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Immunogenetics of leishmanial and mycobacterial infections: the Belem Family Study

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SUMMARY

In the 1970s and 1980s, analysis of recombinant inbred, congenic and recombinant haplotype mouse strains permitted us to effectively 'scan' the murine genome for genes controlling resistance and susceptibility to leishmanial infections. Five major regions of the genome were implicated in the control of infections caused by different *Leishmania* species which, because they show conserved synteny with regions of the human genome, immediately provides candidate gene regions for human disease susceptibility genes. A common intramacrophage niche for leishmanial and mycobacterial pathogens, and a similar spectrum of immune response and disease phenotypes, also led to the prediction that the same genes/candidate gene regions might be responsible for genetic susceptibility to mycobacterial infections such as leprosy and tuberculosis. Indeed, one of the murine genes (*Nramp1*) was identified for its role in controlling a range of intramacrophage pathogens including leishmania, salmonella and mycobacterium infections. In recent studies, multicase family data on visceral leishmaniasis and the mycobacterial diseases, tuberculosis and leprosy, have been collected from north-eastern Brazil and analysed to determine the role of these candidate genes/regions in determining disease susceptibility. Complex segregation analysis provides evidence for one or two major genes controlling susceptibility to tuberculosis in this population. Family-based linkage analyses (combined segregation and linkage analysis; sib-pair analysis), which have the power to detect linkage between marker loci in candidate gene regions and the putative disease susceptibility genes over 10–20 centimorgans, and transmission disequilibrium testing, which detects allelic associations over 1 centimorgan (*ca.* 1 megabase), have been used to examine the role of four regions in determining disease susceptibility and/or immune response phenotype. Our results demonstrate: (i) the major histocompatibility complex (MHC: H-2 in mouse, HLA in man: mouse chromosome 17/human 6p; candidates class II and class III including TNFα/β genes) shows both linkage to, and allelic association with, leprosy *per se*, but is only weakly associated with visceral leishmaniasis and shows neither linkage to nor allelic association with tuberculosis; (ii) no evidence for linkage between *NRAMP1*, the positionally cloned candidate for the murine macrophage resistance gene *Ity/Lsh/Bcg* (mouse chromosome 1/human 2q35), and susceptibility to tuberculosis or visceral leishmaniasis could be demonstrated in this Brazilian population; (iii) the region of human chromosome 17q (candidates *NOS2A*, *SCYA2-5*) homologous with distal mouse chromosome 11, originally identified as carrying the *Scl1* gene controlling healing versus nonhealing responses to *Leishmania major*, is linked to tuberculosis susceptibility; and (iv) the 'T helper 2' cytokine gene cluster (proximal murine chromosome 11/human 5q; candidates IL4, IL5, IL9, IRF1, CD14) controlling later phases of murine *L. major* infection, is not linked to human disease susceptibility for any of the three infections, but shows linkage to and highly significant allelic association with ability to mount an immune response to mycobacterial antigens. These studies demonstrate that the 'mouse-to-man' strategy, refined by our knowledge of the human immune response to infection, can lead to the identification of important candidate gene regions in man.

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1. INTRODUCTION

There are three main routes to identifying candidate gene regions for human susceptibility to parasitic infection: (i) by analysis of regions showing conserved synteny with regions of the murine genome known to be involved in disease susceptibility; (ii) through our knowledge of the types of immune response that are important in development of innate and acquired protective immune responses; and (iii) through total genome scans, which use multicase families to test for genetic linkage between disease susceptibility and *ca*. 300 highly informative microsatellite markers evenly distributed across the genome. This review will examine the use of strategies (i) and (ii) in studying candidate gene regions for human susceptibility to leishmaniasis and the mycobacterial diseases, tuberculosis and leprosy, in multicase families from north-eastern Brazil.

The background for these studies began in the 1970s and 1980s when we 'scanned' the mouse genome for genes involved in susceptibility to various forms of leishmaniasis (Bradley *et al*. 1979; Blackwell *et al*. 1980, 1985; Blackwell 1983; Roberts *et al*. 1989, 1990, 1993; Mock *et al*. 1993). Our ability to map genes in mice was largely dependent on the availability of recombinant inbred strains of mice, which were already typed for hundreds of markers across the genome. If differences in susceptibility to disease were present in the progenitor strains, then phenotypic typing of the recombinant inbred strains provided an immediate location for the gene(s) responsible. Classical backcross linkage analysis was then used to verify the location, and tighten up the linkage. Five major gene regions were identified purely by genetic mapping of disease phenotypes, and candidate genes identified within them either by positional cloning or through our knowledge of the immune response to infection. (i) Proximal mouse chromosome 1/human 2q35, carrying the *Ity/Lsh/Bcg* gene (reviewed Blackwell 1996) controlling innate resistance and susceptibility to *Salmonella typhimurium*, *Leishmania donovani* and mycobacterial species including *Mycobacterium bovis*, *M. lepraemurium*, *M. intracellulare* and *M. avium*. This gene has now been identified through positional cloning (Vidal *et al*. 1993) and renamed the natural resistance associated macrophage protein (*Nramp1* in mice; *NRAMP1* in man) gene. (ii) Distal mouse chromosome 11/human 17q (candidates *Nos2*, *Sigje*, MIP1α, MIP1β, RANTES), carrying the *Scl1* (susceptibility to cutaneous leishmaniasis) gene controlling healing versus nonhealing responses to *L. major* (Mock *et al*. 1993; Roberts *et al*. 1993). (iii) Proximal murine chromosome 11/human 5q (candidates *Il4*, *Il5*, *Il9*, *Il13*, *Irf1*, CD14), the 'T helper 2' cytokine gene cluster, controlling later phases of murine *L. major* infection (Roberts *et al*. 1993). (iv) Mouse chromosome 4/human 9p (candidate *Jak2* kinase), carrying the *Scl2* gene controlling a unique 'no lesion growth' phenotype in DBA/2 mice infected with *L. mexicana* (Roberts *et al*. 1990). (v) Mouse chromosome 17/human 6p, the major histocompatibility complex (MHC: H-2 in mouse, HLA in man), including class II molecules, which influence all forms of leishmanial infection (Blackwell *et al*. 1980; Howard *et al*. 1980; Roberts *et al*. 1989), and the class III genes encoding tumour necrosis factor α and β (TNFα/β), which are also candidates for disease susceptibility. Of these, *Scl2* (Roberts *et al*. 1990) could provide an important parallel to the now overwhelming evidence that most people who become infected with leishmania parasites remain asymptomatic. At present we know nothing further about this gene in mice, other than to suggest a possible candidate gene (*Jak2*), and have not yet targeted the homologous region in human family studies. Hence, this review will be confined to analysis of the other four regions of the genome in human family studies of tuberculosis, leprosy and visceral leishmaniasis.

In looking for genes in man, we might anticipate a situation analagous to that which we found in mice for two compelling reasons. First, as both leishmanial and mycobacterial pathogens occupy an intramacrophage niche in the host, protection against both types of disease relies on activation of macrophages for intracellular killing, and therefore on the induction of a T helper 1 immune response. T helper 2 responses are generally associated with exacerbation of disease (Pirmez *et al*. 1990, 1993; Yamamura *et al*. 1991, 1992; Caceres-Dittmar *et al*. 1993; Sieling & Modlin 1994; Surcel *et al*. 1994). Hence, we might find common genes controlling leishmanial and mycobacterial infections, and we might expect that these genes act somewhere in the pathway to macrophage activation and/or in induction of T helper 1/T helper 2 immune responses. A gene identified in mice as a candidate gene for human leishmanial disease susceptibility therefore becomes, *a priori*, a candidate for susceptibility to human mycobacterial infection as well. Second, just as we have identified different genes controlling different disease phenotypes in different combinations of inbred mouse strains, different human populations might segregate for polymorphisms in different major genes controlling infection. Hence, in South America we might find a different region of the human genome involved in susceptibility to cutaneous leishmaniasis caused by members of the *Leishmania mexicana* complex, compared to genes controlling *L. major* in the Middle East or visceral leishmaniasis caused by *L. donovani* in India. The overall picture is of polygenic control of susceptibility to leishmaniasis or mycobacterial infection, but within one population it may be possible to identify major single gene effects.

2. THE 'BELEM FAMILY STUDY': THE RESOURCE AND DATA ANALYSIS

The 'Belem Family Study' was based at the Instituto Evandro Chagas in Belem in the north-east of Brazil. Multicase families for leprosy (72 families; 389 individuals) and tuberculosis (98 families; 731 individuals) were collected within Belem city. Families for visceral leishmaniasis (89 families; 638 individuals) were collected from Santerem, San Luiz and Teresina. A total of 1758 individual blood samples were collected, Epstein–Barr virus (EBV)-transformed B cells prepared, and cells cryopreserved for transport to the UK. In Cambridge, EBV cells were expanded and DNA prepared for genetic analysis.

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(*a*) *Methods of linkage analysis*

In family studies of this type, various forms of analysis can be performed to determine whether there is linkage between a putative disease susceptibility locus (DSL) and marker genes in the region of interest. Such tests can detect linkage over 10–20 centimorgans (cM). In man, 1 cM approximates to 1 megabase of DNA. The most popular form of genetic linkage analysis used is identity-by-descent affected sib-pair analysis (Risch 1990). This is a robust non-parametric test for linkage between a putative DSL and known markers or candidate genes. It uses only affected sib pairs and their parents (whose disease status is not relevant to the analysis), and requires no knowledge of the mode of inheritance of disease susceptibility. The test examines whether there is any deviation from the expected Mendelian ratio of 1:2:1 for sharing of zero, one or two alleles identical by descent in the affected sib pairs. As unaffected sibs are not used, there is no problem with regard to penetrance of the disease phenotype relative to an individual's genotype (see below). In our laboratory we use a form of identity by descent sib pair analysis developed by Holmans and Clayton (1995) and executed within the computer program SPLINK. This uses additional information from unaffected sibs and probabilities to assign unknown parental genotypes (although the unaffected sibs do not contribute to information about the putative DSL). This is ideal for our type of study where it was not always possible to obtain samples from all parents, but a number of unaffected sibs were generally available. Within our total family study population, we have 125 affected sib pairs for visceral leishmaniasis, 176 for leprosy and 149 for tuberculosis. Comparing these numbers to the total numbers of samples and families for each disease, it is immediately obvious that the ratio of sib pairs:families is highest for leprosy. This is because our collection strategy for this disease was targeted towards the affected sib pair form of analysis (figure 1*a*). For visceral leishmaniasis, and especially for tuberculosis (figure 1*b*), the collection strategy focused more on large and/or multigeneration pedigrees. Hence, we needed to find other forms of analysis that might make more use of the genetic information provided by all the members of these pedigrees.

Probably the next most robust form of analysis we have employed is a form of complex sib-pair analysis recently developed by Professor Newton Morton and his colleagues in Southampton (Morton 1996; Collins *et al.* 1996*b*; Lio & Morton 1997), and executed in the computer program BETA. The β model has greater power to detect family resemblance or linkage than the more general Δ model used in SPLINK (Holmans & Clayton 1995). BETA also has the capacity to use all combinations of concordant and discordant sib pairs, i.e. affected sib pairs, unaffected sib pairs and all pairs of affected/unaffected sibs. It is robust in the sense that no knowledge of the mode of inheritance of disease susceptibility is required, but when using unaffecteds, it may need to take account of problems associated with penetrance, i.e. some of the unaffected sibs will have a susceptible genotype but may not have contracted the disease (see below). For BETA analyses presented here, all combinations of affected and unaffected sib pairs have been employed, but we have not taken penetrance into account.

Problems of penetrance have also to be taken into account in performing more stringent forms of parametric linkage analysis in which the mode of inheritance (dominant, recessive, additive) for disease susceptibility has to be specified. The current computer programs for the lod score method of linkage analysis (e.g. LINKAGE; Ott 1991) assume a monogenic inheritance for the trait to be mapped. One approach is to simply test for linkage between a marker and a putative DSL under different modes of inheritance and variable penetrances. Computer simulation analyses show that analysing oligogenic traits (where a small number of loci are jointly responsible for disease occurrence) under an assumed monogenic inheritance retains much of the information on linkage between one of the trait loci and the marker linked to it (Risch *et al.* 1989; Vieland *et al.* 1992). Maximizing lod scores over penetrance values will tend to inflate the lod score, although this is minimal compared to maximizing over different diagnostic systems (Weeks *et al.* 1990). Ott (1991) concludes that it is possible to analyse a disease susceptibility trait for linkage under a rather simple model and still find evidence for linkage when it is present, but one should limit analyses to a small number of models and be aware that the estimates of the recombination fraction obtained under a wrong model tend to be much too high (Clerget Darpoux *et al.* 1986).

An alternative strategy for analysis of linkage between a putative DSL and markers in our large pedigrees has been to use combined segregation and linkage analysis, specifically that executed in the program COMDS, again developed by Professor Newton Morton and colleagues (Morton *et al.* 1991). This performs linkage analysis under the model of inheritance (single or two gene; recessive, additive or dominant inheritance at each locus) determined by complex segregation analysis. The linkage test is performed by maximizing the likelihood for segregation model parameters assuming no linkage to the marker (recombination fraction = 0.5) and then with the recombination fraction estimated. The difference in the −2 log likelihood values under these two models is a chi-squared distribution with 1 degree of freedom. COMDS also takes problems associated with penetrance into account. These can be due to lack of exposure to the pathogen, or reduced virulence of the infecting organism, and are usually age-related. In the analyses presented here, an underlying scale of liability takes into account age at observation, and the risk of disease for individuals within that age range (e.g. 5–10-year-olds) in the family study population relative to the number of 5–10-year-olds in the total study area (i.e. the prevalence of disease in this age-group). Hence, some basic demographic information was required to assign liability classes by age. The only disadvantage of COMDS at present is that it is

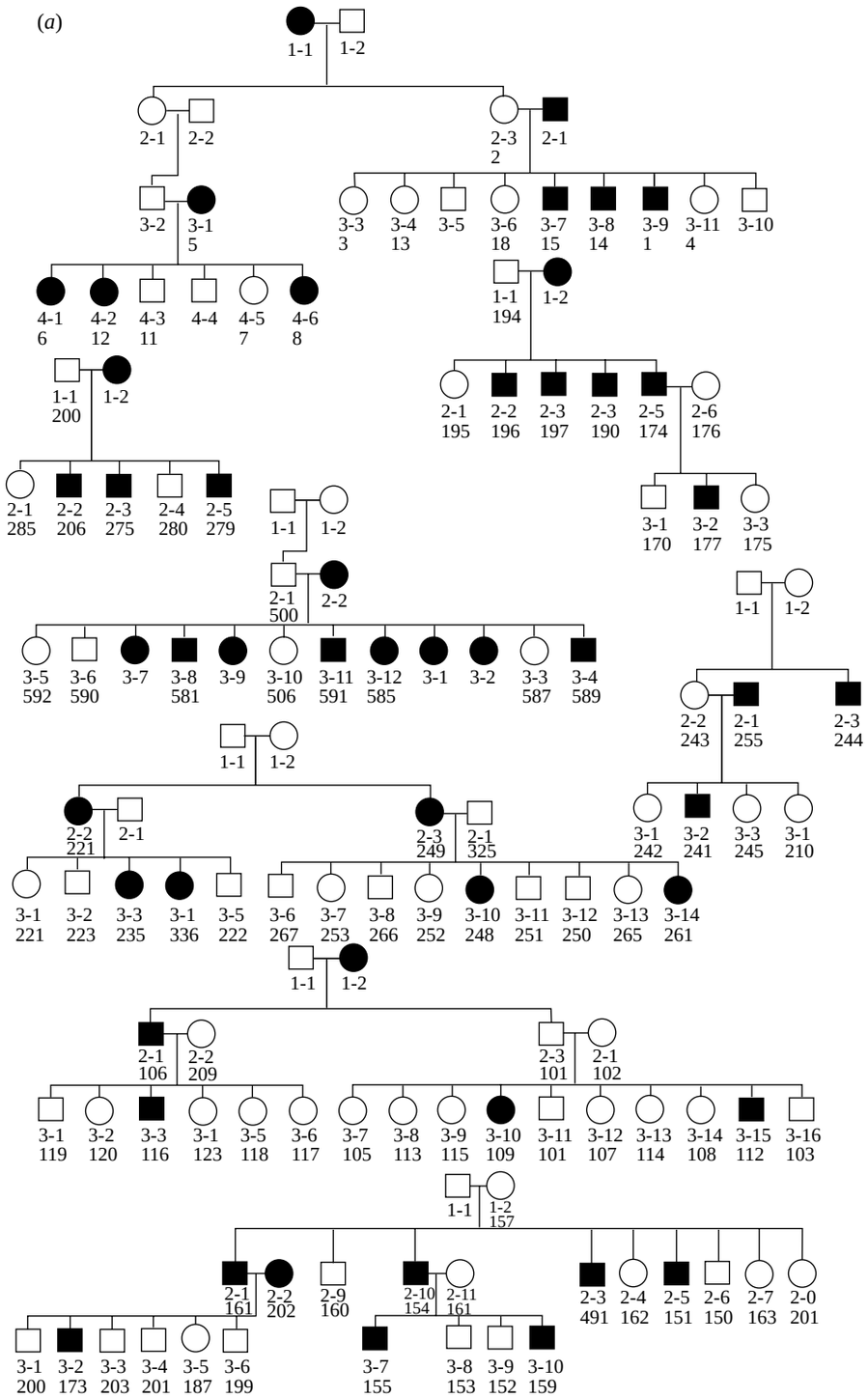

Figure 1. Examples of multicase pedigrees for (*a*) tuberculosis and (*b*) leprosy from the Belem Family Study. Most of the tuberculosis pedigrees were large multigeneration pedigrees for which both affected and unaffected members of the family were sampled. For leprosy, some larger pedigrees were obtained, but the collection strategy focused on affected sib pairs plus parents, as depicted here.

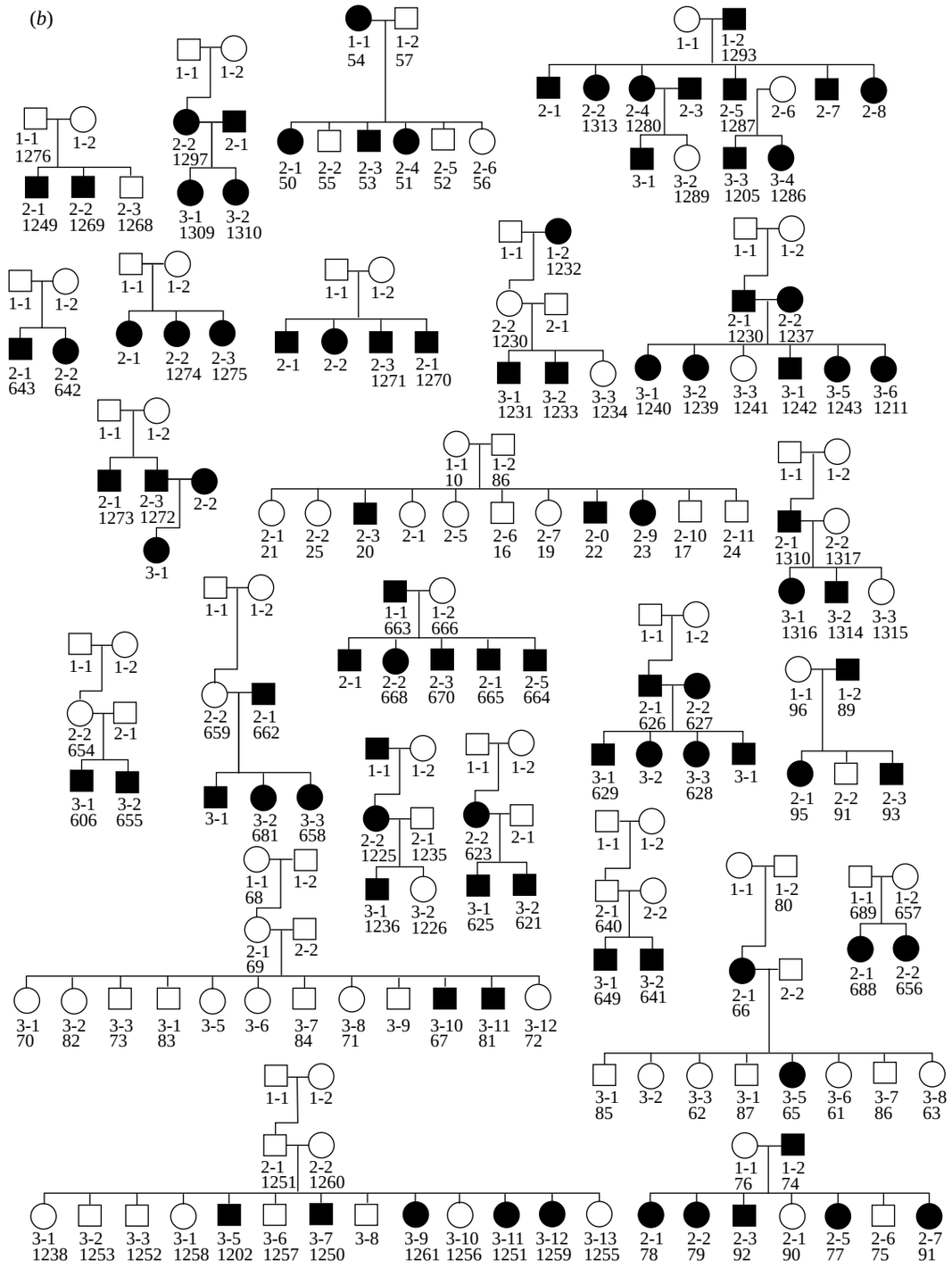

Figure 1. (*cont.*)

limited to analysis of markers with nine or less alleles, thus precluding its use for many of the microsatellite markers we routinely employ. To derive some confidence in this form of linkage analysis, we needed to examine first the results of using complex segregation analysis to determine the most likely mode of inheritance of disease susceptibility in our family study population. As an example, results are presented for the larger set of tuberculosis multicase families.

(*b*) *Evidence for one or two major genes controlling susceptibility to tuberculosis*

For complex segregation analysis of the Belem tuberculosis families, two forms of analysis have been performed using the computer programs POINTER (Lalouel & Morton 1981) and COMDS (Morton *et al.* 1991). Full details of these analyses are given elsewhere (Shaw *et al.* 1997). POINTER is capable of testing

sporadic, polygenic and multifactorial models against major single gene models. The parameters of the model are q, the frequency of the deleterious allele; t, the distance, on the underlying scale of genetic liability, between the two homozygote means; d, the degree of dominance (where $d = 0$ when the deleterious allele is recessive, and $d = 1$ when the deleterious allele is dominant); H, the polygenic heritability; and Z, the ratio of adult:child heritability. Nuclear families were analysed under conditional likelihood, which is less sensitive to ascertainment bias than joint likelihood. Results obtained showed that all single-locus models tested, as well as polygenic and multifactorial models, provide a better fit to the data than does a sporadic model. In addition, all single-locus models are significantly ($p < 0.001$ in all cases) favoured over polygenic or multifactorial models of inheritance of disease susceptibility, and an additive single-locus model favoured ($p < 0.001$ in all cases) over dominant or recessive models. Using COMDS, the analysis was extended to consider various two-locus models, where qm, tm, dm are the parameter estimates for the second locus. A general two-locus model was marginally favoured ($0.01 < p < 0.05$ in all cases) over models for a general single locus, a single locus plus modifier, or a single locus plus pseudopolygenic modifier (which approximates a polygenic background by holding $q = d = 0.5$ or $qm = dm = 0.5$). The data therefore favour the hypothesis that one or two major loci are controlling susceptibility to tuberculosis in this population. When COMDS was used to perform combined segregation and linkage analysis between marker loci and susceptibility to tuberculosis (see below), the general two-locus model was employed. However, as predicted by computer simulation data (Risch *et al.* 1989; Vieland *et al.* 1992), essentially similar results were obtained under an additive single-gene model.

(*c*) *What constitutes significant evidence for linkage?*

The lod score method for linkage analysis (Ott 1991) tests the null hypothesis of free recombination ($\theta = 0.5$) versus the alternative hypothesis of linkage over a range of values for the recombination fraction $\theta < 0.5$. Results are expressed as the likelihood ratio for linkage over nonlinkage at a given value of θ. The lod score (denoted by $Z_{\max}$) is the logarithm (to the base 10) of the likelihood ratio. The recombination fraction at which the maximum lod score occurs is a measure of the distance between the loci being tested. Lod scores can be summed over numbers of families. If linkage really exists, the lod score gets larger and larger as more families are added. However, if there is heterogeneity amongst families (e.g. a particular putative DSL is an important susceptibility gene for infectious disease in some but not all families examined), problems can arise in summing lod scores over multiple families. It is possible to test for homogeneity in the sample before summing lod scores. The likelihood ratio has loosely been termed the odds for linkage. Hence, at a lod score of 3, the odds for linkage over nonlinkage are 1000:1. Under the null hypothesis of absence of linkage, $4.6 \times Z_{\max}$ follows a chi-squared distribution with 1 degree of freedom. In the early days of linkage analysis, the human genetics community adopted stringent standards for reporting linkage data (Morton 1955). Lod scores of 3 were required to declare linkage in official reports and to assign a gene to the linkage map, but weaker evidence could be shared in more informal communications. The asymptotic significance level for this cut-off is $\chi^2 = 4.6 \times 3 = 13.8$; $p = 0.0001$ (one-sided). This is much smaller than the customary significance levels of 0.05 or 0.01. The problem is that there is an *a priori* chance that a pair of loci will be on the same chromosome of about 5%, and a 2% chance that they are within measurable distance of each other. When this is taken into account, it can reduce the probability of linkage associated with a lod score of 3 to 95%. Hence the more rigorous standards required.

The whole issue of proposing standards for reporting linkage data for putative DSL has recently been revisited in relation to data obtained from genome scans (Lander & Kruglyak 1995). With around 300 markers being analysed across the genome, this produces the ultimate multiple testing problem. In searching for disease genes, Lander & Kruglyak (1995) point out that it is important to distinguish between pointwise significance levels and genome-wide significance levels. The pointwise (also called the nominal) significance level is the probability value obtained in a single test of the null hypothesis of no linkage at a specific locus. The genome-wide significance level is the probability of encountering a deviation somewhere in a whole genome scan. For data from a genome scan, Lander & Kruglyak (1995) propose establishing a classification based on the number of times one would expect to see a result at random in a dense, complete genome scan. They propose the following: (i) suggestive linkage—statitistical level equivalent to linkage being detected one time at random in a genome scan, equivalent to a pointwise lod score of 2.2 ($p = 7 \times 10^{-4}$); (ii) significant linkage to occur 0.05 times in a genome scan, equivalent to a pointwise lod score of 3.6 ($p = 2 \times 10^{-5}$); and (iii) highly significant linkage to occur 0.001 times in a genome scan, equivalent to a pointwise lod score of 5.4 ($p = 3 \times 10^{-7}$). Confirmed linkage would require significant linkage from one or a combination of initial studies confirmed in a further sample. These criteria are again quite stringent, but Lander & Kruglyak (1995) believe that suggestive linkage results are worth reporting provided that they are accompanied by an appropriate warning as to their tenuous status, and that they are not used to assign gene names for a putative DSL.

When there is strong prior evidence for restricting the search for a DSL to a particular region, for example a true single-point analysis of a highly relevant candidate gene, a case can be made for some reduction in the lod scores required to propose significant linkage. For the results presented in this paper, we have strong *a priori* evidence from our murine studies that the regions we are targeting will contain putative DSL. In the case of *NRAMP1* and HLA class II molecules, we are targeting specific candidate genes. For the other

regions, we are targeting a candidate gene region, which often contains more than one possible candidate gene. In such cases, the linkage data is backed up by use of linkage disequilibrium testing (see below), which often has the power to identify susceptibility loci even when the probability for linkage falls short of the 'suggestive' criteria (e.g. Copeman *et al.* 1995). The use of linkage disequilibrium testing can also help to sort out which (if any) of the candidate genes within a region are more likely to be the putative DSL.

(*d*) *Tests for allelic association*

If a strong functional candidate gene already exists within a region of interest, or linkage studies indicate very tight linkage between marker genes and the putative DSL, allelic association tests can be used to demonstrate an association between polymorphisms in the putative susceptibility gene (or close marker) and disease phenotype. These can include population-based case–control analysis as well as family-based transmission disequilibrium testing (e.g. Sham & Curtis 1995) or analysis of haplotype relative risk (e.g. Terwilliger & Ott 1992). Such tests rely on the type of linkage disequilibrium (Ott 1991) caused by the slow decay of tight linkage as particular combinations of allelic variants at linked genes travel together through time in pedigrees and in the population. They are most reliable over a distance of 1 cM, i.e. *ca.* 1 megabase. Population-based case–control analysis can provide false positive associations due to population admixture in the study area. As our study was a family-based study, we have used various forms of transmission disequilibrium testing (TDT). This type of test looks for a bias in transmission of particular alleles from heterozygous parents to affected offspring. In the case of a simple biallelic polymorphism the result is clear. When multiallelic loci are used, such as the microsatellite markers routinely employed in linkage studies, problems of multiple testing arise. If we consider one allele at a time and test for deviation from 50% transmission to affected offspring from heterozygous parents, each allele yields a chi-squared test with 1 degree of freedom. A correction factor for the number of tests needs to be applied, but as the tests are not independent, the Bonferroni correction is likely to be conservative. In attempting to deal with this problem, Sham & Curtis (1995) propose two additional forms of analysis. Firstly, a 'genotype-wise' test, which considers every heterozygous parental genotype to determine whether each allele of the genotype is transmitted to affected offspring on 50% of occasions. The deviation of observed from expected for each genotype can be summed to give one chi-squared statistic with degrees of freedom equal to the number of genotypes. The disadvantage is that there may be a large number of genotypes, some of which may occur only rarely and increase the degrees of freedom without contributing much information. Also, no attention is paid to whether particular alleles are preferentially transmitted across different genotypes. A second 'allele-wise' test is therefore proposed by Sham & Curtis (1995), which attempts to establish a pattern of preferential transmission of certain alleles across genotypes, e.g. AB or BC parents at a locus may preferentially transmit allele B to affected offspring, but AC parents may transmit A and C equally. Sham & Curtis model this distortion using logistic regression, to arrive at a chi-squared statistic having $n-1$ degrees of freedom, where n is the number of alleles at the locus. These three forms of TDT—allele-wise, genotype-wise and individual allele transmission testing—are executed within the package ETDT (Extended TDT) developed by Sham & Curtis (1995).

3. HLA AND SUSCEPTIBILITY TO LEPROSY, TUBERCULOSIS AND VISCERAL LEISHMANIASIS

Our initial analysis of genetic susceptibility in the Belem Family Study has focused on polymorphisms in class II (DR/DQ) and class III (TNFα/β) region genes (see figure 2 and table 1) for two reasons: (i) in analysis of autoimmune disease susceptibility genes, the influence of class II polymorphisms has been so strong as to require stratification by DR genotype in identifying a role for non-MHC genes (e.g. Davies *et al.* 1994); and (ii) the class III region gene product TNFα plays such a key role in the macrophage activation pathway that it provides an important candidate for disease susceptibility in its own right.

For 17 multicase leprosy families (32 nuclear families; 204 individuals) analysed using COMDS, significant linkage has been demonstrated between a putative DSL and DR ($\chi^2 = 6.49$, d.f. 1; $p < 0.01$), the -308 bp polymorphism in the TNFα promoter region ($\chi^2 = 6.01$, d.f. 1; $p < 0.01$), and the TNF locus associated microsatellite marker TNFb ($\chi^2 = 5.72$, d.f. 1; $p < 0.05$). All linkages are at a recombination fraction of zero, emphasizing the difficulty in dissecting out the roles of class II and class III genes, which span a physical distance of *ca.* 1 megabase. These families did not contain sufficient numbers of affected sib pairs to obtain significant linkage using SPLINK. However, even in this relatively small dataset, a highly significant ($\chi^2 = 10.28$, d.f. 1; $p < 0.001$) allelic association was observed between leprosy *per se* and the -308 bp polymorphism at the TNFα locus. Allelic associations were not significant for DR, TNFβ, TNFa or TNFb. Analysis of the larger dataset (Shaw *et al.* 1998) is required to confirm these data and to enable us to stratify by DR in determining the influence of TNFα, and vice versa. Despite the numerous population-based case–control studies showing allelic association between DR/DQ and susceptibility to leprosy, this is one of the few studies in which significant linkage of leprosy susceptibility to HLA has been observed, demonstrating the power of COMDS relative to other forms of analysis for these larger multicase family pedigrees. The results for leprosy contrast sharply with our analysis of TNF locus (TNFα, TNFβ) polymorphisms in 37 Belem tuberculosis families (59 nuclear families; 299 individuals), in which we have failed (Shaw *et al.* 1997) to demonstrate linkage using SPLINK, BETA or COMDS, or allelic association using TDT. Although we have yet to type this set of families for DR/DQ, failure to detect linkage to class III genes suggests that HLA is not as strong a component of

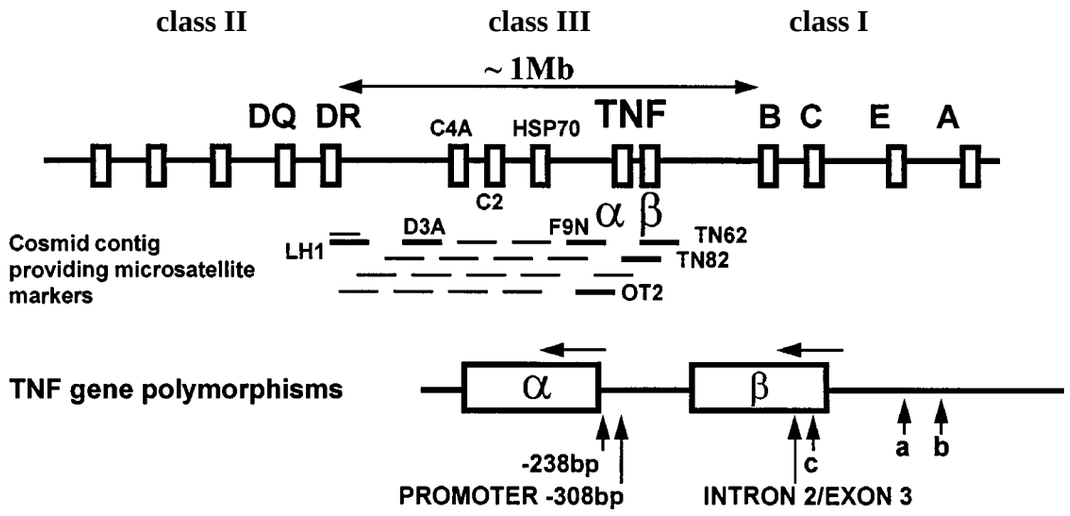

Figure 2. Organization within the HLA–A map HLA showing relative locations for the class II (DR/DQ) and class III (TNFα) loci which form the major candidate genes for susceptibility to leprosy, tuberculosis and visceral leishmaniasis. Beneath the main map are (i) a diagrammatic representation of a cosmid contig, showing the approximate locations for the cosmids (heavy lines) carrying additional microsatellite markers developed in the laboratory of our collaborator Dr Duncan Campbell, Oxford, UK and used in our analysis of visceral leishmaniasis families; and (ii) an expanded diagram of the TNF locus showing the relative positions of point mutations in the TNFα promoter region (Wilson *et al*. 1993), an intronic restriction fragment length polymorphism in intron 2 of the TNFβ gene (which is always found in association with a single base pair conservative substitution in exon 3) (Messer *et al*. 1991), or microsatellite markers (a,b,c) within or distal to TNFβ (Nedospasov *et al*. 1991).

Table 1. *Comparison of results of analyses for linkage between HLA and a DSL for visceral leishmaniasis using SPLINK, BETA or COMDS.*

(Linkage analyses HLA and VL.)

SPLINK	negative for all 11 markers*	
BETA	DQA	LOD = 0.368
	TNFα	LOD = 0.085
	TNFβ	LOD = 0.410
COMDS	DQA	$\chi^2 = 0$, d.f. 1; n.s.
	TNFα	$\chi^2 = 1.6$, d.f. 1; n.s.
	TNFβ	$\chi^2 = 3.6$, d.f. 1; $0.05 < p < 0.1$

* See figure 2.

genetic susceptibility to tuberculosis in this Brazilian population as it is for susceptibility to leprosy, and that it is probably not one of the major genes suggested by the complex segregation analysis.

In mice, the strongest influence of H-2 on leishmanial infections was observed for visceral infection with *L. donovani* (Blackwell *et al*. 1980). In this case, polymorphisms within the class II genes themselves appeared to be responsible, as administration of monoclonal antibodies to IA or IE molecules could reverse the influence of particular allelic gene products at these loci on disease progression (Blackwell & Roberts 1987). In man, high levels of circulating TNFα associated with clinical disease (Barral-Netto *et al*. 1991) suggested that, as we had demonstrated for American mucocutaneous leishmaniasis (Cabrera *et al*. 1995), regulatory polymorphisms in the TNFα locus might also influence disease susceptibility independently of DR/DQ genotype. In an initial crude analysis of gene frequencies for the TNFα−308 bp polymorphism among all affected versus all unaffected offspring in our family study population, we observed a twofold higher relative risk of disease in individuals heterozygous for the rare mutant allele 2 ($\chi^2 = 6.31$, d.f. 1; $p < 0.02$). In an attempt to pinpoint the influence of MHC genes on human disease susceptibility (Peacock *et al*. 1998) 86 visceral (545 individuals) leishmaniasis families were genotyped for DR, DQ, TNFα, TNFβ, TNFa and a range of new microsatellites (collaboration with Drs Duncan Campbell and Ruth March, Department of Biochemistry, Oxford University), from a cosmid contig spanning the region from DR to TNF loci (figure 2). Table 1 compares the lod scores obtained for HLA markers using SPLINK, BETA and COMDS. Only weak evidence for linkage is demonstrated. Similarly, no consistent evidence (table 2) for allelic association between any of these markers and disease phenotype was observed. Hence, HLA class II and class III genes do not appear to play a major role in controlling susceptibility to visceral leishmaniasis in this population. These results are in stark contrast to the detection of linkage to HLA for autoimmune diseases, for example a lod = 8 for linkage to HLA was obtained in a genome-wide scan of 96 affected sib pairs for insulin-dependent diabetes (Davies *et al*. 1994).

4. *NRAMP1* AND SUSCEPTIBILITY TO INFECTIOUS AND AUTOIMMUNE DISEASE

The gene (*Nramp1*) encoding the natural resistance associated macrophage protein is the positionally cloned candidate for the murine macrophage resistance

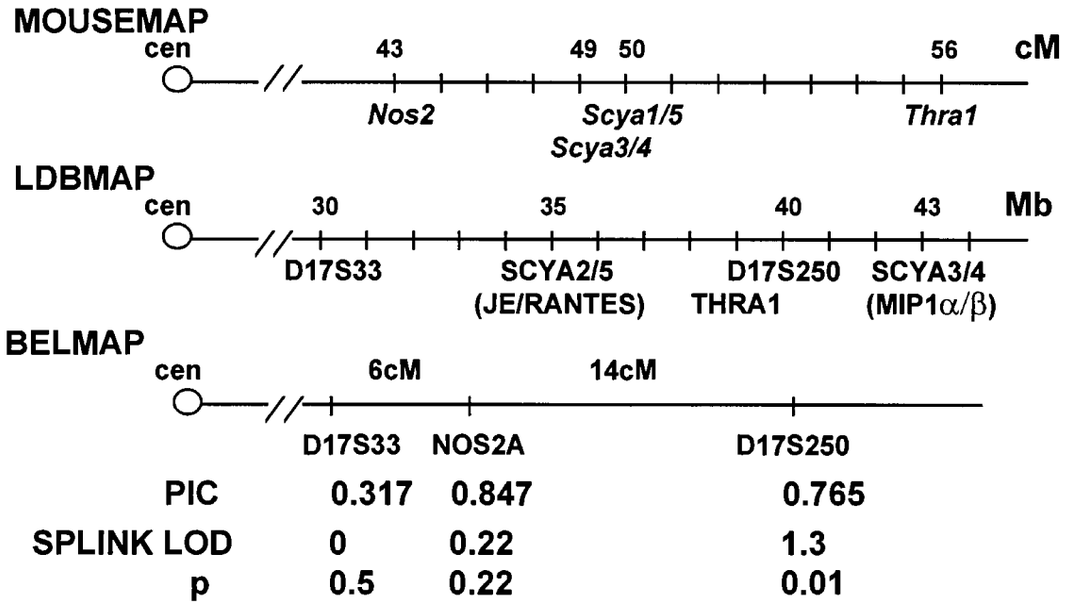

Figure 3. Comparative maps for mouse chromosome 11 and human 17q11.2-q12. Data for the MOUSEMAP are from Mouse Genome (1997), and are given as centimorgans (cM) from the centromere. Data for LDBMAP are the composite locations (comp) for markers in megabases (Mb) from the p telomere as displayed in the the genetic location database LDB (Collins *et al*. 1996*a*). The locations for D17S33 and D17S250 are based on combined linkage and *in situ* hybridization data. The locations for SCYA2/5 amd SCYA3/4 are approximate and based only on *in situ* hybridization data. BELMAP was constructed from two-point linkage analyses for the three markers typed for the Belem tuberculosis and leprosy families. The PIC values for these markers are indicated, along with the SPLINK generated lod scores and *p* values for linkage between the marker and a DSL for tuberculosis.

Table 2. *Allelic association (ETDT) for HLA and VL*

markers*		allele-wise			genotype-wise			individual (alleles)		
	N	χ^2	d.f.	*p*	χ^2	d.f.	*p*	χ^2	d.f.	*p*
TNFa	119	18	11	0.06	34	27	0.10	7.0	1	0.008 (2)
Tnfα	73	—	—	n.s.	—	—	n.s	—	—	n.s.
TNFβ	23	—	—	n.s.	—	—	n.s	—	—	n.s.
TN62	127	—	—	n.s.	—	—	n.s	—	—	n.s.
TN82-1	146	—	—	n.s.	—	—	n.s	—	—	n.s.
TN82-2	144	—	—	n.s.	—	—	n.s	—	—	n.s.
TN82-3	139	—	—	n.s.	—	—	n.s	—	—	n.s.
OT2	163	—	—	n.s.	—	—	n.s	5.0	1	0.03 (6)
F9N1	143	—	—	n.s.	45	23	0.004	—	—	n.s.
D3A	139	—	—	n.s.	—	—	n.s	—	—	n.s.
LH1	141	—	—	n.s.	—	—	n.s	—	—	n.s.

*See figure 2.

gene *Ity/Lsh/Bcg*. This gene was identified > 20 years ago for its role in controlling the early phases of infection with *S. typhimurium*, *L. donovani* or various mycobacterial species including *M. bovis* and *M. lepraemurium*. Following its identification by positional cloning (Vidal *et al*. 1993), full length cDNA sequence was obtained (Barton *et al*. 1994), and its candidacy as *Ity/Lsh/Bcg* confirmed by functional analysis of transfected macrophage cell lines *in vitro* (Barton *et al*. 1995), and by analysis of gene disrupted ('knock-out'; Vidal *et al*. 1995) and transgenic (Govoni *et al*. 1996) mice *in vivo*. Computer-assisted analysis of the deduced amino acid sequence showed that the *Nramp1* gene encoded a 53 kDa protein with homology to bacterial and lower eukaryotic membrane bound-transporter proteins (Vidal *et al*. 1993). The precise molecular function of the Nrampl protein in mice remains unknown. Antibody studies (Searle *et al*. 1997) demonstrate that the Nrampl protein localizes to

an intracellular late endosomal/lysosomal compartment, with further computer-assisted analysis of structure (Cellier *et al.* 1996) suggesting that it functions as an ion channel/transporter, which in some way influences the macrophage activation pathway and antimicrobial activity.

Functional studies utilizing congenic mouse strains and *Nramp1* transfected macrophage lines have shown that the *Nramp1* gene plays an important role early in the macrophage activation pathway, and has many pleiotropic effects on macrophage function including regulation of KC, IL-1β, iNOS, MHC class II molecules, TNFα, NO release, L-arginine flux, oxidative burst and tumoricidal as well as antimicrobial activity (reviewed Blackwell 1989, 1996; Blackwell *et al.* 1991, 1994; Skamene 1994; Radzioch *et al.* 1995). Macrophages carrying the mutated infection susceptible *Nramp1* allele also have a defect in ability to process antigen (Lang *et al.* 1997), which is compounded by the influence of the gene on molecules regulating (TNFα, IL1β) or directly involved in (MHC class II) antigen presentation. This results in an *in vivo* bias towards development of a T helper 1 response in mice bearing the wildtype (infection resistant) allele, with a T helper 2 response elicited in *Nramp1* mutant (infection susceptible) mice (Kaye & Blackwell 1989; Kramnik *et al.* 1994). Interestingly, not all mycobacterial strains can elicit enhanced *Nramp1*-regulated macrophage activation responses (Roach *et al.* 1994), and hence, resistance *in vivo* (Orme 1985; Medina & North 1996). This macrophage-activating property resides in the structure of mycobacterial surface glycoconjugates, principally the lipoarabinomannans (LAMs) (Roach *et al.* 1993, 1994, 1995). The arabinose-terminated LAMs (AraLAMs) of avirulent mycobacterial strains elicit a strong macrophage activation response, whereas the mannosylated LAMs (ManLAMs) of virulent tuberculosis and some strains of *M. bovis* BCG do not. This leads to two predictions, first we might expect micro- or macro-geographic variation in association between human *NRAMP1* and tuberculosis according to the genotype of the infecting bacillus; and second that avirulent mycobacteria may trigger chronic macrophage activation associated with autoimmune disease.

The human *NRAMP1* gene is located on human chromosome 2q35 (White *et al.* 1994), and polymorphisms providing both intragenic and an extended 2q35 haplotype, have been identified (White *et al.* 1994; Blackwell *et al.* 1995; Buu *et al.* 1995; Liu *et al.* 1995; Lewis *et al.* 1996) and used in human genetic studies (Abel *et al.* 1996; Shaw *et al.* 1996). The current status of *NRAMP1* as a human disease susceptibility locus is as follows: (i) weak linkage (LOD score 1.01; $p < 0.02$) for *NRAMP1* and the 2q35 haplotype *NRAMP1-IL8R-VIL1*, and allelic association for a functional promoter region polymorphism in *NRAMP1* ($\chi^2 = 3.89$, d.f. 1; $p < 0.05$), has been demonstrated in a British study of rheumatoid arthritis (Shaw *et al.* 1996); (ii) weak linkage ($t = 2.3$; $p < 0.02$) has been demonstrated for an intragenic *NRAMP1* and an extended 2q35 haplotype in a study of Vietnamese multicase leprosy families (Abel *et al.* 1996); (iii) allelic association ($\chi^2 = 4.12$, d.f. 1; $0.01 < p < 0.05$) for the functional promoter region *NRAMP1* polymorphism and tuberculosis has been demonstrated in 72 genetically independent sex- and age-matched Brazilian cases and controls (J. M. Blackwell, unpublished observation). The latter result is not, however, supported (Shaw *et al.* 1997) by family-based linkage (combined complex segregation and linkage analysis using COMDS; identity-by-descent sib-pair analysis using SPLINK) or allelic association (TDT) methods of analysis of 36 multicase families (287 individuals; 33 affected sib pairs after weighting for multiple sibs within a family; 40 transmissions from heterozygous parents to affected offspring) from the same geographic region. This could mean either that sample size for the family-based analyses is too small (less than half the Belem families have been studied to date), and hence less powerful than the case–control analysis, or that the population-based case–control analysis has provided a false positive result due to population admixture. It is of interest that preliminary evidence for a similar allelic association between the *NRAMP1* promoter region polymorphism and tuberculosis has recently been obtained in a study of 60 cases and controls from Capetown, South Africa (P. van Helden, Stellenbosch University, personal communication). Negative associations have been found in other population-based case–control analyses: (i) in 12 genetically independent tuberculosis cases and 18 controls from Hong Kong and Canadian tuberculosis pedigrees (Liu *et al.* 1995); and (ii) in a study of 184 tuberculosis cases and 191 controls from The Gambia, West Africa (A. V. S. Hill, Oxford University, personal communication). In Belem, we have also failed to show significant linkage or allelic association between *NRAMP1* (or the haplotype *NRAMP1-IL8R-VIL1* known to lie within 150 kb) and susceptibility to visceral leishmaniasis (46 families; 206 individuals; 48 affected sib pairs after weighting for multiple sibs within a family). Hence, our results to date suggest that *NRAMP1* is not one of the major genes for susceptibility to tuberculosis or visceral leishmaniasis in this population, although we cannot yet rule out some weaker influence which might come to light when the study is completed. The Belem leprosy families have still to be analysed for 2q35.

5. HUMAN 17q11.2-q12 AND SUSCEPTIBILITY TO TUBERCULOSIS

The *Scl1* gene in mice mapped to a region of mouse chromosome 11 carrying the *Nos2* gene encoding the inducible nitric oxide synthase (iNOS) gene. Considering the role of iNOS in the generation of nitric oxide for macrophage antimicrobial activity, NOS2A in man became a 'hot candidate' for a DSL for tuberculosis, leprosy and visceral leishmaniasis within the homologous region of human 17q11.2-q12. In the absence of a known polymorphism in NOS2A itself, our analysis (38 families; 287 individuals; 36 affected sib pairs after weighting) focused initially on two microsatellite markers within the region: D17S250 (Weber *et al.* 1990) and D17S33 (Hoff *et al.* 1988). Using SPLINK, we obtained significant evidence (maximum lod score = 1.3; $\chi^2 = 5.98$, d.f. 1; $p = 0.01$) for linkage between a putative

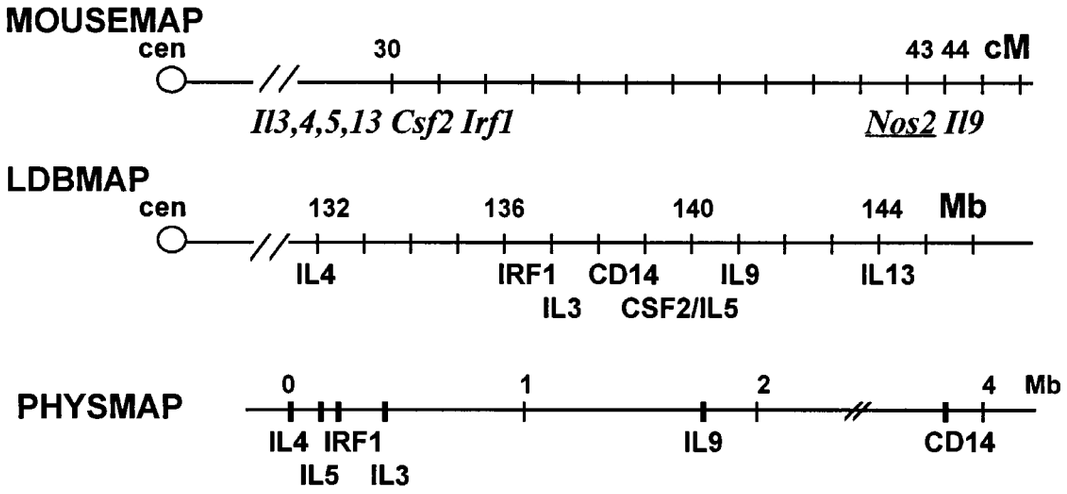

Figure 4. Comparative maps for mouse chromosome 11 and human 5q23-q32. Data for the MOUSEMAP are from Mouse Genome (1996, 1997), and are given as centimorgans (cM) from the centromere. Data for LDBMAP are the composite locations (comp) for markers in megabases (Mb) from the p telomere as displayed in the the genetic location database LDB (Collins *et al*. 1996*a*). Data for the physical map (PHYSMAP) were generated from two-colour fluorescence *in situ* hybridization of cosmids positive for the markers hybridized to interphase chromosomes (Saltman *et al*. 1993), and are shown as Mb from IL4.

DSL for tuberculosis and D17S250, but no evidence (lod score = 0) for linkage to D17S33. This could be explained by the difference in polymorphic information content (PIC) between the two markers (for D17S33, PIC = 0.317; for D17S250, PIC = 0.765), and hence a different effective sample size. However, two-point LINKAGE analysis between the two markers indicated that they lay some 20 cM (sex-averaged distances) apart in our families (compared with a sex-averaged distance of 10 cM on the genetic location database or LDB (Collins *et al*. 1996*a*); see figure 4), suggesting that the putative DSL might lie closer to D17S250. In collaboration with Dr Weiming Xu (University College, London) we were subsequently able to examine a microsatellite marker within the NOS2A locus (47 families; 450 individuals; 51 affected sib pairs after weighting), which resulted in a SPLINK-generated lod score of 0.22 (n.s.). BETA provided parallel differences in the magnitude of lod scores (0, 0.013, 0.541) for linkage between the three markers (D17S33, NOS2A, D17S250) and a putative DSL in this region, although interestingly did not 'improve' the lod score for linkage to D17S250 by using the β model with unaffected sibs incorporated into the analysis. Two-point LINKAGE analysis revealed that NOS2A lies closer to D17S33 than to D17S250 (figure 3). As the PIC for NOS2A (0.847) is equivalent to D17S250, the evidence again favours a gene closer to D17S250 as the putative DSL.

Other candidates within this region are members of the small inducible cytokine family (SCYA1-6), including JE (SCYA2), RANTES (SCYA5), MIP1α (SCYA3) and MIP1β (SCYA4). SCYA2 and SCYA5 are produced by activated macrophages, SCYA3 and SCYA4 by T cells. Chemotaxis is among the earliest events leading to the recruitment of blood monocytes into inflammatory sites. It is a chronic delayed type hypersensitivity (DTH) inflammatory response to *M. tuberculosis*, which causes granuloma formation and the caseous necrotic tissue characteristic of tuberculosis pathology. A mechanism that may account for the accumulation of discrete cell subsets, e.g. T memory cells and macrophages, at the centre of DTH granulomas is the production of specific cytokines or chemokines. RANTES (SCYA5) was shown to be preferentially chemoattractive for these cell types (Schall *et al*. 1990). Subsequently Devergne *et al*. (1994) found strong RANTES gene expression and protein production in lymph nodes presenting typical DTH lesions related to either sarcoidosis or tuberculosis. In the mouse, bacterial lipopolysaccharide, mycobacterial lipoarabinomannan, or leishmanial lipophosphoglycan act synergistically with interferon γ to induce JE (SCYA2) messenger RNA. Dramatic differences in message regulation by these stimuli have been observed in macrophages from the progenitor *Scl1* susceptible and resistant mouse strains BALB/c and C57BL/10ScSn, reinforcing *Sigje* (*Scya2*) as a candidate for *Scl1* (T. I. A. Roach & J. M. Blackwell, unpublished data). Human SCYA2 has been shown to induce monocyte infiltration on intradermal injection into rats and rabbits (Zachariae *et al*. 1990; Van Damme *et al*. 1992), and also elicits the expression of β2 integrins involved in leucocyte migration and a respiratory burst in human monocytes (Jiang *et al*. 1992). Hence, regulatory polymorphisms in these inducible C-C chemokine genes provide plausible candidates for both mycobacterial and leishmanial disease susceptibility loci.

Although not precisely localized, the current LDB public chromosome 17 map indicates that SCYA2/5 and SCYA3/4 lie on either side of D17S250 (see figure 3). Our current strategy is: (i) to complete the analysis of D17S33, NOS2A and D17S250 in the tuberculosis family set to ensure that significant linkage within the

Table 3. *Allelic association (ETDT) for IL4 and IR to PPD/MLSA*

response/ marker*	N	allele-wise χ^2	allele-wise d.f.	allele-wise p	genotype-wise χ^2	genotype-wise d.f.	genotype-wise p	individual (alleles) χ^2	individual (alleles) d.f.	individual (alleles) p
high SI PPD:										
II4Rp1	91	24	12	0.02	25	17	0.10	—	—	—
Il4RP2	53	4.3	1	0.04	4.3	1	0.04	4.24	1	0.04 (2)
high SI MLSA:										
II4RP1	59	14	10	0.17	17	15	0.32	5.16	1	0.02 (4)
IL4RP2	30	9	1	0.002	9	1	0.002	8.53	1	0.003 (2)
low SI PPD:										
II4RP1	50	13	10	0.20	16	13	0.22	6.54	1	0.01 (14)
IL4RP2	18	3.8	1	0.05	3.8	1	0.05	3.55	1	0.06 (1)
low SI MLSA:										
II4RP1	37	14	10	0.16	18	14	0.19	—	—	—
IL4RP2	26	0.2	1	0.69	0.2	1	0.60	—	—	—

*IL4RP1 = microsatellite (17 alleles), Mout *et al.* 1991; IL4RP2 = variable number tandem repeat (2 common alleles), Mout *et al.* 1988.

region is retained; and (ii) if so, to saturate our analysis with other microsatellite markers known to map within this region. Using ETDT, no overall allele-wise or genotype-wise significance was obtained for NOS2A or D17S250 allelic associations, although significant bias ($\chi^2 = 7.26$, d.f. 1; $p < 0.01$) in transmission of allele 3 at D17S250 in an individual allele test survives the application of a multiple testing correction factor of 5 (for the five alleles at which sufficient transmissions were observed to carry out individual chi-squared tests). Further extension of TDT analysis across multiple markers should help to pinpoint the precise location of the gene in this region controlling susceptibility to tuberculosis. This region will also be of interest in further analysis of leprosy and visceral leishmaniasis families.

6. IL4 POLYMORPHISMS AND IMMUNE RESPONSE TO MYCOBACTERIAL ANTIGENS

In studies of other diseases where strong biases in the T helper 1:T helper 2 ratio are important, attention has focused on the region of human chromosome 5q carrying a cluster of T helper 2-related cytokine genes, including IL4, IL5 and IL9 (see figure 4). Studies in man have identified polymorphisms in the IL4 and IL9 genes associated with atopy and bronchial hyperresponsiveness (Doull *et al.* 1996), conditions strongly associated with IL4/IL9-driven IgE responses. Quantitative trait analysis of total serum IgE levels had already been mapped to 5q31.1 (Xu *et al.* 1995), and allelic association with IL4 polymorphism demonstrated. A recent genome scan for genes controlling susceptibility to schistosomiasis, a disease for which T helper 2-mediated IgE responses are thought to be protective, also localized a major single gene controlling infection intensity to this region of 5q (Marquet *et al.* 1996). In human tuberculosis, patients with active disease have been found to have higher IgE levels than age, sex, ethnic, and socioeconomically matched controls in northern India (Papiha *et al.* 1985). Further, in a study from Karachi, Pakistan, patients with pulmonary tuberculosis showed depressed T-cell responses to *M. tuberculosis* antigen in studies of blastogenesis and elevated levels of IgG4, a subtype that is T helper 2 dependent (R. Hussain and H. Dockrell, personal communication).

In the Brazilian study, analysis of two polymorphic markers (Mout *et al.* 1991) within introns of the gene encoding IL-4 in 48 multicase tuberculosis families (402 individuals), 39 leprosy families (208 individuals), and 86 visceral leishmaniasis families (545 individuals) has failed to provide any evidence for linkage to (SPLINK, BETA or COMDS), or allelic association with (TDT, ETDT), susceptibility to disease (G. Black, C. S. Peacock, M.-A. Shaw and J. M. Blackwell, unpublished observations). For visceral leishmaniasis, the analysis included a polymorphic microsatellite marker within the IL9 gene (figure 4), which also failed to provide evidence for linkage or allelic association. This region therefore appeared to be distinctly uninteresting with respect to disease susceptibility for leishmanial and mycobacterial infections in this Brazilian population. Interestingly, however, quantitative trait genetic analysis (Black *et al.* 1998) using LINKAGE, strongly supported by family-based allelic association (ETDT) tests, provide compelling evidence for a gene in this region controlling immune response to mycobacterial antigens. LINKAGE analysis between a low responder phenotype based on stimulation indices for proliferative responses to *M. tuberculosis* purified protein derivative (PPD) or *M. leprae* soluble antigen (MLSA) and polymorphic markers within the IL4 gene provided a peak lod score of 1.9 ($\chi^2 = 8.74$, d.f. 1; $p < 0.01$) under dominant inheritance. An independent analysis for linkage between a high responder phenotype and IL4 markers gave a peak lod score of 0.95 ($\chi^2 = 4.37$, d.f. 1; $p < 0.05$) under recessive inheritance. ETDT (Sham & Curtis 1995) (table 3) showed significant allele-wise (p values ranging from 0.02 to 0.002) and genotype-wise associations (p values ranging from 0.04 to 0.002) between the IL4 polymorphisms and both high and low responder phenotypes, with high

responder status showing a specific allelic association with an allele4/allele2 haplotype for the two intronic IL4 polymorphic markers examined. In a further analysis of PMA/ionophore elicited IL4 production by PPD expanded peripheral blood mononuclear cells from family members carrying a range of IL4 haplotypes, individuals homozygous for the allele4/allele2 haplotype showed low IL4 production, i.e. the inverse of the allelic association obtained for the proliferative response phenotype. Together with the strong allelic association with polymorphisms within the IL4 locus, these data suggest that regulatory polymorphisms at the IL4 locus may directly regulate this difference in immune response to mycobacterial antigens. Other workers (Guler *et al*. 1996) have suggested, however, that the gene in this region (figure 5) encoding the DNA binding protein interferon regulating factor 1 (IRF1) may regulate T-cell responses. IL4 and IRF1 lie within *ca*. 300 kb of each other (Saltman *et al*. 1993). It is also possible that polymorphism at the locus encoding CD14, which is involved in the binding of mycobacterial products and lipopolysaccharide to macrophages, might also influence this response through its effect on macrophage activation and antigen presentation. However, CD14 lies >2 Mb from IL4 (Saltman *et al*. 1993) making it less likely to be in linkage disequilibrium with IL4. It is interesting that the gene in this region regulating immune response to mycobacterial antigens does so independently of mycobacterial disease phenotype, and hence does not appear to contribute to disease susceptibility *per se*. This region may, however, be important in determining response to BCG vaccination, an hypothesis which is currently being pursued.

7. CONCLUSIONS

This review has focused on candidate genes/regions identified through analysis of disease susceptibility phenotypes in murine models of infectious disease as a lead to identification of susceptibility genes in man. Although the Belem Family Study is not complete, results to date have shown: (i) that HLA class II and class III genes appear to be more important in susceptibility to leprosy than for tuberculosis or visceral leishmaniasis; (ii) the role of *NRAMP1* appears to be weak or negative for all three diseases in this population; (iii) human 17q11.2-q12 appears to contain a tuberculosis susceptibility gene which is probably not *NOS2A*; and (iv) while the IL4 gene region is not linked to disease susceptibility to tuberculosis, leprosy or visceral leishmaniasis *per se*, strong allelic associations have been observed between polymorphic alleles at the IL4 locus and immune response to mycobacterial antigens. These studies demonstrate that the 'mouse-to-man' strategy, refined by our knowledge of the human immune response to infection, can lead to the identification of important candidate gene regions in man. The Belem Family Study also provides a valuable resource for genome scanning approaches to identification of other genes influencing human susceptibility to disease.

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