SCIENCE CHINA
Life Sciences

• RESEARCH PAPER •

September 2012 Vol.55 No.9: 800–806
doi: 10.1007/s11427-012-4368-x

Immunotherapy using IL-2 and GM-CSF is a potential treatment for multidrug-resistant *Mycobacterium tuberculosis*

ZHANG YongRong[1], LIU Jian[1], WANG Yong[2], XIAN QiaoYang[2], SHAO LingYun[3], YANG Zhong[4*] & WANG XiaoNing[1,4*]

[1]*State Key Laboratory of Bioreactor Engineering, School of Bioengineering, East China University of Science and Technology, Shanghai 200237, China;*
[2]*Center for Animal Experiment & ABSL-III Lab, Wuhan University, Wuhan 430071, China;*
[3]*Department of Infectious Diseases, Huashan Hospital, Fudan University, Shanghai 200040, China;*
[4]*Department of Microbiology and Microbial Engineering, School of Life Sciences, Fudan University, Shanghai 200433, China*

Received June 27, 2012; accepted August 20, 2012

This study investigated the therapeutic effects of interleukin (IL)-2 and granulocyte-macrophage colony-stimulating factor (GM-CSF) co-administrated with antibacterial agents isoniazid (INH) and rifampin (RIF) to treat a mouse model of tuberculosis (TB) infection. A drug-susceptible TB strain, H37Rv was used to infect mice and the effectiveness of IL-2 and GM-CSF was initially evaluated based on survival rate, bacterial counts in lungs and spleens and the pathological condition of the lungs. Next, the therapeutic effect of the immunotherapy regimen was assessed in multidrug-resistant strain OB35-infected mice. In the H37Rv infection model, IL-2 and GM-CSF monotherapies reduced bacterial numbers in the lungs by 0.82 (P<0.01) and 0.58 (P<0.05) lg colony-forming units (CFU), respectively, and in the spleens by 1.42 (P<0.01) and 1.22 (P<0.01) lg CFU, respectively, compared with the untreated group. Mice receiving immunotherapy developed fewer lesions in the lungs compared with mice receiving antibacterial therapy alone. In the OB35 infection model, immunotherapy with either cytokine resulted in a significant reduction of bacterial load in the lungs and spleens and less severe lesions in the lungs compared with the untreated or antibacterial therapy treated mice. Notably, mice receiving immunotherapy with both cytokines had a 30% survival rate which was higher than that in other treated groups, and had significantly less CFUs in the lungs and spleens (1.02 and 1.34 lg CFU) compared with antibacterial therapy alone (P<0.01). This study demonstrated that immunotherapy with both IL-2 and GM-CSF may be useful to treat multidrug resistant tuberculosis (MDR-TB).

tuberculosis, MDR-TB, IL-2, GM-CSF, immunotherapy

Citation: Zhang Y R, Liu J, Wang Y, *et al*. Immunotherapy using IL-2 and GM-CSF is a potential treatment for multidrug-resistant *Mycobacterium tuberculosis*. Sci China Life Sci, 2012, 55: 800−806, doi: 10.1007/s11427-012-4368-x

Tuberculosis (TB) remains a major public health problem worldwide [1,2]. Directly Observed Therapy–Short Course (DOTS) is the main TB control strategy recommended by the World Health Organization and has been used for many years. DOTS, when used properly, can treat ⩾95% of drug-susceptible TB cases. However, the prevalence of multidrug resistant (MDR) and extensively drug-resistant (XDR)-TB and *Mycobacterium tuberculosis*/HIV co-infection make it more difficult to control TB using the DOTS strategy alone. Moreover, drug-drug interactions cause significant problems in *M. tuberculosis*/HIV co-infected patients. Despite the use of second-line drugs, the side effects and cost mean the rational design of new treatment regimens is required to shorten the therapeutic period, and provide a more effica-

*Corresponding author (email: xnwang88@163.com; zhongyang_fudan@163.com)

cious treatment for MDR and XDR-TB, and TB in HIV-positive individuals [3].

Novel adjunctive immunotherapy using immunomodulators can control infectious diseases [4]. Enhancing the immune response of the host may protect the body against invasive bacteria and help to reduce drug resistance. *M. tuberculosis* is the causative agent of TB and preferentially infects macrophages as their host cells. The pathogenesis of tuberculosis involves a dynamic interaction between host and pathogen. Cytokines, immune cells and antibodies are important in sustaining a successful host defense against TB [5–7]. Numerous studies have demonstrated the treatment potential of cytokines in experimental and clinical studies. GM-CSF and IL-2 are immunomodulators that have been widely used for the treatment of other diseases. GM-CSF and IL-2 stimulate the immune system of the host by activating immune cells. GM-CSF induces the differentiation of circulating monocytes into certain types of macrophage *in vivo*. Activated macrophages act as effector cells in host defense by engulfing and killing *M. tuberculosis* and presenting bacterial antigens to T cells. Stimulation with GM-CSF *in vitro* increases phagocytosis by macrophages and inhibits mycobacterial growth in macrophages [8,9]. IL-2 is a Th1 cytokine and is essential for the development of cell-mediated immune responses to intracellular pathogens [10]. Homeostasis of the Th1/Th2 response is likely associated with the progression of disease. Often Th1 responses are not present in TB patients with advanced disease [11,12]. Clinical trials demonstrated that IL-2 production was reduced in MDR-TB patients [13]. Therefore, exogenous GM-CSF and IL-2 may provide a microenvironment that enhances the activation of effector cells and promotes Th1 response *in vivo*.

Previous studies showed that IL-2 or GM-CSF and their delivery by DNA vaccine could enhance the efficacy of treatments for *M. tuberculosis* infection [14–19]. However, most studies used a drug-susceptible TB strain with a single cytokine treatment. In this study, we used recombinant IL-2 and GM-CSF proteins directly as immunotherapeutic agents in combination with the first-line antibacterial drugs isoniazid (INH) and rifampin (RIF) to treat both drug-susceptible and multidrug-resistant TB. We assessed the efficacy of cytokine monotherapy and combination immunotherapy. We found either cytokine could enhance the therapeutic effects of INH and RIF in drug-susceptible *M. tuberculosis* infection and observed novel synergistic effects of IL-2 and GM-CSF in MDR-TB control.

1 Materials and methods

1.1 Antibacterial agents and cytokines

INH and RIF were purchased from Red Flag Pharmacy in Shenyang, China. Lyophilized recombinant IL-2 and GM-CSF were kindly provided by Amoytop Biotech, Xiamen, China. The cytokines were prepared in 0.9% sterile saline every two days and were stored at 4°C before use.

1.2 Mice

Specific pathogen-free female C57BL/6 mice aged 6–8 weeks purchased from Vital River Co., Ltd., Beijing, China were maintained under barrier conditions in the Animal Biosafety Level-III Laboratory (ABSL-III) of Wuhan University, China and were fed a sterile commercial mouse diet.

1.3 *M. tuberculosis* strains

M. tuberculosis laboratory strain H37Rv (ATCC 93009) and multi-drug resistant strain OB35 displaying resistance to both INH and RIF *in vitro* were provided by Wuhan University. The minimum inhibitory concentration of INH and RIF for the OB35 strain was 1 μg mL^{-1} and 50 μg mL^{-1}, respectively, as previously described [20]. Both bacterial strains were subcultured in Middlebrook 7H9-ADC (Becton Dickinson) medium for 10 d at 37°C. Bacteria were collected by centrifugation and were washed with PBS, resuspended in sterile saline, and stored at −80°C until use.

1.4 Infection of mice

The CFU count of the bacterial suspension was determined using Middlebrook 7H10-OADC (Becton Dickinson) agar plates before mouse inoculation. Mice were infected intravenously via tail vein injection with 0.2 mL of bacterial suspension containing approximately 2×10^6 CFU *M. tuberculosis* H37Rv or OB35 [21].

1.5 Treatment of drug-susceptible H37Rv and MDR-TB OB35 infection

Following infection with *M. tuberculosis* H37Rv, mice were divided randomly into 6 groups (Table 1). Each experimental group contained 5 mice. Five healthy mice were maintained in a non-infection room as healthy controls. On day 14 after infection, 5 mice from the untreated group were sacrificed to verify the pretreatment CFU counts in the lungs and spleens. Treatment was initiated on day 15 after infection and lasted 4 weeks. Each regimen was administered once a day 5 days per week. RIF was dissolved in sterile feeding water at a final concentration of 0.1 g L^{-1}. Each mouse drank approximately 2 mL of water per day. The other drugs were dissolved in sterile saline in a total volume of 100 μL and were administered via intramuscular injection. The doses of these drugs were as follows: INH 5 mg kg^{-1}, IL-2 5000 IU/mouse, and GM-CSF 5000 IU/mouse. In the untreated control group, 100 μL of sterile

Table 1 Experimental groups

H37Rv		OB35	
Treatment regimens	Number of mice	Treatment regimens	Number of mice
IL-2	5	HR[a)]	10
GM-CSF	5	HR+IL-2	10
HR[a)]	5	HR+GM-CSF	10
HR+IL-2	5	HR+IL-2+GM-CSF	10
HR+GM-CSF	5	Untreated	15
Untreated	10		

a) HR, isoniazid (INH) and rifampin (RIF).

saline was used as a placebo. Mortality and body weights were monitored throughout the experiment, and the spleen from each mouse was weighed after sacrifice. Surviving mice were sacrificed within 1 week after termination of the treatment. The *in vivo* study was repeated twice.

Following analysis of results from treatment of the H37Rv infection model, only regimens of INH-RIF (HR) in combination with IL-2 or GM-CSF or both cytokines were adopted in the OB35 infection model in addition to antibacterial therapy and saline (Table 1). All treatment parameters were the same as for treatment of the H37Rv infection.

1.6 Bacterial counts

Portions of the lung and the whole spleen from each mouse were removed during autopsy and were weighed before homogenization in sterile saline. The tissue suspension was serially diluted 10-fold, and 0.1 mL of each dilution was plated in triplicate onto Lowenstein-Jensen medium and incubated at 37°C for 4 weeks [22]. Bacterial colonies were enumerated, and the results were expressed as lg CFU per gram of the organs.

1.7 Histopathology

The remaining portions of the lungs were fixed in 10% neutral formaldehyde, and after dehydration, transparent, waxing, embedding, and slicing, the tissue sections were prepared. The sections were stained with hematoxylin and eosin and were analyzed by a certified pathologist [21,23]. Additionally, acid-fast staining was performed as previously described for the visual observation of mycobacteria in the lungs [24,25].

1.8 Statistical analysis

Data were expressed as the mean plus standard deviations. Statistical significance between each group was analyzed by Kaplan-Meier survival analysis with Log-rank test, ANOVA and *t*-test using Prism statistical software program. $P<0.05$ was considered to be statistically significant.

2 Results

2.1 Efficacy of treatment in the H37Rv infection model

2.1.1 Survival rate and mean spleen weight

In the saline control group, 1 mouse (1/5) died at week 6 post-infection, and the rest were severely sick. All the treated mice survived until the end of the experiment. As shown in Figure 1, after 4 weeks of treatment the mean spleen weight of mice receiving HR or HR plus a single cytokine combination regimen was significantly reduced compared with the untreated group. However, no difference in mean spleen weight was observed between mice treated with HR or immunotherapy regimens alone. For mice receiving either cytokine alone, spleen weights were the same as those of untreated mice.

2.1.2 Measurement of CFU in the lungs and spleens

The bacterial burden in the lungs and spleens from each group was determined after 4 weeks of incubation on culture plates. Overall, bacterial numbers in all HR treated groups, single cytokine treated groups and HR plus IL-2/GM-CSF treated groups were significantly reduced compared with the early or late saline treated control groups. HR significantly inhibited bacterial growth in lungs and spleens. Bacterial numbers in lungs and spleens were reduced by at least 1 lg CFU after antibacterial therapy or immunotherapy with HR plus IL-2 or GM-CSF (Table 2). When compared with the late untreated group, IL-2 and GM-CSF monotherapy regimens reduced the bacterial number in the lungs by 0.82 ($P<0.01$) and 0.58 ($P<0.05$) lg CFU, respectively, and in the spleens by 1.42 ($P<0.01$) and 1.22 ($P<0.01$) lg CFU, respectively (Table 2). Given that the two cytokines are not bactericidal, the reduction of the bacterial loads was likely due to the enhanced host protective immune response. However, reduced CFU in the immuno-

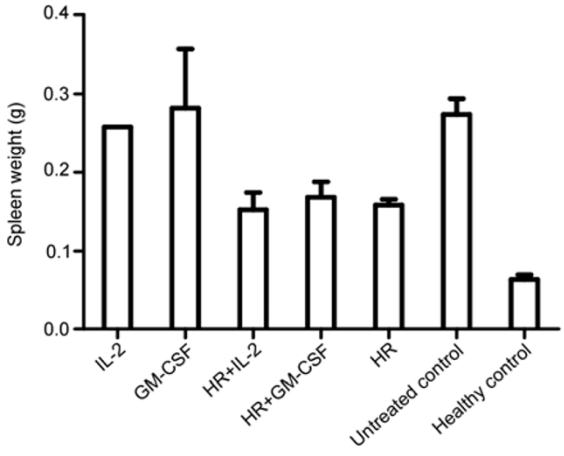

Figure 1 Mean±SD weight of spleens from mice infected with H37Rv *M. tuberculosis* on day 42 post-infection.

therapy groups were not statistically significant when compared with the antibacterial therapy group (P>0.05) (Table 2).

2.1.3 *Histopathological examination*

Lungs from the untreated control group exhibited severe lesions (Figure 2A). Alveolar structural damage and the infiltration of numerous mononuclear phagocytes were also observed in the untreated control group. Lungs from the IL-2 and GM-CSF monotherapy groups had fewer lesions, consistent with the CFU data (Figure 2B and C). Mice receiving HR+IL-2 or GM-CSF exhibited no apparent lesions (Figure 2D and E). Although differences in the CFU counts between the antibacterial therapy and cytokine-assisted immunotherapy groups were not significant, lungs from the cytokine-assisted immunotherapy groups displayed fewer lesions or were close to normal in appearance (Figure 2D–G). The histopathological results suggested that exogenous cytokine treatment could accelerate the recovery of hosts from TB.

Table 2 CFU counts in the lungs and spleens of mice infected with the H37Rv strain after 4 weeks of treatment[a)]

Treatment	Lg CFU/g organ (mean±SD)			
	Lung on day 14	Lung on day 43	Spleen on day 14	Spleen on day 43
Untreated	7.44±0.39	7.60±0.22	6.68±0.25	7.76±0.17
IL-2		6.78±0.05**		6.34±0.13**
GM-CSF		7.02±0.15*		6.54±0.13**
HR+IL-2		6.34±0.08**		5.75±0.12**
HR+GM-CSF		6.09±0.08**		5.90±0.06*
HR		6.18±0.18**		5.92±0.13**

a) Significant differences were observed between all treated groups and the untreated control group; *, P<0.05; **, P<0.01. No significant difference was observed between the HR+IL-2, HR+GM-CSF and HR groups. CFU, colony-forming units; HR, isoniazid (INH) and rifampin (RIF).

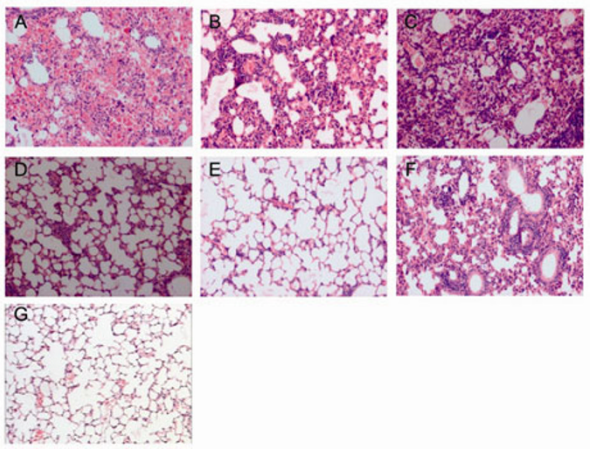

Figure 2 Histopathology of lung sections from mice infected with H37Rv *M. tuberculosis* on day 42 post-infection. A, Untreated control. B, IL-2. C, GM-CSF. D, HR+IL-2. E, HR+GM-CSF. F, HR. G, Healthy control (shown at 100× magnification).

2.2 Efficacy of treatment in the multidrug-resistant strain OB35 infection model

2.2.1 *Survival rate and mean body weights*

Eight untreated mice (8/10) rapidly died within 25 days post-infection. All untreated mice had died by the end of the experiment (Figure 3A). In contrast, up to 25 days post-infection, 3 mice (3/10) in each of the HR and HR+IL-2+GM-CSF groups and 6 mice (6/10) in the HR+IL-2 group died, whereas only 1 mouse (1/10) died in the HR+GM-CSF group (Figure 3A). However, most of the treated mice died three weeks after treatment. By the end of the treatment, 3 (3/10) mice in the HR+IL-2+GM-CSF group and 1 (1/10) mouse in each of the HR and HR+IL-2 groups survived (Figure 3A). Analysis of the survival curves by log-rank test demonstrated that HR+GM-CSF and HR+IL-2+GM-CSF survival was statistically significant from the untreated group (P<0.05). The results suggested that administration of antibacterial drugs combined with single cytokine regimens protected the host from early death, whereas a regimen containing drug treatment plus both cytokines reduced the mortality rate of the MDR-TB infection and prolonged survival.

The mean body weight of mice from each group was monitored as an indicator of disease progression. All infected mice exhibited similar weight loss 2 weeks after infection. After the second week of treatment, the weight of mice receiving HR or HR+IL-2 or GM-CSF treatment stabilized. In contrast, mice receiving HR+IL-2+GM-CSF exhibited significant weight gain at the same time point. All treated mice gained weight gradually during the entire course of treatment (Figure 3B). Although the mice receiving HR exhibited weight gain, the mean body weight curve of the HR group was below that of the others, which suggested that HR treatment alone was less effective for host recovery. Moreover, the mean body weight curve in the HR+IL-2+GM-CSF group was above that of the other treatment groups, which also suggested that the regimen of HR plus both cytokines was the most efficacious treatment in terms of weight gain.

2.2.2 *Measurement of CFU in the lungs and spleens*

After treatment, bacterial counts from the lungs and spleens of all 10 mice per group, including dead and surviving mice were measured. As shown in Table 3, all the immunotherapy regimens significantly inhibited the growth of multidrug-resistant *M. tuberculosis* in the lungs and spleens (P<0.01) compared with the early and late untreated groups. Although the bacterial loads in the lungs and spleens were reduced in the HR regimen compared with the late untreated group, HR did not appear to inhibit bacterial growth efficiently in the lungs when compared with the CFU of the early untreated group. Co-administration of a single cytokine and HR had greater efficacy than HR alone. The HR+IL-2 and HR+GM-CSF treatments resulted in a

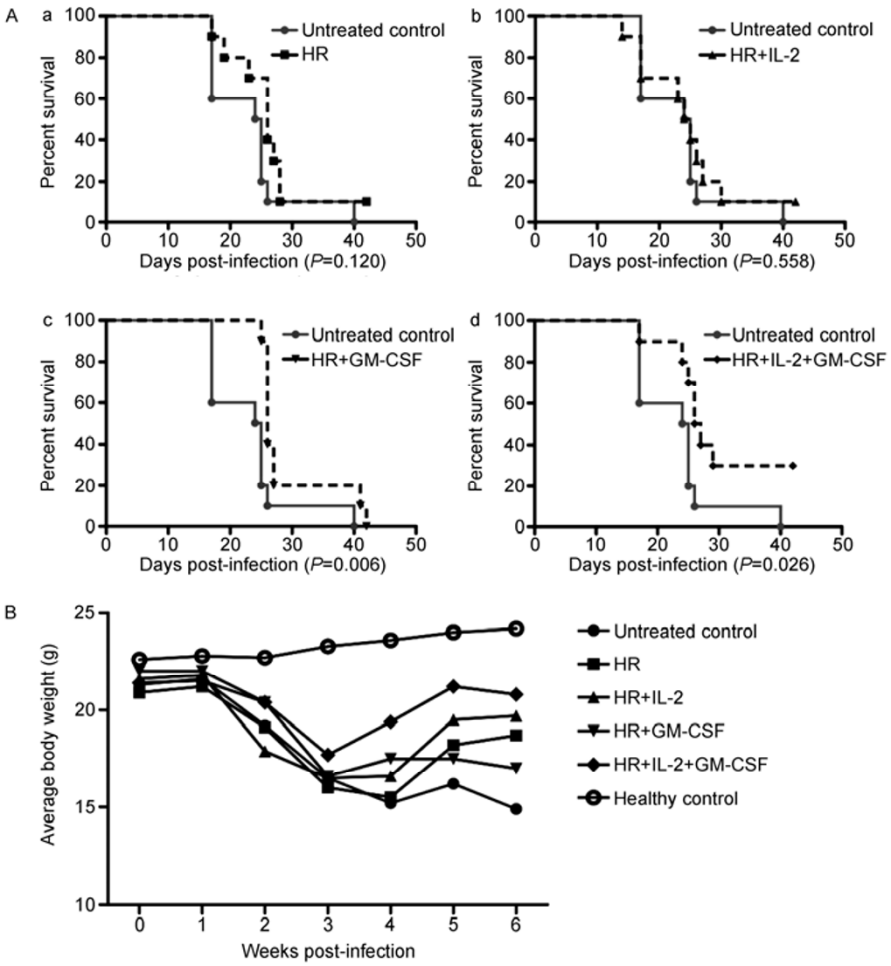

Figure 3 Survival curves and weight loss of treated or untreated mice infected with OB35 *M. tuberculosis*. A, Kaplan-Meier survival curves for each group of treated mice (n=10). a, HR; b, HR+IL-2; c, HR+GM-CSF; d, HR+IL-2+GM-CSF. B, The mean body weight.

Table 3 CFU counts in the lungs and spleens of mice infected with the OB35 strain after 4 weeks of treatment[a)]

Treatment	Lg CFU/g organ (mean±SD)			
	Lung on day 14	Lung on day 43	Spleen on day 14	Spleen on day 43
Untreated	8.38±0.06	9.23±0.05	7.29±0.15	7.97±0.03
HR		8.78±0.08$^{\#\#}$		6.70±0.06$^{\#\#}$
HR+IL-2		8.11±0.20$^{**\#\#}$		6.41±0.14$^{**\#\#}$
HR+GM-CSF		8.05±0.39$^{*\#}$		6.46±0.14$^{*\#\#}$
HR+IL-2+GM-CSF		7.76±0.33$^{**\#\#}$		5.36±0.23$^{**\#\#}$

a) Significant differences were observed between the treated groups and the untreated group (#), or between the immunotherapy groups and HR group (*). #, P<0.05, ##, P<0.01; *, P<0.05, **, P<0.01. CFU, colony-forming units; HR, isoniazid (INH) and rifampin (RIF).

greater reduction of CFU in lungs (0.67 and 0.73 lg CFU, respectively, P<0.01) and spleens (0.29 and 0.24 lg CFU, respectively, P<0.05) compared with HR alone. Moreover, HR+IL-2+GM-CSF treatment induced the greatest reduction of lung and spleen bacterial loads (1.02 and 1.34 lg CFU, respectively), when compared with HR (P<0.01).

2.2.3 Histopathological examination of the lungs

Histopathological results are shown in Figure 4. Extensive lung lesions and hyperemia congestion in the alveoli with damaged construction were observed in lung sections from untreated mice (Figure 4A). Antibacterial therapy did not result in a significant improvement although the pulmonary bacterial loads decreased when compared with untreated mice (Figure 4A and B). The administration of HR plus single cytokine regimens resulted in clearer alveoli structures and fewer lesions (Figure 4C and D). In contrast, the HR+IL-2+GM-CSF regimen appeared to be the most efficacious treatment because the lesion area of the lungs from surviving mice in this group was greatly reduced

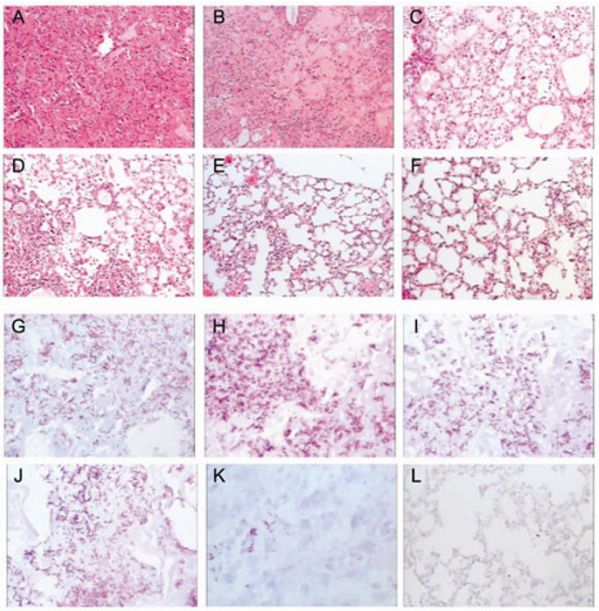

Figure 4 Histopathology of lung sections from treated or untreated mice infected with OB35 on day 42 post-infection. A–F, Hematoxylin and eosin-stained lung tissue sections (shown at 100× magnification). A, Untreated control. B, HR. C, HR+IL-2. D, HR+GM-CSF. E, HR+IL-2+GM-CSF. F, Healthy control. G–L, Acid-fast stain of *M. tuberculosis* in lungs (shown at 400× magnification). G, Untreated control. H, HR. I, HR+IL-2. J, HR+GM-CSF. K, HR+IL-2+GM-CSF. L, Healthy control.

(Figure 4E). Furthermore, we examined bacteria in the lungs of infected mice using acid-fast staining, and the results confirmed the CFU data from each matched group (Figure 4G–L).

3 Discussion

Disorders of cytokine networks are closely associated with disease [26,27]. Cytokines produced by immune cells can influence the outcome of mycobacterial infection. The aim of this study was to determine whether exogenous cytokines could improve the efficacy of first-line drugs during active *M. tuberculosis* infection.

Using the *M. tuberculosis* H37Rv infection model, both IL-2 and GM-CSF alone reduced the bacterial load in the lungs and spleens of mice. The primary function of IL-2 is to stimulate the proliferation of T cells, whereas GM-CSF stimulates macrophage differentiation, proliferation, and activation, which can improve antigen-presenting efficiency. Both cytokines are important for eliciting a protective immune response to *M. tuberculosis* infection. The bacterial inhibitory rates following either cytokine monotherapy were ⩾80% in the lungs and ⩾90% in the spleens compared with the untreated group. The reduction of CFU in the absence of antibacterial drugs was likely due to cytokine-induced activation of host immunity. However, the therapeutic effects observed when using antibacterial drugs and a single cytokine was not significant when compared with antibacterial drugs alone in terms of CFU and spleen weight. This may be due to the bactericidal effects of the HR regimen overwhelming the host immune clearance of the bacteria, although a relatively low dose of HR was intentionally used throughout the entire experiment. However, it was reported that specific cytokines had an essential protective role in preserving alveolar structure of *M. tuberculosis* infected mice [28].

Based on results using the H37Rv model, we investigated the effect of immunotherapy with IL-2 and/or GM-CSF in the MDR-TB model. Given the virulence of the OB35 strain, only the combination regimens were used for treating mice infected with *M. tuberculosis* OB35 strain. All the combination regimens prolonged the survival time and decreased the bacterial load of infected mice. Eight untreated mice (8/10) died rapidly between day 20 and day 25 because of the high virulence of the MDR-TB strain, whereas the number of mice that died in the treated group was less than 6, suggesting that the combination regimens slowed disease progression after the initial treatment. The effects of immunotherapy were revealed by further analysis. The use of combination HR and cytokine treatment further enhanced the inhibitory effect of the two antibacterial drugs in terms of CFU counts, in contrast to the H37Rv infection model. As a result, moderate numbers of lung lesions were observed in the cytokine-treated groups.

Notably, the synergistic effect of IL-2 and GM-CSF was demonstrated clearly in the MDR-TB model as follows: (i) the most dramatic increase in mean body weight was observed in mice treated with HR+IL-2+GM-CSF; (ii) the reduction of lung and spleen CFU counts under the HR+IL-2+GM-CSF regimen was one lg CFU greater than in the antibacterial therapy alone regimen, which demonstrated the advantage of the two cytokine combination immunotherapy; (iii) the synergistic effect of these cytokines prevented the formation of lesions in the lungs; and (iv) mice receiving the HR+IL-2+GM-CSF regimen had a higher survival rate and statistically significant survival curve compared with untreated mice. Indeed, the synergistic effect of IL-2 and GM-CSF in enhancing the immune response was also reported in a vaccine study [29,30]. IL-2 stimulates the immune response by activating effector cells, whereas GM-CSF enhances the immune response by increasing antigen presentation through macrophages. The different mechanisms of these two cytokines in enhancing the host protective response may account for the synergistic effect observed when both cytokines with antibacterial drugs are used to treat MDR-TB. The results of the current study demonstrated that IL-2 and GM-CSF could improve the efficacy of anti-tuberculosis drugs leading to a significant decrease in the bacterial load. Therefore, the use of IL-2 and GM-CSF may be used to shorten the treatment duration and improve the treatment outcome for MDR-TB. It will be of interest to screen other cytokines to enhance the therapeutic

effect of TB drugs in future studies.

However, there are limitations to this study. Because of its high virulence and high dose of infection, a high mortality rate was observed in the OB35-infected mice. A reduced infection dose, different doses of cytokines, and longer treatment duration will be tested in future studies to find a more significant efficacy of protection. As OB35 is resistant to both INH and RIF, other first-line or second-line drugs will be evaluated to assess the immunotherapeutic effects of cytokines.

In summary, the use of IL-2 and/or GM-CSF as immunomodulators improved the efficacy of conventional antibacterial drugs in drug-sensitive and MDR-TB murine models. This study is the first report to describe the synergistic effects of combined IL-2 and GM-CSF for MDR-TB treatment. The cytokine network is complex because of the synergism and suppression among different cytokines. Thus, further studies of the mechanisms involved and clinical data are needed to promote new opportunities for therapeutic intervention in TB.

We thank Dr. Zhang Ying for helping revising the manuscript. This work was supported in part by the Key Technologies Research and Development Program for Infectious Diseases of China (Grant No. 2012ZX10003001), the Key Project of Science and Technology of Shanghai (Grant No. 10411955000), and the Shanghai Science and Technology Development Funds (Grant No. 10XD1400900).

1 World Health Organization. Global Tuberculosis Control: Epidemiology, Strategy, Financing. WHO, 2009
2 World Health Organization. Global Tuberculosis Control 2010. WHO, 2010
3 Nuermberger E L, Spigelman M K, Yew W W. Current development and future prospects in chemotherapy of tuberculosis. Respirology, 2010, 15: 764–778
4 Masihi K N. Fighting infection using immunomodulatory agents. Expert Opin Biol Ther, 2001, 1: 641–653
5 Urdahl K B, Shafiani S, Ernst J D. Initiation and regulation of T-cell responses in tuberculosis. Mucosal Immunol, 2011, 4: 288–293
6 Cooper A M, Mayer-Barber K D, Sher A. Role of innate cytokines in mycobacterial infection. Mucosal Immunol, 2011, 4: 252–260
7 Abebe F, Bjune G. The protective role of antibody responses during *Mycobacterium tuberculosis* infection. Clin Exp Immunol, 2009, 157: 235–243
8 Coleman D L, Chodakewitz J A, Bartiss A H, *et al.* Granulocyte-macrophage colony-stimulating factor enhances selective effector functions of tissue-derived macrophages. Blood, 1988, 72: 573–578
9 Suzuki K, Lee W J, Hashimoto T, *et al.* Recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) or tumour necrosis factor-alpha (TNF-alpha) activate human alveolar macrophages to inhibit growth of *Mycobacterium avium* complex. Clin Exp Immunol, 1994, 98: 169–173
10 Berretta F, St-Pierre J, Piccirillo C A, *et al.* IL-2 contributes to maintaining a balance between $CD4^+Foxp3^+$ regulatory T cells and effector $CD4^+$ T cells required for immune control of blood-stage malaria infection. J Immunol, 2011, 186: 4862–4871
11 Yamamura M, Uyemura K, Deans R J, *et al.* Defining protective responses to pathogens: cytokine profiles in leprosy lesions. Science, 1991, 254: 277–279
12 Wnr R C, Liu Y M, Schluger N W. Local immune responses correlated with presentation and outcome in tuberculosis. Am J Respir Crit Care Med, 1998, 157: 729–735
13 Tan Q, Xie W P, Min R, *et al.* Characterization of Th1- and Th2-type immune response in human multidrug-resistant tuberculosis. Eur J Clin Microbiol Infect Dis, 2012, 31: 1233–1242
14 Cai H, Yu D H, Tian X, *et al.* Coadministration of interleukin 2 plasmid DNA with combined DNA vaccines significantly enhances the protective efficacy against *Mycobacterium tuberculosis*. DNA Cell Biol, 2005, 24: 605–613
15 Nambiar J K, Ryan A A, Kong C U, *et al.* Modulation of pulmonary DC function by vaccine-encoded GM-CSF enhances protective immunity against *Mycobacterium tuberculosis* infection. Eur J Immunol, 2010, 40: 153–161
16 Zhuang Y, Li G, Zhang X, *et al.* Study of recombinant IL-2 on *Mycobacterium tuberculosis* infected mice. Xian Dai Mian Yi Xue, 1992, 12: 3
17 Chun N H, Zhu L Z, Yie Z Z, *et al.* A controlled clinical study on the efficacy of recombinant human interleukin-2 in the treatment of pulmonary tuberculosis. Zhonghua Jie He He Hu Xi Za Zhi, 2003, 26: 548–551
18 Bermudez L E, Martinelli J, Petrofsky M, *et al.* Recombinant granulocyte-macrophage colony-stimulating factor enhances the effects of antibiotics against *Mycobacterium avium* complex infection in the beige mouse model. J Infect Dis, 1994, 169: 575–580
19 Pedral-Sampaio D B, Netto E M, Brites C, *et al.* Use of Rhu-GM-CSF in pulmonary tuberculosis patients: results of a randomized clinical trial. Braz J Infect Dis, 2003, 7: 245–252
20 Lu J, Yue J, Wu J, *et al. In vitro* and *in vivo* activities of a new lead compound I2906 against *Mycobacterium tuberculosis*. Pharmacology, 2010, 85: 365–371
21 Watson V E, Hill L L, Owen-Schaub L B, *et al.* Apoptosis in *Mycobacterium tuberculosis* infection in mice exhibiting varied immunopathology. J Pathol, 2000, 190: 211–220
22 Liang Y, Wu X, Zhang J, *et al.* Treatment of multi-drug-resistant tuberculosis in mice with DNA vaccines alone or in combination with chemotherapeutic drugs. Scand J Immunol, 2011, 74: 42–46
23 Liang Y, Wu X, Zhang J, *et al.* The treatment of mice infected with multi-drug-resistant *Mycobacterium tuberculosis* using DNA vaccines or in combination with rifampin. Vaccine, 2008, 26: 4536–4540
24 Aslanzadeh J, de la Viuda M, Fille M, *et al.* Comparison of culture and acid-fast bacilli stain to PCR for detection of *Mycobacterium tuberculosis* in clinical samples. Mol Cell Probes, 1998, 12: 207–211
25 Gebre N, Karlsson U, Jonsson G, *et al.* Improved microscopical diagnosis of pulmonary tuberculosis in developing countries. Trans R Soc Trop Med Hyg, 1995, 89: 191–193
26 Arnaud L, Gorochov G, Charlotte F, *et al.* Systemic perturbation of cytokine and chemokine networks in Erdheim-Chester disease: a single-center series of 37 patients. Blood, 2011, 117: 2783–2790
27 Katsikis P D, Mueller Y M, Villinger F. The cytokine network of acute HIV Infection: a promising target for vaccines and therapy to reduce viral set-point? PLoS Pathog, 2011, 7: e1002055
28 Szeliga J, Daniel D S, Yang C H, *et al.* Granulocyte-macrophage colony stimulating factor-mediated innate responses in tuberculosis. Tuberculosis (Edinb), 2008, 88: 7–20
29 Toubaji A, Hill S, Terabe M, *et al.* The combination of GM-CSF and IL-2 as local adjuvant shows synergy in enhancing peptide vaccines and provides long-term tumor protection. Vaccine, 2007, 25: 5882–5891
30 Ahlers J D, Dunlop N, Alling D W, *et al.* Cytokine-in-adjuvant steering of the immune response phenotype to HIV-1 vaccine constructs: granulocyte-macrophage colony-stimulating factor and TNF-alpha synergize with IL-12 to enhance induction of cytotoxic T lymphocytes. J Immunol, 1997, 158: 3947–3958