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Review Article

Macrophage Models for Tuberculosis Studies, A Systematic Review And Meta-Analysis Study

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Abstract

Setting: Macrophage models create an environment for the growth of mycobacterium tuberculosis and can be applied to studies on the intracellular pathogen.
Objective: To review macrophage models for tuberculosis studies in terms of the cell sources and applications.
Methods: The online English database PubMed, Chinese databases such as CNKI, SinoMed, and Wanfang were searched up to December 2010 for published articles. Two groups of terms were used for the information retrieval, one of which includes macrophage model and macrophage; the other group of terms comprises *mycobacterium tuberculosis*. For PubMed, the two groups of English terms were applied, while for the Chinese databases, related Chinese terms were used.
Results: Cell sources of macrophage models include murine bone marrows, human peripheral blood, human lungs, and cell lines. The screened antituberculosis drugs are Western drugs such as ciprofloxacin and ofloxacin, and traditional Chinese medications like *pittosporum brevicalyx* (oliv.) gagnep and *Ranunculus ternatus* thunb. The regulation of SigB and SigF was described in the pathogenesis of tuberculosis, while pknE mutant modifying the innate immune response in immuno-mechanism of tuberculosis.
Conclusions: There are four major cell sources for developing a macrophage model, which can be applied to studies on screening both Western and Chinese anti-TB drugs, figuring out the pathogenesis and immune-mechanism of TB with some key issues remaining to be dealt with.

1. Introduction

Mycobacterium tuberculosis is still one of the most significant human pathogens since it was discovered[1]. It is an intracellular pathogen surviving and replicating within macrophages[2]. Knowledge of the bacteria-macrophage interaction can help develop novel measures to combat tuberculosis. Macrophage models are of great importance for studies on tuberculosis because the environment can be simulated for the growth of mycobacterium tuberculosis with macrophage models. In this article we review cell sources and applications of macrophage models for tuberculosis studies.

2. Methods

The online English database PubMed, Chinese databases CNKI, SinoMed, and Wanfang were searched up to December 2010 for published articles. Two groups of terms were used for the information retrieval, one of which includes macrophage model and macrophage; the other group of terms are composed of comprises *mycobacterium tuberculosis*. For PubMed, the two groups of English terms were applied, while Chinese terms were used.

Inclusion criteria were set up for this review, by which articles on cell sources, screening of anti-tuberculosis drugs, pathogenic mechanisms of tuberculosis or immunomechanism of tuberculosis of macrophage models are collected. Exclusion criteria were also developed, by which articles about other aspects of macrophage models, such as vaccination of tuberculosis, were excluded.

3. Results

Thirty-seven articles met the inclusion criteria, most of which are from PubMed. The topics in these articles are cell sources, screening of anti-tuberculosis drugs, pathogenesis and immunomechanism of tuberculosis with the application of macrophage models.

2.1. Development of Macrophage Models

Slayden *et al* established a macrophage model in the way described below: the marrow of six- to eight-week-old female specific-pathogen-free C57BL/6 mice was flushed from their femurs. The marrow plugs were disrupted by gentle pipetting, washed twice, and plated at tissue culture treated plates. After 48 hours of incubation, the nonadherent cells were removed and new sDMEM was added. The cells were washed after a further 4 days, and antibiotic-free medium was added and incubated for 2 more days. Eight days after plating, macrophages were infected with *M. tuberculosis* Erdman in 200 µl of medium for 2 hours, and then extensively washed to remove extracellular bacteria[3].

Zhao *et al* developed a macrophage model in the following steps: cells of human monocytic cell line THP1 were cultured without antibiotics. THP1 cells were seeded into the wells of tissue culture plates. Phorbol 12-myristate 13-acetate was added to each well at a final concentration of 100 nM after incubation. Finally, mycobacteria were added after one additional hour of incubation[4].

According to Eddine *et al*, a macrophage model was made as follows: bone marrow-derived macrophages were extracted from C57/BL6 mice and seeded in culture dishes. They were allowed to differentiate for 5 to 6 days in Dulbecco's modified essential medium.

These cells were then seeded in 96-well plates and incubated overnight for adhesion[5].

2.2. Cell Sources of Macrophage Models

2.2.1 Murine Bone Marrows

Murine bone marrows are one of the cell sources for macrophage models. Bone marrow-derived macrophages can be obtained from C57BL/6 female mice[6]. Mice can be killed by exposure to CO_2 with the femur bones dissected out. Their bones are trimmed at each end, and the marrow is washed out using Dulbecco's minimal essential medium (DMEM) supplemented with fetal calf serum, L-929 fibroblast conditioned supernatant, HEPES buffer, nonessential amino acids, L-glutamine, and antibiotics. Cell suspensions is then washed twice and plated in supplemented DMEM.

According to Pichugin *et al*, macrophages were isolated from femurs and tibias of male mice for macrophage models by centrifugation at room temperature on a NycoPrep gradient, washed, and cultured in tissue culture flasks in supplemented DMEM. The nonadherent cells were harvested and transferred into tissue culture flacks. Nonadherent cells were harvested, washed, and plated to tissue culture plates in the same medium without IL-3. Finally the cells were maintained by feeding with fresh medium to form monolayer[7].

BALB/c mice can also be the source for building macrophage models. Bashir N *et al* reported that RAW 264.7 macrophages were washed with wash buffer twice and then incubated with rRv2626c for various times on ice. These macrophages were incubated with the anti-Rv2626c antibody followed by incubation with anti-mouse fluorescein isothiocyanate conjugate after washing, suspended in sheath fluid, and analysed on a fluorescence-activated cell sorter machine after a final washing[8].

2.2.2 Human Peripheral Blood

Another cell source of macrophage models is human peripheral blood. Nair *et al*[9] reported that, in order to obtain a macrophage model, monocyte-derived macrophages were isolated from heparinized blood from the vein of the healthy participants. Cells were collected from the interface. Adherent monolayers were harvested by incubating the peripheral blood monocytes in tissue culture plates. Nonadherent cells were removed with a transfer pipette and used for purifying T cells[9]. There are some other scholars who also used human peripheral blood for setting up macrophage models[10][11].

2.2.3 Human Lungs

Bronchoalveolar cells are also a source of macrophage models. According to Juarez *et al* the bronchoscope was used, and 0.9% sterile saline fluid was instilled into each of two adjacent lung subsegments. Bronchoalveolar lavage fluid was centrifuged. The bronchoalveolar cells were resuspended in culture medium and their viability was assessed using Trypan blue exclusion[12].

2.2.4 Cell Lines

In addition, cell lines are one of the cell sources for macrophage models. According to Tominaga *et al* a macrophage cell line with activated characters and unique morphology is isolated from the human monocytic cell line THP-1. The original THP-1 cells have been cultured for years. When they appear to be cells with a different morphology, the cells adhere to the bottoms of the culture flasks. The adherent cells are selected by discarding floating nonadherent cells at every subculture. Enrichment of adherent THP-1 cells with long processes proceeds. Adherent THP-1 cells indicate phenotypic changes, not only morphologically, but also functionally. The adherent cell line is taken as activated-THP-1 (A-THP-1), because it shows characteristics of activated macrophages continuously without extra stimulation, providing a good model for understanding of activation mechanisms of macrophages and multinucleation[13]. Some other researchers also reported that cells of human monocytic cell line THP1 were cultured and then seeded into tissue culture plates to set up a macrophage model[4][9][14].

2.3. Applications of Macrophage Models to Tuberculosis Studies

2.3.1 Screening Antituberculosis Drugs

Crowle described a technique to treat with chemotherapy human macrophages infected ex vivo with tubercle bacilli. The infected phagocytes interacted with such drugs as streptomycin, ethambutol, pyrazinamide, isoniazid, and ceforanide[15].

Rastogi *et al* investigated the actions of ciprofloxacin and ofloxacin against *M. tuberculosis* with a TB-infected macrophage model in which the J-774 macrophage cell line was infected with the H37Rv type strain of *M. tuberculosis*. The two tested drugs were added after 2 days of intracellular growth of the bacteria. They came to a conclusion that both drugs did not affect macrophage viability, and were effective against the virulent tubercle bacilli[16].

A study by Sbarbaro *et al* finds a higher bacteriostatic effect when low, nonbactericidal levels of rifampin are combined with pyrazinamide but not with higher bactericidal levels of rifampin. The way to introduce the drugs affects the result. Giving pyrazinamide after the introduction of rifampin increased the killing effect, while adding rifampin after the introduction of pyrazinamide resulted in a weaker activity than giving the agents simultaneously[17].

Rifabutin, clarithromycin, and ethambutol were tested by Furney *et al* for their capacities to inhibit the growth of two isolates of Mycobacterium avium in mice and *in vitro* in a macrophage model. In the latter model, rifabutin and clarithromycin showed modest level of activities against strain 101 and somehow better activities against strain 2-151. When all of the three drugs were given in combination, they indicated the best results against strain 101, but no significant improvement compared with the result of clarithromycin given alone[18].

Sbarbaro *et al* quantified the intramacrophage antimycobacterial effect of pyrazinamide (PZA) with ofloxacin. As a result, a clinically achievable level of PZA strengthens the antimycobacterial effect of low, non-bactericidal levels of ofloxacin and does not hinder the bactericidal effect of a higher level of ofloxacin[19].

Kelly *et al* tested four rifamycins, which includes rifampin, rifabutin, rifapentine, and KRM-1648, in an *in vitro* murine macrophage model and then in the low-dose aerosol infection model, for their effects against *M. tuberculosis*. In both models, KRM-1648 showed the highest level of activity among all the tested drugs. In the infected-lung model, rifabutin, rifapentine, and KRM-1648 all sterilized the bacterium when given orally at 5 mg/kg per day. With a daily dose of 2.5 mg/kg, KRM-1648 exerted the highest level of activity among these four rifamycins, reducing the bacterial load[20].

Thiolactomycin (TLM) has in vivo antimycobacterial activity against the virulent strain *M. tuberculosis* Erdman, shwoing complete inhibition of growth on solid media at 25 micrograms/ml. In an *in vitro* murine macrophage model, the killing of viable intracellular *M. tuberculosis* in a dose-dependent manner was also seen[3].

In a human macrophage model infected by wild-type *Mycobacterium bovis* BCG, the C-8 methoxyl group decreased survival 20- to 100-fold compared with the same concentration of a C-8-H fluoroquinolone, improving fluoroquinolone action against both quinolone-susceptible and -resistant clinical isolates. Therefore, a C-8 methoxyl group strengthens the bactericidal activity of quinolones with N1-cyclopropyl substitutions, implying that further refinement of fluoroquinolones could be done as antituberculosis agents[4].

Isoxyl (ISO), a thiourea (thiocarlide; 4,4′-diisoamyloxythiocarbanilide), showed effective activity against the clinical isolates of *M. tuberculosis* from different geographical areas with various drug resistance patterns. In a murine macrophage model, ISO indicated bactericidal killing of viable intracellular *M. tuberculosis* in a

dose-dependent manner, inhibiting the synthesis of both fatty acids and mycolic acids at its MIC for *M. tuberculosis*, while both isoniazid and ethionamide demonstrated similarity in inhibition of the synthesis of all kinds of mycolic acids only. In addition, a homologous series of ISO derivatives were synthesized, most of which were as effective as or more effective than the parent compound. Therefore, these thioureas demonstrate promise in counteracting a big variety of strains of *M. tuberculosis*[21].

Two of the isoniazid derivatives with MIC < 3.13 microg/ml and SI > 10 were tested by Szymańska *et al* for efficacy *in vitro* in a TB-infected macrophage model. As a result, none of them demonstrated satisfactory activity[22].

Foroumadi *et al* evaluated a series of piperazinyl quinolones for their antituberculosis activity against *M. tuberculosis* H37Rv with the BACTEC 460 radiometric system and BACTEC 12B medium. It was indicated that compounds 1a, 1e and 1g were efficient antimycobacterial agents. In their study, compound 1a was also examined for efficacy in a macrophage model[23].

Qian Zhongqing established macrophage models to test the efficacy of Isoniazid, extract of Pittosporum Brevicalyx (Oliv.) Gagnep against *M. tuberculosis*. It is concluded that the two tested drugs are effective[24].

Maccari *et al* tested antimycobacterial activities of cobalt (II) and copper (II) complexes of fluorinated isonicotinoylhydrazones in *M. tuberculosis*-infected macrophage model. They came to a conclusion that all metalcomplexes indicated great activity against *M. tuberculosis* Erdman and single-drug-resistant *M. tuberculosis* strains[25].

Maccari *et al* reported that some hydrazides and isonicotinoylhydrazones (ISNEs) are more effective antimycobacterial agents than their parental isoniazid in a TB-infected macrophage model[26].

One of the six carbazole alkaloids from the CH2Cl2 extract of the stem bark of Micromelum hirsutum, which is also called micromolide. It indicated potent *in vitro* anti-TB activity against H37Rv, and exhibited activity against the Erdman strain of *M. tuberculosis* in a J774 mouse macrophage model[27].

In a study by Boyne *et al* two high-affinity alkyl-substituted diphenyl ethers, 6PP and 8PP, were tested for their *in vitro* activity against clinical isolates of *M. tuberculosis*. The two substances showed enhanced activity against bacteria in a macrophage model of infection[28]. Eddine AN *et al* reported the inhibition of the growth of *M. tuberculosis* by 4,4'-dihydroxybenzophenone (DHBP) in a mouse macrophage model, while no cell toxicity was detected for DHBP up to 200 μm[5].

In vitro, morphine did not show any direct anti-mycobacterial activity up to $1x10^{(-4)}$ M concentration, which was assessed by radiometric BACTEC method. In a macrophage model of infection, morphine indicated maximal killing at $1x10^{(-7)}$ M concentration, but was blocked by naloxone and aminoguanidine. These results suggest that morphine has a dose-dependent effect in murine tuberculosis, while its protective effect is naloxone-reversible and may involve macrophage-mediated protective mechanisms[29].

Wu Guodong *et al* established peritoneal macrophage models for screening anti-TB drugs such as Rifampin, Ranunculus Ternatus Thunb, and Xinlaoning Capsule. As a result, the three drugs showed anti-mycobacterial effects while Ranunculus Ternatus Thunb and Xinlaoning Capsule indicated weaker bactericidal effects than Rifampin[30].

2.3.2 Pathogenic Mechanisms of Tuberculosis

Horgen *et al* examined the postantibiotic effects (PAEs) of four agents against Mycobacterium avium with a macrophage model. They created two different experimental conditions. For postantibiotic leukocyte enhancement (PALE), the bacteria were exposed to antibiotics before phagocytosis. For pulsed exposure (PE), antibiotics were used after phagocytosis. The drugs were applied at their peak concentrations in serum in both cases. It was indicated that even a brief exposure of *M. avium* to peak concentrations of some agents in serum may lead to prolonged and persistent suppression of bacterial growth in human macrophages[31].

As an intracellular pathogen, *M. tuberculosis* suppresses macrophage apoptosis to support survival and replication within the host cell. Jayakumar *et al* demonstrated that the functional serine/threonine kinase, PknE, is essential for survival of *M. tuberculosis* which enhances macrophage viability by inhibiting apoptosis. A promoter of PknE was shown to respond to nitric oxide stress. Deletion of pknE in *M. tuberculosis* resulted in an increased-resistance strain to nitric oxide donors and a more-sensitive strain to reducing agents. The deletion mutant caused by specialized transduction induced apoptosis while inhibiting necrosis[32].

According to Basler *et al* murine macrophage cell lines are a suitable system to examine M. avium ssp. patho-mechanisms and could dispaly that MAP, but not MAA, specifically inhibited the antigen-specific stimulatory capacity for CD4(+) T-cells[33].

Lee *et al*[1] examined the roles of SigB and SigF in sigma factor regulation in *M. tuberculosis* in terms of their physiological effects of transcriptional activation by testing the growth of the sigB and sigF KI strains during macrophage infection. It was indicated that baseline sigB and sigF expressions in the KI strains were higher than those in the control strains because of the increased gene dosage and leakiness of the acetamide promoter system. Even with the acetamide-free conditions, *M. tuberculosis* recombinants overexpress sigB and sigF. The sigB KI strain demonstrated a growth defect in macrophages, because it could not replicate at the same rate as the control strain. Similarly, the normalized CFU counts showed a reduced growth rate for the sigB-overexpressing strain compared with the control strain. The CFU counts for the sigF-overexpressing strain also displayed a slowed intracellular growth rate.

Although macrophages are effective in internalizing and clearing most of the bacteria, *M. tuberculosis* H37Rv has evolved some effective survival strategies, which include inhibiting phagosome-lysosome fusion, inhibiting phagosome acidification, recruiting and retaining tryptophan-aspartate with coat protein on phagosomes to prevent their delivery to lysosomes and expressing members of the host-induced repetitive glycine-rich protein family of proteins[34].

Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the first key step of gluconeogenesis. Marrero *et al* applied genetic analyses and (13) C carbon tracing to show that PEPCK is crucial for growth of *M. tuberculosis* on fatty acids and increases the rate of carbon flow from tricarboxylic acid cycle-derived metabolites to gluconeogenic intermediates. It was further shown that PEPCK is a must for growth of *M. tuberculosis* in a murine macrophage model and in mice. In addition, *M. tuberculosis* without PEPCK failed to replicate in mouse lungs, and could not survive, while PEPCK depletion in chronic phase of infection caused mycobacterial clearance. *M. tuberculosis* is therefore based on gluconeogenesis during the infection. PEPCK depletion weakens *M. tuberculosis* in IFN gamma-deficient mice, suggesting that this enzyme could be a target for chemotherapy[35].

Bordbar *et al* developed a cell-specific alveolar macrophage model, iAB-AMØ-1410, and then integrated it with an *M. tuberculosis* H37Rv model, iNJ661, to construct an integrated host-pathogen genome-scale reconstruction, iAB-AMØ-1410-Mt-661. The integrated model allows the simulation of the metabolic changes during infection which shows three distinct pathological states[36].

2.3.3 Immunomechanism of Tuberculosis

According to Jayakumar *et al* the pknE mutant alters the innate immune response as displayed by the decrease in the pro-inflammatory cytokines in a macrophage model[32].

In terms of the pathophysiological functions of proline-glutamic acid (PE)/proline-proline-glutamic acid (PPE) family of proteins of *M. tuberculosis*, Nair *et al* demonstrated in their study that one of the PPE proteins, PPE18, can stimulate macrophages to secrete IL-10 which favors a Th2 type response. When macrophages were infected with a mutant *M. tuberculosis* strain lacking the PPE18, less IL-10 was produced as compared with those infected with the wild-type strain. The evidence suggests that the PPE18 protein may initiate an anti-inflammatory response by stimulating IL-10 production[9].

Pichugin *et al* used a mouse model of infection with *M. tuberculosis* to figure out the functioning of the genetic locus sst1 in hosts developing pulmonary tuberculosis. It is indicated that sst1 can control necrosis within tuberculosis lesions in the lung, which is independent of both the route of infection and the host's genetic background. What for more, sst1-dependent necrosis took place at low bacterial loads in the lung after anti-tuberculosis drug therapy was stopped. As a result, sst1-susceptible mice with tuberculosis-resistant and -susceptible genetic backgrounds can be used to reproduce different types of clinical pulmonary tuberculosis and may be applied to predict the efficacy of anti-tuberculosis drugs for different human populations[7].

T cell immunoglobulin and mucin domain 3 (Tim3) is a negative regulatory molecule that suppresses effector T(H)1-type responses. Such inhibitory signals stop unintended tissue inflammation, but can be harmful if they result in premature T cell exhaustion. Despite the fact that the role of Tim3 in autoimmunity has been extensively explored, whether Tim3 regulates antimicrobial immunity is still unknown. According to Jayaraman *et al* it is demonstrated that Tim3 expressed on T(H)1 cells interacts with its ligand, galectin-9 (Gal9), which is expressed by *M. tuberculosis*-infected macrophages to inhibit intracellular bacterial growth. Tim3-Gal9 interaction activates macrophages and increases bactericidal activity by stimulating caspase-1-dependent IL-1β secretion. The T(H)1 cell surface molecule Tim3 evolves to restrict the growth of intracellular pathogens via its ligand Gal9, which suppresses expansion of effector T(H)1 cells to stop further tissue inflammation[37].

Secretory proteins of *M. tuberculosis* are the important immunomodulators of the host immune response. Open reading frame (ORF) Rv2626c, which encodes a conserved hypothetical protein inducing a strong humoral immune response in patients with tuberculosis (TB), is up-regulated upon infection in mice on hypoxic conditions. Bashir *et al* demonstrate that recombinant Rv2626c protein (rRv2626c) can bind to the surface of murine macrophages and lead to the type-1 immune response by nitric oxide (NO) secretion and expression of inducible nitric oxide synthase (iNOS). Together with rRv2626c, significant induction of pro-inflammatory cytokines, which include interleukin (IL)-12 and tumour necrosis factor (TNF)-alpha, can stimulate murine macrophages and peripheral blood mononuclear cells isolated from patients with active TB disease. Stimulation with rRv2626c also increases the expression of costimulatory molecules like B7-1, B7-2 and CD40 on murine macrophages. Furthermore, the production of NO and pro-inflammatory cytokines in response to rRv2626c is mediated by the transcription factor NF-KB, which is confirmed by using pyrrolidine dithiocarbamate, a specific pharmacological inhibitor of NF-KB. Rv2626c therefore seems to regulate macrophage effector functions by eliciting immune responses[8].

IL-32 is a cytokine stimulated by *M. tuberculosis* in a big variety of cell types such as human monocytes and macrophages. Bai X *et al* examined the biological significance of IL-32 in an *in vitro M. tuberculosis* infected macrophage model. It is shown that, in THP-1 cells infected with *M. tuberculosis* and stimulated with rIL-32, a higher level of apoptosis was seen compared with that with *M. tuberculosis* infection alone. On the other hand, significant abrogation of apoptosis induced by *M. tuberculosis* and a concomitant decrease in caspase-3 activation was observed in cells depleted of endogenous IL-32. The rIL-32gamma decreased the number of viable *M. tuberculosis* bacteria, which was abolished with a caspase-3 inhibitor. They came to a conclusion that IL-32 plays a host defense role against *M. tuberculosis* in a macrophage model[14].

The glutathione-redox balance, which is expressed as the ratio of intracellular reduced glutathione and oxidized glutathione, plays an essential role to regulate cellular immune responses. Alam *et al* demonstrated in their study that modification of glutathione-redox balance in macrophages can differentially regulate the production of IL-12 cytokine. It is indicated that redox balance of glutathione is an important factor that adjusts IL-12 induction in native macrophages, while N-acetyl-L-cysteine can tailor macrophages to stimulate enhanced Th1 response which may be useful to control tuberculosis and other pathophysiological disorders[11].

Toll-like receptors (TLRs) are important components in the regulation of pulmonary immune responses and the recognition of respiratory pathogens like *M. Tuberculosis*. Juarez *et al* examined human alveolar macrophages to define the expression profiles of TLR2, TLR4 and TLR9. They came to a conclusion that the TLR expression profile of autologous human alveolar macrophages and monocytes is not identical, which may contribute to compartmentalized immune responses. The dissimilarities may have some important implications for the evaluation of vaccines with TLR-stimulating adjuvants for the respiratory tract[12].

3. Conclusion

In regard to cell sources of macrophage models, murine bone marrows, human peripheral blood, human lungs, and cell lines are the major resources of macrophages for tuberculosis studies.

The screened antituberculosis drugs include streptomycin, ethambutol, pyrazinamide, isoniazid, ceforanide, ciprofloxacin, ofloxacin, rifampin, pyrazinamide, rifabutin, clarithromycin, ethambutol, ofloxacin, rifapentine, KRM-1648, thiolactomycin, C-8 methoxyl group, C-8-H fluoroquinolone, isoxyl, compound 1a of piperazinyl quinolones, pittosporum brevicalyx (oliv.) gagnep, cobalt (II) and copper (II) complexes of fluorinated isonicotinoylhydrazones, hydrazides, isonicotinoylhydrazones, micromolide, 6PP, 8PP, 4,4'-dihydroxybenzophenone, morphine, ranunculus ternatus thunb, and Xinlaoning capsule.

As to the pathogenesis of tuberculosis, it is indicated from the results of studies with macrophage models that, *M. tuberculosis* H(37)Rv has evolved some effective survival strategies, including inhibition of phagosome-lysosome fusion and phagosome acidification, recruitment and retention of tryptophan-aspartate containing coat protein on phagosomes, and expression of the host-induced repetitive glycine-rich proteins. The roles of SigB and SigF in sigma factor regulation in *M. tuberculosis* were studied, as a result, it was shown that baseline sigB and sigF expressions in the KI strains were higher than those in the control strains because of the increased gene dosage and leakiness of the acetamide promoter system. Phosphoenolpyruvate carboxykinase (PEPCK) is required for growth of *M. tuberculosis* in isolated bone marrow-derived murine macrophages and in mice. *M. tuberculosis* relies on gluconeogenesis throughout the infection. Genes were expressed at a much lower level in MAP-infected macrophages than in MAA-infected macrophages. Among these were the genes for IL-1beta, IL-1alpha, CXCL2, PTGS2 (COX2), lipocalin (LCN2) and TNF, which are essential pro-inflammatory factors. Infection with MAH also indicated strong induction of IL-1beta, CXCL2, COX2, LCN2 and TNF. It is evident that the functional serine/threonine kinase is crucial for *M. tuberculosis* to survive by inhibiting apoptosis of macrophages. In addition, it is revealed that a short exposure of *M. avium* to peak concentrations of drugs in serum may result in long suppression of bacterial growth in macrophages.

The results about immuno-mechanism of tuberculosis from studies with macrophage models include: (a)the pknE mutant which modifies the innate immune response as shown by the marked decline in the pro-inflammatory cytokines in a macrophage model of infection, (b)the T(H)1 cell surface molecule Tim3 which inhibits growth of intracellular pathogens and expansion of effector T(H)1 cells to stop further tissue inflammation, (c) Rv2626c which modulates macrophage effector functions by stimulating immune responses, (d) IL-32 playing a host defense role against *M. tuberculosis*, (e) redox balance of glutathione playing an essential role in regulating IL-12 induction in macrophages, (f) N-acetyl-L-cysteine tailoring macrophages to stimulating enhanced Th1 response helpful to control tuberculosis, (g) TLR expression of alveolar macrophages and monocytes, (h) PPE18 protein triggering an anti-inflammatory response by inducing IL-10 production, and (i) sst1 controlling necrosis within tuberculosis lesions in the lungs.

What could we do for the next step? Here are some questions without exact answers. For instance, what is the mechanisms by which *M. tuberculosis* H(37)Rv enters the host cell, circumvents host defenses and spreads to neighboring cell? Given the propensity of DHBP for nonspecific interactions with proteins, further studies are required to figure out a mechanism of DHBP action and to distinguish between the bacteriostatic and bactericidal nature of the drug.

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