NRAMP1 (SLC11A1) and vitamin D receptor genes: disease associations

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ABSTRACT

Resistance to infectious diseases is complex, being regulated by multiple genes, and so far no one locus has been shown to dominate the susceptibility in outbred human populations. Multi-case families have contributed greatly to defining important host immune response pathways using a candidate gene approach. It is likely that genome scans will identify novel genes for resistance to disease that could help define new approaches to drug and vaccine development.

INTRODUCTION

Susceptibility to infectious diseases is under polygenic control, as exemplified by genetic susceptibility to malaria. Severe *Plasmodium falciparum* malaria exerts enormous selective pressure on populations living in malaria-endemic areas. In the era before the advent of antimalarial medicines, those who could not survive malaria and develop effective immunity were wiped out at a young age and only those who survived were able to pass on their genes to the next generation. The sickle-cell haemoglobin mutation (S) confers protection against malaria in its single-dose heterozygote form (AS) and provides a selective advantage, despite the disadvantage conferred by the two-dose (SS) form in homozygotes.[1] Many genes have now been described as providing resistance against malaria, including various haemoglobin variant genes giving rise to thalassaemia[2] and glucose-6-dehydrogenase deficiency, as well as class I and II HLA molecules. HLA B53 is found in 30% of the Gambian population whereas it is uncommon in Europe. The nine-amino-acid peptide that fits best in the groove of HLA-B*53 codes for a portion of the malarial liver-specific antigen LSA-1, presumably demonstrating that the selective advantage of B*53 identifies a vulnerable target in the parasite life cycle. Following on from this work in The Gambia, new malaria vaccine constructs have been designed to include this and other liver-stage antigens not previously considered as vaccine candidates. The promise that identification of susceptibility/resistance alleles can lead to the development of new approaches to vaccines or therapeutic agents has encouraged us in The Gambia to pursue studies of the genetics of disease susceptibility. We have focused on important endemic infectious diseases in Africa including malaria, tuberculosis, hepatitis B, chlamydia and HIV. This paper briefly summarises our efforts on two candidate genes, NRAMP 1 and vitamin D receptor, to define susceptibility to tuberculosis.

GENETIC SUSCEPTIBILITY TO TUBERCULOSIS

Heritability of tuberculosis has been suggested by various studies in twins,[3] by racial differences in resistance to TB[4,5] and by animal studies in rabbits and inbred strains of mice.[6,7] Families expressing a high rate of mycobacterial disease have been important in defining various Mendelian susceptibilities in the interferon-γ and IL-12 pathways.[8-11]

Most people who breathe in aerosol droplets containing *M. tuberculosis* become infected. However, only 5 to 10% of close contacts of a case of open pulmonary TB go on to develop tuberculosis, and it has been assumed that this susceptibility is related to the effectiveness of the immune system. Many factors including genetic background influence the innate and acquired arms of the immune response. Poor nutrition, alcoholism and immunosuppression due to drugs or other infections, including HIV and measles, have been implicated in an increased susceptibility to TB.

An increasing number of genes have been implicated in susceptibility to tuberculosis. HLA is perhaps the best documented, with conflicting positive and negative associations reported. An increased frequency of HLA-DR2 is the most consistent association but has not been found in all populations examined, nor was there clear linkage to the MHC in a genome-wide screen carried out on patients from The Gambia and South Africa.[12]

When the Scottish missionary Dr David Livingstone travelled through Central Africa in the mid 19th century he did not see a lot of TB, though he would have observed plenty in Britain. Senegalese troops who fought in the 1914-18 war contracted TB at a higher rate than European soldiers, suggesting that they were nonimmune and had not been exposed to *M. tuberculosis* previously, or perhaps were more susceptible genetically. Stead reported that black Americans in Arkansas nursing homes had twice the risk of acquiring *M. tuberculosis* infection compared with white subjects.[4] Crowle confirmed a similar trend *in vitro*, showing that macrophages from blacks were more easily infected and less activated by vitamin D.[5]

VITAMIN D RECEPTOR AND TUBERCULOSIS

Historically, there has been circumstantial evidence for an association between vitamin D and tuberculosis. Prior to effective chemotherapy, patients were sent off to sanatoria where they were exposed to fresh air and sunlight, which was thought to provide the best environment for recovery. Patients with skin TB or lupus vulgaris responded well to vitamin D therapy but some patients with pulmonary tuberculosis fared badly. Rook showed that macrophages stimulated with vitamin D were activated, providing a rationale for these anecdotal clinical observations.[13] The active form of vitamin D, 1,25 dihydrocholecalciferol (1,25D3), binds the vitamin D receptor (VDR) in the cytoplasm of macrophages and activated lymphocytes. VDR is a zinc-finger DNA-binding transcription factor which migrates to the nucleus, resulting in the expression of a number of hormone-sensitive genes.

VDR maps to chromosome 12q13-14, and belongs to the steroid/thyroid hormone receptor family with 11 exons. Polymorphisms within the VDR gene include a Taq I site at codon 352, an Apa I site and a Bsm I site in intron 8 and a Fok I site in exon 2 which leads to an alternative transcription site. Although the association between VDR polymorphisms and bone mineral density has been hotly debated,[14] the homozygous Taq I genotype 'tt' has been associated with decreased bone mineral density (osteoporosis). Bellamy *et al.* found this genotype less commonly in 400 TB cases in The Gambia than in the 400 ethnically matched controls (odds ratio 0.53, 95% confidence limits 0.31-0.88, p=0.01).[15] Wilkinson *et al.* undertook a case-control study of Gujarati Indians in West London, demonstrating an association between TB and a combination of VDR genotype and serum 1,25D3 concentration.[16] Another study in India did not confirm an association with VDR, though the TT genotype was overrepresented in female cases compared with controls.[17] Vitamin D receptor polymorphism has been shown to be relevant to susceptibility to leprosy in Calcutta, where 'tt' was overrepresented in tuberculoid cases and 'TT' was associated with lepromatous disease.[18] Interestingly, 'tt' has also been associated with enhanced clearance of Hepatitis B virus, suggesting that 1,25D3 and VDR may play a more central role in regulating immune responses against infections.[15]

NATURAL RESISTANCE ASSOCIATED MACROPHAGE PROTEIN 1

Natural resistance associated macrophage protein 1 (NRAMP1) has recently been renamed SLC11A1 (Solute Carrier family11 member1). Originally described in inbred mouse strains, this locus was confirmed to be a single gene on chromosome 1 when a knockout mouse proved NRAMP1 was responsible for resistance to several intracellular infections.[19] A single amino acid change on the 4th transmembrane domain of the protein resulted in an absence of the protein in susceptible mice.[7,20] This naturally occurring mutation led to a Gly-Asp substitution at amino acid 169, which enhanced susceptibility in mice to intracellular infections with leishmania, salmonella, toxoplasma, candida and mycobacteria.[19]

In humans the NRAMP1 gene is located on chromosome 2q 35[21] and has been associated/linked to various infectious diseases (tuberculosis, leprosy, [?meningococcal meningitis], leishmaniasis and HIV) and autoimmune diseases (rheumatoid arthritis, diabetes, sarcoidosis, and Crohn's disease).[22-26]

In 400 cases and controls in The Gambia, Bellamy and colleagues have shown that four variants of the NRAMP1 gene are significantly associated with an increased risk of developing cavitary pulmonary tuberculosis.[23] In the patient group there was an increased risk (odds ratio 4.08, 95% confidence interval 1.86-9.12, p<0.001) of TB in individuals heterozygous for both a single nucleotide polymorphism (SNP) in intron 4 and a 'TGTG' deletion in the 3'untranslated region (3'UTR).[23] A family-based study from Guinea-Conakry confirmed the association with the intron 4 polymorphism[27] and a case-control study has confirmed the association with the 3'UTR deletion in Koreans.[28] Gao *et al.* have found associations with a 5' promoter region polymorphism in

Japanese patients.[29] Linkage to a chromosome 2 region, which includes the NRAMP1 gene, has been documented in a Canadian aboriginal family.[30] However, the results from a linkage study in a Brazilian population showed no evidence of NRAMP1 involvement in susceptibility to TB.[22] Moreover, a genome-wide screen demonstrates that NRAMP1 is not a major susceptibility gene in Gambians, in whom only a weakly positive linkage was shown to 2q 35.[12]

Slc11a1 is found in late endosomes and lysosomes but not in early endosomal membranes. It is unclear how Slc11a1 functions in the maturation of the phagolysome nor in the reduced acidification found in mycobacterium-containing lysosomes. Slc11a1 is a proton/divalent cation transporter (Fe, Zn, Mn) which can move divalent cations in either direction against a proton gradient. Thus cations can be delivered from the cytosol to the acidic compartment of late endosomes/lysosomes, where they catalyse the production of reactive oxygen intermediates (ROI) including hydroxyl radicals. Killing of mycobacteria probably involves both ROIs as well as reactive nitrogen intermediates (RNI) generated by nitric oxide synthetase NOS2, and by apoptosis of macrophages induced by ATP through the P2X7 receptor.[31]

Blackwell has defined four alleles containing different numbers of DNA repeats at positions (i)203, (ii)201, (iii)199 and (iv)189, with allele frequencies of (ii) and (iii) being 0.25 and 0.75 respectively.[26] Using a reporter gene system, LPS drives five to eightfold higher gene expression by allele (iii) and significant reduction in expression by allele (ii). They proposed that the higher expressing allele (iii) with (GT)n polymorphism would be associated with autoimmune disease and the low-expressing allele (ii) with infection. Allele frequencies for SLC11A1 allele(ii) are in the range 0.14 to 0.20 in West and Southern Africa, compared with 0.25 to 0.29 in Northern Europe and 0.36 in Brazil. Individuals homozygous for iii/iii produced higher concentrations of TNF and were at significantly higher risk of severe clinical meningococcal disease (Hibbard, Levin & Blackwell).

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Discussion following lecture by K.P.W.J. McAdam

Fenton: Are there any data suggesting that similar susceptibility loci are involved in other populations and racial groups?

McAdam: Quite a large number of different places have been looked at, for instance for HLA. The NRAMP work has been done in South America, the USA and in Canadian aboriginals.

De Vries: For the known four associations – HLA, the vitamin D receptor, the NRAMP1 and interleukin-1 – there are several ways to get an idea to what extent they explain the genetic variability. Has that been studied?

McAdam: My understanding is that the heritability has been derived from the twin studies. In terms of the individual genes, not one of those comes up on the whole genome scan as a susceptibility area for linkage. So I suspect the contribution is quite small.

Ottenhoff: There was a recent paper by Adrian Hill's group showing that there is a major locus at chromosome 10 estimated to contribute up to about 40% of the total genetic component in leprosy.[1] So that is actually quite a large contribution for a single locus.

De Groot: The paper by Wilkinson *et al*. on West Gujarati Indians clearly showed the gene-environment interaction in an infectious disease.[2] Do you know what happened in the follow-up of this population? Are they prescribing vitamin D for these people, and does it have an effect on the susceptibility to tuberculosis?

McAdam: I am told that the authors are not using vitamin D therapy or suggesting any intervention. I would actually like to know whether anyone else is considering vitamin D therapy and how this would be tested. Vitamin D has been used historically and in the book by Dubos & Dubos it is recorded that quite a lot of people did worse with vitamin D therapy.[3] They seemed to lyse their lungs. So I'm not sure about this intervention, except for skin tuberculosis – there vitamin D is very effective.

Van der Meer: We would rather use vitamin A and zinc supplementation in view of a placebo-controlled study in Indonesia in which we were involved.[4] After two months the supplement had a distinct effect on both wellbeing and the X-ray signs of the tuberculosis. This effect was lost after six months, probably because there was no resistant tuberculosis among the patients, and all of them responded well to the antituberculous drugs which were given concomitantly.

McAdam: And the people on isoniazide, did they do as well as the people on isoniazide plus zinc and vitamin A?

Van der Meer: The latter group did better. All this has still to be confirmed in a larger independent study.

McAdam: How are we, as a clinical investigating community, going to respond to genes that are going to be accumulative in terms of their effect? If you select patients with a particular vitamin D receptor genotype, you do not actually know – unless you type their genes – what other susceptibility loci they have. Ottenhoff's suggestion of using the array technology to develop a haplotype might have to be followed before one can actually look at functional genomics.

El-Omar: Quite a number of populations that are predisposed to tuberculosis also have a high incidence of starvation, malnutrition, malabsorption enteropathies and so forth.

To what extent do you think these environmental factors are modifying the genetic constitution in terms of expression of the disease, either towards susceptibility or towards responding to the infectious agent?

McAdam: That is a very pertinent question, to which I do not know the answer. Common sense says it must make a huge difference, and if you look at animal studies, it does make a dramatic difference. When you look at guinea pigs that are fed a protein-deficient diet and then are challenged with aerosol tuberculosis they die very rapidly, whereas protein-sufficient animals do not. Of course, many of these patients, by the time you see them, have a nutritional problem. With tuberculosis they have lost weight, they have had fever, they have got lots of inflammatory cytokines on board. We are doing a prospective case-contact study at the moment trying to analyse that, but it is not easy to investigate.

Appelmelk: I am confused about how vitamin D works, if it works. You stated that it causes macrophage activation and then bacteria are killed, but at the same time, as you said, it causes a shift from Th1 to Th2, meaning the opposite.

McAdam: It originates from studies done by Graham Rook and the supposition is that vitamin D modulates the cytokine profile, leading to proinflammatory cytokine production.[5]

Netea: I wonder whether we are on the right track if we are not taking into account that susceptibility to mycobacteria may vary depending on the strain. This would hold for *M. tuberculosis* versus atypical mycobacteria but perhaps also within *M. tuberculosis* isolates. I am thinking for instance of the Beijing strains.

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