Experimental *Mycobacterium tuberculosis* infection in the Chinese tree shrew

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**Abstract**

In recent years, the Chinese tree shrew has been considered to be a promising experimental animal for numerous diseases. Yet the susceptibility of *Mycobacterium tuberculosis* (MTB) in Chinese tree shrew is still unknown. We infected Chinese tree shrews with a high dose (2.5 × 10⁶ CFU) or a low dose (2.5 × 10³ CFU) of the H37Rv strain via the femoral vein to cause severe or mild disease. Disease severity was determined by clinical signs, pathologic changes and bacteria distribution in organs. Furthermore, among lung samples of the uninfected, mildly and seriously ill Chinese tree shrews, differentially expressed protein profiles were analyzed through iTRAQ and validated by qPCR. Tuberculous nodules, skin ulceration, pleural effusion and cerebellum necrosis could be observed in seriously ill animals. Regulation of the actin cytoskeleton was newly defined as a possible MTB-related pathway correlated with disease progression. This comprehensive analysis of the experimental infection and the depiction of the proteomics profiles in the Chinese tree shrew provide a foundation for the establishment of a new animal model of tuberculosis and provide a better understanding of the mechanism of tuberculosis.

**Introduction**

Chinese tree shrews are mainly distributed across southwest China (including the Yunnan, Guizhou, Sichuan, Guangxi and Hainan Provinces) and have been classified as *Tupaia belangeri*. Phylogenetic analysis of the Chinese tree shrew supports its close affinity to primates (Nie et al., 2008; Fan et al., 2013), and it is more similar to humans than rodent species such as rats, mice and marmots in terms of its genomics (Fan et al., 2013). Moreover, Chinese tree shrews have other advantages, such as ease of availability, good cost effectiveness and easy maintenance. Thus, the Chinese tree shrew may be a promising animal for developing disease models.

Various significant findings have been obtained in Chinese tree shrew models of reproductive, immunologic and social-psychological diseases (Yu et al., 2004; Keuker et al., 2005; Wang et al., 2011). This animal is also used for research on diseases caused by viruses, such as hepatitis A/ B/C/D (Guitart et al., 2005; Zhao et al., 2005; Amako et al., 2010), HIV and influenza (Zi-Feng Yang et al., 2013), as well as diseases caused by bacteria, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Li et al., 2012). However, it is unknown whether *Mycobacterium tuberculosis* (MTB) could infect Chinese tree shrews and cause disease.

In this study, we infected Chinese tree shrews with high and low doses of the H37Rv tuberculosis strain and investigated the clinical parameters (temperature and body weight), laboratory parameters [erythrocyte sedimentation rate (ESR)], pathologic changes and bacteria loads in the tissues or organs. Chinese tree shrews with serious and mild disease, latent infection, skin ulcerations, pleural effusion and tuberculous encephalitis were observed. Differentially expressed proteins (DEPs) were analyzed using iTRAQ among the lung samples obtained from normal, mildly and seriously ill tree shrews and were further validated by qPCR. MTB-related DEPs and signaling pathways were analyzed to elucidate the possible mechanism of MTB infection in Chinese tree shrews.
Materials and methods

Ethics statement
The animals were housed and maintained in an Animal Biosafety Level 3 (ABSL-3) facility. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Institute of Laboratory Animal Science, Peking Union Medical College ( Permit Number: ILAC-PC-2012-003). All surgeries were performed under sodium pentobarbital anesthesia, and efforts were made to minimize animal suffering.

Chinese tree shrew
Twenty-four clean outbred Chinese tree shrews were purchased from Kun-Ming Institute of Zoology, Chinese Academy of Sciences. All the animals were 1-year-old adult males with weights ranging from 130 to 140 g, housed in a 25 × 30 × 40 cm³ stainless steel cage with a 15 × 15 × 20 cm³ PE plastic sleeping box, fed commercial Chinese tree shrew chow (purchased from Vital River Laboratory Animal Technology Co. Ltd, China) and water ad libitum. Animals were located in the ABSL-3 facility for 2 weeks before they were challenged with the MTB H37Rv strain to adapt to the environment.

Strain
The MTB H37Rv strain [CMCC(B)95052] was obtained from the National Center for Medical Culture Collections (CMCC, Beijing, China). The H37Rv strain was passaged in mice and harvested and then subpackaged and stored at −80 °C. An aliquot of bacilli was plated on L–J plates for CFU enumeration with a concentration of 1 × 10⁷ CFU (colony-forming units) mL⁻¹.

Infection of the Chinese tree shrew with MTB
Aliquots of frozen MTB strains were thawed and then kept undiluted or were diluted 1000-fold in 0.9% NaCl to obtain high and low doses. The Chinese tree shrews were divided randomly into three groups: the high infectious dose group (n = 9, H1-9), the low infectious dose group (n = 9, L1-9), and the blank control (n = 6, C1-6). After being fixed in the triangle bag, the animals were injected through the inguinal vein with 2.5 × 10⁶ (high dose) or 2.5 × 10⁵ CFU MTB (low dose) in 0.25 mL 0.9% NaCl. The concentrations of the remaining bacteria were confirmed by bacteria culture on L–J medium. Control animals were injected with 0.25 mL of 0.9% NaCl. All procedures were performed in a biosafety cabinet in an ABSL-3 laboratory.

Clinical sign observation
The activity and mental state of the Chinese tree shrews were observed daily. Body weight and temperature (taken by infrared thermometer) were monitored once a week. At necropsy at the 5th, 8th and 11th week, 1.0 mL of blood was collected in an EDTA anticoagulation tube for routine testing. Another 1.6 mL of blood was placed into a blood sedimentation tube, and the ESR was detected after 1 h.

Pathologic evaluation
At each time point post-infection, three animals for each group were euthanized randomly; for the high- and low-dosage groups, the 5th, 8th and 11th week were included, but for the control group, the 8th week was not included. At necropsy, gross lesions in the tissues and organs were observed by a veterinary pathologist. Organs, including heart, liver, spleen, lung, kidney, cerebri pavementum, testis, epididymis, colon, ileum, jejunum, stomach, adrenal gland, salivary glands, bladder, and pancreas, were removed from the animals. Each organ was divided into three parts: one for pathologic analysis, one for bacterial distribution analysis, and one for proteomic or mRNA analysis. The sample for pathologic analysis was placed in 10% normal buffered formalin and then paraffin-embedded. The sections were cut and stained with hematoxylin and eosin or subjected to acid-fast staining. Histologic changes were reviewed by a veterinary pathologist.

Bacteria culture in organs and tissues
Organs and tissues were washed in 4% H₂SO₄ and 0.9% NaCl and then homogenized in glass pestle and mortal with 1 mL of 0.9% NaCl. Pleural effusion was also collected for the bacterium culture. The homogeneous sample fluids and pleural effusions were plated into L–J medium tubes and incubated at 37 °C and 5% CO₂, and the bacteria culture in the tubes was observed after 4 weeks.(Yuan et al., 2012).

Proteomic analysis
Lung tissues were obtained from the following three groups: Chinese tree shrews with serious lesions (TH-A group), Chinese tree shrews with mild lesions (TH-B), and blank controls (TH-C). The tissues were loaded into Eppendorf tubes and boiled in water for 5 min to inactivate the MTB. The inactivated samples were then sent to BGI (the Beijing Genomic Institute) at 4 °C for iTRAQ proteomic analysis (see Supporting Information, Data S1).
**Database search and bioinformatics**

The resulting MS/MS spectra were searched against the International Protein Index Chinese tree shrew sequence databases (50,461 sequences) using MASCOT software version 2.2 (Matrix Science, London, UK). The logical algorithm for set operations was applied to further screen for DEPs. Based on the KEGG database, the DEPs were mapped in the MTB-related pathways, and homologous proteins were identified (for details see Table S1).

**Validation by quantitative reverse transcriptase-PCR (qRT-PCR)**

Total RNA was extracted with Trizol reagent from the lung tissue. Some DEPs (fold change ≥ 1.5) were selected for validation through quantitative reverse transcriptase-PCR at the mRNA level. β-actin was used as the internal control gene. qRT-PCR was performed using Power SYBR Green PCR Master Mix (ABI) according to the protocol described previously (Zhan et al., 2012), and the data were calculated using the relative ΔΔC\textsubscript{T} method. The primer pairs are listed in Table 1.

**Results**

**Clinical signs**

After infection, all of the animals were euthanized at the designated time points. The animals in the high-dosage groups appeared to exhibit clinical changes, whereas few clinical changes were observed in the low-dosage group. For the high-dosage group, at the 5th week, H4-5 lost 40–50 g of weight, was diagnosed as dying and was euthanized. At the 8th week, H5-8 was moribund and exhibited less activity and was in a depressed mental state. For all of the animals in the high- and low-dosage groups, body temperature fluctuated by < 0.5 °C; the ESRs were < 5 mm, as were the blank controls; routine blood examination indicated that the total number of leukocytes and the ratio of lymphocytes increased during infection (Table 2).

**Pathological changes**

Among the nine Chinese tree shrews in the high infectious dose group, four animals, H4-5, H5-8, H3-8 and H7-11, developed serious disease, and the other three animals (H1-5, H2-5, H6-8) with mild disease. H8-11, H9-11 and L1-L9 exhibited an inflammatory response (Table 2). Thus, the outcomes were partially determined by the infectious dosage.

Of the four animals with serious disease, H4-5 was dying at the 5th week and was promptly autopsied. Numerous nodules with diameters from 1 to 5 mm were observed in the intercostal space near the spinal column, the posterior peritoneum and the mediastinum, and gray lesions were observed in the kidneys (Fig. 1). Histopathology analysis showed that most areas of lung and spleen had tuberculosis granulomas, with caseous necrosis in the center and a large number of inflammatory cells outside the center (Fig. 2). Focal necrosis with inflammatory cells was distributed in the adrenal cortex and adrenal capsula, and there was focal inflammatory cell infiltration in the liver and kidney. Large necrotic areas were observed in the muscles, which were infiltrated with inflammatory cells. The tuberculosis-related lesions in the above organs were confirmed to contain MTB, as confirmed using MTB acid-fast staining (Fig. 3, Fig. S1).

**Table 1. Primer pairs for quantitative PCR**

<table>
<thead>
<tr>
<th>gene ID (Chinese tree shrew)</th>
<th>Protein name (human)</th>
<th>Primer pair sequences (F/R 5\textsuperscript{0}–3\textsuperscript{0})</th>
</tr>
</thead>
<tbody>
<tr>
<td>gi</td>
<td>379803280</td>
<td>gb</td>
</tr>
<tr>
<td>gi</td>
<td>379806493</td>
<td>gb</td>
</tr>
<tr>
<td>gi</td>
<td>379820736</td>
<td>gb</td>
</tr>
<tr>
<td>gi</td>
<td>379790773</td>
<td>gb</td>
</tr>
<tr>
<td>gi</td>
<td>379801388</td>
<td>gb</td>
</tr>
<tr>
<td>gi</td>
<td>379801739</td>
<td>gb</td>
</tr>
<tr>
<td>gi</td>
<td>379798528</td>
<td>gb</td>
</tr>
<tr>
<td>gi</td>
<td>379782627</td>
<td>gb</td>
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<tr>
<td>gi</td>
<td>379813433</td>
<td>gb</td>
</tr>
<tr>
<td>gi</td>
<td>379799947</td>
<td>gb</td>
</tr>
<tr>
<td>AF110103.1</td>
<td>beta-actin</td>
<td>GCCACTCCTCCAGCAGATCTGATGAG/GGCGCGAGGCGAGGCTTCA</td>
</tr>
</tbody>
</table>

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For H3-8 and H7-11, pleural effusion was observed in the gross pathology and was confirmed to contain MTB by bacterial culture. Tuberculosis nodules (diameter 1–5 mm) were also observed in all of the lung lobes; the number of nodules in the left lung lobes was greater than that in the right lung lobes. Through histology, a large area of consolidation and necrosis was found in the left lung, and inflammatory cells were found to have infiltrated these areas (Figs 2 and 3).

Large skin ulcerations were observed around the inoculated site of H1-5 and H2-5 at the 5th week, H6-8 at the 8th week and H7-11 at the 11th week. MTB was confirmed around the ulceration area by acid-fast staining (Figs 1 and 3).

The animals H8-11, H9-11 and L1-L9 developed mild diseases, without any obvious abnormalities in clinical signs and gross pathology. However, inflammatory cell infiltrations were observed in the kidney and in the portal area of the liver, and the alveolar septa was thickened (Fig. 2, Fig. S2). Although no tuberculosis-specific lesion was found, the pathologic changes were confirmed by acid-fast staining to be the results of MTB infection (Fig. S1).

### Table 2. Clinical signs of MTB-infected Chinese tree shrews

<table>
<thead>
<tr>
<th>Groups</th>
<th>High-dose group (n = 9)</th>
<th>Low-dose group (n = 9)</th>
<th>Control group (n = 6)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinic signs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ESR</strong></td>
<td>≤5 mm h⁻¹</td>
<td>≤5 mm h⁻¹</td>
<td>≤5 mm h⁻¹</td>
</tr>
<tr>
<td><strong>Blood routine examination¹</strong></td>
<td>Leukocyte number</td>
<td>Leukocyte number</td>
<td>Leukocyte number</td>
</tr>
<tr>
<td></td>
<td>9.43 ± 4.38</td>
<td>10.35 ± 4.18</td>
<td>3.17 ± 1.18</td>
</tr>
<tr>
<td><strong>Temperature fluctuation</strong></td>
<td>9.5 ± 0.5 °C</td>
<td>10.5 ± 0.5 °C</td>
<td>17.4 ± 3.4 °C</td>
</tr>
<tr>
<td><strong>Body weight change</strong></td>
<td>H4-5 and H5-8 decreased 40–50 g; Seven animals with no obvious decrease</td>
<td>No obvious change</td>
<td>With a 5–10 g increase</td>
</tr>
<tr>
<td><strong>Mental state and activity</strong></td>
<td>At the eighth week, H5-8 was depressed, with less activity</td>
<td>Without evident abnormality</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Death</strong></td>
<td>At the fifth week, H4-5 was dying; at the eighth week, H5-8 was moribund</td>
<td>All alive</td>
<td>All alive</td>
</tr>
<tr>
<td><strong>Gross pathology</strong></td>
<td>H4-5, H5-8, H3-8 and H7-11 with tuberculosis nodules in lung, kidney, intercostal space near spinal column, and posterior peritoneum; H1-5, H2-5, H6-8 and H7-11 developed skin ulceration around the inoculation site; H3-8 and H7-11 with pleural effusion (positive for MTB); necrosis in cerebellum was observed in H5-8</td>
<td>No evident change</td>
<td>No evident change</td>
</tr>
<tr>
<td><strong>Histopathology</strong></td>
<td>TB granuloma with caseous necrosis, inflammatory cells were infiltrated outside lung and spleen; large area of consolidation in the lung, inflammatory cell infiltration in the liver and kidney; necrosis in muscle, dermis, and subcutaneous tissue, epencephalon</td>
<td>No TB granulomas, but numerous inflammatory cell infiltrations were observed in liver and kidney. Alveolar septum was thickened</td>
<td>No evident change</td>
</tr>
</tbody>
</table>

*For the control group, animals were dissected at the fifth week and the eleventh week, three for each time point.

¹Leukocyte number: 10⁶/μL, and Ratios of lymphocyte (%).
Bacteria distribution in tissues

Tissue samples were collected separately from the Chinese tree shrews with serious and mild diseases. The samples were ground and inoculated into L–J medium tubes. For the high-dose animals, the lung, spleen, kidney, liver, salivary gland, adrenal gland, testis, ileum and cerebri pavimentum were positive for MTB culture, but the heart, epididymis, colon, jejunum, stomach, bladder and pancreas were negative. For the animals in the low-dose group, the animals exhibited no evident lesions, but the same organs as those of high-dose group were also positive for MTB (Table 3).

DEPs in Chinese tree shrews with or without previous association with tuberculosis

Based on the KEGG database, the DEPs were mapped in the MTB-related pathways, and homologous proteins were identified (Berry et al., 2010; Pan et al., 2012). Some homologous proteins of DEPs had not been reported to be related to MTB infection.

The threshold values for up- and down-regulated proteins in this study, which were considered strict criteria for judging the DEPs, were $\geq 1.5$ or $\leq 0.667$ (≥ 1.5-fold) with $P$ values of $< 0.05$. A total of 134, 144, and 9 up-regulated proteins were identified in the TH-A/TH-C, TH-A/TH-B, and TH-B/TH-C groups, respectively. The corresponding numbers of identified down-regulated proteins were 53, 76, and 20 (Tables S1–S3).

Regulation of actin cytoskeleton might be an MTB-related pathway

Among the 1697 detected proteins, 1450 were mapped onto 243 pathways in the KEGG database (Fan et al., 2013). To elucidate possible tuberculosis mechanisms, MTB-related pathways were summarized based on the
tuberculosis pathway (map05152) (Fig. 4) (Shui et al., 2009).

Regulation of the actin cytoskeleton, phagosome, calcium signaling pathway, Jak-STAT signaling pathway, and antigen processing and presentation pathway were involved in the Chinese tree shrew. Among these, regulation of the actin cytoskeleton pathway was newly defined as a possible MTB-related pathway. DEPs in the regulation of the actin cytoskeleton included PFN, MLC, GSN, Arp2/3, Actin, Vav, VASP, LOM7, ZO-1 and Ponsin.

Validated altered proteins at the transcriptional level

The randomly selected DEPs used for qPCR validation included STAT-1, CD45, ITGB2, Cathepsin D, SHP-1, GSN, Coronin1, ASC, V-ATPase, Rab7, HSP90 and CANX. The primers for the DEPs were designed according to the nucleotide sequences in the Chinese tree shrew database (Table 1).

STAT1 was up-regulated in our study, a characteristic that has been previously confirmed in tuberculosis; there-
Fig. 3. Acid-fast staining of lung and skin in MTB-infected Chinese tree shrews. Serial sections of lung at 5th week were analyzed by H&E and acid-fast staining. The first line of panels (from top to bottom) is the lung of a high-infectious-dosage animal. (a) H&E staining, 100×. (b,c) Acid-fast staining; 100× (b), 1000× (c). The second line of panels is the lung of a low-infectious-dosage animal. (d) H&E staining, 100×. (e,f) Acid-fast staining; 100× (e), 1000× (f). The third line of panels is the lung of a normal animal. (g) H&E staining, 100×. (h,i) Acid-fast staining; 100× (h), 1000× (i). The fourth line of panels is the skin ulcer at 5th week. (j) H&E staining, 100×. (k,l,m) Acid-fast staining; 100× (k), 1000× (l,m). MTB is indicated with an arrow.
fore, we used this as a reference in qPCR validation. The randomly selected candidate proteins SHP-1, Coronin 1, ASC, CD45, CTSD, GSN, ITGB2 and V-ATPase were demonstrated to be up-regulated by qRT-PCR at the mRNA level, which agreed with the iTRAQ results. However, the qPCR results for HSP90 and CANX were not consistent with iTRAQ. So, except for HSP90 and Calnexin, the DEPs are regarded as statistically significant DEPs (Student’s t-test, P < 0.05). These results should be validated on the protein level by Western blot analysis once antibodies have been developed against them (Fig. 5).

Discussion

During infection, some Chinese tree shrews exhibited evident tuberculosis-related clinical signs and pathologic characteristics similar to those of human tuberculosis. Most organs were positive for MTB culture. These findings indicated that Chinese tree shrews are susceptible to MTB.

After infection, animals with serious diseases partially exhibited the same clinical signs of active tuberculosis as those observed in humans. Some animals with mild disease had no evident abnormalities, which is similar to sub-clinical or latent MTB infection in humans (Table 2).

The pathologic changes in MTB-infected Chinese tree shrews exhibited unique characteristics. Tuberculosis nodules were found in multiple organs and tissues of Chinese tree shrews, and the tuberculosis granulomas were characterized by caseous necrosis in the center and by lymphocytes surrounding the necrosis, but lacked Langhans giant cells and fibrocytes in the rim. The pathologic features were more typical of tuberculosis in Chinese tree shrew than those of the tuberculosis mouse model, in which no macroscopic tuberculosis nodules are found except in the lung, and the tuberculosis granulomas in most strains of

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Table 3. Distribution of MTB in the infected Chinese tree shrew

<table>
<thead>
<tr>
<th>Organ</th>
<th>Heart</th>
<th>Liver</th>
<th>Spleen</th>
<th>Lung</th>
<th>Kidney</th>
<th>Cerebri pavimentum</th>
<th>Testis</th>
<th>Epididymis</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria culture</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Organ</td>
<td>Colon</td>
<td>Ileum</td>
<td>Jejunum</td>
<td>Stomach</td>
<td>Adrenal gland</td>
<td>Salivary glands</td>
<td>Bladder</td>
<td>Pleural effusion</td>
<td></td>
</tr>
<tr>
<td>Bacteria culture</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

‘+’ indicates a positive result, ‘−’ indicates a negative result.

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Fig. 4. Signaling pathways correlated with MTB infection in the Chinese tree shrew. The green square represents the DEPs shared by TH-A/TH-C and TH-A/TH-B that have been previously correlated with MTB infection; the red squares are the DEPs shared by TH-A/TH-C and TH-A/TH-B that are not correlated with MTB infection; the square with words in green represents the DEPs in TH-A/TH-C or TH-A/TH-B that have been correlated with MTB infection. The square with words in red represents the DEPs in TH-A/TH-C or TH-A/TH-B that are not correlated with MTB infection.

Fig. 5. Western blot analysis of the DEPs in TH-A/TH-C and TH-A/TH-B.
mice do not exhibit caseous necrosis, except for the C3HeB/FeJ mouse strain, in which the Langhans giant cell and the fibrocytes in the rim are also seldom observed (Vilaplana et al., 2013). Notably, evidence of tuberculosis-related pleural effusion, skin ulcerations and necrosis in the cerebellum was found by acid-fast staining in our Chinese tree shrews. These lesions are seldom observed in monkeys and mice (Tsenova et al., 2006; Sun et al., 2012). These rare lesions indicated that Chinese tree shrew had some specific characteristics, and might be applied in future research in the above areas (Figs 1 and 2).

Although the outcome of the high-infectious-dosage group was evidently different from that of the low-infectious-dosage group, the bacteria distribution in organs/tissues did not exhibit significant differences between the two groups from the 5th to the 11th week (Table 3).

The DEPs with previous associations might indicate the agreement of this infection with other tuberculosis models (Converse et al., 1996; Taborda & Casadevall, 2002; Bulut, 2005; Evans et al., 2008; Capparelli et al., 2009; Seto et al., 2009; Constantoulakis et al., 2010; Rhee & Veillette, 2012), and DEPs without previous associations might imply unique characteristics of this species. Signaling pathway analysis based on DEPs could help to interpret the mechanism of tuberculosis infection. Among the involved pathways in Fig. 4, the Jak-STAT phagocytosis signaling pathway was activated in the Chinese tree shrew, which has been confirmed in tuberculosis patients and other tuberculosis animals models (Rojasa et al., 2002). The regulation of the actin cytoskeleton might play a vital role in MTB infection and has not been investigated in tuberculosis previously. Thus, this would be a new direction for tuberculosis mechanism research. Since the protein-related reagents for tree shrew are being developed, the regulation of the actin cytoskeleton signaling pathway could be demonstrated in other species.

However, some confirmed pathways of innate immunity in tuberculosis, such as the Toll-like receptor signaling pathway, the NOD-like receptor signaling pathway and the Mincle/Dectin-1/DC-SIGN-mediated necrosis factor (NF)-κB signaling pathway (Fig. 4), were not indicated in our data from Chinese tree shrew MTB infection.

In summary, Chinese tree shrews were susceptible to MTB infection, and the infection has its own characteristics. Tuberculosis in Chinese tree shrew could easily cause lesions in lung, spleen and other organs, with typical caseous necrosis in granulomas (Vilaplana et al., 2013). It could also cause some rare tuberculosis lesions such as skin ulceration, pleural effusion and cerebellum necrosis. Shrews could exhibit serious or mild disease, which makes them suitable for active or latent tuberculosis research, especially for the study of tuberculosis pathogenesis, pathology, immunology and therapy.

Acknowledgements

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Authors’ contribution

L.Z. and H.D. contributed equally to this work.

References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. MTB stained in tissues of high-dose animals.

Fig. S2. Histologic changes and MTB stained in organs/tissues of lowdose animals.

Table S1.1. Altered proteins in TH-A/TH-C and TH-A/TH-B comparison.

Table S1.2. Altered proteins in TH-A/TH-C and TH-A/TH-B comparison.

Table S2.1. Altered proteins in TH-A/TH-C comparison.

Table S2.2. Altered proteins in TH-A/TH-C comparison.

Table S3.1. Altered proteins in TH-A/TH-B comparison.

Table S3.2. Altered proteins in TH-A/TH-B comparison.

Data S1. Proteomic analysis.