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Are mouse models of human mycobacterial diseases relevant? Genetics says: 'yes!'

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Immunology welcome responses to this commentary.

Summary

Relevance and accuracy of experimental mouse models of tuberculosis (TB) are the subject of constant debate. This article briefly reviews genetic aspects of this problem and provides a few examples of mycobacterial diseases with similar or identical genetic control in mice and humans. The two species display more similarities than differences regarding both genetics of susceptibility/severity of mycobacterial diseases and the networks of protective and pathological immune reactions. In the opinion of the author, refined mouse models of mycobacterial diseases are extremely useful for modelling the corresponding human conditions, if genetic diversity is taken into account.

Keywords: genetic homology; humans; immunity; mice; mycobacteria

All animals are equal, but some animals are more equal than others.

George Orwell, Animal Farm

Introduction

Much of what we know about the pathogenesis of and immune response to mycobacteria we have first learned in the rationally established, fine-tuned experimental models in inbred laboratory mice. Nevertheless, disputes about the relevance of experimental mouse models of tuberculosis (TB) are commonplace.[1–3] Considering the differences between *Mus musculus* and *Homo sapiens* in body size, lifespan and population structure, along with the endless list of species-specific physiological peculiarities, intuitive doubts in the validity of mouse models are quite accountable. As always in biology, the unifying link is provided by genetics: mouse and man have $\sim$ 85% genomic similarity, and many genes involved in the disease control are shared by the two species.

Contrary to a commonly expressed judgement that pathogenic features of TB infection substantially differ between mice and humans,[1–5] the spectrum of lung pathology caused by TB infection is similar in the two species (although not identical, e.g. the mouse lifespan does not provide enough time for calcification of fibrotic zones and formation of true cavities), if one takes into account genetic diversity. In recently published reviews compelling supportive evidence has been provided,[6,7] and it was stated that 'there clearly are major differences between experimental infection of mice and natural infection of humans, but this overarching dogma (mouse is not a good model for TB – AA) is non-conducive to fruitful research'.[7] To specifically advocate those mouse models of human mycobacterial diseases in which the genetic aspect is seriously taken into account, in this review I will provide examples of pathological conditions that display clear phenotypic parallels in mice and humans and are controlled by the orthologous genes. These findings were largely based upon comparisons between rare Mendelian disorders in humans and knockout mutations in mice, i.e. combination of segregation analysis, genetic mapping and the 'reverse genetics' approach. I will discuss also the irrefutable value of the 'forward genetic approach' (from phenotype to gene) for identification of complex patterns of genetic interactions between quantitative trait loci (QTL) in the control of disease spectra.

Straightforward cases for interspecies comparisons: genetic knock outs and monogenic disorders with Mendelian inheritance

Similar diseases of mice and humans caused by single, highly penetrating, Mendelian-inherited mutations affect-

ing immune functions have been known for decades. The X-linked immunodeficiency mutation, first mapped and phenotypically characterized in mice in 1970s,[8] followed by mapping and identification of mutations in the *btk* gene in mice and humans,[9–11] as well as phenotypic similarities between the Di George syndrome in humans and the *nude* mutation in mice,[12] provide good examples. However, only the introduction of principle genetic knockout techniques, based upon achievements in pluripotent embryonic stem cell culture systems and reciprocal genetic recombination in mammals (reviewed in refs 13,14), into common laboratory practice made it possible to systematically address the role of particular genes in the manifestation of disease phenotypes. How productive is this interspecies comparative approach in the field of mycobacterial diseases is exemplified below.

Humans and mice are similar in the main features of their innate and adaptive immune responses to mycobacteria, including the protective role of $CD4^+$ T cells, interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α).[15] Early phenotypic observations, which established these key features of anti-TB response in mice with non-manipulated genetics,[16–18] were supported by seminal studies in the 1990s performed in mice with genetically disrupted genes encoding IFN-γ, TNF-α, interleukin-12 (IL-12), their receptors and inducible nitric oxide synthase (reviewed in ref. 19). Overall, the data clearly demonstrated that genetic interference with the type 1 cytokine response profoundly exacerbates the course of TB in the murine host.

In parallel, several studies reported cases of severe clinical diseases caused by both bacillus Calmette–Guérin (BCG) vaccine[20–22] and low-virulence environmental mycobacteria[23,24] in children with inherited errors in immunity (reviewed in detail in ref. 25). Based upon segregation in kindreds, a corresponding syndrome was designated Mendelian susceptibility to mycobacterial diseases. The candidate gene approaches, combined with the phenotyping of family members, provided clear evidence that the genetic reason for an exceptionally high susceptibility to mycobacteria in otherwise healthy children was loss-of-function mutations in a few key genes involved in the type 1 response pathway, e.g. genes encoding IFN-γ receptors, IL-12 receptors, Stat-1 and some others.[24–27] Mendelian predisposition to mycobacteria as the result of mutations in the IL-12–IFN-γ pathway was not limited to low-virulence species because several cases of flourishing TB caused by classical *Mycobacterium tuberculosis* in children with defective alleles in corresponding genes were reported.[28,29]

A large international research team has reported on familial cases of Mendelian susceptibility to mycobacterial diseases, including TB, caused by non-sense mutations in the X-chromosome *CYBB* human gene encoding the $gp91^{phox}$ NADPH oxidase, the enzyme involved in respiratory burst in phagocytes. Interestingly, these mutations selectively impaired respiratory burst within macrophages, i.e. the key effector cells in anti-mycobacterial defence.[30] In the context of this review, it is important that this human genetic disorder resembles the phenotype of mice with knock outs of the homologous *Cybb* or $gp47^{phox}$ NADPH oxidase subunit genes.[31,32] The fact that the expression of newly discovered human mutations is limited to mature macrophages and does not affect monocytes and neutrophils[30] is a challenge for establishing adequate, extremely restricted conditional knockout mutations in mice, a tool that is requisite for the further molecular analyses.

Paramount reactions of protective immunity to mycobacteria in mice and humans depend upon identical genes, the rude disruption of which by experimental manipulations in mice or natural 'knockout experiments' in humans leads to strong defects resulting in severe mycobacterial infections. Moreover, in many aspects the mouse appears to be an adequate tool with which to experimentally study human TB, and so is a valuable model.

Complex cases for interspecies comparisons: allelic variants and polygenic control of infections

Although murine experimental systems based upon genetic disruption of genes encoding central elements of host defence have proved to be extremely useful analytical tools, they possess at least one major intrinsic disadvantage for modelling the spectrum of genetic and immune variability existing in the general population. Gene targeting, which results in the complete abrogation of any key function of the immune system, leads to a defect in protection against infection which is extreme. Given that such defects are normally rapidly eliminated from a population by natural selection, they could hardly account for the much more common modestly susceptible phenotypes.

Genetic and immunological mechanisms underlying the expression of the latter could be more rationally studied by the traditional means of segregation genetic analysis and forward genetic approach. However, both in mice and humans, quantitative differences between strains and individuals in, for example, the level of a cytokine production or the degree of cell activation, are under polygenic control. Therefore, the interpretation of results obtained by studying genetically non-manipulated mouse strains and general human populations is usually far more ambiguous than of those obtained in experiments with gene-targeted animals and Mendelian human disorders. By definition, this should impede building direct mouse–human parallels for quantitative traits; however, it is enough to mention that 'forward genetics' allowed the discovery of key pathways of immune response, including the Toll-like receptor family[33] and

several others,[34] to demonstrate the utmost importance of this approach. Some useful insights into genetic control and pathogenesis of important human diseases provided by quantitative genetic studies in the two species are discussed below.

Slc11a1 (Nramp1) in mice and humans

The famous *Nramp1* gene (former *Bcg*, presently *Slc11a1*), the first one shown to be involved in the control of genetic susceptibility to several intracellular bacteria, was mapped about 30 years ago and identified by positional cloning about 20 years ago.[35,36] Its phenotypic expression, as well as the molecular and functional characteristics of the corresponding protein, which functions as an efflux pump for divalent cations at the membrane of phagosome,[37] were recently reviewed,[25] so these data will not be included here. In the context of this review, relevance of the disease phenotypes observed in mice differing in *Slc11a1* alleles to those observed in humans with *SLC11A1* diversity are of interest.

Combining the phenotypic variants determined by the *Slc11a1* alleles and genetic approaches used for their identification, one can nicely bridge two main types of genetic analysis discussed above, that is, reverse and forward genetics. On the one hand, distribution of a single functional versus non-functional (G169D substitution) allelic variant of this gene in a large panel of inbred mouse strains, respectively, resistant or susceptible to low-virulence and mildly virulent intracellular parasites,[38] as well as a Mendelian dominant inheritance of the functional *r* (resistance) allele, closely mimics the monogenic disorders described above. On the other hand, the relatively weak influence on susceptibility to highly virulent mycobacteria, the generally intact immune system of the *s* (susceptibility) allele carriers, and the need for development of *Nramp1*-congenic mouse strains carrying a very short *Nramp1*-containing interval of the chromosome 1 for positional cloning[36] all resemble QTL-driven conditions.

Identification of *Slc11a1* allelic variants in mice stimulated studies in humans to assess the involvement of the *SLC11A1* homologue in the control of susceptibility to mycobacterial diseases. As non-functional alleles of *SLC11A1* were (and still are) not found, research in human populations was based on case–control and family genetic approaches. In case–control studies, significantly increased frequencies of a few polymorphisms, mainly in non-coding regions of *SLC11A1*, were discovered in TB[39–42] and leprosy[43,44] patients in ethnically distant populations, demonstrating that the gene is involved in the control of mycobacterial infections. The clearest evidence for the role of *NRAMP1* in susceptibility to TB *per se* was provided by a family genetic study in a very large Canadian aboriginal family experiencing a TB outbreak: strong linkage to the gene (Log of Odds > 3) was discovered.[45] Taken together, these studies provide ample evidence of the *NRAMP1* involvement in mycobacterial infection control. Not surprisingly, the influence of its allelic variants on the infectious course in humans is much milder than that of a complete switch-off of the genes in the type 1 immune response pathway, e.g. IL-12 or IFN-γ, but is comparable with that provided by natural allelic variability in the latter.[46,47]

Of course, it was important to find out whether or not these findings in humans – inspired by discovery of the *Nramp1* gene in mice – are supported by experimental mouse models of 'real' mycobacterial diseases, given that experiments with the almost avirulent *Mycobacterium bovis* BCG strain led to *Nramp1* identification. Experiments in mice congenic for *Nramp1 r* and *s* alleles using fully virulent *M. tuberculosis* strains provided conflicting results. Whereas R. North with colleagues found no influence of *Nramp1* on susceptibility to and severity of TB infection,[48] in our hands *Nramp1*^{s} mice following a low-dose intravenous challenge controlled multiplication of virulent mycobacteria in spleens significantly less effectively than their *Nramp1*^{r} congenic counterparts.[49] For the disease caused by a less virulent pathogen, *Mycobacterium avium*, the role of *Nramp1* is far more evident: strong regulation of mycobacterial multiplication in organs by *Nramp1* alleles was demonstrated not only in *Nramp1* congenic mice,[50] but, more recently, by means of segregation genetic analysis.[51] Organisms of the *M. avium* complex are intracellular human pathogens in the absence of the normal T-cell immunity,[52,53] present in approximately 70% of patients with advanced untreated AIDS and are considered the major killer in this cohort.[54] In addition, *M. avium*-triggered lung disease in mice is considered as a useful model of lung granuloma formation in humans.[55] Recently, we have shown that only mice genetically susceptible to *M. avium* because of the *Nramp1*^{s} genotype follow the 'correct', human-like pattern of granuloma development. A mirror pattern of granuloma formation is evident during *M. tuberculosis*-triggered disease: necrotizing hypoxic granulomata also developed in genetically susceptible mice, but irrespective of their *Nramp1* genotype, because the host genetic control of TB largely depends upon other genes.[51,56]

Overall, these results suggest that: (i) *Nramp1* allelic polymorphisms influence, albeit weakly, TB susceptibility in mice; (ii) *NRAMP1* allelic polymorphisms have a moderate effect on genetic TB susceptibility in humans; and (iii) the role of *NRAMP1* polymorphisms in immunocompromised humans infected with *M. avium* is worth studying because *Nramp1* is a key gene determining susceptibility/resistance to this agent. Referring to clinical cases of non-TB, non-BCG mycobacterial infections in patients with Mendelian susceptibility to mycobacterial diseases,[23,24] it would be interesting to find out whether such individuals are specifically predisposed to atypical mycobacterial diseases.

Ipr1 in mice, *SP110* in humans and polygenic quantitative traits

The group of I. Kramnik studied the genetic control of TB susceptibility using a set of recombinant congenic strains derived from the C3H (susceptible) and C57BL6/J (resistant) mouse strains.[57] They identified the C3HeB/FeJ substrain that was especially susceptible to TB,[58] despite the lack of known immune deficiencies. The major TB phenotype in the C3HeB/FeJ mice was rapid development of necrotic lung lesions. Using linkage analysis in the (B6 × C3HeB/FeJ) F_2 hybrids, a locus on mouse chromosome 1 was mapped and named *sst1* for super-susceptibility to TB.[58] In the newly developed *sst1*-congenic mouse strains the locus was reduced to an interval containing 22 known and predicted genes, and the analysis of expression of the interval-encoded genes in *M. tuberculosis*-infected macrophages identified *ifi75* (IFN-inducible-75) gene as a top candidate. Further functional and genetic characterization of the *sst1* locus, in the first instance the assessment of the expression of *ifi75* in lungs and macrophages (see ref. 59 for details), led to positional cloning of the *Ipr1* (for *Intracellular pathogen resistance 1*) gene,[60] as it was demonstrated that expression of the $Ipr1^r$ allele in the $Ipr1^s$ macrophages increased their ability to control multiplication of *M. tuberculosis*[60] and *Listeria monocytogenes*.[61] Deleterious effect of the $Ipr1^s$ allelic variant on TB manifestation was comparable with that of genetic knockout in the type 1 response pathway.[59]

The ability of the mouse *Ipr1* gene to control the formation of necrotic TB lesions in the lungs suggested that its human homologue might also be involved in the control of pulmonary TB, as necrosis of lung TB foci, with their subsequent caseation, are typical for the human disease. The SP110 protein is the closest human homologue of mouse Ipr1. It is encoded by the *SP110* gene orthologous to *Ipr1* and located in a highly conserved region of human chromosome 2q37. As for mouse *Ipr1*, *SP110* is strongly regulated by interferons,[62] suggesting its possible role in microbial immunity. However, there is still no convincing evidence that *SP110* polymorphisms influence susceptibility to human TB. Analysis of single nucleotide polymorphisms (SNP) within the *SP110* sequence in large cohorts of patients with TB and healthy controls of Russian origin revealed no significant associations.[63] A recent study of patients with TB and healthy controls in China claims significant associations between a few *SP110* SNP and elevated incidence of TB;[64] however, the statistical details of this work, published in Chinese, are unclear. Although expression of *SP110* mRNA in peripheral blood cells was found to be higher in patients with TB than in healthy controls,[65] the most likely explanation for this effect is up-regulation of the expression because of elevated IFN-γ levels in infected individuals,[59,62] rather than genuine genetic association. A supportive evidence of the role of *SP110* in mycobacterial infections control was provided by the study in cattle, in which a single SNP in the bovine *SP110* orthologue was associated with the difference in susceptibility to John's disease, a chronic granulomatous condition caused by *M. avium* ssp. *paratuberculosis*.[66] More work is needed to reach definitive conclusions.

Complex phenotypes of mycobacterial infections result from epistatic interactions between several loci. Earlier, we have reported on inter-locus genetic interactions when studying the body weight loss control after TB infection in [(I/St × A/Sn) F_1 × I/St] backcross mice[67] segregating for the alleles of three QTL located on chromosomes 3, 9 and 17.[67,68] Another example is a strong epistatic interaction between *Nramp1* and an *MHC*-located QTL in the control of *M. bovis* BCG infection in mice derived from the wild.[69] Generally speaking, both penetrance and expressiveness of a certain QTL appeared to be dependent upon allelic variation in another QTL. Work on identification of QTL involved in TB control in the B6–C3H strain combination also contributed to the list of interacting genetic loci. A strong influence of the *Ipr1* alleles on severity of TB infection masked the input of other QTL segregating in the (B6 × C3HeB/FeJ) F_2 mice. However, after fixing the effect of the *sst1* locus by developing two panels of *sst1*-congenic strains on both parental genetic backgrounds, six additional QTL involved in TB control polymorphic between B6 and C3H mice have been mapped by Kramnik's group on chromosomes 2, 7, 12, 13, 15 and 17.[59,70] An important practical outcome of this work was the creation of a panel of mouse strains combining *s* and *r* *Ipr1* alleles with *s* and *r* alleles in other QTL. As suggested by I. Kramnik,[59] variations of *r* and *s* allelic combinations in *Ipr1* and the Chr. 7 QTL (yet to be cloned) are sufficient to roughly model many major clinical TB manifestations: caseous pneumonia, miliary TB, chronic TB with necrotizing lesions, and chronic persistent TB. Given that all three different mouse strain combinations systemically studied so far with regard to the TB susceptibility/severity provided unique combinations of the QTL involved[67–72] (except the *MHC*-located QTL(s), which is not a surprise), a conclusion that the whole spectrum of TB manifestations can be reproduced in a rationally developed and subtly selected panel of strains congenic for key TB-controlling QTL looks realistic.[6]

Involvement of the *MHC* region in TB control in mice and humans is a separate problem which requires a separate review. There is ample evidence that polymorphisms in the *MHC* region in both species have modest effects on TB susceptibility (reviewed in refs. 22,59), and profound effects on the immune response to mycobacterial antigens.[73–75] However, the number of polymorphic genes located within the *MHC* region and potentially involved in the anti-mycobacterial immune response is very large,

even if classical Class I and Class II antigen-presenting molecules and TNF-like products are left aside. Hence, by establishing a panel of intra-*H2* recombinant congenic strains in B6–I/St strain combination, we recently narrowed the segment of interest to $\sim 1{\cdot}1$ Mb and excluded the distal part of the *H2* complex, i.e. *IE*, *Class III*, *TNF* and *H2-D*, *L*, *Q* regions, from TB control in our system.[76] However, the presence of several genes with non-synonymous mutations in their coding sequences, as well as the mode of expression of TB-related phenotypes (N. Logunova, A. Apt, unpublished observations), suggest that more than one QTL is located even within this short fragment of the *H2* complex.

From quantitative genetics of mycobacterial infections to systemic immune disorders: the *Icsbp/IFR8* gene

Among other approaches, comprehensive studies of *Nramp1* gene included studies on *M. bovis* BCG infection control in a panel of recombinant inbred congenic strains derived from B6/J and C3H/HeJ progenitors (the BXH panel). It was noted that mice of the BXH-2 strain were permissive for BCG growth despite their *Nramp1*^{r} genotype.[35] More recently, it was demonstrated that the ability of BXH-2 mice to control *M. tuberculosis* infection is also severely impaired as the result of an abrogated immune response against the parasite along the type 1 axis.[77] These mice also displayed a myeloproliferative syndrome, characterized by uncontrolled infiltration of lymphoid organs with granulocyte precursors.[78] Segregation analysis mapped the trait involved in both defects to the distal mouse chromosome 8, and positional cloning identified the gene *Icsbp/Irf8* encoding a transcriptional factor from the interferon response factor (IRF) family, participating in regulation of transcription of IFN-inducible genes.[78,79] The BXH-2 mice carry an impaired-function (hypomorphic) mutation R294C and do not respond with IL-12 and IFN-γ to external activating stimuli. Extreme susceptibility to mycobacteria in these mice is apparently explained by the importance of IRF8 for systemic immunity: not only activation but proliferation of myeloid cells depends on transactivation of the *Il12b* gene after IRF8 binding to its promoter. Consequently, IRF8-deficient BXH-2 mice are depleted of CD8α^{+} lymphoid and CD103^{+} myeloid dendritic cells, and IRF8-devoided *Irf8*$^{-/-}$ knockout mice also lack plasmacytoid dendritic cells.[80,81]

These data have helped to establish the cause of a newly identified syndrome in humans.[82] A large international team leaded by P. Gros and J.-L. Casanova evaluated an infant with early-onset disseminated BCG disease, who did not respond to antibiotic therapy, required haematopoietic stem cell transplantation and closely resembled mouse *Irf8*$^{-/-}$ phenotype regarding both BCG susceptibility and monocyte/dendritic cell content. The candidate genes were sequenced, and it appeared that the K108E mutation in *IRF8* caused autosomal recessive severe immunodeficiency with a complete lack of circulating monocytes and dendritic cells. In addition, two subjects with a history of disseminated but curable BCG disease in childhood, and lacking mutations in previously identified immunodeficiency genes, were studied. Both individuals carried the T80A substitution in *IRF8*, associated with a moderate autosomal dominant immunodeficiency and a selective depletion of CD11c^{+} CD1c^{+} circulating dendritic cells, closely resembling the BXH-2 phenotype. Structural analysis demonstrated that both K108E and T80A mutations impaired IRF8 transcriptional activity by disrupting the interaction between IRF8 and DNA.[82] Taken together, these results not only prove that mutations in orthologue genes in mice and humans cause similar immunological disorders, but also provide an example of parallel variations in their penetrance.

In the context of this review, four deductions readily occur. First, studies on susceptibility to a mycobacterial infection in a panel of specifically established and genetically defined mouse strains led to the conclusion that yet another transcriptional factor (see above about the *Ipr1* gene) is important for anti-mycobacterial immunity. Second, studies applying forward quantitative genetics of infectious diseases are not inferior to those using knockout mutations in discovering loss-of-sense mutations and deciphering gene functions (*Nramp1*, *Ipr1*, *Icsbp* – what follows?). Third, by analysing the genetics of susceptibility to infection we may discover genes with very broad physiological functions. Finally, many disorders with similar phenotypic expression in mice and humans are determined by mutations in orthologous genes, and genetically refined mouse models of diseases provide deep insights and valuable tools to study corresponding conditions in humans. Remarkably, the National Institutes of Health-organized workshop made refining animal TB models in general, and mouse models in particular, its first recommendation.[83]

For an immunologist, the best illustration of how interosculation of mouse and human disease control genetics has led to a better understanding of immune system functioning is the T regulatory cell story. In the early 1990s, a severe disorder of immunity displaying several features of a fatal autoimmnue disease was described in the *scurfy* (*sf*) mouse mutants.[84,85] Ten years later, two concurrent important observations were published. First, genetic analysis in patients with the X-linked hereditary monogenic syndrome (IPEX) including immune dysregulation, polyendocrinopathy and enteropathy discovered the *FOXP3* gene on the X chromosome.[86,87] Second, disruption of the murine homologue *Foxp3* (also X-located) was found to be the causal mutation underlying the *scurfy* phenotype, which included lymphadenopathy, splenomegaly, dermatitis and eczema, closely resembling the human IPEX syndrome. In addition, genetic transduction of

common peripheral T cells, normally showing no *Foxp3* expression, with the Foxp3-encoding genetic constructs resulted in the acquisition of immune regulatory functions; adoptive transfer of such cells into mutant mice abrogated IPEX-like syndrome.[88–90]

These findings not only identified a common genetic basis of a systemic immune disorder in humans and mice, but led to the discovery of a previously unknown $CD4^+$ $CD25^+$ $Foxp3^+$ T-cell population, commonly appreciated now as T regulatory cells. Studies on the physiology, maintenance, trafficking and education of these cells have been performed in the 8 years since their discovery, and now transfer of the knowledge obtained largely in mouse models into medical practice is in prospect (reviewed in ref. 91). Hence, regarding T regulatory cells, mouse models appeared to be absolutely adequate, and their application was essential for the discovery of molecular mechanisms underlying a whole class of immune reactions. Will *Icsbp* eventually develop into a full-size *Foxp3* twin?

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Disclosures

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