrophages and epithelial cells during the early phase of respiratory mycobacterial infection. Lcn2 inhibits *in vitro* growth of Mtb by binding the mycobacterial siderophore carboxymycobactin, thereby sequestering iron uptake. Moreover, Lcn2-deficient mice are highly susceptible to intratracheal infection with Mtb. Lcn2 is internalized into alveolar epithelial cells by endocytosis and colocalized with mycobacteria within the cells. Therefore, Lcn2 presumably sequesters iron uptake of mycobacteria within epithelial cells and thereby inhibits their intracellular growth. Within macrophages, the endocytosed Lcn2 and mycobacteria show distinct patterns of subcellular localization, which might allow growth of mycobacteria within macrophages [60]. Thus, Lcn2, which is secreted into the alveolar space during the early phase of mycobacterial infection, is endocytosed into alveolar epithelial cells, thereby inhibiting mycobacterial growth [61].

5. Autophagy in Mycobacterial Infection

Phagocytosis of myobacteria and PRR-dependent recognition of mycobacteria activate several effector functions in macrophages (Figure 4). Maturation of phagosomes is a crucial step in the elimination of intracellular bacteria. The natural-resistance-associated macrophage protein (Nramp1), which is encoded by Slc11a1, is thought to mediate transportation of divalent cations in the phagosomal membrane and thereby sequesters iron (Fe^{2+}) from mycobacteria to enhance bacterial killing by macrophages [62]. Polymorphisms of the *SLC11A1* gene have been associated with susceptibility to several infectious diseases,

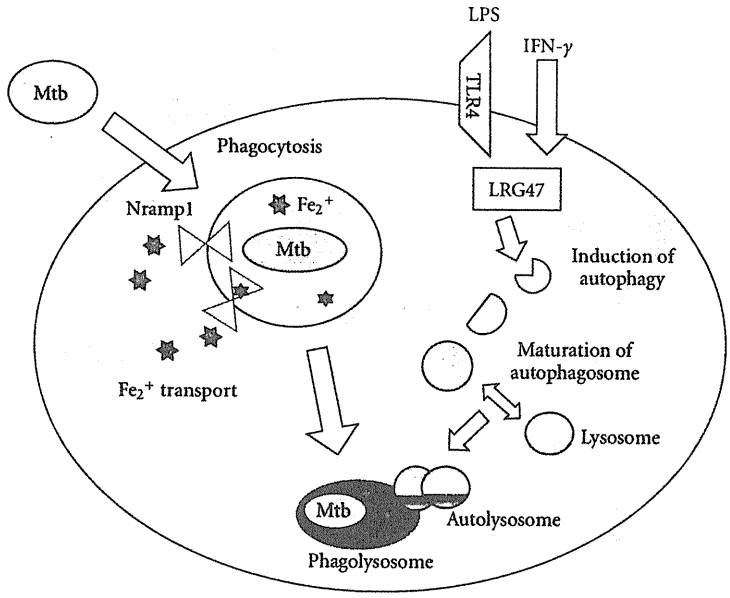

FIGURE 4: Effectors that mediate mycobacterial killing in macrophages. Macrophages eliminate invading mycobacteria by activating several effector functions, such as phagosomes and autophagy. Nramp1 is expressed in the phagosomal membrane and presumably mediates mycobacterial killing by sequestering iron uptake. IFN-γ and the TLR4 ligand induce expression of LRG47, which in turn stimulates autophagy in macrophages. Autophagy is responsible for mycobacterial killing by promoting fusion of mycobacterial phagosomes to lysosomes.

including tuberculosis [63, 64]. However, *in vivo* studies have shown that Nramp1-deficient mice are not more susceptible than wild-type mice to infection with virulent Mtb [65]. Thus, the role of Nramp1 in mycobacterial infection is still controversial. This might be due to the presence of other killing mechanisms for mycobacteria in macrophages. Indeed, autophagy has recently been shown to be involved in host defense against several intracellular pathogens that reside within phagosomes [66]. Autophagy was originally identified as a homeostatic mechanism for the catabolic reaction of cellular constitutes [67, 68]. It has been demonstrated that autophagy mediates innate immune responses against mycobacteria by promoting phagolysosomal maturation within macrophages [69, 70]. Autophagy is induced by IFN-γ-dependent induction of a member of the immunity-related p47 guanosine triphosphatases (IRG) family, LRG47 (also known as Irgm1) in murine macrophages [69]. The importance of LRG47 in resistance to Mtb infection was demonstrated in LRG47-deficient mice, which show high susceptibility to infection [71]. A subsequent study demonstrated that stimulation of macrophages with the TLR4 ligand LPS leads to the MyD88-independent induction of autophagy, which enhances mycobacterial colocalization with the autophagosomes. Since LPS stimulation induces expression of LRG47, the TLR signaling establishes a close relationship between innate immunity and autophagy in mycobacterial infection [72]. In humans, the most equivalent gene to murine Irgm1 is IRGM. IRGM has also been implicated in the induction of autophagy in mycobacteria-infected human macrophages [73]. Irgm1 has been shown to associate with the mycobacterial phagosome by interacting with phosphatidylinositol-3,4-bisphosphate (PtdIns(3,4)P(2)) and PtdIns(3,4,5)P(3) [74]. The connection of the IRG family of proteins with autophagy has been further demonstrated in an alternative intracellular infection model. In this study, Irgm3 (also known as IGTP) has been implicated in autophagy induction in macrophages infected with *Toxoplasma gondii* [75].

p62 (also called A170 or SQSTM1) directly binds to cytosolic polyubiquitinated proteins and thereby induces their autophagic clearance [76, 77]. It has also been shown that p62 targets intracellular *Salmonella typhimurium* decorated by ubiquitinated proteins to induce autophagy [78]. In the case of mycobacteria residing in the phagosome, p62 delivers cytosolic ubiquitinated proteins to autophagolysosomes where they are proteolytically processed to products that are able to kill mycobacteria [79]. In accordance with this finding, it has been shown that mycobacterial killing by ubiquitin-derived peptides is enhanced by autophagy [80].

As described above, 1, 25 $(OH)_2D_3$ mediates antimycobacterial activity via induction of cathelicidin. A recent report demonstrated that 1, 25 $(OH)_2D_3$-mediated expression of cathelicidin induces autophagy [81]. Thus, several innate immune effectors are closely interacted.

6. Human Genetics in Tuberculosis

In addition to the intensive studies using murine models, considerable advances have been made in our understanding of the susceptibility to Mtb infection in humans through the identification of mutations and polymorphisms of

innate immunity-related genes in tuberculosis patients. As described above, polymorphisms of the *SLC11A1* gene are associated with tuberculosis. Subsequent studies identified a significant distinction between tuberculosis patients and healthy controls in *TLR2* Arg753Gln polymorphism genotype, indicating that the *TLR2* polymorphism influences the susceptibility of Mtb infection [82]. *VDR* polymorphisms have also been implicated in the susceptibility of Mtb infection [83]. These studies suggest that several genes, which have been revealed to be critical in innate responses in mouse models of Mtb infection, regulate Mtb infection in humans.

7. Conclusion

Since the discovery of TLRs at the end of the 20th century, rapid advances have been made in our understanding of the mechanisms for activation of innate immunity. Accordingly, innate immunity has been revealed to have a pivotal role in host defense against mycobacteria. The TLR-independent mechanisms for the innate immune response to mycobacteria have also been elucidated. The emergence of multidrug-resistant Mtb is now a major public health problem all over the world. In this context, it is highly critical to develop a new strategy for the treatment of Mtb-infected patients that supplements the conventional antimycobacterial chemotherapeutic drugs. More precise understanding of the innate immune response to Mtb will pave the way for the development of an effective drug that targets the host innate immunity for the treatment of tuberculosis.

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