GENETIC EPIDEMIOLOGY OF LEPROSY AND MITSUDA REACTION

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SUMMARY - *The genetic mechanisms that may act on the susceptibility/resistance to leprosy are reviewed, and it is stressed that although the mouse model is very attractive to be transferred to humans, particularities of this disease make difficult any extrapolation. Several recent studies based on the familial distribution of leprosy did not find a clear genetic mechanism responsible for leprosy per se, nor for the polar types of the disease. Nevertheless, the Mitsuda reaction was shown to be a phenotype determined by a major gene with a high level of dominance. It is emphasized that the research should now be focused on mapping the gene responsible for the variability exhibited by the late reaction to lepromin inradermally injected.*

Key-words: *Genetics; Hansen's disease; Mitsuda Reaction.*

1. INTRODUCTION

It is a principle of general pathology that three factors should always by taken into account when an infectious disease is under consideration: the pathogenic agent, the degree of host resistance to infection, and the environmental conditions. However, in the particular case of leprosy, it is accepted beyond doubt that the degree of tissular resistance of human beings to *Mycobacterium leprae* plays the most important role among the factors which interfere in the manifestations of the disease.

This acceptance is due, on the one hand, to the fact that the majority of individuals exposed to leprosy bacilli do not manifest the disease (Godal and Negassi, 1973). On the other hand, leprosy is not a monomorphic disease, but includes, among various forms, two types - lepromatous and tuberculoid leprosy - which are antithetical from the clinical, pathological and immunological points of view (polar types of leprosy).

Since no phenotypic manifestations can be produced without the commitment of some genetic entity, it seems obvious that the degree of tissular resistance/susceptibility to *M. leprae* infection should depend, to some extent, upon host inherited factors. However little is known about them, at the presente time, as is shown in the following pages.

2. ANIMAL MODEL

The variation in the host's resistance to leprosy infection has been shown to be genetically controlled in animal models (Skamene et al., 1982). Experimental infections in mice have demonstrated that a gene located on chromosome 1 regulates the resistance/susceptibility to *M. lepraemurium* (Brown et al., 1982; Skamene et al., 1984) and *M. intracellulare* (Goto et al., 1984)

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among isogenic strains. The acquired resistance, occurring at a later stage and related to the specific immune cell-mediated response, seems to depend on more complex genetic mechanisms, among which the major histocompatibility complex (MHC) has been shown to play a role (Curtis et al., 1984). The natural resistance of mice to infections with *Salmonella typhimurium* (Plant and Glynn, 1974, 1976) and *Leishmania donovani* (Bradley, 1977) is regulated by chromosome 1 gene(s) designated *Ity* and *Lsh,* respectively (Plant and Glynn, 1979; Bradley et al., 1979). Also, innate resistance of inbred mice to infection with *Mycobacterium bovis* (BCG) is regulated by a single autosomal gene termed *Bcg,* which is expressed in two allelic forms: the dominant for resistance and the recessive for susceptibility (Gros et al., 1981).

A high resolution genetic map of the *Bcg* region on proximal mouse chromosome 1 has revealed that a 35cM fragment around the murine *Bcg* locus has been conserved between the murine chromosome 1 and the telomeric end of human chromosome 2q, region 2q32-2q37 (Schurr et al., 1990). Although other studies (Blackwell 1992; Shaw et al., 1993; Levee et al., 1994) could not provide conclusive evidence of linkage between a putative "leprosy" or"tuberculosis" gene and DNA markers on chromosome 2q35 (Morgan et al., 1994), which is believed to have regions homologous to the mouse chromosome 1 (Vidal at ai., 1993). This candidate gene forBcg encodes an integral membrane protein that has structural homology with known prokaryotic and eukariotic transport systems, suggesting a macrophage-specific membrane transport function (Vidal et al., 1993).

3. HUMAN LEPROSY

Despite accumulating evidence that genetic factors play a significant role in the susceptitility to human leprosy, no definite conclusion could be reached. Procedures to uncover genetic mechanisms affecting the susceptibility to leprosy, in humans, have been carried out basically in two sets of studies. The first one is the association and linkage studies of leprosy with genetic markers, especially HLA at either the population or the familial level (De Vries et al., 1980; Miyanaga et al., 1981; Searjeantson 1983; Van Eden and De Vries et al., 1984; Ottenhoff et al., 1984; Van Eden et al., 1985; Schauf et al., 1985; Ottenhoff and De Vries, 1987; Gorodezky et al., 1987; Abel et al., 1989). The other set of studies comprises familial aggregation of leprosy, including various twin studies (reviewed in Beiguelman 1972, 1983; Smith, 1979) and recently, complex segregation analyses (Serjeantson et al., 1979; Demenais et al., 1985; Haile et al., 1985; Shields et al., 1987; Abel and Demenais, 1988; Wagener et al., 1988; Abel et al., 1989, 1995; Feitosa et al., 1995).

Whereas segregation of a major gene either for leprosy or for its subtypes has been suggested by some studies, it is not supported by others. Smith (1979) investigated families from Philippines, in a classical segregation analysis, and found an autosomal recessive gene for susceptibility to lepromatous leprosy, but the authors also argued in favor a multifactorial hypothesis with heritability of about 80%. Searjeantson et al. (1979) analyzed 340 leprosy probands from Papua, New Guinea, applying multifactorial and single-gene models of inheritance, and foundthatthe familial distributions of both lepromatous and nonlepromatous cases are compatible with the multifactorial model. Haile et al. (1985) studying 72 multiplex families in South India, throughout the mixed model, suggested an autosomal recessive mode of inheritance for tuberculoid leprosy. Demenais et al. (1985), with 16 multigenerational pedigrees from Desirade Island, rejected the hypothesis of Mendelian transmission of a major gene under the transmission-probability model (Elston and Stewart 1971), using the joint likelihood of pedigrees. Abel and Demenais (1988), with a larger sample (27 multigenerational pedigrees), proposed the presence of a recessive major gene controlling susceptibility to leprosy *per se* and nonlepromatous leprosy, respectively.

Recent studies carried out in two groups of families of Chinese and Vietnamese, residing in Vietnam (Abel et al., 1995) showed that a single Mendelian gene could not account for the familial distribution of leprosy *per se* and its subtypes in the whole sample. Nevertheless, in the Vietnamese

subsample, there was evidence for a codominant major gene with residual familial dependence for the leprosy per se and rejection of the Mendelian transmission for nonlepromatous leprosy. In Chinese families, rejection of Mendelian transmission was obtained for leprosy per se, and no evidence for a familial component in the distribution of the nonlepromatous leprosy was detected. Similar results were obtained from a sample residing in Vietnam and in a Brazilian sample (Feitosa et al., 1995), after applying complex segregation analysis (Lalouel etal.,1983). The later results suggested the presence of a recessive major gene controlling susceptibility to leprosy perse. Nevertheless there were deviations from the expected Mendelian segregation proportions. For lepromatous leprosy and tuberculoid leprosy there were suggestions for a segregating major effect, but Mendelian transmission could not be demonstrated in either case. Consequently, a single Mendelian gene could not account for the familial distribution of leprosy and its subtypes.

The discrepancies among these results might have come about due to several reasons, such as genetic heterogeneity and the differing methodological approaches used. Environmental and/or behavioral factors on the transmission of the disease play an important role and may obscure a major genetic mechanism (if indeed is exists).

4. MITSUDA REACTION

When 0.1 ml of a sterile suspension of heat-killed leprosy bacilli (lepromin) is intradermally injected it may provoke a late reaction (Mitsuda reaction) which is macroscopically read at four weeks. This reaction is a consequence of events that follow the phagocytosis of the leprosy bacilli contained in lepromin by the histiocytes (macrophages) of the skin. If the phagocytized bacilli are destroyed by the macrophages, these cells transform themselves into epithelioid elements. Therefore, a positive Mitsuda response is histologically defined by the presence of epithelioid cells assuming a tuberculoid or tuberculoid-like structure where acid-fast bacilli are absent or scarcely found, while the absence of these picture will characterize a histologically negative Mitsuda reacion (Bechelli et al., 1959).

Taking into account that lepromatous leprosy patients are negative Mitsuda reactors, and that a positive Mitsuda reaction manifested by healthy individuals indicates resistance at least to lepromatous leprosy, it becomes very attractive to investigate if Mitsuda reaction would be a genetic polymorphism, which could explain inherited susceptibility/resistance to lepromatous leprosy.

Earlier studies have shown that this reaction exhibits familial aggregation either in samples free of leprosy (Beiguelman, 1962, 1971; Beiguelman and Quagliato, 1965) or in a sample of families with at least one parent affected by a polar form of leprosy (Beiguelman, 1965). Data on Mitsuda reaction of families tested by the late Dr. Reynaldo Quagliato in Campinas, SP, were analyzed by segregation analysis (Feitosa et al., 1996). The results suggested the segregation of a major gene with a frequency of q = 0.47, since the premises of transmission frequencies were satisfied, i.e., the hypotheses of non-Mendelian transmission and nontransmission of major gene were rejected (model 1 vs. model 3: x^2_3=0.07-0.0 = 0.07, P > 0.99; model 3 vs. model 2: x^2_3= 198.28 - 0.0 = 198.28, P > 0.0001, respectively) while a model with major gene with partial recessive effect (d = 0.811) fitted well to the data (Table).

5. FURTHER RESEARCH

Instead of focusing the search of an important genetic mechanism acting on disease or its forms, the results of Feitosa et al. (1996) attest that efforts should be deviated to the Mitsuda reaction due to its apparently more homogeneous phenotypes and clear genetic pattern. These approaches should emphasize the search for the physical localization of the gene responsible for the variability of the Mitsuda reaction. Undoubtedly the long arm of chromosome 2, around the position 32, should be the first candidate for linkage studies. In the case of failure to assign this gene to chromosome 2, different strategies should be devised, in order to assign to a specific chromosome the first infectious disease associated gene in the human species.

Table. Segregation analysis of Mitsuda reaction

Model	q	H	-2lnL + c	χ^2	D.F.
1. Mixed Mendelian τAA = 0.0, τAa = 0.5, τaa = 0.0	0.474	0.0*	0.07		
2. Free τ_s τAA = 1.0*, τAa = 0.492, τaa = 0.0*	0.473	0.0*	0.00	0.07	3
3. Equal τ_s τAA = τAa = τaa = 1.0*	0.173	0.0*	198.28	198.28	2

(*) reached its bound Source: Feitosa et al. (1996)
d = degree of dominance q = gene frequency H = multifactorial component

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