FACTORS AFFECTING SUSCEPTIBILITY AND RESISTANCE TO TUBERCULOSIS

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Introductory article

Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study

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*Background: Susceptibility to disease after infection by Mycobacterium tuberculosis is influenced by environmental and host genetic factors. Vitamin D metabolism leads to activation of macrophages and restricts the intracellular growth of M tuberculosis. This effect may be influenced by polymorphisms at three sites in the vitamin D receptor (VDR) gene. We investigated the interaction between serum vitamin D (25-hydroxycholecalciferol) concentrations and VDR genotype on susceptibility to tuberculosis. Methods: This study was a hospital-based case-control analysis of Asians of Gujarati origin, a mainly vegetarian immigrant population with a high rate of tuberculosis. We typed three VDR polymorphisms (defined by the presence of restriction endonuclease sites for Taq1, Bsm1, and Fok1) in 91 of 126 untreated patients with tuberculosis and 116 healthy contacts who had been sensitised to tuberculosis. Serum 25-hydroxycholecalciferol was recorded in 42 contacts and 103 patients. Findings: 25-hydroxycholecalciferol deficiency was associated with active tuberculosis (odds ratio 2.9 [95% CI 1.3–6.5], p=0.008), and undetectable serum 25-hydroxycholecalciferol (<7 nmol/l) carried a higher risk of tuberculosis (9.9 [1.3–76.2], p=0.009). Although there was no significant independent association between VDR genotype and tuberculosis, the combination of genotype TT/Tt and 25-hydroxycholecalciferol deficiency was associated with disease (2.8 [1.2–6.5]) and the presence of genotype ff or undetectable serum 25-hydroxycholecalciferol was strongly associated with disease (5.1 [1.4–18.4]). Interpretation: 25-hydroxycholecalciferol deficiency may contribute to the high occurrence of tuberculosis in this population. Polymorphisms in the VDR gene also contribute to susceptibility when considered in combination with 25-hydroxycholecalciferol deficiency. (**Lancet** 2000;355:618–21)*

THE GLOBAL INCIDENCE OF TUBERCULOSIS

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Tuberculosis is one of the principal infectious causes of disease and death worldwide, yet there are very marked differences in its incidence from region to region. At one extreme there is an incidence of disease of less than 5/100 000 per annum in the population of Western Europe,[1 2] while at the other extreme the incidence among miners in South Africa is in excess of 800/100 000.[3] Intermediate incidences of 50–100/100 000 and 100–200/100 000 are reported, respectively, in Eastern Europe and the Indian subcontinent. These variations are usually due to obvious factors such as lifestyle, economic status, occupational exposure, and HIV infection, but there may well be genetic and ecological factors that contribute to the observed variations.

Although tuberculosis is so prevalent that it has been declared a global emergency, most people do not develop disease after infection. The ratio of infection to disease—the disease ratio—is about 10%. Thus, approximately one third of the world population—two billion people—have been infected with the tubercle bacillus,[4] but the great majority only have latent tuberculosis infection (LTBI) demonstrable by a positive tuberculin skin test. From this infected third of mankind, 8–10 million new cases of tuberculosis arise every year and 2–3 million of these eventually die of the disease. There has been much discussion over what factors allow persistence of infection and infection to progress to overt disease.

In particular, the question of the relative importance of genetic and environmental factors in determining the changes in prevalence over time of tuberculosis in a community has been raised

Some putative determinants of virulence of Mycobacterium tuberculosis.

- Factors that enhance the entry of bacilli into cells including complement receptors and terminal mannose on cell wall lipoarabinomannan side chains.
- Factors that prevent phagosome-lysosome fusion within the macrophage.
- Toxic lipids in the cell wall: acidic sulpholipids and trehalose dimycolate ("cord factor").
- Factors that protect the parasites against bactericidal enzymes and reactive oxygen and nitrogen metabolites within the macrophages, e.g. superoxide dismutase.
- Secreted antigens such as the 19 kd antigen that interfere with antigen recognition required for the development of protective immune responses.
- Mycobacterial iron chelating agents such as mycobactins and exochelins.
- Factors regulating the ability of tubercle bacilli to persist in an inactive state in tissues: "wake up genes" and inducers of the dormant state, e.g. isocitrate lyase.

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by observations on the steady decline in the incidence of the disease seen in England during Victorian times.[5] While the number of cases of tuberculosis declined by around 1.7% annually between 1850 and 1910, no other indicator of mortality (total mortality, infant mortality or death from cholera or typhoid) declined in a statistically significant way over that period. The only significant change occurring over that period was an increase in income of 1.2% annually, although this was not parallelled by a reduction in overcrowding.

An analysis of factors determining the risk of overt disease following infection must take into consideration both the virulence of the causative organism and the immune defences of the infected person. Louis Pasteur termed these factors "the seed and the soil". Until recently, immunological studies on tuberculosis focused on immune reactivity in those with the disease, but attention is now turning to why the majority of infected persons remain healthy. In this context it is important to determine whether those who remain healthy have a genetically endowed high level of resistance to tuberculosis or whether resistance is affected by environmental or other exogenous factors that are subject to change and, perhaps, to modification or correction.

The seed

The mechanisms of virulence of the tubercle bacillus are poorly understood and, although determinants of virulence have been described, it has proved very difficult to establish the relative importance of these in human tuberculosis.[6] Some putative determinants of virulence are shown in the box. Although experimental animals such as the guinea pig or rabbit have been used to quantify the virulence of tubercle bacilli, much doubt has been shed on the relevance of these studies to human infection. For example, some strains of *Mycobacterium tuberculosis* originating from South India and others that are resistant to isoniazid are of very low virulence in the guinea pig yet, apparently, fully virulent in humans.[7]

Mycobacterium bovis, the causative agent of tuberculosis in cattle, illustrates the difficulty in determining the virulence of a variant of the tubercle bacillus in humans. It is generally assumed that *M bovis* is of lower virulence than *M tuberculosis* to humans but firm evidence for this is elusive. A mathematical model based on the ratio of infection to disease in urban and rural communities, on the somewhat dubious assumption that rural communities would largely be infected by *M bovis*, established that the risk of developing late post-primary pulmonary tuberculosis after infection by *M bovis* was 2–10 times less than after infection by *M tuberculosis*.[8 9] It was, however, emphasised in these studies that this difference might reflect the route of infection rather than difference in virulence between the two bacilli. In addition, any difference might be attributable to many environmental differences between urban and rural populations, as discussed below.

Notwithstanding, despite the difficulties in assessing the virulence of tubercle bacilli, some recent reports from the USA raise the possibility of the emergence of strains of *M tuberculosis* with unusually high virulence for humans. In one such example a strain causing active tuberculosis in five of 18 infected persons after brief and casual exposure to the source cases was shown to replicate more rapidly than the reference strain (the Erdman strain) in the lungs of mice.[10] In another example a strain responsible for a large outbreak of tuberculosis in Los Angeles replicated significantly more rapidly in human macrophages in vitro than strains responsible for small clusters or single cases.[11] This, of course, raises the question as to whether the experimental models used really reflect virulence differences in humans. Nevertheless, the findings are a cause of concern as we might be at the dawn of an era of extensive outbreaks of tuberculosis due to highly virulent bacilli.[12] If they do indeed exist, such bacilli will obviously spread through the community more rapidly than strains of standard virulence and vigilance is required.

The soil

The relative impact of innate, largely genetically determined, specific and non-specific immune defences and the impact of the environmental factors must be considered in relation to the disease ratio.

Natural selection

The concept that the process of natural selection was responsible for the sharp decline in the incidence of tuberculosis in the developed nations over the 20th century has been widely debated. While some argue that better social conditions were responsible for the changes,[13] others, citing the evidence presented above, maintain that social improvements do not give the complete explanation for the observed declines.[5] The possibility that natural selection played at least some role in this decline in the pre-chemotherapeutic era cannot therefore be dismissed.

It has been postulated—on the basis of a hypothetical model of the rise and fall of tuberculosis in a previously uninfected population—that, following the introduction of the disease into a community, the incidence would increase rapidly over the first half century to several hundred cases per hundred thousand of the population annually.[14] It would thereafter decline to around 5/100 000 some 150 years after the peak of incidence, a decline of 4.7% annually. This predicted course of the disease closely reflects the observed epidemiological changes in Western Europe over the previous two centuries. In the United Kingdom, for example, case rates reached a peak between 1800 and 1850, when the disease caused a quarter of all deaths, before declining, initially at a rate of less than 2% annually but increasing to almost 10% annually after the introduction of BCG vaccination and specific chemotherapy in the 1950s.

The global spread of tuberculosis

The urbanisation associated with the industrial revolution prevalent in Western Europe corresponded with the time of increased world trade and empire building. This led to the export of tuberculosis to the rest of the world where case rates peaked some time later—around 1880 in North America, 1900 in India and Africa, and as late as 1950 among the First Nation peoples of Canada and Papua New Guinea.

It would therefore appear that different populations across the globe are at different stages in the pandemic of tuberculosis. The white population of Western Europe, after a 200 year history of the disease, would appear to be at or near the end of the epidemic, but the populations of the developing nations are only half way down the slope with a "natural" annual incidence of tuberculosis of 100–200/100 000.

Although only hypothetical, the above pandemic model based largely on a process of natural selection accurately describes the changing trends observed in tuberculosis up to the present time. This suggests that nature rather than nurture determines the trend of the disease. Against this argument, however, is the skeletal evidence of tuberculosis in the dispersed indigenous populations in pre-Columbian America. It has been suggested therefore that tuberculosis occurred sporadically in dispersed populations well adapted to their environment but increased substantially in prevalence when these peoples were subjected to stress and overcrowding by their European colonisers.[16 17] Major social change may therefore be more important than natural genetic selection in determining the observed behaviour of tuberculosis in a population.

Genetic basis of variations in resistance

There have been many claims that populations and racial groups vary in their resistance to tuberculosis but it has been very difficult to separate the genetically determined factors from environmental ones. Nevertheless, several studies on monozygotic and dizygotic twins clearly indicate that inheritable factors are involved in determining susceptibility and resistance to overt tuberculosis after infection.[18] The human immune response to tuberculosis is multifactorial, involving the uptake of tubercle bacilli by macrophages, class II HLA determined antigen presentation, activation of macrophages by T cell derived cytokines and vitamin D,[19 20] granuloma formation, and apoptosis of bacteria laden cells. These processes are genetically controlled and several of the genes involved have been identified. Although much interest focused on the HLA genes (notably the HLA-DR2 and HLA-DQB1 loci) that determine which mycobacterial antigens are presented to the helper T cells,[21 22] interest has now largely shifted to non-HLA genes. Of these, two are of particular interest—the genes coding for the vitamin D receptor[19] and the natural resistance associated macrophage protein (NRAMP1) gene.[23] The latter is the human equivalent of a gene found to determine resistance to intracellular parasites such as *Leishmania* and *Salmonella* in the mouse and it clearly affects human resistance to tuberculosis. Its function is unknown but there is evidence that it regulates the concentration of iron and perhaps other cations in the phagosomes of the macrophage.

Other genes affecting immune function have also been associated with resistance to tuberculosis.[24] These include genes determining the allotypes of the Km1 light chain immunoglobulin allotype[25] and of haptoglobin,[26 27] but it is not clear whether these are of prime importance or merely reflect gene linkage. Other genes currently under investigation include those affecting vitamin D levels (see below), the mannose binding lectin protein,[28] and the various cytokines involved in protective immunity.[29]

Table 1 Factors involved in susceptibility or resistance to tuberculosis and subject to genetically determined variation.

Factor or gene	Function or mode of action
Natural resistance associated macrophage protein (NRAMP 1)	?Regulation of phagosome cation levels
Mannose binding lectin protein	Entry of mycobacteria into cells
HLA-D	Antigen presentation
Vitamin D	Macrophage activation
Locus on chromosome 15	Unknown
Locus on X chromosome	Unknown, may account for higher rates of tuberculosis in males
Haptoglobin	?Regulator of lymphocyte function
Km1 immunoglobulin allotypes	?Related to autoimmune tissue damage
Various cytokines	T cell maturation and patterns of immune reactivity

Although there have been a few examples of susceptibility to tuberculosis and other mycobacterial diseases within families associated with specific defects in gamma-interferon and other cytokines, family based linkage studies and population based case-control studies clearly indicate that resistance to tuberculosis is generally the result of a large number of genes inherited in a complex way.[24] The factors involved in susceptibility and resistance to tuberculosis that have been shown to vary according to genotype are summarised in table 1.

Acquired factors affecting susceptibility to tuberculosis

Any condition which compromises the integrity of the immune responses predisposes to the development of active tuberculosis and to an adverse outcome. In recent years, HIV infection has emerged as the most important and prevalent predisposing factor for tuberculosis worldwide. Other immunocompromising factors include congenital immunodeficiencies, high dose steroid therapy, cytotoxic drugs, immunosuppressive drugs, protein-calorie malnutrition, acute viral infections especially measles in children, schistosomiasis, renal failure, liver failure, haematological malignancies and other cancers, diabetes mellitus, and local lung damage due to smoking or industrial dust disease.

Protective immunity and delayed type hypersensitivity

In addition to genetic factors, there is increasing evidence that the pattern of immune reactivity in tuberculosis, leading to protection or tissue destroying hypersensitivity, is influenced by environmental factors. Several studies have shown that the outcome of infection by *M tuberculosis* is dependent on the maturation pattern, Th1 and Th2, of the helper T cells.[30] Protective immunity is associated with Th1 cytokine mediated macrophage activation and granuloma formation but a superimposed Th2 response elicits tissue destroying delayed hypersensitivity reactions. This crucial difference in immune reactivity is related to the cytokine regulated effect of tumour necrosis factor (TNF)α on the tissues at the site of the lesions.[31] Thus, while TNFα plays a key role in the development of the granuloma in a Th1 mediated response, Th2 associated cytokines, by a direct or indirect mechanism, render tissues at the site of the immune reaction extremely sensitive to killing by TNFα. This pattern of reactivity causes massive tissue necrosis and pulmonary cavity formation.

It is therefore important to determine what factors are responsible for the undesirable superimposition of the Th2 component in the immune responses in tuberculosis. The Th2 component may be induced by endogenous hormonal factors, particularly the balance between the two major adrenocortical hormones, glucocorticoids and dehydro-epiandrosterone (DHEA).[32] Glucocorticoids favour Th2 maturation and, as levels are increased by stress, this could provide an explanation for the claims that stress reduces resistance to tuberculosis.[33] In addition, there is evidence that Th1 and Th2 responses to *M tuberculosis* are determined by immunologically effective contact with antigens of environmental mycobacteria. The nature and extent of such contact varies from region to region and is thought to be responsible for the very significant regional differences in the protective efficacy of BCG.

Bovine tuberculosis

An example of the effect of contact with a mycobacterium of relatively low virulence on subsequent immunity to infection by *M tuberculosis* is provided by the bovine tubercle bacillus, *M bovis*. Tuberculosis in cattle became prevalent throughout Europe in the mid 19th century following the introduction of intensive dairy farming methods. In the early 20th century up to 40% of dairy herds contained cows with tuberculosis and, although the udder was involved in the disease in only 1% of cows, the risk of milk-borne infection increased by the bulk mixing of milk from several herds to facilitate transport to urban areas.

Milk-borne *M bovis* was a serious cause of tuberculosis in children, notably non-pulmonary lesions including tuberculous cervical lymphadenitis (scrofula), but epidemiological studies showed that the risk of developing overt disease following infection by *M bovis* was 2–10 times less than after infection by *M tuberculosis*. There was also epidemiological evidence—though, by modern standards, rather dubious—that self-healing scrofula in childhood afforded protection against pulmonary tuberculosis in adult life, the so-called "Marfan's law".[34] This concept was supported by a negative correlation between the prevalence of bovine tuberculosis in cattle and the morbidity and mortality due to tuberculosis in the human population of Scandinavia.[35] More recently, and perhaps for the same reason, it has been observed that the incidence of human tuberculosis in the northern sector of Burkino Faso (Upper Volta), where bovine tuberculosis is uncommon, is five times higher than in the southern sector where the latter is common.[36]

Accordingly, the changing prevalence of bovine tuberculosis and the risks of human infection may have had a profound impact on the incidence of all forms of tuberculosis in the human population. The supposed immunising effect of *M bovis* was even used as an argument against the bovine tuberculosis eradication programmes. Fortunately, the immunising effect of infection by *M bovis* could be replaced by the administration of its attenuated derivative, BCG vaccine.

Variations in the incidence of tuberculosis within geographical regions

In addition to the genetic and environmental factors contributing to resistance and susceptibility to tuberculosis, consideration must be given to the socioeconomic factors responsible for the very great differences in the incidence of tuberculosis within countries. Tuberculosis has long been considered a disease of the poor and socially disadvantaged. A radiographic survey of homeless people seeking temporary shelter over two Christmas periods in London revealed active tuberculosis in 2% of them.[37] A study in Liverpool showed that tuberculosis was closely associated with deprivation throughout the 33 electoral wards of the city,[38] and in Birmingham tuberculosis was found to be linked to poverty in the indigenous white population but not among the Asian population.[39]

Tuberculosis is also associated with migration, as has been well illustrated by successive groups of migrants to the UK since the end of the Second World War. Very high rates of tuberculosis were recorded in immigrants from the Indian subcontinent in 1958,[40] and subsequent surveys have revealed rates of pulmonary tuberculosis in this population 20–30 times higher than in the indigenous white population. All types of non-pulmonary tuberculosis, with the notable exception of renal tuberculosis, are particularly common in immigrant groups with rates up to 150 times higher than in the indigenous population.[1 41]

In general, higher rates of tuberculosis are seen in recent immigrants than in those who have been resident in the UK for longer periods. Within the Indian subcontinent ethnic group, rates in those aged 35 years or over and resident in the country for less than 5 years before presenting with disease were over 1000/100 000, and a recent survey has shown that rates in those classified as Black African now exceed those from the Indian subcontinent ethnic group.[2]

Reasons for high rates and diversity of presentations of tuberculosis in immigrant groups

It is not easy to determine the cause of the high rates of tuberculosis in immigrant populations. While innate differences in resistance may occur, these must be considered in the light of numerous non-inherited lifestyle and social factors.[42 43] In particular, it is difficult to separate immigration per se from poverty. Thus, while the immigration index had the strongest explanatory power in accounting for tuberculosis rates in relation to deprivation indices across the metropolitan districts of England,[44] a study of the recent increase in tuberculosis in England in relation to the deprivation indices showed that the rise had occurred exclusively within the poorest 30% of the residential areas.[45] It was therefore concluded that high rates of tuberculosis are related to poverty rather than to the ethnic origins of the patients, although the study did not take into account the fact that the great majority of those in the ethnic minority groups live in poor areas.[46]

In this respect, studies in the USA have led to the conclusion that low socioeconomic status accounts for much of the risk of tuberculosis previously thought to be related to race or ethnicity.[47] Thus, the risk of tuberculosis in all racial and ethnic groups was related to low income, poverty, need for public assistance, unemployment, poor education and, most strongly, with overcrowding.

Other lifestyle factors also affect the risk of tuberculosis. A case-control study of 112 patients who had had tuberculosis and controls matched for age, sex, and ethnic origin was conducted in Liverpool.[48] On multivariate analysis, controls were 4.0 times more likely than tuberculosis patients to have more than one bathroom—an indicator of higher socioeconomic status—and 3.8 times more likely to have consumed dairy products every week, suggesting that dietary factors such as vitamins might have enhanced protective immunity. Controls were also 2.3 times less likely to have

Learning points

- The current world pandemic of tuberculosis probably originated in Europe 200 years ago with the Industrial Revolution.
- The difference in risks across the world is partly related to the speed at which the disease has increased and decreased as it is moved across the world.
- Tuberculosis declined in the UK over a 150 year period before drugs were introduced; this may be related to a process of natural selection.
- Current specific genes such as HLA-DR2 may be related to susceptibility to tuberculosis.
- The Th1/Th2 responses can alter the pattern of the host immune response to tuberculosis.
- Within the UK poverty has been shown to be closely associated with tuberculosis but, in areas with a large population of ethnic minority subjects, being from an ethnic minority group is a greater risk.

smoked more than 20 cigarettes a day which could reflect differences in socioeconomic status or point to a detrimental effect on pulmonary immune defence mechanisms. In this context, an extensive study on risk factors among health workers in China showed that smoking was the leading risk factor for tuberculosis.[49]

The relatively high incidence of extrathoracic manifestations of tuberculosis in the Indian subcontinent ethnic group has been referred to above. This has not been adequately explained. As a similar high proportion of non-respiratory disease is observed in AIDS patients, it has been postulated that immigration might be associated with some form of acquired immunodeficiency.[50] One distinct possibility is the lowering of serum vitamin D (25 hydroxycholecalciferol) levels up to tenfold that occurs in immigrants from developing countries after arrival in the relatively sunless United Kingdom.[51] This vitamin is an important mediator of macrophage activation which is central to protective immunity in tuberculosis[52]; indeed, this explains the claimed success of cod liver oil and sunlight in the treatment of tuberculosis in the pre-chemotherapeutic era.[53] Thus, infected people might have latent tuberculosis while in their own country but develop active disease when their vitamin D levels fall.

The relationship between vitamin D and susceptibility to tuberculosis is, as mentioned above, affected by genetic factors. In India a study of the wives of men with tuberculosis suggested that the homozygous TT vitamin D receptor (VDR) genotype was associated with resistance and the tt genotype with susceptibility to the disease.[54] The expression of the impact of VDR genotype may be accentuated by vitamin D deficiency. This was the case in a predominantly vegetarian population of Gujarati Asians resident in west London. Those with serum vitamin D levels too low for detection had an almost tenfold increase in their risk of active tuberculosis.[20] Although there was no independent association between the VDR genotype and the risk of tuberculosis, the combination of the TT/Tt VDR genotype and vitamin D deficiency was associated with disease and a combination of the tt genotype and undetectable serum vitamin D had a fivefold stronger association with disease.

Factors influencing bacillary persistence

One of the most important factors in the development of tuberculosis is the poorly understood ability of the tubercle bacillus to persist in the tissues for long periods of time. Very recent evidence has cast interesting new light on the factors which may influence bacillary persistence. The use of polymerase chain reaction (PCR) to detect DNA specific for *M tuberculosis* in cadaveric lungs has shown that this bacterium can persist intracellularly without histological evidence of tuberculosis lesions. *M tuberculosis* DNA is situated, not only in macrophages, but also in other cells not normally regarded as phagocytic such as type II pneumocytes, endothelial cells, and fibroblasts. These cells, rather than the healed granuloma, might offer protected sites for persistent bacilli. These findings contradict the dominant view that latent organisms exist in old classic tuberculosis lesions and have relevance to strategies aimed at eliminating latent and persistent bacilli.[55 56]

Experiments in the mouse model have shown that the enzyme isocitrate lyase (ICL) is necessary for the persistence of *M tuberculosis*. Intracellular pathogens require essential nutrients from the host to survive. Fatty acids provide a source of carbon and energy for *M tuberculosis*. ICL is one of the glyoxylate shunt enzymes necessary for the production of fatty acids, so its reduction may result in early elimination of the bacteria. When mice are infected with the wild strain of *M tuberculosis* there is an initial rise in bacterial number in the lungs followed by a plateau in bacterial burden as a result of acquired immunity. The mice eventually die months later from gradual destruction of lung tissue. This plateau phase may be equivalent to the latent infection phase in human tuberculosis. New evidence shows that a strain of *M tuberculosis* with a mutation in the ICL gene grows normally in the mouse tissues for 2 weeks of infection but than shows a late stage defect and is cleared from the lungs. Thus, the development of ICL inhibitors offers hope for a new form of chemotherapy to remove LTBI and eliminate persistent bacilli.[57 58]

Conclusions

Both nature and nurture must be contributing to the risk of tuberculosis. If undue emphasis is put on inheritable factors, the humanitarian need to improve the lot of the poorest of

the world who run the greatest risk of disease will be overlooked. On the other hand, an emphasis on nurture may well have contributed to the initial failure to detect and acknowledge the resurgence of the disease that has occurred in the developed countries in recent years and its threat to all levels of society. The "nature-nurture" debate will continue but, thanks to the recent mapping of the human genome, fact may soon replace speculation.

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