Clin Exp Immunol 1997; **107**:11–14

**EDITORIAL REVIEW**

# Tuberculosis and immunodeficiency—of mice and men

D. S. KUMARARATNE *Departments of Immunology, The Medical School Birmingham and City Hospital NHS Trust, Birmingham, UK*

*(Accepted for publication 29 October 1996)*

In a recent issue of this journal, Vordermeier *et al*. [1] reported that immunoglobulin $\mu$-chain gene-disrupted mice, which lack B cells, exhibit decreased resistance to experimental *Mycobacterium tuberculosis* infection. This is a surprising result, as immunity against *M. tuberculosis* cannot be passively transferred to recipient animals by injecting serum from immune donors, and is therefore not antibody-mediated, although protection can be adoptively transferred using T cells from *M. tuberculosis* or *M. bovis* bacille Calmette-Guérin (BCG)-primed donors [2]. Vordermeier *et al*. [1] showed that the recognition of some mycobacterial epitopes by T cells was defective in B cell 'knock-out' mice [1]. B cells can take up antigen via their specific immunoglobulin receptors, and present processed epitopes to T cells [3]; thus it is possible to speculate that the absence of such antigen presentation may impair the development of T cell responses to some mycobacterial epitopes.

How significant are these findings to the pathogenesis of human tuberculosis? Primary or secondary antibody deficiency does not result in increased susceptibility to tuberculosis, atypical mycobacterial infection or disseminated BCG infection [4–6]. Patients with common variable immunodeficiency or secondary antibody deficiency due to myelomatosis, chronic lymphocytic leukaemia and other types of non-Hodgkin's lymphoma show an increased susceptibility to extracellular bacterial sepsis, but not to mycobacterial disease [7]. Human X-linked antibody deficiency (XLA) is caused by mutation of the Bruton's tyrosine kinase (Btk) gene, resulting in B cell developmental arrest at the pre-B cell stage and the absence of B cells and serum immunoglobulin [8]. In that sense, patients with XLA are the human equivalent of B cell knock-out mice [8]. These patients do not exhibit increased susceptibility to intracellular bacterial infection including mycobacterial infection. In mice, naturally occurring point mutations of the Btk gene, or its inactivation by homologous recombination, results in an X-linked immunodeficiency (XID), which is significantly milder than XLA in humans [8]. XID mice are not globally B cell-deficient but have a selective deficit of a $CD5^+$ (B1) B cell subset, indicating that mice but not humans have a kinase that is capable of partially compensating for Btk deficiency. XID mice have normal antibody responses to proteins and to lipopolysaccharide (LPS) but do not respond to type II T cell-independent antigens [8]. This comparison strikingly illustrates the paradigm that while the immune systems of mice and men are broadly similar, there are significant differences in detail which impose limitations in extrapolating findings from murine models to explain the pathogenesis of human disease.

Correspondence: Dr D. S. Kumararatne, Department of Immunology, City Hospital NHS Trust, Dudley Road, Birmingham B18 7QH, UK.

The X-linked hyper-IgM syndrome (HIGM), which is the consequence of deficiency of a T cell surface molecule, CD40-ligand, is another form of antibody deficiency. While some patients with CD40-ligand deficiency develop opportunistic infections (e.g. pneumocystis infection or cryptosporidiosis), typically seen in patients with impaired cell-mediated immunity, there are no reports of increased susceptibility to mycobacterial infection [9].

Table 1a summarizes the effect of immunological deprivation on the susceptibility of mice to the *M. bovis* BCG or to virulent *M. tuberculosis*. Table 1b summarizes the effect of human immunodeficiency states on susceptibility to mycobacterial disease caused by *M. tuberculosis*, *M. bovis* BCG and atypical mycobacteria. In mice the expression of optimum anti-mycobacterial immunity requires the coordinated activity of a whole range of T cell and macrophage functions, including those of TCR $\alpha\beta^+$ T cells [10], TCR $\gamma\delta^+$ T cells [11], MHC class 1-dependent T cell activation (CD8 T cell function) [12], MHC class II-dependent T cell function (CD4 function) [11], interferon-gamma (IFN-$\gamma$) [13], tumour necrosis factor-alpha (TNF-$\alpha$) [14], IL-12 [15] and the induction of nitric oxide (NO) production within murine macrophages [16]. In experimental mycobacterial infections of mice, lack of any of the above defence mechanisms cannot be fully compensated for by alternative immune mechanisms. These immune mechanisms appear to have a cascade effect on each other, leading to the generation of an optimal response. Even in mice, the immune mechanisms critically important for controlling infection by *M. tuberculosis* in contrast to the relatively avirulent BCG, may be different. For example, in mice, function of the *Nramp*1 gene, coding for a macrophage-specific membrane protein (natural resistance associated macrophage protein), is relevant to the control of the early phases of infection with *M. bovis* BCG [17], but plays no role in the control of *M. tuberculosis* infection [18]. The gene homologous to *Nramp*1 is expressed by human macrophages [19]. However, the *NRAMP* locus has not to date been shown to strongly influence human susceptibility to tuberculosis or leprosy [20]. Furthermore, even BCG-susceptible strains of mice ($Nramp1^{-/-}$ or $Nramp^{Asp169}$) can rapidly control this organism once T cell responses are initiated [17].

The main conclusion from Table 1b is that antibody production, and the functions of B cells, complement [21] and neutrophils [4], do not play any significant role in human anti-mycobacterial immunity; T cell and macrophage function are clearly important [22,23].

IFN-$\gamma$ receptor (IFN-$\gamma$R1) deficiency due to a mutation of the high-affinity binding chain of this receptor is a cause of fatal disseminated BCG infection, or infection with *M. avium* and salmonella species [24]. This is indeed similar to the situation

**Table 1.**

**a.** Effect of immunological manipulation in mice

| Pathogen | Defect/treatment/genotype | Susceptibility |
|---|---|---|
| *M. bovis* BCG | TCR $\alpha/\beta$ or $\gamma$ GKO | Increased |
| | Recombinase activating gene-1 GKO | Increased |
| | $\beta_2$-M GKO (secondarily CD8 T cell-deficient) | Increased |
| | IFN-$\gamma$ GKO | Increased |
| | IFN-$\gamma$ receptor GKO | Increased |
| | IFN-$\gamma$ regulatory factor 1 GKO | Increased |
| | *Nramp*1$^{Asp169}$ GKO | Increased |
| | Treatment with anti-TNF-$\alpha$ antibody | Increased |
| *M. tuberculosis* | $\beta_2$-M GKO | Increased |
| | MHC class II GKO (secondary CD4 T cell deficit) | Increased |
| | IgH GKO | Increased |
| | Treatment with rIL-12 | Reduced |
| | Treatment with iNOS inhibitors | Increased |
| | BCG susceptibility locus (including *Nramp*1) | No effect |

**b.** Effect of immunodeficiency in humans on mycobacterial infection

| Physiological mechanism | Abnormality | Result |
|---|---|---|
| Circulating antibody | Primary and secondary antibody deficiency | No effect |
| Mucosal antibody | IgA deficiency | No effect |
| Complement | Congenital deficiency of classical pathway or regulatory components: C1q, C2, C4, C3, Factor I or Factor H | No effect |
| | Congenital deficiency of alternative pathway: Properdin, Factor D | No effect |
| | Congenital deficiency of terminal components: C5, C6, C7, C8, C9 | No effect |
| Mannose binding lectin | Inherited deficiency | ? decreased susceptibility to TB |
| Phagocyte number/ function | Neutropenia | No effect; no susceptibility to MAI or non-tuberculous mycobacteria |
| | Leucocyte adhesion deficiency type 1 (CD18/CD11a,b,c deficiency) | No mycobacterial infections reported |
| | Chronic granulomatous disease | Eleven cases of disseminated BCG infection reported; no susceptibility to MAI or non-tuberculous mycobacteria |
| | Chediak Higashi syndrome | No mycobacterial infections reported |
| Cell-mediated immunity: Primary T cell defect | Severe combined immunodeficiency: all forms | Increased, including disseminated BCG infection |
| Secondary T cell defect | HIV; immunosuppressive therapy | Increased (TB, MAI, other atypical mycobacteria; disseminated BCG infection) |
| MHC class II | Inherited deficiency | No mycobacterial infections reported |
| TAP transporter (indirect MHC class 1 deficiency) | Inherited deficiency | No mycobacterial infections reported |

**Table 1b.** continued

| Physiological mechanism | Abnormality | Result |
|---|---|---|
| Macrophage function | Defective pulmonary macrophage function in: | |
| | (a) Silicosis | Progressive cavitatory pulmonary tuberculosis; no systemic dissemination as seen in T cell deficiency |
| | (b) Pulmonary alveolar proteinosis | Pulmonary tuberculosis as in silicosis; pulmonary nocardial infection |
| Cytokine production | Reduced IL-12 production | Familial progressive MAI infection |
| IFN-$\gamma$ receptor | Inherited deficiency | Progressive BCG, MAI or atypical mycobacterial infection |

GKO, Gene knock-out.

observed in IFN-$\gamma$R1 gene-deleted mice [25]. However, this gene defect is seen in only a small proportion of all patients with disseminated BCG infection (J. L. Casanova, personal communication). Thus far, IFN-$\gamma$R1 deficiency appears only to cause human susceptibility to attenuated or environmental mycobacteria and salmonella species. *Mycobacterium tuberculosis* infection has not been reported in these patients; nor do they develop any other types of microbial infection [24]. This is different from observations in IFN-$\gamma$R1 knock-out, mice which are susceptible to a broad range of pathogens, including intra- and extracellular bacteria, some viruses and parasites [26].

Some patients with familial disseminated *M. avium* infection (not associated with HIV disease) or disseminated BCG infection, not responding to anti-mycobacterial chemotherapy, recovered from their mycobacterial infection when treated with IFN-$\gamma$ ($50\,\mu g/m^2$, administered subcutaneously, three times a week) ([27] and A. Segal and D. S. Kumararatne, unpublished information). This indicates that IFN-$\gamma$ contributes to human immunity against relatively avirulent mycobacteria. However, unlike murine macrophages, human monocytes and monocyte-derived macrophages cannot be activated *in vitro* with rIFN-$\gamma$ to control the intracellular growth of virulent *M. tuberculosis* [28]. It is possible that *in vivo* IFN-$\gamma$ may have a much more complex effect in promoting immunity to *M. tuberculosis* (cf. the ability of IFN-$\gamma$ to reduce bacterial and fungal infections in patients with chronic granulomatous disease (CGD), even though phagocytic cells from CGD patients who are treated do not show enhanced anti-microbial effects *in vitro* [29]). Data from trials in progress using IFN-$\gamma$ and other cytokines to treat patients with multi-drug-resistant tuberculosis, are eagerly awaited. Recently, monocytes from patients with familial susceptibility to severe, disseminated *M. avium* infection have been reported to exhibit defective IL-12 production *in vitro* [30].

NO production by murine macrophages activated by cytokines like IFN-$\gamma$ is essential for the control of *M. tuberculosis* infection, as indicated by exacerbation of this infection by the administration of inhibitors of inducible nitric-oxide synthetase (iNOS) [16] or the reduced NO production with a concomitant increased susceptibility to mycobacterial infection seen in mice deficient in IFN-$\gamma$ [31] or its receptor [25], or the IFN-$\gamma$ regulatory factor 1 [32]. A careful review of published data including an audit of the metabolism of L-arginine (precursor of NO) with human macrophages concluded that NO synthesis was not relevant to antimicrobial activity of human mononuclear phagocytes [33]. Most of the studies failing to detect a role of inducible NO synthesis in antimicrobial immunity have used blood monocyte-derived human macrophages [34]. Recently, Nicholson *et al*. [34] demonstrated that alveolar macrophages obtained after broncho-pulmonary lavage in patients with tuberculosis could be stained with an antibody specific for human iNOS. Using an indirect marker of NO synthesis, viz. diaphorase cytochemistry, they showed that diaphorase-positive alveolar macrophages were also increased in patients with tuberculosis, approximating to the proportion of those expressing iNOS. However, in other studies human macrophages expressing detectable iNOS were unable to generate NO [35]. It is argued that precise signals which induce human mononuclear phagocytes to produce NO have yet to be clearly defined [34]. Some support for this view is provided by studies of the promoter regions of human and mouse iNOS genes indicating that nucleotide substitutions found in the human iNOS promoter region made it unresponsive to up-regulation initiated by LPS and IFN-$\gamma$ [35]. Furthermore, human macrophages appear to lack nuclear factors (e.g. LPS-inducible nuclear factor-kappa B/Rel complex) that are required for maximum expression of iNOS [35]. In summary, the role of NO in human anti-mycobacterial immunity is possible but unproven.

In conclusion, experimental studies in healthy and immunologically deficient mice have highlighted the main components of immunity against mycobacteria. The broad components of human anti-mycobacterial immunity are similar. However there are significant differences in detail, as exemplified by the enhanced growth of *M. tuberculosis* in B cell-deficient mice contrasted to the irrelevance of antibody and of B cells to the expression of human mycobacterial immunity. This may be due, in part, to an overestimation of the importance of putative immune mechanisms as assessed by experimental in contrast to natural infection. For example, in the experiments by Vordermeier *et al*. [1], mice were infected by the i.v. injection of $10^6$ live bacteria. In contrast, human pulmonary tuberculosis is caused by small droplet nuclei containing one to three bacilli being inhaled into the alveoli, with 10–50 inhaled 'nuclei' on average, being required to establish infection in an individual recipient [36]. Therefore there is a need to design animal experiments, where possible, to mimic the pathogenesis of human tuberculosis. In addition, ideas generated using animal

experimental models should be tested for validity by comparison with conclusions gleaned from observations in immunodeficient humans.

## REFERENCES


1 Vordermeier HM, Harris DP, Venkatprasad N *et al*. Increase in tuberculosis infection in the organs of B cell-deficient mice. Clin Exp Immunol 1996; **106**:312–6.

2 Lefford MJ. Transfer of adoptive immunity to tuberculosis in mice. Infect Immun 1975; **11**:1174.

3 Lanzavecchia A. Antigen specific interaction between T and B cells. Nature 1985; **314**:537–9.

4 Johnston RB. Recurrent bacterial infection in children. NEJM 1984; **310**:1237–43.

5 Primary Immunodeficiency diseases. Report of a WHO Scientific Group. Immunodef Rev 1992; **3**:195.

6 Buckley RH. Humoral immunodeficiency. Clin Immunol Immunopathol 1986; **40**:13.

7 Chapel HM, Bunch C. Mechanisms of infection in chronic lymphocytic leukaemia. Semin Haematol 1987; **24**:291.

8 Satterthwaite AB, Witte ON. Lessons from human genetic variants in the study of B-cell differentiation. Curr Opin Immunol 1996; **8**:454.

9 Laman JD, Claassen E, Noelle RJ. Immunodeficiency due to a faulty interaction between T-cells and B-cells. Curr Opin Immunol 1984; **16**:636–41.

10 Mombaerts P, Alnoldi J, Russe D *et al*. Different roles of $\alpha/\beta$ and $\gamma/\delta$ T-cells in immunity against an intracellular bacterial pathogen. Nature 1993; **365**:53–56.

11 Kaufmann SHE. Bacterial and protozoal infections in genetically disrupted mice. Curr Opin Immunol 1994; **6**:518–25.

12 Flynn JL, Goldstein MM, Treibold KJ. Major histocompatability complex class 1-restricted T-cells are required for resistance to *M. tuberculosis* infection. Proc Natl Acad Sci USA 1992; **89**:12013.

13 Flynn JL, Chan L, Triebold KJ *et al*. An essential role for interferon gamma in resistance to tuberculous infection. J Exp Med 1993; **178**:2249–54.

14 Kindler V, Sappino AP, Cran GE *et al*. The inducing role of tumour necrosis factor in the development of bacterial granulomas during BCG infection. Cell 1989; **56**:731–40.

15 Cooper AM, Roberts AD, Rhoadis ER *et al*. The role of IL-12 in immunity to *M. tuberculosis*. Immunology 1995; **84**:423–32.

16 Chan JJ, Tanaka D, Carroll J *et al*. Effects of NO synthetase on murine infection with *M. tuberculosis*. Infect Immun 1995; **63**:736–40.

17 Vidal S, Tremblay ML, Govoni G *et al*. The Ity/Lsh/Bcg locus. Natural resistance to infection with intracellular parasites is abrogated by disruption of the *Nramp*1 gene. J Exp Med 1995; **182**:655–66.

18 Medina E, North RJ. Evidence inconsistent with the role for the Bcg gene (*N ramp* 1) in resistance of mice to infection with virulent *M. tuberculosis*. J Exp Med 1996; **183**:1045–51.

19 Cellier MG, Govoni S, Vidal T *et al*. Human natural resistance-assiciated in macrophage protein. J Exp Med 1994; **180**:1741–52.

20 Blackwell JM, Barton CH, White JK *et al*. Genetic regulation of leishmanial and mycobacterial infection—the LSH/ITY/BCG gene story continues. Immunol Letters 1994; **43**:99–107.

21 Figueroa JE, Densen P. Infectious diseases associated with complement deficiencies. Clin Microbiol Rev 1991; **4**:359.

22 Selwyn PA, Hartel D, Lewis VA *et al*. A prospective study of the risk of tuberculosis among intravenous drug users with human and immunodeficiency virus infection. NEJM 1989; **320**:545–50.

23 Kumararatne DS, Pithie AD, Bassi EOE. Mycobacterial immunity and mycobacterial disease in relation to HIV infection. In Bird AG, ed. Immunology of HIV infection. Oxford: Kluwer Academic Publishers, 1992:113.

24 Jouanguy E, Altase F, Lamhamedi S *et al*. Interferon gamma deficiency associated with idiopathic lethal BCG infection. NEJM 1996; in press.

25 Kamijo R, Le J, Shapiro D *et al*. Mice that lack the interferon gamma receptor have profoundly altered responses to infection with BCG and subsequent challenge with lipopolysaccharide. J Exp Med 1993; **178**:1435–40.

26 Dalton DK, Pitts-Meek S, Kershaw S *et al*. Multiple defects of immune cell function in mice with disrupted interferon gamma genes. Science 1993; **259**:1739–42.

27 Holland SM, Eiseinstein EM, Kuhus DM *et al*. Treatment of refractory disseminated nontuberculous mycobacterial infection with interferon gamma. NEJM 1994; **330**:1348–55.

28 O'Brian L, Roberts B, Andrew PW. *In vitro* interaction of *M. tuberculosis* and macrophages. Curr Topics Microbiol Immunol 1996; **215**:97–130.

29 International Chronic Granulomatous Disease cooperative study group. A controlled trial of interferon gamma to prevent infections in chronic granulomatous disease. NEJM 1991; **324**:509.

30 Frucht DM, Holland SM. Defective monocytes costimulation for IFN-$\gamma$ production in familial disseminated *Mycobacterium avium* complex infection. J Immunol 1996; **157**:411–6.

31 Cooper AM, Dalton DK, Stewart TA *et al*. Disseminated tuberculosis in interferon gamma gene disrupted mice. J Exp Med 1993; **178**:2243–7.

32 Kamijo R, Husada H, Matsuyama T *et al*. Requirement for transcription factor IRF-1 in NO synthetase induction in macrophages. Science 1994; **263**:1612–5.

33 Schoedon G, Schuerman R, Walter *et al*. Nitric oxide and infection: another view. Clin Infec Dis 1995; **21** (Suppl. 2):5151–7.

34 Nicholson S, Bonencini-Almeida M, Silva JRL *et al*. Inducible nitric oxide synthetase in pulmonary alveolar macrophages from patient with tuberculosis. J Exp Med 1996; **183**:2293–302.

35 Chang XO, Laubach VE, Alley EW *et al*. Transcriptional basis for hyporesponsiveness of the human inducible nitric oxide synthetase gene to LPS/interferon-gamma. J Leuc Biol 1996; **59**:575–85.

36 Riley RL, Mills CC, Nyaka W *et al*. Aerial dissemination of pulmonary tuberculosis. Am J Hyg 1959; **70**:185.