Mycobacterial Diseases
ISSN: 2161-1068

Nikonenko et al., Mycobact Dis 2017, 7:4
DOI: 10.4172/2161-1068.1000251

Research Article Open Access

Efficacy of Isoniazid Therapy in Mice with Different Genetic Susceptibility to Infection

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Rec date: September 18, 2017; **Acc date:** October 18, 2017; **Pub date:** October 23, 2017

Abstract

The question about possible influence of genetic susceptibility to Tuberculosis (TB) infection on the efficacy of its antibiotic treatment remains unanswered, and the results of scarce studies on the topic look contradictory. In the present work we studied the efficacy of short-term INH therapy against *M. tuberculosis* in hyper-susceptible I/St, relatively resistant BALB/c and highly resistant (I/St x BALB/c) F1 mice by comparing lung and spleen CFU counts and lung histopathology after 1- and 2-mo therapy. Our results indicate that the efficacy of INH therapy, as evaluated at the early phase of infection, is more effective in genetically susceptible hosts. Possible reasons for contradictions between studies applying early *VS*. late evaluation of the efficacy of treatment are discussed.

Keywords: TB mouse genetics; INH treatment; Lung pathology

Introduction

Among forty inbred mouse strains studied by our lab during last decades, mice of the I/St Strain demonstrated the highest of susceptibility to infection with *M. tuberculosis* strain H37Rv [1-6]. Unlike the majority of conventional mouse strains, after *M. tuberculosis* infection I/St mice form necrotic and hypoxic lung granulomas [6,7], similar to those in humans [8], and in another hyper-susceptible mouse strain C3HeB/FeJ [9]. In numerous studies dissecting TB susceptibility genetic control by segregation analyses, most often mice of the C57BL/6 strain were used as a TB-resistant partner [10-13], although other strains appeared to be also helpful [2,3]. Taking into account dozens of studies in mice bearing knockout mutations in genes encoding key cytokines, chemokines and their receptors [14], one may conclude that mouse genetics of susceptibility to *M. tuberculosis* infection is relatively well characterized.

On the other hand, dependence of anti-TB drugs' performance upon genetics of the host remains very poorly studied, and the question about possible influence of genetic susceptibility to infection on the efficacy of treatment remains unanswered. The study performed in C3HeB/FeJ "Kramnik mice" demonstrated that more resistant BALB/c mice responded to chemotherapy better than mice of this hyper-susceptible strain [15]. The authors attributed this result to the fact that C3HeB/FeJ mice formed necrotic granulomata in their lungs, inside which the *Bacilli* were less accessible for drugs. Another possible explanation is that the tissue surrounding necrotic granuloma provides more stressful conditions for mycobacteria leading to down-regulation of their metabolism and making them less susceptible to drug activity. Our study in susceptible DBA/2 and resistant C57BL/6 mice [16] demonstrated higher efficacy of treatment in a more susceptible strain. Arguing by contradiction, this somewhat paradoxical result probably means that under less stressful conditions provided by inefficient host response a larger proportion of mycobacterial population remains in metabolically active state and is susceptible to the drug action. These two studies, performed in different strain combinations and not validated independently by other research teams, basically, exhaust the list of publications on the topic. Thus, it remains unclear which pattern is consistent with TB susceptibility level determined by different genes and on different genetic backgrounds.

To address this issue and accumulate more data on the subject, in the present work we studied the efficacy of short-term INH therapy against *M. tuberculosis* in hyper-susceptible I/St, relatively resistant BALB/c and highly resistant (I/St x BALB/c) F1 hybrids infected via intravenous route. Our specific interest to the I/St mouse strain was dictated by the fact that these mice are extremely susceptible to TB challenge and this susceptibility is underlined by impaired mechanisms of both innate and acquired immunity: ineffective mycobacterial killing by lung macrophages [17] and impaired H2-dependent INF-γ production by CD4+ T-cells [15].

Materials and Methods

M. tuberculosis strain

M. tuberculosis strain H37Rv was originally obtained from the Institute Pasteur, Paris, France. Mycobacteria were passage through C57BL/6 mice to increase virulence. The final mycobacterial culture was washed in phosphate-buffered saline (PBS) with 0.05% Tween 80, resuspended in PBS with 0.01% BSA and 0.05% Tween 80, dispensed in aliquots into polypropylene vials, and frozen at -80°C. CFU of the frozen aliquots were determined after thawing by plating serial 10-fold dilutions on 7H10 agars [18].

Animals

I/St, BALB/c and (I/St x BALB/c) F1 female mice 22-23 g of body weight were used. Mice were bread under conventional conditions at the Animal Facility of the Central Institute for Tuberculosis (CIT), Moscow, Russia in accordance with the guidelines from the Russian Ministry of Health #755, INH Office of Laboratory Animal Welfare (OLAW). Water and food were provided at libitum. All experimental

procedures were approved by CIT animal care committee (IACUC protocols #4, 7, 8).

Infection and CFU counts

In an acute preliminary experiment confirming different genetic susceptibility to infection (Figure 1), mice were inoculated i. v. with 106 CFU of *M. tuberculosis* in 0.2 ml of PBS containing 0.05% Tween 80. In the experiments assessing efficacy of INH treatment I/St mice received 5 x 104 CFU, BALB/c and F1 – 5 x 105 CFU. After week 3 of infection, treatment with INH (25 mg/kg, PO) was initiated and continued for 2 months. 0.2 ml of INH dissolved in water (2.5 mg/ml) was administered by gavages daily 6 times per week. Lungs and spleens were extracted at the day of chemotherapy initiation and at month 1 and 2 of therapy (51- and 81-day after TB challenge, respectively). Serial dilutions of organ homogenates in PBS with Tween 80 were plated on Dubos agar, and CFU were counted after 3-wk incubation at 37°C. Statistical significance was assessed by ANOVA and Student's t-tests.

Histological analysis

Lung tissue samples from the middle-right lobes were frozen across the −60°C to −20°C temperature gradient of in the electronic CryotomeH system (ThermoShandon, Runcorn, United Kingdom), and serial 6–8-mm-thick sections were made across the widest area of the lobe. Lung cryosections were fixed with acetone and stained with hematoxylin-eosin. The slides were examined by an experienced pathologist and photographed using an Axioskop 40 microscope and AxioCamMRc 5 camera (Carl Zeiss, Berlin, Germany).

Results and Discussion

Survival curves presented in Figure 1A demonstrate that after infection with the identical high dose of mycobacteria mice of the three groups substantially differed by susceptibility to infection: mean survival time (MST) in I/St, BALB/c and F1 mice was, respectively, 22.5 ± 3.7, 64.5 ± 14.6 and 124 ± 23.9 days (P<0. 001 for all combinations, ANOVA). Obviously, development of infection in the lungs of these mice has different dynamics and provides different CFU counts at any given time point, which interferes with formal evaluation of the efficacy of identical chemotherapy. To standardize the dynamics of infectious process, susceptible I/St mice were challenged with a 10-fold lower dose of mycobacteria than relatively resistant BALB/c and F1 hybrids. As shown in Figure 1B, this approach resulted in equal lung CFU counts (~107 per organ) at week 3 of infection (P>0.2 between all groups, ANOVA), i. e, at the starting point of chemotherapy initiation.

1-mo INH therapy resulted in appearance of significant differences in the lung CFU counts between groups (Figure 2). Whereas in I/St mice lung CFU counts dropped up to 5.32-logs and in BALB/c mice-5.84 logs, in the most resistant F1 hybrids CFU counts decreased only up to 6.29 logs (P<0.01 compared to I/St mice, ANOVA).

After 2 months of therapy lung CFU counts further decreased in all groups (4.50, 4.61 and 5.28 logs in, respectively, I/St, BALB/c and F1 mice), again demonstrating the lowest efficacy of treatment in more TB-resistant F1 animals (P<0.01 compared to parental mice, ANOVA).

Results of lung CFU estimation were confirmed by evaluation of lung pathology. As shown in Figure 3, after 2-mo treatment F1 lungs contained substantially more TB foci, some of which showed clear signs of confluence, whereas in I/St animals lungs were less severe affected by TB inflammation.

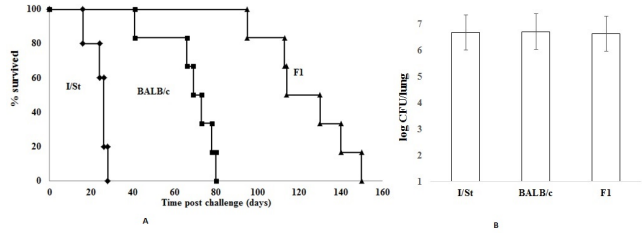

Figure 1: Development of TB infection in mice with genetically different susceptibility is possible to equalize by varying the size of challenging inoculum. (A) Survival of I/St, BALB/c and (I/St x BALB/c) F1 mice after i.v. infection with 106 *M. tuberculosis* H37Rv. P<0001 between all groups, ANOVA. (B) Decreasing of challenging dose 20-fold for I/St and 2-fold for BALB/c and F1 mice (see M&M) results in identical (P>0.2 between all groups) lung CFU content at week 3 of infection.

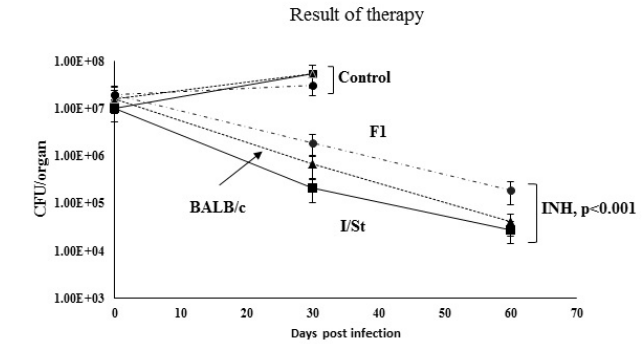

Figure 2: Effect of INH therapy on the lung CFU content in infected mice. Solid lines -I/St, hyphen lines-BALB/c, hyphen-dotted lines-F1 mice. ** P<0.01 between I/St and F1 mice. See text for details.

This is in a sharp contrast with histological picture in the I/St TB-affected lungs in the absence of therapy that we repeatedly observed earlier [19,20]. Thus, mycobacterial growth in the lungs, as well as lung pathology, was more effectively inhibited by INH in genetically susceptible mice. However, there were no differences in splenic CFU counts between groups (data not shown).

The results presented herein show that TB-resistant F1 mice are characterized by the lowest efficacy of treatment in the lungs. This is consistent with our earlier observation obtained in DBA/2 and C57BL/6 mice [16]. One possible explanation of these results may be a higher pressure onto *M. tuberculosis* in the resistant host compared to the susceptible one. This may cause a more rapid transition to dormancy [21] and hence development of relative drug resistance in a larger proportion of the parasite population.

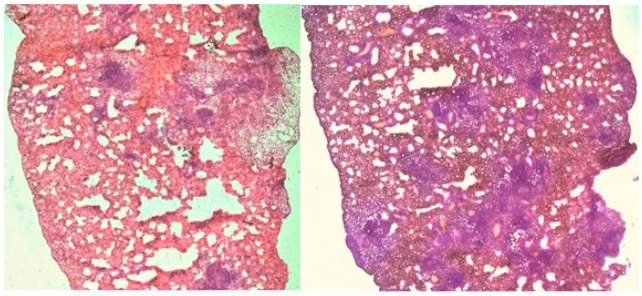

Figure 3: Lung pathology in I/St and F1 mice after 2-mo INH treatment. Lung sections were prepared from three mice in each group providing consistent results. Representative photos demonstrating profound differences in numbers and shape of TB foci in I/St and F1 mice are presented. Hamatoxylin-eosin staining, magnification: x37.

Results of our study are contradictory to the data obtained by Driver et al. [15] in "Kramnik mice": in our experiments, hyper-susceptible I/St mice readily responded to the INH therapy, whilst hyper-susceptible C3HeB/FeJ mice demonstrated an "attenuated efficacy" of treatment. The most likely reason for this discrepancy is substantial difference between the protocols of the two studies. In the study of Driver et al., the effect of treatment was followed up to the late stages of infection, at which C3HeB/FeJ mice developed fibrotic, encapsulated lung granulomata with central necrosis containing abundant extracellular *Bacilli*, whereas BALB/c mice formed non-necrotic lesions containing predominantly intracellular bacilli. We used the scheme of INH chemotherapy often used for testing the efficacy of new drug candidates, when treatment is started at week 3 post-challenge and is followed for two months only. Although I/St mice are prone to form necrotic and hypoxic lung lesions [6], the duration of experiment was not long enough for their development. It seems reasonable to argue that at the late stages of infection, when residual granuloma in I/St mice, despite their paucity after treatment, start to develop hypoxia and necrosis due to genetic peculiarities of the host, continuation of treatment would be more effective in more resistant F1 mice with their infection-restricting type of inflammation in the lungs. We plan to study further this issue in prolonged experiments.

Acknowledgements

This study was financially supported by the Russian Science Foundation (grant 15-15-30020).

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