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Man and mouse TB: contradictions and solutions

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We be of one blood, ye and I

Rudyard Kipling, The Jungle Books

As anyone who has attended tuberculosis research meeting in recent years can attest, disputes about validity of experimental animal models of tuberculosis (TB) erupt frequently, but mostly deteriorate into eloquence matches failing to produce satisfactory conclusions. Funding agencies also join the debate, since translating research into effective measures of TB control in humans is critically dependent on reliable testing of new interventions in animal models. Concerns about the validity of the most popular and accessible mouse model arouse as in some studies robust performance of a vaccine or a drug combination in mice failed to correlate with their efficacy in other species[1]. Here we address controversies that surround the mouse model of tuberculosis and offer a genetic perspective on how to make use of its full power for testing anti-tuberculosis interventions and dissecting pathogenesis of the disease.

Much of the information on TB pathogenesis, genetic control and the immune response to infection was obtained in experiments using inbred laboratory mice, which demonstrated that humans and mice are similar in the main features of the innate and adaptive immune responses to mycobacteria, including the protective role of $CD4^+$ T cells, IFN-γ, and TNF-α [2]. As in humans, in the mouse model the pathogen primarily targets the lungs causing a range of pathologies. Availability of unsurpassed genetic resources, which include hundreds of inbred, congenic, recombinant, mutant and genetically engineered strains, and abundance of immunological reagents and methodologies allow in-depth analysis of virtually any aspect of TB pathogenesis in mice, whereas their assortment is scarce for other animal species. Moreover, experiments in mice are less expensive as compared to other species, while genetic standardization further reduces the cost by decreasing individual variation and, hence, numbers of animals in experimental groups. In spite of these advantages, mouse experimental models of TB were repeatedly subjected to substantial criticism as mimicking the human disease with insufficient accuracy. Before we consider the soundness of the statement that TB course in the mouse lung poorly reflect the clinical disease in humans, a simple preamble on how principles of biological diversity are applied to humans and mice in TB studies is needed.

Genetic heterogeneity is a fundamental property of all animal species, and it accounts for a considerable fraction of intraspecies variation in susceptibility to and severity of mycobacterial infections typical for all mammals (reviewed in [3, 4]). Introductory sections of virtually all TB papers contain trivial sentences that: (i) only a small per cent of individuals infected with virulent *M. tuberculosis* develop clinical signs of infection; and (ii) infection can result in divergent outcomes in different individuals, ranging from spontaneous eradication, latent non-

symptomatic, mild chronic to rapidly progressing primary or reactivation diseases. Clearly, here a sober attitude to the fact that variability of the human host is something really important is displayed. Surprisingly, as soon as the mouse models of TB are addressed, often this realistic point of view is replaced by an abstract "mouse model" and the implanted verdict that mice are generally non-adequate as a model to study TB because they do not develop a human-like course of pathology. Meanwhile, reasoning abstractedly, such a statement implies that the genetic variability in *Mus musculus* is substantially lower than that in *Homo sapience*, given that pathological manifestations of pulmonary TB in humans are extremely variable [5, 6]. A TB professional should be aware of the fact that, except scarce information available from very early studies [reviewed in [7]], we know virtually nothing about the appearance of tuberculous granuloma in genetically resistant humans, simply because such foci either do not develop at all, or disappear long before the moment when lung tissue obtained from a TB-resistant individual during surgery or autopsy can be examined by a pathologist majoring in TB. As the result, lung pathology in TB-susceptible humans is correctly considered as a typical for this disease, but in the most of studies its features are inappropriately superposed with those observed in TB-resistant mice, without paying attention to the fact that a substitution of the concept occurred.

What observations and conclusions put considerable scepticism on the value of the mouse TB models? First, it was stated that, in contrast to humans, there is no central necrosis in lung granulomata of mice infected via the respiratory tract [8, 9]. Second, it was noted that the locations of macrophages and T cells in tuberculous foci in humans and mice are not identical [10, 11]. Third, a point was made that granulomatous zones remain aerobic in the lungs of mice, in contrast to humans, since staining for hypoxia did not reveal substantially hypoxic zones in mouse lung TB lesions [10, 12]. Remarkably, these results were obtained using mice of just two most popular inbred mouse strains: C57BL/6 (B6), one of the most resistant genetically to airborne *M. tuberculosis* infection, and also a relatively resistant BALB/c [13]. Meanwhile, there is a significant variation in tuberculosis susceptibility even among a small number of the most commonly used standard inbred mouse strains, and classical well-characterized strains CBA, DBA/2, A/J and C3H are more susceptible to tuberculosis than B6 and BALB/c[13–17]. A less well known SWR mouse is also highly susceptible, and a florid inflammatory cell response leading to degeneration, necrosis and consolidation of a large percentage of the lung surface area was observed in this strain after a low-dose aerosol infection[18].

Evaluation of pathology caused by *M. tuberculosis* in another TB-susceptible inbred mouse strain I/St performed by Apt's group showed a feature often considered as characteristic for the human as opposed to the murine disease: in addition to necrotic granuloma, development of highly hypoxic zones around TB lesions in the lung tissue was observed [19]. This is in sharp contrast with observations made by others in TB-resistant B6 mice [10, 12]. Importantly, a broader superposition of mycobacterial infections in B6 and I/St mice provides "the rule of contraries", further emphasizing the importance of host genetics in pathogenesis. Infection of B6 mice with *M. avium* has been suggested as a good surrogate mouse model for the studying TB pathology, since general appearance and cellular composition of lung granulomata were similar to those observed in TB patients [20]. As we showed recently, TB-susceptible I/St mice are genetically resistant to *M. avium*, and after infection with this agent they do not develop necrotizing granuloma with the regular co-axial structure, whilst in *M. avium*-susceptible B6 mice lung pathology indeed resembles human TB lesions [21]. Exactly as observed in TB models, the level of genetic susceptibility of the host to a specific lung pathogen determined characteristics of developing pathology.

In addition to these major similarities of lung TB pathology in humans and genetically susceptible mice, more subtle specific features of TB inflammation shared by the two species were noticed in some mouse TB models. For example, we and others [10, 19, 26] described the

appearance of B cell follicles in the infected mouse lung tissue and formation of structures closely resembling tertiary lymphoid organs, only recently reported for individuals suffering advanced TB[27]. In another recent study direct comparison of post-primary human TB and reactivation-like murine TB under Cornell model conditions revealed several common pathological features, e.g., lipid pneumonia, in the two species [28]. Taken together, characteristics of *M. tuberculosis* infection obtained in genetically susceptible mice and the diversity of TB-susceptible strains allow us to conclude that rationally selected murine models are reliable and adequate tools that reproduce various forms of the disease caused by virulent *M. tuberculosis*.

Although B6 mice are among the most TB-resistant, even they develop slowly progressing chronic lung inflammation and eventually succumb to infection. A clinical isolate of MTB was shown to induce necrosis within lung lesions of B6 mice[22]. Thus, these mice represent neither truly resistant, nor susceptible human phenotype. Their ascendance to a status of the general "mouse model" of TB within past decades was mainly due to a convention according to which genetically engineered mutations are generated on or transferred onto the B6 genetic background. Experiments using genetically engineered knockout mice include a so-called "wild type" control, which is in fact almost universally represented by B6. This, of course, eliminates the "genetic noise", but creates an incorrect notion of a typical course of TB in mice ignoring substantial natural variability.

We wish to emphasize that a considerable number of immunocompetent but TB-susceptible mouse strains whose lungs are primarily targeted by the pathogen is available. Remarkably, within this group the rate of the bacterial growth, severity of lung inflammation and tissue damage varies substantially, even between genetically closely related animals. One of us has found that a particular C3H substrain, namely, C3HeB/FeJ, is more susceptible to infection than other C3H substrains [23, 24], and described well-organized necrotic lung granulomas that developed in the lungs of these mice after a low-dose aerosol challenge with *M. tuberculosis* Erdman [23, 25] and H37Rv (Sanjay Jain, personal communication).

So far, forward genetic analysis (from phenotype to gene) consistently revealed complex genetic architecture of tuberculosis susceptibility in mice and identified quantitative genetic loci (QTL) specifically controlling such traits as cachexia[29], bacterial multiplication in the lungs[30] and necrosis within pulmonary lesions[25]. Epistatic gene interactions were shown to play a significant role in shaping these quantitative traits. For example, Kramnik's group demonstrated that not only the *sst1* locus plays a dominant and specific role in pathogenesis of necrotizing lung granulomas, but its phenotypic expression depends upon genetic background, i.e. genetic interactions[31, 32]. Animals bearing susceptible allele of the *sst1* locus ($sst1^S$) on the highly TB-susceptible C3HeB/FeJ background developed caseous pneumonia and rapidly succumbed, while the B6.C3H-$sst1^S$ congenic mice that carry the C3HeB/FeJ-derived $sst1^S$ allele on the resistant B6 background survived significantly longer and displayed the phenotype resembling chronic cavernous tuberculosis with extensive tissue remodeling and fibrosis (Pichugin et al, submitted for publication). The progress of TB-caused cachexia is controlled by certain combinations of alleles in three independently segregating QTL[33]. Thus, phenotypic diversity can be increased via combinatorial genetics. In fact, the existing independent inbred mouse strains carry unique combinations of genetic variants that were fixed as a result of almost a century of inbreeding. None of these combinations can be considered as a typical representative of the species, and the vast majority of allelic combinations were never intentionally selected for any particular phenotype[34]. Identification of major susceptibility/resistance loci by forward genetics allows generation and selection of novel genetic combinations via directed breeding in laboratory, thereby increasing the range of clinically relevant phenotypes. Rapidly developing tools for the genetic analysis of complex phenotypes

and sequencing of complete genomes of individual mouse strains ensure that genetic basis of these phenotypes can be elucidated.

As anti-tuberculosis strategies must protect genetically heterogeneous populations, their efficacy should be more properly assessed using a set of genetically diverse mouse strains developed and characterized to represent specific types of latent or active tuberculosis infection. Indeed, earlier studies have demonstrated that outcomes of vaccination [24, 35–37] and the rate of relapse after chemotherapy [38, 39] are determined by genetic background of the mouse hosts. As no single human represents a genetically diverse population, neither does an individual mouse strain. From this perspective, even a hypothetical ideal "Tuberculomouse" cannot be solely used to recapitulate the breadth of host responses and diverse outcomes of infection in humans. Therefore, a notion of generic "mouse TB model" should be, probably, eliminated. Instead, natural genetic variation in a diverse pool of inbred strains, their derivatives, and genetically segregating hybrids, which carry both ancestral polymorphisms and novel genetic combinations, should be employed to produce a spectrum of relevant human-like phenotypes. Within diverse but well-defined genetic context of the host the efficacy of anti-TB vaccines and drugs can be assessed more accurately, while their failures in a particular mouse strain may be correlated with specific defects of host resistance, anatomy of tuberculosis lesions and clinical forms of the disease. When combined with sophisticated analytical tools available exclusively for the mouse, the advanced genetic mouse TB model will have greater predictive power and help accelerate the development of anti-tuberculosis strategies effective in human populations.

As follows from the above discussion, the laboratory mouse represents an extremely valuable resource for TB research. However, more work is needed to generate and characterize mouse models that adequately represent the whole spectrum of relevant human phenotypes. In fact, TB resistance observed in the majority of humans has proved to be more difficult for reproduction in experimental settings, as compared to the disease. Paradoxically, focusing on the disease phenotypes temporary foreshadowed the lack of convenient mouse model of latent TB (LTB), although understanding the LTB mechanisms and the development of efficient treatments are considered high scientific and public health priorities. Recently guinea pig[40] and rabbit[41] models of LTB have been developed. However, unparalleled wealth of mouse genetic recourses and cost effectiveness makes mouse a species of choice for this task as well.

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References

1. Baldwin SL, D'Souza C, Roberts AD, Kelly BP, Frank AA, Lui MA, Ulmer JB, Huygen K, McMurray DM, Orme IM. Evaluation of new vaccines in the mouse and guinea pig model of tuberculosis. Infect Immun 1998;66:2951–9. [PubMed: 9596772]
2. North RJ, Jung YJ. Immunity to tuberculosis. Annu Rev Immunol 2004;22:599–623. [PubMed: 15032590]
3. Fortin A, Abel L, Casanova JL, Gros P. Host genetics of mycobacterial diseases in mice and men: forward genetic studies of BCG-osis and tuberculosis. Annu Rev Genomics Hum Genet 2007;8:163–92. [PubMed: 17492906]
4. Schurr, E.; Kramnik, I. Genetic Control of Host Susceptibility to Tuberculosis. In: Kaufmann, S.; Britton, W., editors. Handbook of Tuberculosis: Immunology and Cell Biology. Weinheim: WILEY-VCH Verlag; 2008. p. 295-336.
5. Dannenberg, A., Jr; Tomashevski, J. Pulmonary tuberculosis. In: Fishman, A., editor. Pulmonary Diseases and Disorders. New York: McGraw-Hill Book Co; 1988. p. 1821-42.

6. Ulrichs T, Kosmiadi GA, Jorg S, Pradl L, Titukhina M, Mishenko V, Gushina N, Kaufmann SH. Differential organization of the local immune response in patients with active cavitary tuberculosis or with nonprogressive tuberculoma. J Infect Dis 2005;192:89–97. [PubMed: 15942898]
7. Medlar E. The pathogenesis of minimal pulmonary tuberculosis. American Rev Tuberculosis 1948;24:558–82.
8. Flynn JL. Lessons from experimental Mycobacterium tuberculosis infections. Microbes Infect 2006;8:1179–88. [PubMed: 16513383]
9. Rhoades ER, Frank AA, Orme IM. Progression of chronic pulmonary tuberculosis in mice aerogenically infected with virulent Mycobacterium tuberculosis. Tuber Lung Dis 1997;78:57–66. [PubMed: 9666963]
10. Tsai MC, Chakravarty S, Zhu G, Xu J, Tanaka K, Koch C, Tufariello J, Flynn J, Chan J. Characterization of the tuberculous granuloma in murine and human lungs: cellular composition and relative tissue oxygen tension. Cell Microbiol 2006;8:218–32. [PubMed: 16441433]
11. McMurray DN, Collins FM, Dannenberg AM Jr, Smith DW. Pathogenesis of experimental tuberculosis in animal models. Curr Top Microbiol Immunol 1996;215:157–79. [PubMed: 8791713]
12. Aly S, Wagner K, Keller C, Malm S, Malzan A, Brandau S, Bange FC, Ehlers S. Oxygen status of lung granulomas in Mycobacterium tuberculosis-infected mice. J Pathol 2006;210:298–305. [PubMed: 17001607]
13. Medina E, North RJ. Resistance ranking of some common inbred mouse strains to Mycobacterium tuberculosis and relationship to major histocompatibility complex haplotype and Nramp1 genotype. Immunology 1998;93:270–4. [PubMed: 9616378]
14. Turner J, Gonzalez-Juarrero M, Saunders BM, Brooks JV, Marietta P, Ellis DL, Frank AA, Cooper AM, Orme IM. Immunological basis for reactivation of tuberculosis in mice. Infect Immun 2001;69:3264–70. [PubMed: 11292749]
15. Kramnik, I.; Demant, P.; Bloom, BB. Novartis Found Symp. Vol. 217. 1998. Susceptibility to tuberculosis as a complex genetic trait: analysis using recombinant congenic strains of mice; p. 120-31.discussion 32–7
16. Watson VE, Hill LL, Owen-Schaub LB, Davis DW, McConkey DJ, Jagannath C, Hunter RL Jr, Actor JK. Apoptosis in mycobacterium tuberculosis infection in mice exhibiting varied immunopathology. J Pathol 2000;190:211–20. [PubMed: 10657021]
17. Cardona PJ, Gordillo S, Diaz J, Tapia G, Amat I, Pallares A, Vilaplana C, Ariza A, Ausina V. Widespread bronchogenic dissemination makes DBA/2 mice more susceptible than C57BL/6 mice to experimental aerosol infection with Mycobacterium tuberculosis. Infect Immun 2003;71:5845–54. [PubMed: 14500506]
18. Turner OC, Keefe RG, Sugawara I, Yamada H, Orme IM. SWR mice are highly susceptible to pulmonary infection with Mycobacterium tuberculosis. Infect Immun 2003;71:5266–72. [PubMed: 12933873]
19. Radaeva TV, Kondratieva EV, Sosunov VV, Majorov KB, Apt A. A human-like TB in genetically susceptible mice followed by the true dormancy in a Cornell-like model. Tuberculosis (Edinb) 2008;88:576–85. [PubMed: 18667358]
20. Ehlers S, Benini J, Held HD, Roeck C, Alber G, Uhlig S. Alphabeta T cell receptor-positive cells and interferon-gamma, but not inducible nitric oxide synthase, are critical for granuloma necrosis in a mouse model of mycobacteria-induced pulmonary immunopathology. J Exp Med 2001;194:1847–59. [PubMed: 11748285]
21. Kondratieva EV, Evstifeev VV, Kondratieva TK, Petrovskaya SN, Pichugin AV, Rubakova EI, Averbakh MM Jr, Apt AS. I/St mice hypersusceptible to Mycobacterium tuberculosis are resistant to M. avium. Infect Immun 2007;75:4762–8. [PubMed: 17664269]
22. Guirado E, Gordillo S, Gil O, Diaz J, Tapia G, Vilaplana C, Ausina V, Cardona PJ. Intragranulomatous necrosis in pulmonary granulomas is not related to resistance against *Mycobacterium tuberculosis* infection in experimental murine models induced by aerosol. Int J Exp Pathol 2006;87:139–49. [PubMed: 16623758]
23. Pan H, Yan BS, Rojas M, Shebzukhov YV, Zhou H, Kobzik L, Higgins DE, Daly MJ, Bloom BR, Kramnik I. Ipr1 gene mediates innate immunity to tuberculosis. Nature 2005;434:767–72. [PubMed: 15815631]

24. Yan BS, Pichugin AV, Jobe O, Helming L, Eruslanov EB, Gutierrez-Pabello JA, Rojas M, Shebzukhov YV, Kobzik L, Kramnik I. Progression of pulmonary tuberculosis and efficiency of bacillus Calmette-Guerin vaccination are genetically controlled via a common sst1-mediated mechanism of innate immunity. J Immunol 2007;179:6919–32. [PubMed: 17982083]
25. Kramnik I, Dietrich WF, Demant P, Bloom BR. Genetic control of resistance to experimental infection with virulent Mycobacterium tuberculosis. Proc Natl Acad Sci U S A 2000;97:8560–5. [PubMed: 10890913]
26. Yan BS, Kirby A, Shebzukhov YV, Daly MJ, Kramnik I. Genetic architecture of tuberculosis resistance in a mouse model of infection. Genes Immun 2006;7:201–10. [PubMed: 16452998]
27. Ulrichs T, Kosmiadi GA, Trusov V, Jorg S, Pradl L, Titukhina M, Mishenko V, Gushina N, Kaufmann SH. Human tuberculous granulomas induce peripheral lymphoid follicle-like structures to orchestrate local host defence in the lung. J Pathol 2004;204:217–28. [PubMed: 15376257]
28. Hunter RL, Jagannath C, Actor JK. Pathology of postprimary tuberculosis in humans and mice: contradiction of long-held beliefs. Tuberculosis (Edinb) 2007;87:267–78. [PubMed: 17369095]
29. Lavebratt C, Apt AS, Nikonenko BV, Schalling M, Schurr E. Severity of tuberculosis in mice is linked to distal chromosome 3 and proximal chromosome 9. J Infect Dis 1999;180:150–5. [PubMed: 10353873]
30. Mitsos LM, Cardon LR, Ryan L, LaCourse R, North RJ, Gros P. Susceptibility to tuberculosis: a locus on mouse chromosome 19 (Trl-4) regulates Mycobacterium tuberculosis replication in the lungs. Proc Natl Acad Sci U S A 2003;100:6610–5. [PubMed: 12740444]
31. Kramnik I. Genetic dissection of host resistance to Mycobacterium tuberculosis: the sst1 locus and the Ipr1 gene. Curr Top Microbiol Immunol 2008;321:123–48. [PubMed: 18727490]
32. Sissons J, Yan BS, Pichugin AV, Kirby A, Daly MJ, Kramnik I. Multigenic control of tuberculosis resistance: analysis of a QTL on mouse chromosome 7 and its synergism with sst1. Genes Immun. 2008Epub Sep 11
33. Sanchez F, Radaeva TV, Nikonenko BV, Persson AS, Sengul S, Schalling M, Schurr E, Apt AS, Lavebratt C. Multigenic control of disease severity after virulent Mycobacterium tuberculosis infection in mice. Infect Immun 2003;71:126–31. [PubMed: 12496157]
34. Taft RA, Davisson M, Wiles MV. Know thy mouse. Trends Genet 2006;22:649–53. [PubMed: 17007958]
35. Apt AS, Avdienko VG, Nikonenko BV, Kramnik IB, Moroz AM, Skamene E. Distinct H-2 complex control of mortality, and immune responses to tuberculosis infection in virgin and BCG-vaccinated mice. Clin Exp Immunol 1993;94:322–9. [PubMed: 8222323]
36. Nikonenko BV, Apt AS, Mezhlumova MB, Avdienko VG, Yeremeev VV, Moroz AM. Influence of the mouse Bcg, Tbc-1 and xid genes on resistance and immune responses to tuberculosis infection and efficacy of bacille Calmette-Guerin (BCG) vaccination. Clin Exp Immunol 1996;104:37–43. [PubMed: 8603530]
37. Medina E, North RJ. Genetically susceptible mice remain proportionally more susceptible to tuberculosis after vaccination. Immunology 1999;96:16–21. [PubMed: 10233673]
38. Radaeva TV, Nikonenko BV, Mischenko VV, Averbakh MM Jr, Apt AS. Direct comparison of low-dose and Cornell-like models of chronic and reactivation tuberculosis in genetically susceptible I/St and resistant B6 mice. Tuberculosis (Edinb) 2005;85:65–72. [PubMed: 15687029]
39. Lecoeur HF, Lagrange PH, Truffot-Pernot C, Gheorghiu M, Grosset J. Relapses after stopping chemotherapy for experimental tuberculosis in genetically resistant and susceptible strains of mice. Clin Exp Immunol 1989;76:458–62. [PubMed: 2502336]
40. Kashino SS, Napolitano DR, Skobe Z, Campos-Neto A. Guinea pig model of Mycobacterium tuberculosis latent/dormant infection. Microbes Infect 2008;10:1469–76. [PubMed: 18817888]
41. Manabe YC, Kesavan AK, Lopez-Molina J, Hatem CL, Brooks M, Fujiwara R, Hochstein K, Pitt ML, Tufariello J, Chan J, McMurray DN, Bishai WR, Dannenberg AM Jr, Mendez S. The aerosol rabbit model of TB latency, reactivation and immune reconstitution inflammatory syndrome. Tuberculosis (Edinb) 2008;88:187–96. [PubMed: 18068491]