

# Human Genetic Susceptibility to Tuberculosis

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## Abstract

**Introduction:** Recent advances in the understanding of human susceptibility to tuberculosis have resulted from the application of molecular techniques to answer mechanistic questions on genetic regulation of host defense. **Methods:** This review is based upon a MEDLINE search of articles relevant to human genetic susceptibility to mycobacteria. **Results:** The impact of natural selection in shaping susceptibility to infectious disease should be taken into account when different ethnic groups are compared. Molecular typing has improved the accuracy of HLA analysis. However, most of the recent advances have come from research in non-HLA genes. Multiple non-HLA genes controlling susceptibility to tuberculosis have been identified by family-based linkage studies and population-based case-control studies. Comparative genomic studies based upon a mice model have identified a new resistance-associated protein in human macrophages. Several rare inherited disorders of mycobacterial immunity are caused by isolated gene mutations in the interferon  $\gamma$ -interleukin 12 pathways. Allelic variations in the vitamin D receptor have also been related to tuberculosis. A locus on human chromosome 17q have been linked to tuberculosis in a family-based genomic screening study. **Conclusion:** Susceptibility to tuberculosis involves large number genes inherited in a complex manner. Further discoveries are expected in the wake of rapid progress in the human genome project.

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**Key words:** Genetic susceptibility, Immunogenetics, Natural resistance-associated macrophage protein 1, Tuberculosis

## Introduction

The World Health Organization has declared that the recent resurgence of tuberculosis (TB) is a global emergency.<sup>1</sup> Southeast Asia, which is seeing the greatest increase in new TB patients, is at the epicentre of this pandemic.<sup>1</sup> Singapore, despite its relatively low incidence of new cases and rigorous screening for active disease among foreign workers, has not avoided this global re-emergence of an ancient foe.<sup>2</sup> This resurgence has happened over a short period of time and is the result of multiple environmental factors and not related to decline in genetically determined host susceptibility. Factors such as HIV, multidrug-resistant TB, population migration, neglect of TB control programmes and the financial crisis are converging in this region and increasing the complexity of the epidemic, making it far more difficult to contain.

This renewed interest in TB, however, has resulted in improved research funding and the widespread application of new and innovative molecular techniques to investigate key mechanistic questions in this disease. In particular, genetic regulation of host defense mechanisms has been an important field of research. Genetic manipulation of the

immune response in order to augment anti-bacterial drugs and vaccines has therapeutic potential.

Racial variations in susceptibility to TB<sup>3</sup> and classic studies in twins<sup>4</sup> which show greater TB rates in monozygotic versus dizygotic twins substantiate the importance of genetic factors in TB. Epidemiological studies merely provide the background but do not yield any clues on underlying mechanisms. The aim of this review is therefore to examine recent advances in the understanding of genetic susceptibility to mycobacteria based upon molecular techniques.

## Method

This review is based upon a systematic search of the literature on the Medline (on CD-Rom and Pub Med on the Internet) resources. The literature search was from 1970 to 1999 with the key words/MESH headings of "mycobacteria-immune response-genetic susceptibility-immunogenetics". The search was restricted to human studies in English and included reviews. Only positive studies on mycobacterial infections in humans were reviewed. Some relevant animal models will also be cited.

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## The Immune Response To Tuberculosis (Fig. 1)

Several recent reviews have addressed comprehensively, current concepts of the immune response to tuberculosis.<sup>5-8</sup> Thus, only the key elements of host defence against *Mycobacterium tuberculosis* have been outlined in this article. Figure 1 shows a network which integrates innate immunity (natural resistance) with acquired (adaptive) immunity. Genetic variations which regulate most of the pathways described in this figure have been linked to disease susceptibility in humans.

Innate immunity<sup>9,10</sup> or natural resistance is not antigen specific and non-clonal (i.e. not associated with immunological memory). It comprises of macrophages<sup>6</sup> and cytotoxic lymphocytes<sup>7</sup> (NK cells, CD8 +ve T cells and CD4 +ve T cells with  $\gamma\delta$ T cell receptors) which inhibit growth and kill mycobacteria probably by direct lysis. The main role of natural immunity in tuberculosis is usually assigned to macrophages. Macrophage phagosome-lysosomal fusion is a key event in natural immunity. Anti-bacterial processes include enzyme lysis, acidification, generation of reactive oxygen and nitrogen species and the participation of membrane transport proteins such as natural resistance-associated macrophage protein 1 (Fig. 1, Step 2\*:Nrpml).<sup>5-7,11</sup> The fate of mycobacteria is critically dependent upon the interactions between macrophages and other effector cells (lymphocytes in particular) and soluble mediators such as cytokines, chemokines, hormones and pro-hormones.<sup>5,8,12</sup>

Phagocytosis is a non-specific trigger for interleukin 12 (IL-12) secretion by macrophages.<sup>5,8,12</sup> Interleukin 12 is a pivotal cytokine which links the innate and adaptive arms of the immune response.<sup>5,8,12</sup> With regards to innate immunity, interleukin 12 induces lymphocytes (NK cells in particular) to secrete interferon  $\gamma$  (IFN $\gamma$ ). IFN $\gamma$  further activates macrophages and enhances their ability to inhibit mycobacteria via nitrogen radicals and vitamin D3.<sup>5,8,12,13</sup> Both IFN $\gamma$  and vitamin D3 have autocrine activities which serve to amplify this process.<sup>5,13</sup>

Adaptive immunity and immunological memory are acquired following MHC restricted encounter between newly processed antigen on dendritic cells<sup>14</sup> and specific T cells. In the presence of appropriate co-stimulating signals, CD4+ T cell proliferation occurs. In this context, IL-12 and IFN $\gamma$  are powerful determinants of the TH<sub>1</sub> (T helper 1) phenotype in proliferating CD4 +ve T cells.<sup>5,8,15,16</sup> Recent studies have correlated the presence of TH<sub>1</sub> phenotype (high levels of IL-12 and IFN $\gamma$  expression) with more favourable clinical presentation in pulmonary tuberculosis.<sup>17,18</sup>

Immune suppressor cells and inhibitory cytokines probably influence disease susceptibility and presentation as much as anti-bacterial responses.<sup>19</sup> There is very little

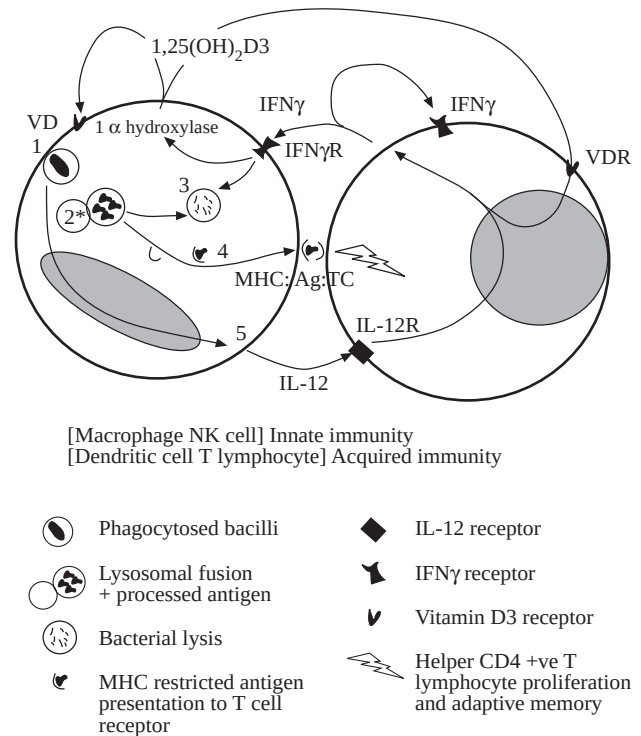


Fig. 1. The immune response to *M. tuberculosis* consists of cooperation between phagocytic, lytic, antigen presenting cells (macrophages and dendritic cells) and cytokine secreting, proliferating cells (natural killer cells and T lymphocytes). Phagocytosis (1) is followed by fusion with the lysosomal-endosomal compartments (2), which result in either (3) bacterial lysis and/or co-localisation of mycobacterial antigens with MHC class II molecules for antigen presentation to T cells (4). Secretion of IL-12 by phagocytes (5) is an early event following antigen exposure. IL-12 induces IFN $\gamma$  secretion by NK cells (initially) and T lymphocytes. IFN $\gamma$  has a positive feedback on both phagocytes and lymphocytes. It enhances bacterial lysis via reactive oxygen, nitrogen radicals and 1,25(OH)<sub>2</sub> vitamin D<sub>3</sub>. Natural resistance associated macrophage protein 1 (\*: Nrpml) is an iron transport membrane glyco-protein of the endo-lysosomal compartment which also inhibits mycobacteria. Variations in the genetic regulation of most of the pathways described in this figure have been linked to disease susceptibility in humans.

information however, on the genetic regulation of immunosuppression relevant to disease susceptibility in human tuberculosis.

## Effect of Natural Selection on Susceptibility and Clinical Presentation

The clinical spectrum and susceptibility patterns observed in any population for an infectious disease such as TB depend upon the history of host-pathogen relations. Humans are the obligate hosts for *M. tuberculosis* and their relationship is an ancient one, having lasted for at least 5000 years.<sup>20</sup> The disease can only be sustained in densely settled populations. Thus, it has been described in all ancient centres of civilization from the Middle East, ancient Greece, India to China.<sup>20</sup> However, it only reached epidemic proportions as the "white plague" in European cities during the 18th century following mass urban migration, when infection was universal and TB accounted for up to 25% of

deaths.<sup>21</sup> This epidemic was sustained in Europe by the Industrial Revolution and spread to previously naïve populations of sub-Saharan Africa and the New World by the slave trade and colonisation.<sup>22</sup> Indeed, we are seeing the resurgence of this very epidemic today.<sup>1,23</sup> Tuberculosis is a highly contagious infection with a pre-antibiotic mortality rate of over 50% among the young. The highest ever recorded mortality rate for TB was among native Americans crowded into reservations and barracks, reaching 9000 per 100,000 population.<sup>23</sup> This must have a major impact in shaping the immune response of subsequent generations to *M. tuberculosis*.

In urbanised societies like Singapore, the majority of people infected with *M. tuberculosis* show a fully developed cell-mediated immunity response [detected as a positive delayed skin reaction to purified protein derivative (PPD)]. Indeed a positive PPD test in a previously healthy and naïve individual is considered the most reliable index of recent infection. Cohort studies have shown that this acquired immune response is highly effective and merely 5% to 10% of recent PPD converters go on to develop frank clinical disease in their lifetime.<sup>23</sup> This high prevalence of resistance against TB is probably a direct outcome of the role of TB in natural selection. Another feature of the late phase of a TB epidemic is the predominant clinical presentation with “post-primary TB” or “reactivation TB”. This is the familiar pattern of chronic cavitary pulmonary TB seen among older patients presenting as an indolent illness or the “phthisis” syndrome.

By contrast, when a previously naïve population encounters TB for the first time, the consequences are very different. There will be a low level of herd immunity against TB and clinical disease is of the “primary TB” pattern. This is a progressive, subacute “typhoidal” illness with high mortality.<sup>22,23</sup> It is characterised by anergy (low intensity of cell mediated response), wide spread extra-pulmonary dissemination and prominent lymph node involvement. This was the main pattern of TB described in native Americans, during their early internment in camps; African slaves; Black African regiments deployed by the British in Ceylon; Senegalese recruits in the French Army in World War I; and most recently, among Yononami Indians in Brazil.<sup>22-24</sup> In urban westernised populations, “primary” TB is seen mostly in young children and patients with HIV infection.

Thus, the phase of the TB epidemic and its effect on the process of human natural selection must be taken into account when we compare disease susceptibilities between ethnic groups and even within populations. Since resistant individuals and families have a clear survival advantage, it may be predicted that stable urban populations during the matured stage of the epidemic may show little variations in

TB susceptibility. Indeed, several of the recent breakthroughs in elucidating the mechanisms of genetic susceptibility to TB have come out of sub-Saharan Africa where the epidemic is relatively “newer”.

### HLA Associations

The human leukocyte antigens (HLA) are products of the major histocompatibility gene complex in humans. They are key immuno-modulatory molecules which prevent autoimmunity and mediate specific immune response to a foreign antigens. The HLA D locus on the short arm of chromosome 6 encodes MHC Class II molecules which are expressed strongly on cells (usually dendritic cells) responsible for presentation of exogenous antigens.<sup>25</sup> Thus, *M. tuberculosis* antigens must first be processed and bound to MHC class II molecules before they can be recognised by T lymphocytes (Fig. 1, step 4). The MHC molecules are extremely varied with polymorphisms occurring at multiple sites which bind to antigen.<sup>26</sup> This variable binding of mycobacterial antigen to HLA molecules may affect the intensity of the adaptive immune response and influence susceptibility to TB.<sup>27,28</sup>

Thus, early studies of genetic susceptibility to TB have focused on MHC antigens from the HLA D region. McPeck et al<sup>29</sup> showed that monocytes from white donors expressed HLA-DR molecules more effectively (against protein synthesis inhibition with cyclohexamide) than from black donors. This might account for the racial differences in relative permissiveness of macrophages for mycobacteria observed by Crowle et al.<sup>30</sup> More recently, Agrewala et al<sup>31</sup> showed that host MHC can strongly influence the phenotype of CD4+ helper T cells. They found that antigen presenting cells which express HLA DRB1\*0101, \*1501 and \*0401 induced the most profound proliferation and highest IFN $\gamma$  secretion in CD4+ T cells in response to mycobacteria antigen.

The HLA system was initially typed by serological methods. Results of serotyping studies were inconsistent but suggested that susceptibility to TB was associated with HLA DR2 in Indian, Indonesian and Russian populations but not among the Chinese.<sup>32,33</sup> However, Rajalingam et al<sup>34</sup> were not able to confirm the association of TB with HLA DR2 in Indians by using more accurate molecular methods. Goldfeld et al,<sup>35</sup> using a two-staged molecular typing study in rural Cambodia, reported association between a HLA DQ allele (B1\*0503) and progressive TB. They noted that the DQB1\*0503 allele encodes for a change at amino acid position 57 of the beta chain (beta57), which influences the charge (more negative) in the putative peptide binding pocket, P9, of the DQ molecule. They further suggest that MHC-restricted presentation of peptides by TB-infected macrophages may be affected in patients who express this

particular P9 pocket. The negatively charged P9-binding pocket may bind TB antigens less effectively or elicit a diminished immunogenic response. Teran-Escandon et al,<sup>36</sup> in a similar study of Mexican patients, found significantly increased frequencies of HLA DQA1\*0101, HLA DQB1\*0501 and HLA DRB1\*1501 alleles and reduced frequencies of DQB1\*0402 alleles in patients with TB compared with controls. Thus, two recent studies using molecular typing concur that some alleles of the HLA belonging to the locus DQ1 may influence the susceptibility to TB in humans. However, a study by Blackwell,<sup>37</sup> using family-based linkage analysis in Brazil has failed to show either linkage or allelic association between HLA and TB.

### Natural Resistance-associated Macrophage Protein 1

*M. tuberculosis* is an obligate intracellular pathogen and phagocytosis by pulmonary macrophages is the very first event in the host-pathogen relationship (Fig. 1, step 1). The ability of these macrophages to eliminate mycobacteria in the early pre-immune phase of infection is the hallmark of innate resistance. Thus, the factors which determine the viability of *M. tuberculosis* within pulmonary macrophages are the most extensively researched aspects of the host response. An important breakthrough in the understanding of resistance against *M. tuberculosis* has arisen from comparative genomic studies using a mouse model of susceptibility to TB.<sup>38</sup>

Vidal and colleagues<sup>39</sup> were able to show that natural resistance of in-bred mice to BCG, *M. lepraemurium*, *Salmonella* and *Leishmania*, all obligate intracellular parasites, was controlled by a single Bcg gene. Bcg is inherited as an autosomal dominant Mendelian trait in mouse chromosome 1. Using high resolution positional gene mapping techniques followed by a single disruption (aspartic acid for glycine at position 169), they were able to prove conclusively that a point mutation of the natural resistance-associated macrophage protein 1 (Nramp1) is the cause of mouse susceptibility to Bovis, *S. typhimurium* and *L. donovani*.

Nramp1 is an integral structural glycoprotein of the late endosomal/lysosomal compartment in macrophages (Fig. 1, step 2 \*).<sup>38</sup> It is a member of an ancient and very highly conserved family of cellular proteins found in all living organisms from bacteria to man. While the exact mechanism of Nramp1 in bacterial defence remains undefined, recent evidence suggest that it may be a free iron transport protein with a possible regulatory role in apoptosis.<sup>40-42</sup>

The human Nramp1 gene has been mapped to chromosome 2 q35.<sup>11</sup> It has a high degree of sequence homology with murine Nramp1 and is strongly expressed in the lung. Polymorphisms in the human Nramp1 gene

have been used to study the association between Nramp1 and susceptibility to mycobacterial infections. Abel et al,<sup>43</sup> using segregation analysis in 20 multiplex families comprising 168 individuals from South Vietnam, were able to show that Nramp1 gene was associated with leprosy. Bellamy et al,<sup>44</sup> in a microsatellite analysis of Nramp1 polymorphisms in 410 adults in Gambia with smear positive TB and 417 matched controls, showed significant associations between 4 Nramp 1 polymorphisms and susceptibility to TB. There is, however, variation in the role of Nramp 1 between ethnic groups. For example, Blackwell et al<sup>37</sup> found no evidence for linkage between Nramp 1 and susceptibility to TB or leishmaniasis in Brazil. They suggest instead that allelic variations in the promoter of human Nramp1 gene may influence susceptibility to infective versus autoimmune disease.<sup>45,46</sup>

### Cytokines and Hormones

The genetic basis for susceptibility to human mycobacterial (and salmonella) infections have been most clearly proven for IFN $\gamma$  and IL-12 mediated immunity.<sup>47</sup> Several families, with rare Mendelian disorders of autosomal recessive inheritance which predispose subjects to overwhelming infections by relatively avirulent mycobacteria such as BCG and non-tuberculous mycobacteria, have been elucidated.<sup>47,48</sup> Recessive mutations in four genes encoding: IFN $\gamma$ R1, IFN $\gamma$ R2, IL-12 p40 and IL-12 R $\beta$ 1 have been identified.<sup>47-49</sup> These are isolated defects along a common positive feedback pathway for macrophage stimulation involving IFN $\gamma$  and IL-12 (Fig. 1, step 5). These defects are thus genetically distinct but immunologically related. The clinical phenotype depends upon the nature and severity (partial versus complete) of the defect. Most patients present in early childhood and idiopathic disseminated BCG infection is a common presenting problem. Complete IFN $\gamma$ R1 and IFN $\gamma$ R2 deficiencies result in severe, usually fatal mycobacterial infections with impaired granuloma formation.<sup>48</sup> The phenotype for complete IL-12 p40 and IL-12 R $\beta$ 1 deficiency is less severe with curable infections and well-developed BCG granulomas.<sup>49</sup> Partial IFN $\gamma$ R deficiency is similarly a milder illness. These single gene defects highlight the important roles of IFN $\gamma$  and IL-12 in the immunity to mycobacteria. However, they are rare disorders. While disseminated BCG infections have been reported in Indians and Pakistanis, it has not been reported in Chinese or Singaporeans despite only 98% compliance with BCG vaccination in infancy. Thus, such rare single gene defects are unlikely to account for most of the variations in susceptibility in the general population.

Allelic variations in the gene for the vitamin D3 receptor have been reported to be associated with susceptibility to leprosy in Bengali patients from Calcutta and TB in

Gambia.<sup>50,51</sup> There have been earlier reports of associations between such variations (at codon 353) and osteoporosis.<sup>52</sup> The notion that vitamin D supplementation may prevent both TB and osteoporosis is an intriguing one.

The production of early phase pro-inflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ), tumour necrosis factor  $\alpha$  and interleukin-8 and their down regulation by cytokines such as transforming growth factor- $\beta$ , interleukin-10 and the IL-1 receptor antagonist (IL-1Ra) may also play important regulator roles in the early stages of TB.<sup>5</sup> Polymorphisms in the IL-1Ra gene have been directly associated with variations in IL-1Ra protein production. Individuals positive for allele 2 (IL-1Ra A2+) produce more IL-1Ra. Wilkinson et al<sup>53</sup> conducted a case controlled study on the influence of polymorphism in the genes for IL-1 $\beta$  and IL-1Ra on TB susceptibility and clinical presentation in a population of Gujarati Hindus from Harrow, England. They found that, among 89 patients with TB and 114 controls (mostly non-consanguineous spouses), the IL-1Ra A2+ haplotype was associated with a reduced Mantoux skin reaction while the IL-1Ra A2- haplotype (more pro-inflammatory) was more common in patients with pleural TB and less common in extrapulmonary TB.<sup>53</sup> Thus, genetic polymorphisms which regulate IL-1 activity may determine both the strength of delayed-type hypersensitivity and clinical disease expression in TB.

### Human Chromosome 17q

Blackwell and colleagues used complex segregation analysis in multi-case families in a genome wide screen for gene regions which segregate with TB.<sup>37,45</sup> They combined family-based linkage analysis with transmission disequilibrium testing in 98 pedigrees, 704 individuals and 205 nuclear families from Brazil.<sup>45</sup> They concluded that TB susceptibility was under oligogenic control (the data best fitted either a 1 or 2 locus model) and identified a region of chromosome 17q (candidates NOS2A, SCYA2-5) homologous with mouse chromosome 11 as the likely location.<sup>37</sup>

### Conclusion

In the past decade, major advances in the understanding of genetic susceptibility to TB have resulted from the application of molecular techniques. They include family-based linkage studies to locate major susceptibility genes<sup>37,45</sup> and population-based case-control studies to identify less influential candidate genes.<sup>54-56</sup> These are complimentary methods and have been augmented by comparative genomic studies using animal models.<sup>38</sup> Comprehensive studies have so far only been conducted by a small group of researchers in a few well-defined populations.<sup>37,54-56</sup> Because there is much variation in the results between populations, these studies should be repeated for different geographic

locations and ethnic groups.<sup>54-56</sup> This process may be facilitated by more efficient methods of gene mapping and sequencing such as using sequence tagged sites as common genetic landmarks.<sup>57</sup> It is also very likely that new susceptibility genes will be discovered by comprehensive cataloging of known genes and their variations in sequence by the Human Genome Project<sup>57</sup> and the identification of single-nucleotide polymorphisms within several thousand candidate genes by the National Cancer Institute's Genetic Annotation Initiative<sup>58</sup> using a combination of gel and silicon chip-based techniques.<sup>59-61</sup>

At this present moment it may be reasonable to speculate that further research in genetic regulation of the early events in the encounter between *M. tuberculosis* and the human immune system, such as the IL-1 feedback loop and Nramp 1 mechanism, are most likely to yield advances in immunotherapy or prophylaxis against TB.

### REFERENCES

1. Dye C, Scheele S, Dolin M P, Pathania V., Ravigione M C, for the WHO Global Surveillance and Monitoring Project, World Health Organization. Global burden of tuberculosis estimated incidence, prevalence, and mortality by country. JAMA 1999; 282:677-86.
2. Lim T K. Emerging pathogens for pneumonia in Singapore. Ann Acad Med Singapore 1997; 26:651-8.
3. Stead W W, Lofgren J P, Senner J W, Reddick W T. Racial differences in susceptibility to infection with *M. tuberculosis*. N Engl J Med 1990; 322:422-7.
4. Comstock G W. Tuberculosis in twins: a re-analysis of the Proffit survey. Am Rev Respir Dis 1978; 117:621-4.
5. Schluger N W, Rom W N. The host immune response to tuberculosis. Am J Respir Crit Care Med 1998; 157:679-91.
6. Fenton M J. Macrophages and tuberculosis. Curr Opin Hematol 1998; 5:72-8.
7. Stenger S, Modlin R L. T cell mediated immunity to *Mycobacterium tuberculosis*. Curr Opin Microbiol 1999; 2:89-93.
8. Beyers A D, van Rie A, Adams J, Fenhalls G, Gie R, Beyers N. Signals that regulate the host response to *Mycobacterium tuberculosis*. Novartis Found Sym 1998; 217:145-57.
9. Fearon D T. Seeking wisdom in innate immunity. Nature 1997; 388: 323-4.
10. Medzhitov R, Janeway C A Jr. Innate immunity: the virtues of a nonclonal system of recognition. Cell 1997; 91:295-8.
11. Skamene E, Schurr E, Gros P. Infection genomics: Nramp 1 as a major determinant of natural resistance to intracellular infections. Ann Rev Med 1998; 49:275-87.
12. Unanue E R. Studies in listeriosis show the strong symbiosis between the innate cellular system and the T-cell response. Immunol Rev 1997; 158:11-25.
13. Rook G A W, Hernandez-Pando R, Lightman S L. Hormones, peripherally activated prohormones and regulation of Th1/Th2 balance. Immunol Today 1994; 15:301-3.
14. Henderson R A, Watkins S C, Flynn J L. Activation of human dendritic

- cells following infection with *Mycobacterium tuberculosis*. *J Immunol* 1997; 159:635-43.
15. Fulton S A, Cross J V, Toossi Z T, Boom W H. Regulation of interleukin-12 by interleukin-10, transforming growth factor-beta, tumor necrosis factor-alpha, and interferon-gamma in human monocytes infected with *Mycobacterium tuberculosis* H37Ra. *J Infect Dis* 1998; 178:1105-14.
  16. Zhang M, Gong J, Presky D H, Xue W, Barnes P F. Expression of the IL-12 receptor beta 1 and beta 2 subunits in human tuberculosis. *J Immunol* 1999; 162:2441-7.
  17. Casarini M, Ameglio F, Alemanno L, Zangrilli P, Mattia P, Paone G, et al. Cytokine levels correlate with a radiologic score in active pulmonary tuberculosis. *Am J Respir Crit Care Med* 1999; 159:143-8.
  18. Condos R, Liu Y, Rom W, Schluger N. Local immune responses correlate with presentation and outcome in tuberculosis. *Am Respir Crit Care Med* 1997; 157:729-35.
  19. Hirsch C S, Ellner J J, Blinkhorn R, Toossi Z. *In vitro* restoration of T cell responses in tuberculosis and augmentation of monocyte effector function against *M. tuberculosis* by natural inhibitors of transforming growth factor beta. *Proc Nat Acad Sci USA* 1997; 94:3926-31.
  20. Haas F, Haas S S. The origins of *M. tuberculosis* and the notion of its contagiousness. In: Rom W N, Garay S M, editors. *Tuberculosis*. New York: Little Brown & Co, 1996:3-19.
  21. O'Brien S J. Ghetto legacy. *Curr Biol* 1991; 1:209-11.
  22. Stead W W. Genetics and resistance to tuberculosis. *Ann Intern Med* 1992; 116:937-41.
  23. Stead W W, Bates J H. Geographic and evolutionary epidemiology of tuberculosis. In: Rom W N, Garay S M, editors. *Tuberculosis*. New York: Little Brown & Co, 1996:77-83.
  24. Sousa A O, Salem J I, Lee F K, Vercosa M C, Cruaud P, Bloom B, et al. An epidemic of tuberculosis with a high rate of tuberculin anergy among a population previously unexposed to tuberculosis, the Yanomami Indians of the Brazilian Amazon. *Proc Nat Acad Sci USA* 1997; 94:13227-32.
  25. Margulies D H. Interactions of TCRs with MHC-peptide complexes: a quantitative basis for mechanistic models. *Curr Opin Immunol* 1997; 9:390-5.
  26. Ou D, Mitchell L A, Tingle A J. A new categorization of HLA DR alleles on a functional basis. *Hum Immunol* 1998; 59:665-76.
  27. Kumararatne D S, Pithie A, Drysdale P, Gaston J S, Kiessling R, Iles P B, et al. Specific lysis of mycobacterial antigen-bearing macrophages by class II MHC-restricted polyclonal T cell lines in healthy donors or patients with tuberculosis. *Clin Exp Immunol* 1990; 80:314-23.
  28. Vordermeier H M. T-cell recognition of mycobacterial antigens. *Eur Respir J* 1995; 20:657S-667S.
  29. McPeck M, Salkowitz J, Laufman H, Pearl D, Zwilling B S. The expression of HLA-DR black and white donor monocytes: different requirements for protein synthesis. *Clin Exp Immunol* 1992; 87:163-6.
  30. Crowle A J, Elkins N. Relative permissiveness of macrophages from black and white people for virulent tubercle bacilli. *Infect Immunol* 1990; 58:632-5.
  31. Agrewala J N, Wilkinson R J. Influence of HLA-DR on the phenotype of CD4+ T lymphocytes specific for an epitope of the 16-kDa alpha-crystallin antigen of *M. tuberculosis*. *Eur J Immunol* 1999; 29:1753-61.
  32. Sanjeevi C B, Narayanan P R, Prabakar R, Charles N, Thomas B E, Balasubramanian R, et al. No association or linkage with HLA-DR or -DQ genes in south Indians with pulmonary tuberculosis. *Tuber Lung Dis* 1992; 73:280-4.
  33. Hawkins B R, Higgs D A, Chan S L, Lowrie D B, Mitchison D A, Girling D J. HLA typing in Hong Kong Chest service/British Medical Research Council study of factors associated with the breakdown to active tuberculosis of inactive pulmonary lesions. *Am Rev Respir Dis* 1989; 140:1616-21.
  34. Rajalingam R, Mehra N K, Jain R C, Myneedu V P, Pande J N. Polymerase chain reaction-based sequence-specific oligonucleotide hybridization analysis of HLA class II antigens in pulmonary tuberculosis. *J Infect Dis* 1996; 173:669-76.
  35. Goldfeld A E, Delgado J C, Thim S, Bozon M V, Uglierio A M, Turbay D, et al. Association of an HLA-DQ allele with clinical tuberculosis. *JAMA* 1998; 279:226-8.
  36. Teran-Escandon D, Teran-Ortiz L, Camarena-Olvera A, Gonzalez-Avila G, Vaca-Marin M A, Granados J, et al. Human leukocyte antigen-associated susceptibility to pulmonary tuberculosis: molecular analysis of class II alleles by DNA amplification and oligonucleotide hybridization in Mexican patients. *Chest* 1999; 115:428-33.
  37. Blackwell J M. Genetics of host resistance and susceptibility to intramacrophage pathogens: a study of multicase families of tuberculosis, leprosy and leishmaniasis in north-eastern Brazil. *Int J Parasitol* 1998; 28:21-8.
  38. Qureshi S T, Skamene E, Malo D. Comparative genomics and host resistance against infectious diseases. *Emerg Infect Dis* 1999; 5:36-47.
  39. Vidal S, Tremblay M L, Govoni G, Gauthier S, Sebastiani G, Malo D, et al. The Ity/Lsh/Bcg locus: natural resistance to infection with intracellular parasites is abrogated by disruption of the Nramp1 gene. *J Exp Med* 1995; 182:655-66.
  40. Gruenheid S, Canonne-Hergaux F, Gauthier S, Hackman D J, Grinstein S, Gros P. The iron transport NRAMP2 is an integral membrane glycoprotein that colocalizes with transferring in recycling endosomes. *J Exp Med* 1999; 189:831-41.
  41. Zwilling B S, Khun D E, Wikoff L, Brown D, Lafuse W. Role of iron in Nramp1-mediated inhibition of mycobacterial growth. *Infect Immun* 1999; 67:1386-92.
  42. Rojas M, Olivier M, Gros P, Barrera L F, Garcia L F. TNF-alpha and IL-10 modulate the induction of apoptosis by virulent *M. tuberculosis* in murine macrophages. *J Immunol* 1999; 162:6612-31.
  43. Abel L, Sanchez F O, Oberti J, Thuc N V, Hoa L V, Lap V D, et al. Susceptibility to leprosy is linked to the human NRAMP1 gene. *J Infect Dis* 1998; 177:133-45.
  44. Bellamy R, Ruwende C, Corrah T, McAdam K P, Whittle H C, Hill A V. Variations in the NRAMP1 gene and susceptibility to tuberculosis in West Africans. *N Engl J Med* 1998; 338:640-4.
  45. Shaw M A, Collins A, Peacock C S, Miller E N, Black G P, Sibthorpe D, et al. Evidence that genetic susceptibility to *M. tuberculosis* in a Brazilian population is under oligogenic control: linkage studies of candidate genes NRAMP1 and TNFA. *Tuber Lung Dis* 1997; 78:35-45.
  46. Searle S, Blackwell J M. Evidence for functional repeat polymorphism in the promoter of human Nramp1 gene that correlates with autoimmune versus infectious disease susceptibility. *J Med Genet* 1999; 36:295-9.
  47. Altare F, Jouanguy E, Lamhamedi S, Doffinger R, Fischer A, Casanova J L. Mendelian susceptibility to mycobacterial infection in man. *Curr Opin Immunol* 1998; 10:413-7.
  48. Jouanguy E, Doffinger R, Dupuis S, Pallier A, Altare F, Casanova J L. IL-12 and IFN- in host defense against mycobacteria and salmonella in mice and men. *Curr Opin Immunol* 1999; 11:346-51.
  49. de Jong R, Altare F, Haagen I A, Elferink D G, Boer T, van Breda Vriesman P J, et al. Severe mycobacterial and Salmonella infections in interleukin-12 receptor-deficient patients. *Science* 1998; 280:1435-8.
  50. Bellamy R, Ruwende C, Corrah T, McAdam K, Thursz M, Whittle H C,

- et al. Tuberculosis and chronic hepatitis B virus infection in Africans and variation in the vitamin D receptor gene. *J Infect Dis* 1999; 179:721-4.
51. Roy S, Frodsham A, Saha B, Hazra S K, Mascie-Taylor C G, Hill A V. Association of vitamin D receptor genotype with leprosy type. *J Infect Dis* 1999; 179:187-91.
  52. Eisman J A. Vitamin D receptor gene variants: implications for therapy. *Curr Opin Genet Dev* 1996; 6:361-5.
  53. Wilkinson R J, Patel P, Llewelyn M, Hirsch C S, Pasovol G, Snounou G, et al. Influence of polymorphism in the gene for the interleukin-1 receptor antagonist and IL-1 $\beta$  on tuberculosis. *J Exp Med* 1999; 189:1863-73.
  54. Bellamy R. Genetics and pulmonary medicine. 3. Genetic susceptibility to tuberculosis in human populations. *Thorax* 1998; 53:588-93.
  55. Bellamy R, Hill A V S. Genetic susceptibility to mycobacteria and other infectious pathogens in humans. *Curr Opin Immunol* 1998; 10:483-7.
  56. Hill A V. The immunogenetics of human disease. *Ann Rev Immunol* 1998; 16:593-617.
  57. Schuler G D. Electronic PCR: bridging the gap between genome mapping and genome sequencing. *Trends Biotech* 1998; 16:456-9.
  58. Collins F S. 1999. Shattuck lecture-medical and societal consequences of the Human Genome Project. *N Engl J Med* 1998; 341:28-37.
  59. National Cancer Institute Genetic Annotation Initiative. <http://lpg.nci.nih.gov/GAI>
  60. Wang D G, Fan J B, Siao C J, Berno A, Young P, Sploski R, et al. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science* 1998; 280:1077-82.
  61. Gilles P N, Wu D J, Foster C B, Dillon P J, Chanock S J. Single nucleotide polymorphic discrimination by an electronic dot blot assay on semiconductor microchips. *Nat Biotechnol* 1999; 17:365-70.
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