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EDITORIALS

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Immunogenetics of Susceptibility to Leprosy, Tuberculosis, and Leishmaniasis. An Epidemiological Perspective

The phrase "immunogenetics of susceptibility" has a stylish ring to it. But what does it mean, with reference to diseases such as leprosy, tuberculosis, and leishmaniasis? We may find it has an illusory potential of appearing clearer at a distance than close by.

The "genetics" part may be easiest to understand, in its classical sense of referring to the mechanism of control by nuclear genes, DNA, Mendel's rules, and so forth. But the word "immunogenetics" begins to raise problems. Textbooks and dictionaries suggest that the subject of immunogenetics covers, e.g., "factors which control the immunologic responsiveness of the host to foreignness"[1]. This is all very well, except that it may be too narrow a view if one's ultimate interest is not restricted to humoral or cell-mediated responses or to HLA per se but is in the genetic control of how an individual responds when exposed to microorganisms. Some of these responses may be determined by membrane structures or by cellular metabolic factors distinct from classical immunological ones. Certainly very little of the available evidence on genetic determination of responses to mycobacterial or leishmanial infection is sufficient to confirm an immunological mechanism as such. The word "immunogenetic" might thus be too restrictive.

Finally the trouble grows deep with the term "susceptibility." Dictionaries contribute rather little: e.g., "susceptibility is the state of being readily affected . . . ."[2] What does this mean in the context of, say, leprosy? Is it the state of being readily infected with *M. leprae* or of responding to this infection in one or another way? Is susceptibility to tuberculoid leprosy similar to susceptibility to lepromatous leprosy or is it something very different? Is a self-healing tuberculoid lesion evidence of susceptibility at all—or of resistance? Is susceptibility a quantitative or a qualitative (binary?) variable? Let me emphasize that I do not

[1] Bellanti, J. A. *Immunology*. Philadelphia: W. B. Saunders Co., 1971.

[2] *Dorland's Illustrated Medical Dictionary*. 24th ed. Philadelphia: W. B. Saunders Co., 1965.

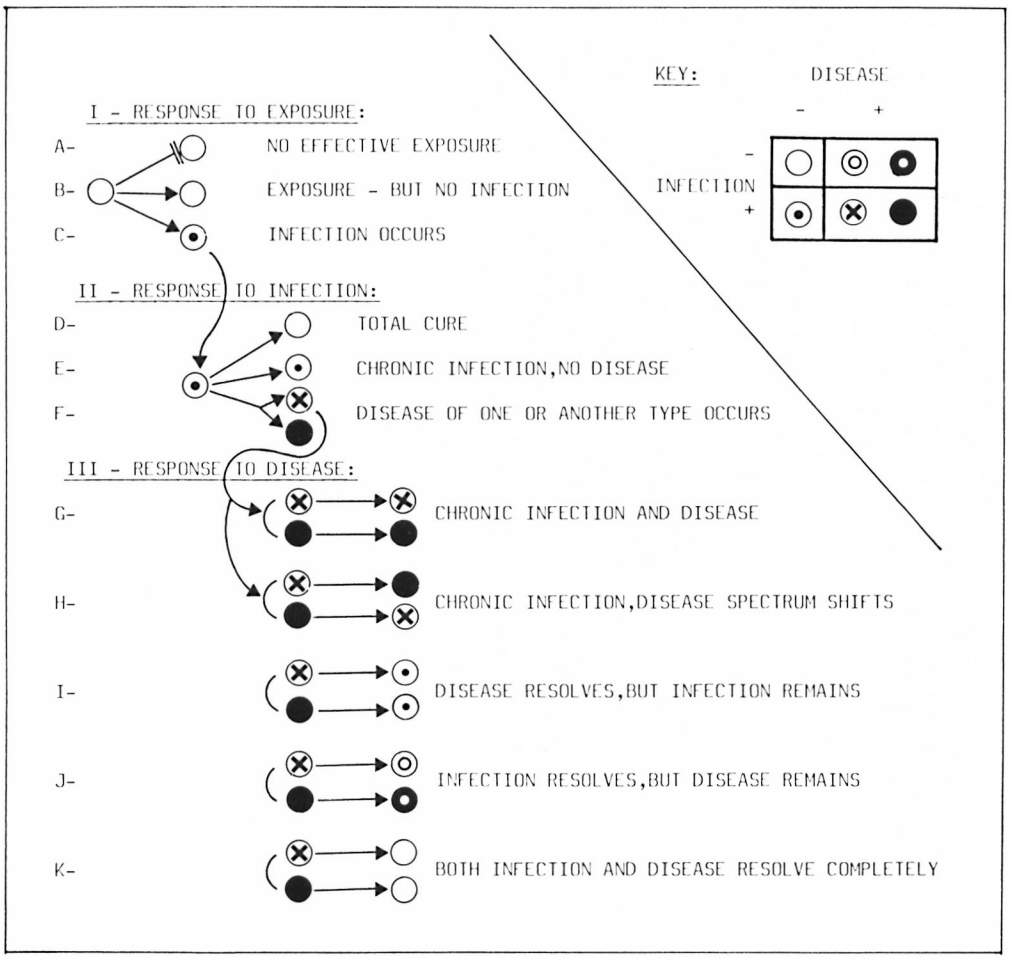

FIG. 1. Schematic diagram illustrating the possible successions of events following exposure of an animal to an infectious agent. Many of these possible transitions have implications for different concepts of "susceptibility."

believe this is an artificial confusion; on the contrary, it is real, widespread, and important. Different authors use the term "susceptibility" to mean different things, and it may be that this important subject is obstructed by self-imposed semantic if not conceptual problems.

Though not intended as a definitive rescue mission, the framework indicated in Figure 1 provides one approach to clarifying this dilemma over the meaning of susceptibility. It illustrates in a logical stepwise fashion the various things which can happen if an animal is exposed to an infectious agent, for example, if man is exposed to *Mycobacterium leprae*. First, no infection may occur because the exposure was inappropriate or inadequate (*A*). Second, no infection may occur despite massive and thorough exposure because of some form of resistance, innate or acquired, in the host (*B*). Third, infection (defined as invasion and proliferation of the agent within the host) may occur. Which of these situations implies host susceptibility? The first (*A*) tells us nothing, since the individual was not effectively exposed (though the concept of susceptibility to exposure should not be entirely dismissed). The second (*B*) may or may not say anything about susceptibility to infection, depending upon whether the failure of infection to occur is due to an innate resistance in a therefore insusceptible person or perhaps to an acquired resis-

tance in an individual who was originally susceptible to the infection. Only the third case (*C*) provides clear evidence of susceptibility—susceptibility to infection—by definition. It would be nice if it were always that simple in practice. Phthisiologists are fortunate in being able to recognize the infected state in tuberculosis on the basis of reasonably specific and sensitive tuberculin testing. But leprologists are less lucky in that they have as yet no highly sensitive let alone specific tool for recognizing infection with *M. leprae*. Recent work on development of a specific lepromin skin test antigen and on fluorescence tests for detection of serum antibodies hold promise but as of now we still lack such a test in leprosy. Diagnostic tests for subclinical leishmanial infection are by no means perfect.

What happens to an infected individual? He may undergo a total cure of the infection with no observable or measurable disease manifestation (*D*). He may maintain the infection within him but with no recognizable disease (*E*). Or he may go on to develop some form of pathology, say clinical tuberculoid or lepromatous leprosy (*F*). Which one is susceptible? Different authors define their own rules, according to the data at hand, and the game they wish to play.

In a similar fashion, logic tells us that there are several possible outcomes for the diseased class: a maintenance of both the infection and the disease (*G*); a maintained infection but shift in disease spectrum (*H*); a resolution of all pathology but maintained infection (*I*); resolved infection but continued overt disease (*J*); or a complete resolution of both the infection and the disease process—the "complete cure" (*K*). My suspicion is that most, if not all, of these possibilities actually occur in leprosy and perhaps in tuberculosis and leishmaniasis as well. Of course this is a very crude view. Immunologists would prefer to reduce the several alternatives to arrows indicating cellular mechanisms and interactions. We will find that some of these transitions are in fact comprised of several steps. On the other hand, these are the basic states which the clinician and geneticist observe as phenotypes, which have thus been widely studied in the literature, and which are conventionally discussed in terms of host resistance or susceptibility.

One way out of the terminology muddle is to carefully and constantly qualify the term "susceptibility"; "susceptibility to infection"; "susceptibility to tuberculoid disease"; "susceptibility to lepromatous disease"; "susceptibility to progressive pulmonary disease." Indeed, one finds these different qualifications of susceptibility discussed throughout the literature. This is all very well, provided it avoids diluting the concept of susceptibility to nothingness, e.g., "susceptibility to default in treatment," and avoids any implication that the basic mechanisms are identical. These different sorts of susceptibilities probably involve a variety of very different mechanisms. Furthermore, not all of them need be genetically determined.

This raises a further paradox for those with interests in genetic mechanisms underlying these phenomena. Though not necessarily genetically fully determined, each observed type of susceptibility must at least indicate an underlying genotype which is consistent with the observed response. In one sense, all biological processes are genetically programmed and all susceptibilities are genetical. On the other hand, this may not be a very interesting perspective. In practice, genetical factors become interesting only when they explain to an appreciable degree the observed differences between individuals in a population. The phrase "to an appreciable degree" has been underlined advisedly because it reflects the crucial issues in considering the immunogenetics of our three diseases. The outstanding questions today are not whether but how, i.e., by what mechanism, and to what degree do genetic factors determine host response to exposure or to infection. This raises issues of polymorphisms or genetic variability within and between populations, of the penetrance of genetic factors, and of the relative risks associated with particular genotypes (to which we must return later).

The evidence in man. We now turn to review available evidence that genetical factors determine differences in host susceptibility or response to infection with mycobacterial or leishmanial organisms. Table 1 summarizes the situation in the form of a matrix showing the different sorts of evidence which have been published for

TABLE 1. *Evidence for genetic control of "susceptibility."*

	Leprosy	Tuber-culosis	Leish-maniasis
In man			
Gossip	++	+	−
Racial differences	+/−	+/−	+/−
Family clustering	+/−	+/−	+/−
Pedigree analysis	+/−	−	−
Twin studies	+/−	+	−
Marker-population	+	+/−	−
Family linkage	++	−	−
Gene identification	−	−	−
In animals			
Strain differences	+	+	++
Linkage	−	−	++
Gene identification	−	−	+/−

each of the infections. The literature on the genetics of the leishmanial infections in man is small compared with that on leprosy and tuberculosis, and thus the several different leishmanial infections are subsumed under a single column heading. In general, the trend is from weaker to stronger levels of inference as one moves from top to bottom within the table. The evidence will be discussed in this order.

1) **Gossip.** Conventional wisdom should not be entirely overlooked. Many traits which we now recognize to be genetically determined had long been popularly recognized to have an hereditary basis. Indeed, one has only to scratch the surface of the literature to find a deep traditional belief that genetic, or at least hereditary, factors are of importance in both leprosy and tuberculosis. Two quotations from important 19th century works illustrate this point nicely:

a) Regarding leprosy:

> "Few facts in the history of tubercular leprosy seem to be more universally admitted by all writers on the disease, both ancient and modern, than the transmission of the predisposition to it from parents to offspring."
>
> (Simpson, 1841)[3]

b) Regarding tuberculosis:

> "That phthisis propagates itself in many families from generation to generation is so much a matter of daily experience, that the severest sceptic can hardly venture to deny a hereditary element in the case: even if we be unable for the present to decide whether it consists in the transmission of a specific poison, . . . or whether it do not rather depend, as seems to me more probable . . . , upon a congenital disposition towards the disease, a disposition that has to be looked for, naturally, in the organization of the respiratory system."
>
> (Hirsch, 1886)[4]

To my knowledge, no such tradition exists in the leishmaniasis literature.

Early opinions on the hereditary nature of leprosy and tuberculosis were challenged by the discoveries of *Mycobacterium leprae* and *M. tuberculosis* in the last century. The new bacteriologists claimed that familial patterns reflected only the transmission of the agents under conditions of close contact within the home rather than some inherent or genetical family trait. However, their challenge did not lead to easy victory but to a debate which is still not fully resolved. It has been a personal, ironic and emotional, as well as a scientific debate.

Both argument and irony began when Armauer Hansen, himself son-in-law to D. Danielssen, one of the most prominent upholders of the theory of leprosy's hereditary nature, discovered the leprosy bacillus and espoused a contagionist view of its epidemiology[5]. The irony was heightened when C. W. Boeck, colleague of Danielssen and a convinced hereditarian, and Armauer Hansen both travelled to the United States to test their hypotheses by studying leprosy among Norwegian immigrants. They looked at the same data but came to opposite conclusions: Boeck found heredity; Hansen found infection[6]. The debate has been com-

[3] Simpson, J. Y. Antiquarian notices of leprosy and leper hospitals in Scotland and England. Part III. Edin. Med. Surg. J. **57** (1841) 394–429.

[4] Hirsch, A. *Handbook of Geographical and Historical Pathology*. Vol. III, 2nd ed. Creighton, C., trans. London: New Sydenham Society, 1886.

[5] Aycock, W. L. Familial susceptibility as a factor in the propagation of leprosy in North America. Int. J. Lepr. **8** (1940) 137–150.

[6] Lie, H. P. Norwegian lepers in the United States. Int. J. Lepr. **6** (1938) 351–356.

plicated by the religious associations and social stigma attached to leprosy in particular, which have led some authors to reject the hereditarians' hypotheses on the grounds of unpleasantness as much as on the basis of their logical content. The fact that the vehicles of at least one leprosy control program in India were carrying banners declaring that "leprosy is not hereditary" in 1979 indicates the persistent and many-sided nature of this controversy.

Once again a major problem has been semantic—a confusion over the meaning of heredity with reference to a condition such as leprosy or tuberculosis. Many of the early authors were careful to suggest that it was the "predisposition" (another word for susceptibility?) which was inherited, but there were many variations on the theme. For example, August Weismann, to whom we owe the concept of the continuity of the germ plasm, wrote:

> "Without wishing to deny existence of such a predisposition to infection, I do not believe that the transmission of tuberculosis is due merely to the inheritance of a greater degree of susceptibility. A large number of facts seem to me, on the contrary, to support the view that *infection of the germ plays the chief part* in the process."[7]

The important message is that the confusion of just what is being inherited, a predisposition, a susceptibility to infection, the infection itself, or some feature of the family environment, is a persistent theme throughout this literature. One may well question whether the much discussed "factor N" hypothesis, first proposed by Rotberg to explain leprosy patterns, is any improvement on the vague arguments of this debate[8,9]. We are still not out from under it.

2) **Racial differences.** Many discussions of leprosy and tuberculosis epidemiology refer to evidence for differences in incidence rates or disease manifestations between different races[10]. Most often cited in this context is the variation in proportion lepromatous among leprosy patients, generally considered to be higher among caucasians than among negroids[11], or in the case fatality rate of tuberculosis, often considered to be higher among blacks than among whites[4,12]. The leishmaniasis literature carries few such anecdotes, though one recent study has suggested a significant difference in leishmaniasis pattern between genetically different but sympatric populations in South America (P. D. Marsden, personal communication, 1980).

Some authors have attempted to draw genetical messages out of this evidence for racial differences in disease patterns. But such evidence is weak. Even if the reported differences were true, and not just artifacts of differences in case ascertainment or diagnostic convention (and one may wonder, for example, as to the implications of skin pigmentation on the recognition and interpretation of skin lesions), they need not be attributed to genetics. Many factors which are likely to vary between racial groups could equally well explain differences in disease patterns: age at infection, level of exposure, nutrition, intercurrent infection, strain of infectious agent involved, access and use of medical care services, and a variety of environmental factors. Indeed, most critical commentators on this literature have tended to disregard the racial difference argument as dangerously weak or even misleading[13,14]. It is extremely difficult to find racially distinct groups which are nonetheless sufficiently comparable in terms of the many nongenetic risk factors to allow confident inferences as to their

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[7] Weismann, A. *The Germ-Plasm: A Theory of Heredity*. Parker, W. N. and Rönnfeldt, H., trans. London: Walter Scott, Ltd., 1893.

[8] Rotberg, A. Some aspects of immunity in leprosy and their importance in epidemiology, pathogenesis and classification of forms of the disease. Rev. Bras. Leprol. **5** (1937) 45–97.

[9] Newell, K. W. An epidemiologist's view of leprosy. Bull. WHO **34** (1966) 827–857.

[10] Spickett, S. G. Genetics and the epidemiology of leprosy: I. The incidence of leprosy. Lepr. Rev. **33** (1962) 76–93.

[11] Spickett, S. G. Genetics and the epidemiology of leprosy. II. The form of leprosy. Lepr. Rev. **33** (1962) 173–181.

[12] Puffer, R. R. *Familial Susceptibility to Tuberculosis: Its Importance as a Public Health Problem*. Cambridge: Harvard University Press, 1946.

[13] Stoner, G. L. Ir genes and leprosy. Int. J. Lepr. **46** (1978) 217–220.

[14] Simonds, B. *Tuberculosis in Twins*. London: Pitman Medical Publ. Co., Ltd., 1963.

genes. The several attempts to do this in the leprosy literature are in general unconvincing. Certainly the leap from such evidence to immunogenetics is chasmatic.

3) **Family clustering.** Genes cluster in families, so genetically determined traits must do so as well. Following this or another logic, many workers have looked for family clusters of leprosy, tuberculosis, and leishmaniasis. Clusters have been found. Especially interesting, however, is the fact that the observed clustering has often been interpreted in different ways.

Though workers in some parts of the world have commented that family clustering of *Leishmania donovani* infections was not apparent (e.g., [15]). Southgate reported and emphasized the household clustering of kala azar cases in northeastern Kenya[16]. His inference was that *L. donovani* was locally transmitted in an anthroponotic rather than a zoonotic cycle; in effect, households living near termite hills harboring the vector *Phlebotomus martini* experienced high incidence rates because of the shared environment. Interestingly, Southgate did not mention genetics at all in his discussion of household clustering. Is it that one sees what one looks for? It is only with the current surge of interest in genetics, and with the evidence for strong genetic control of leishmanial infections in mice, that research and interpretations have begun to focus on this aspect of the human disease (T. C. Jones, personal communication, 1980).

Familial tuberculosis has traditionally been interpreted as reflecting both common exposure and shared genetic susceptibility[4, 12, 14, 17]. The contagious potential of sputum-positive cases has long been recognized and has provided one obvious explanation. The intimacy of contact within the home would be expected to produce family clusters of tubercular disease, even without any genetic differences in the population. On the other hand, there has been a longstanding belief in the hereditary predisposition to phthisis, antedating the bacteriological era. This may explain why familial tuberculosis has generally been interpreted as reflecting both contact and genetical factors.

Family clustering has probably been more persistently studied with reference to leprosy than with reference to either tuberculosis or leishmaniasis[5, 10, 18, 19, 20, 21]. Reading through this literature, I am impressed that the authors have often emphasized genetical factors in their interpretation of observed clustering. Two reasons might be suggested for this: 1) the long-standing tradition of attributing leprosy to hereditary factors; and 2) because of a confusion between concepts of infection and disease and an inability to recognize subclinical infections, *Mycobacterium leprae* has until recently been considered of very low contagious potential. Convinced of low contagion, some workers may have been reluctant to stress the common exposure factor in interpreting familial clusters.

Family clusters have thus been reported for each of our three disease groups, but they have often been interpreted differently. This is only part of the irony, however, for a closer look at the literature may encourage skepticism as to whether much of the reported family clustering is real at all. There are a number of methodological issues which must be met by any convincing study of this problem:

a) Most of the claims of clustering have been based upon an implicit or explicit difference between the frequency distribution of observed numbers of cases per household and expectations based on Poisson or binomial distributions. Many studies have

[15] Taj-eldin, S., Guirges, S. Y. and Almashadani, H. M. On the reservoir host of kala azar in Iraq. Iraqi J. Pediatr. **1** (1971) 21–32.

[16] Southgate, B. A. The structure of foci of visceral leishmaniasis in north-eastern Kenya. Coll. Intern. CNRS **239** (1974) 241–247.

[17] Frost, W. H. Risk of persons in familial contact with pulmonary tuberculosis. Am. J. Public Health **23** (1933) 426–432.

[18] Beiguelman, B. An appraisal of genetic studies in leprosy. Acta Genet. Med. Gamellol. **21** (1972) 21–52.

[19] Noordeen, S. K. and Mohamed Ali, P. A study of 579 families having multiple cases of leprosy: first report. Lepr. India **36** (1964) 176–182.

[20] Rao, P. S. S., Karat, A. B. A. and Karat, S. Epidemiological studies in leprosy in Gudiyatham Taluk, II. Patterns of familial aggregation of leprosy in an endemic area. Lepr. Rev. **40** (1969) 93–98.

[21] Sharma, V. K. The epidemiologic significance of leprosy within the household. Leprosy in India **36** (1968) 1–16.

failed to standardize for family size, though this is clearly crucial: if cases come from large families, they will appear to be clustered. Beiguelman noted that family size standardization is necessary and suggested a simple method for doing this[22]. He fails, however, to emphasize that several other factors can bias such data to an even greater extent.

b) Given a correlation between age and infection or disease prevalence, and between ages and family size, then a comparison between families must consider whether different age distributions could explain the result.

c) Most of the so-called family cluster studies do not deal with familes *per se* but with households. For example a familial aggregation study in South India defined a family as follows: "a group of individuals partaking of food from a common kitchen[20]. Such definitions encompass unrelated individuals and omit related individuals, and they certainly lower the strength of genetic inferences. It may be added that the ascertainment of true families presents a difficult problem in the field and has rarely been achieved.

d) Apparent family clustering may arise if case finding is more intense in some families than in others. In fact, the widespread belief that leprosy clusters in families and institutionalized programs of contact tracing within households have meant that ascertainment is rarely uniform between families and individuals, independent of family history. In this situation, family clustering becomes a self-fulfilling prophecy. This bias is exceedingly difficult to avoid in practice and may itself invalidate most of the published studies of family clustering.

e) Social factors may be responsible for clustering. There may be a tendency for affected individuals to remain unmarried and hence to accumulate within families. Or there may be a tendency for leprosy cases to marry other cases; hence producing an apparent cluster. The only way to avoid this bias is by complete and accurate ascertainment of all relatives, regardless of where they live.

f) One must be wary of the bias of the extraordinary. The unusual families with multiple cases tend to attract attention and may lead to false impressions of case clustering, though they may represent merely the expected extremes of a random distribution.

Avoidance of all these biases presents a considerable challenge for field methodology and analytical procedures. I am aware of no study in the literature which has succeeded in doing so, though the current Lepra Evaluation Project in northern Malawi is at last assembling an appropriate data set. Thus the sceptic may question whether family clustering has ever been convincingly demonstrated. This precedes the crucial question of the reason for any such clustering—for families share beds, air, drinking cups, etc., as well as genes.

Only one study has, to my knowledge, attempted to examine the confounding between genetical and contact relationships within families. White, *et al.* examined the risk of leprosy in families included in the MRC trial of BCG against leprosy in Uganda[23]. They found the geneticists' prediction: the closer the genetic relationship to an infected proband, the higher the incidence rate. But the closest relatives also had closest contact with the probands. When White, *et al.* controlled for proximity of contact, the genetic gradient disappeared; the apparent risk associated with genetic relationship could be completely explained in terms of contact. They thus concluded ". . . if a genetic component of susceptibility existed, its influence was small."

4) **Pedigree analysis.** Genetics textbooks are typically decorated with family trees illustrating near inheritance patterns consistent with Mendel's laws, the best known being the distribution of hemophilia among the descendants of Queen Victoria. In principle this seems an elegant way to test genetical hypotheses. On the other hand, it has severe limitations, as it is widely recognized that pedigree analysis has very poor resolving power unless the genetic factor is a single gene with high penetrance.

[22] Beiguelman, B., Dall'aglio, F. F. and Da Silva, E. Analise da recorrencia familias de lepra. Rev. Paul Med. **72** (1968) 105–110.

[23] White, S. J., Stone, M. M., and Howland, C. Genetic factors in leprosy: a study of children in Uganda. J. Hyg. (Camb.) **80** (1978) 205–216.

Let alone the questionable *a priori* plausibility of expecting a single gene to control the population pattern of leprosy, tuberculosis, or leishmaniasis, we might not expect very high penetrance of such a factor, given that the gene could not be itself sufficient to produce the clinical phenotype. The infection must be present as well.

Despite such difficulties, a few authors have applied conventional pedigree analysis to families with leprosy. Belknap and Hayes thus suggested a dominant gene for susceptibility, with 63% penetrance in a Caucasian population in Louisiana[24]. Spickett suggested a dominant autosomal gene with 83% penetrance controlling susceptibility in a French population in New Brunswick ([10]). Gallant attempts though these were—and Spickett can at least be credited for his attempt to introduce rigorous logic to the leprosy problem—they can carry little weight. Spickett did not distinguish between tuberculoid and lepromatous cases in his pedigrees, thus implying a uniform condition with a single susceptibility factor, an assumption which most contemporary workers would consider unlikely. Then there is the logical problem introduced by the assumption of incomplete penetrance; such models are so flexible as to be almost untestable. This problem of untestability has still not deterred some geneticists from applying highly complex multifactorial models to leprosy data. For example, Sergeantson, *et al.* found that a multifactorial model fitted a New Guinea data set better than did a single gene model[25]. This is neither surprising nor, as the authors admit, is it in any way conclusive.

Pedigree analyses can take other forms as well. Several authors have claimed that spouses of leprosy cases have lower incidence rates than do their children or blood relatives, suggesting that this is due to genetical factors in the case[26,27]. This too is not a simple argument, as any difference could also be due to the late age of exposure of spouses and long incubation periods, to protective immunity (e.g., previous BCG or cross-protecting atypical mycobacterial infection in spouses), or to the fact that much of the leprosy diagnosed in children may be of the ephemeral self-resolving sort, which would have resolved years before in the spouse. No author has as yet presented a convincing argument on this.

Puffer applied an analogous argument for tuberculosis, inferring that the high rate of tuberculosis among spouses of cases was an indication of contact but that a correlation between rates in spouses and rates in spouses' families indicated genetic predisposition as well[12]. Of course the sceptic could note that both associations would be expected even in the absence of any genetically-determined susceptibility.

Another pedigree approach was taken by Beigueman in studies of the lepromin reaction among children of parents, neither, one, or both of whom were lepromin positive[28,29]. He considered the results as favoring a dominant gene for lepromin positivity. Unfortunately, he did not control for age, which is widely recognized to confound lepromin results. Among children of two lepromatous parents, his putative homozygous recessives, 31% were lepromin positive. To infer a single gene from such evidence seems unreasonable.

Though classical pedigree analysis is unlikely to help in sorting out the genetic basis of susceptibility to leprosy, tuberculosis, or leishmaniasis, there is one aspect of pedigree logic which has not yet been sufficiently emphasized. Geneticists in the Mendelian mode generally assume symmetry for pedigree patterns, as is expected for autosomal factors. On the other hand, there are numerous sets of data which indicate an asymmetry to leprosy and tuberculosis distributions within families. In general, though not universally, a slightly higher

[24] Belknap, H. R. and Hayes, W. G. A genetic analysis of families in which leprosy occurs (abstract). Leprosy in India **29** (1961) 375.

[25] Sergeantson, S., Wilson, R. R. and Keats, B. J. B. The genetics of leprosy. Ann. Hum. Biol. **6** (1979) 375–393.

[26] Mohamed Ali, P. A study of conjugal leprosy. Int. J. Lepr. **33** (1965) 223–228.

[27] Quagliato, R. Lepra conjugal. Rev. Bras. Leprol. **25** (1957) 59–68.

[28] Beiguelman, B. and Quagliato, R. Nature and familial character of the lepromin reactions. Int. J. Lepr. **33** (1965) 800–807.

[29] Beiguelman, B. Leprosy and genetics; a review of past research with remarks concerning future investigations. Bull. WHO **37** (1967) 461–476.

TABLE 2. *Summary of results of major twin studies of tuberculosis (adapted from reference [14]).*

Authors	Monozygotic				Dizygotic			
	Total pairs		Concordant pairs		Total pairs		Concordant pairs	
	Number	(%)	Number	(%)	Number	(%)	Number	(%)
Diehl & von Verschuer[34]	80	(39%)	52	(65%)	125	(61%)	31	(25%)
Dehlinger & Künsch[35]	12	(26%)	7	(58%)	34	(74%)	2	(6%)
Kallman & Reisner[36]	78	(25%)	69	(62%)	230	(75%)	83	(18%)
Harvald & Hauge[37]	37	(26%)	14	(38%)	106	(74%)	20	(19%)
Simonds[14] and Comstock[38]	55	(27%)	18	(32%)	150	(73%)	21	(14%)

risk is noted among children of affected mothers than among children of affected fathers[12, 30]. This asymmetry, if real, is less likely to reflect a sex linked genetical factor than to indicate an implication of the special intimacy of contact between mothers and children. It raises problems for pedigree analysis which have not yet been met by any of the gene-theory bound writers on this subject, with the exception of a clever and little known analysis by Stocks and Karn[31, 32].

5) **Twin studies.** We now make a quantum leap in power of experimental design. Monozygous twins share similar chromosomes; dizygous twins share on the average half of their genes. Thus, if a trait is genetically determined, we expect monozygotes to resemble each other for the trait—to have a higher "concordance rate" than do dizygotes. If one can assume that the two types of twins share their environments to an equivalent degree, then a difference in observed concordance rates between the two types of twins may be attributed to their different genetic backgrounds. In theory, the twin study is ideal for testing genetic hypotheses when multiple genes are involved, i.e., in those situations where family pedigree studies fail. In addition, the twin study has the special advantage of allowing a direct measure of penetrance (defined as the proportion of carriers of a genotype who express this phenotypically). Penetrance should be the proband concordance rate, or the proportion affected among co–twins of ascertained affected monozygotes[33]. There is a long history of twin studies in tuberculosis and a rather shorter one for leprosy.

Table 2 presents a summary of results of the better known twin studies in tuberculosis. Note that in each of these studies ⅔ to ¾ of the ascertained twins were dizygotes, which is the proportion expected. Secondly, note that each study found a considerably higher concordance rate between monozygotic pairs. On the surface, this seems good evidence for genetic factors in some aspect of susceptibility to tuberculosis. One might even go so far as to suggest a 30–60% penetrance for the predisposing genotype on the basis of the concordance rates among monozygotes. On the other hand, one must not accept such results uncritically as twin studies are notoriously prone to many biases. Only a few can be mentioned here, and the interested reader

[30] Dharmendra. *Notes on Leprosy*. Delhi: Ministry of Health, Government of India, 1967.

[31] Stocks, P. and Karn, M. N. Fresh evidence on the inheritance factor in tuberculosis. Ann. Eugen. **3** (1928) 84–95.

[32] Fine, P. E. M. Analysis of family history data for evidence of non-Mendelian inheritance resulting from vertical transmission. J. Med. Genet. **14** (1977) 399–407.

[33] Bulmer, M. G. *The Biology of Twinning in Man*. Oxford: Clarendon Press, 1970.

[34] Diehl, K. and Von Verschuer, O. *Der Erbeinfluss bei den Tuberkulose*. Jena: Gustav Fischer, 1936.

[35] Dehlinger, E. and Künsch, M. Uber Zwillingstuberkulose. Beitr. Klin. Tuberk. **92** (1938) 275.

[36] Kallman, F. J. and Reisner, D. Twin studies on the significance of genetic factors in tuberculosis. Ann. Rev. Tuberc. **47** (1943) 549–574.

[37] Harvald, B. and Hauge, M. A catamnestic investigation of Danish twins—a preliminary report. Dan. Med. Bull. **3** (1956) 150–158.

[38] Comstock, G. W. Tuberculosis in twins: a reanalysis of the Prophit survey. Am. Rev. Respir. Dis. **117** (1978) 621–624.

is referred to Bulmer[33] or Simonds[14] for further discussions.

a) Ascertainment is the main problem. In principle, a twin study should include all twin pairs in the population of interest. In many populations, approximately 1% of births are twin deliveries and thus approximately 2% of individuals have twins (though this varies considerably between different races). Of the tuberculosis studies, those by Harvald and Hauge and by Simonds came closest to ascertaining all twins in their populations. In general, failure to ascertain all twins selects in favor of monozygotes and concordant pairs, since it is the usual similarities which are recognized in the community. Thus it is probably no coincidence that the Harvald-Hauge and the Simonds studies revealed lower concordance rates for monozygotes than did the other twin studies cited. Twin study estimates of concordance (and of penetrance) are likely to be exaggerations.

b) Second is the problem of zygosity diagnosis. Accurate distinction of monozygotes and dizygotes is essential. In addition to objective measures such as blood markers, zygosity diagnosis generally involves a subjective general similarity assessment, and this is typically biased in favor of increasing monozygote concordance rates.

c) When studying traits with a considerable environmental component, such as infectious diseases, it is necessary to insure or assume similar contact or exposure histories for both types of twins. This presents practical problems, for in most societies monozygotic twins are socially closer than are dizygotes, and this will bias in favor of environmentally-determined concordance among monozygotes.

d) The careful student must be aware of several other biases in such data. For example, in Kallman and Reisner's tuberculosis series, the prevalence of tuberculosis was much higher among parents of monozygotes than among parents of dizygotes, a difference which may well have exaggerated the risk and hence concordance rates among monozygotic pairs. In another example, Simonds found an excess of female pairs among monozygotic twins in the Prophit survey; the higher incidence of disease among females may thus have increased the concordance among monozygotes in that series[14].

TABLE 3. *Summary of results of twin study of leprosy by Chakravartti and Vogel*[40].

Leprosy distribution	62 monozygotic pairs		40 dizygotic pairs	
Both afflicted	37		8	
Type concordant	32		6	
Both lepromatous		11		2
Both tuberculoid		19		4
Both borderline		2		—
Type discordant	5		2	
One afflicted	25		32	
Lepromatous		6		8
Tuberculoid		18		20
Borderline		1		4

In the face of so many biases, caution is essential in the interpretation of twin data. Indeed, Simonds was so cautious in interpreting the Prophit survey—probably the best available data set—as to question whether the apparent difference in concordance between the two twin types was real at all[14]. However, Comstock was able to reanalyze these data using multivariate methods to standardize for a large number of variables and concluded that the difference was significant, though only at the 5% level[38].

The leprosy twin studies are less convincing. There is the usual series of case reports, generally examples of two affected twins, but such data are notoriously misleading since there is little motive to ascertain or to publish discordant twin pairs[11]. Series of leprosy twins have been assembled by a few workers, but these were in general very poorly documented[11,39]. The only thoroughly documented twin series in leprosy is that of Chakravartti and Vogel, summarized in Table 3[40]. These authors considered that the higher concordance rate for leprosy among monozygotes (37/62) in comparison to dizygotes (8/40) was strong evidence in favor of genetical factors underlying susceptibility to leprosy. On the other hand, ascertainment bias was ob-

[39] Mohamed Ali, P. and Ramanujam, K. Leprosy in twins. Int. J. Lepr. **34** (1966) 405–407.

[40] Chakravartti, M. R. and Vogel, F. A twin study on leprosy. Top. Hum. Genet. **1** (1973) 1–123.

viously huge in this study as they found considerably more monozygotic than dizygotic pairs whereas one expects at least two-thirds of twin pairs should be dizygotes. Ascertainment always favors the discovery of like pairs; thus, these concordance rates are without doubt greatly exaggerated. The authors argued that this bias might affect dizygotes more than monozygotes and thus at least the difference in rates might be real, but this may not convince the critical worker. Furthermore, the comparison of overall leprosy concordance rates (37/62 versus 8/40) implies a susceptibility factor for clinical leprosy per se which many workers would find less likely *a priori* than a factor associated with one or another clinical form. With respect to concordance for clinical type, the rates were similar for monozygotes (32 of 37) and dizygotes (6 of 8). Of course one expects the majority of affected pairs to be similar for type, just by chance, since most leprosy is classified at the tuberculoid end of the spectrum. On the other hand, the 5 type-discordant monozygote pairs in these data are perhaps the most interesting observations in the literature on leprosy genetics. If valid, they firmly establish that the lepromatous response to infection is not determined by genes alone.

6) **Genetic marker studies.** There are two types of studies based on genetic markers. One seeks broad associations between gene markers and phenotypes at the population level, using a conventional case-control design. The other looks within families, taking principles of Mendelian segregation into consideration. Both studies investigate whether a putative genetic factor is identical with, or closely linked to, the marker employed—traditionally a blood group marker, but more recently a variety of serum proteins, enzymes, or HLA haplotypes.

There is a large literature on population marker studies, especially with reference to leprosy. Among the markers which have been examined are: blood groups ABO, Rh, MN, Kidd, Kell, Cellano, Duffy, Lutheran; erythrocyte enzymes phosphoglucomutase 1 and 2, acid phosphatase, adenylate kinase, adenosine deaminase, G6PD; serum proteins Hp, Gc, Gm, Pi, Inv, Tf, atypical pseudocholinesterase; ability to taste phenylthiourea; and histocompatibility antigens at the A, B, C and D loci (e.g., [29, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50]). There have been fewer studies of this sort with reference to tuberculosis, but a number of recent authors have examined HLA antigens in tubercle patients and controls[51, 52, 53].

Several of these studies have found associations which cross the threshold of statistical significance. On the other hand, only one of the associations (between leprosy and HLA-DR2; see below) has proven consistent between different studies on dif-

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[41] Lechat, M. F., Bias, W. B., Guinto, R. S., Cohen, B. H., Tolentino, J. G. and Abalos, R. M. A study of various blood group systems in leprosy patients and controls in Cebu, Philippines. Int. J. Lepr. **36** (1968) 17–31.

[42] Hitzeroth, H. W., Walter, H. and Hilling, M. Genetic markers and leprosy in South African negroes. I. Serum protein polymorphisms. S. Afr. Med. J. **56** (1978) 653–658.

[43] Hitzeroth, H. W., Walter, H., Hilling, M. and Munderloh, M. Genetic markers and leprosy in South African negroes. II. Erythrocyte enzyme polymorphisms. S. Afr. Med. J. **56** (1978) 507–510.

[44] Thomas, M. and Job, C. K. Serum atypical pseudocholinesterase and genetic factors in leprosy. Br. Med. J. **3** (1972) 390–391.

[45] Chan, S. H., Oon, B. B., Kamarudin, A. and Wee, G. B. HLA and leprosy in Chinese. Tissue Antigens **13** (1979) 73–74.

[46] Greiner, J., Schleirmacher, E., Smith, T. Lenaard, V. and Vogel, F. The HLA system and leprosy in Thailand. Hum. Genet. **42** (1978) 201–213.

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[49] Rea, T. H. and Terasaki, P. I. HLA-DR antigens in tuberculoid and lepromatous leprosy. Lepr. Rev. **51** (1980) 117–123.

[50] Sugiyama, K., Izumi, S., Matsumoto, Y., Ohkawa, S., Matsumoto, H., Miyazaki, T. Juji, T. and Maeda, H. Analysis of the immunogenetic background of Japanese leprosy patients by HLA and serum protein allotypes. Int. J. Lepr. **48** (1980) 502.

[51] Al Arif, L. J., Goldstein, R. A., Affronti, L. F. and Janicki, B. W. HLA-Bw15 and tuberculosis in a North American black population. Am. Rev. Respir. Dis. **120** (1979) 1275–1278.

[52] Rosenthal, I., Scholz, I. S., Klimmek, R., Albert, E. O. and Blaha, H. HLA antigens and haplotypes in patients with tuberculosis. Z. Immunitaetsforch. **144** (1973) 424.

[53] Selby, R., Bernard, M. J., Buchler, K. S., Crumley, J., Larsen, B. and Marshall, W. H. Tuberculosis associated with HLA-B8, BfS in a Newfoundland community study. Tissue Antigens **11** (1978) 403–408.

ferent populations[49, 50]. There are two methodological problems which cast considerable doubt on much of this work.

a) Many of the control groups have been inappropriate, representing groups which differed from the patients in ways which could, quite apart from the disease under study, explain any observed genotype frequency differences. The problem of selecting an appropriate control group is not simple, especially for a disease like leprosy with its apparent familial and social clustering. On the other hand, blood donors and hospital employees—all too frequently used as control groups—are likely to be inappropriate and misleading.

b) The second problem is that of assessing the statistical validity of a few apparent associations within a large number of comparisons. Even if there were no real differences between the groups compared, one in every 20 comparisons should reach $p < 0.05$. This problem has been recognized by many authors, and it is now conventional to multiply the "p" value times the number of comparisons examined before assessing its significance. One may wonder whether this is a sufficient precaution, considering the large number of such studies being carried out, some of which never reach publication.

A failure to find consistent associations in population-based studies is not necessarily lethal to genetic hypotheses. The markers studied may not be on the same chromosome as the "susceptibility genes," or they may be on the same chromosome but so far apart as to be in linkage equilibrium. Or the inconsistent associations might be due to linkage disequilibrium, i.e., crossing over between the functional gene loci and the marker loci may have led to different associations in different population groups[54]. This is to be expected. Fortunately, however, we are able to overcome this effect by looking within families and relying on the reasonable assumption that tight linkages should hold between closely related individuals. This family approach has now been applied several times to leprosy, using HLA as the marker system. The facts that the HLA system is intimately related to the immune recognition mechanism and that its analogous locus "H-2" in mice may be linked to important Ir (immune response) genes in some animals have encouraged concentration on these markers in man[55].

One approach has been taken by Stoner and his colleagues, who compared lymphocyte transformation tests between HLA identical and HLA non-identical siblings of leprosy cases[56]. They found little if any difference between the responses of HLA-D identical and non-identical siblings of lepromatous patients and concluded that the specific nonresponsiveness of lepromatous patients is not determined by an HLA-linked genetic defect.

A more powerful research design for testing the linkage hypothesis has been developed by De Vries and his colleagues[57]. Here the comparison is between haplotypes of two or more affected siblings. Mendelian principles dictate that four different haplotype combinations are possible among full siblings, and thus on average the probability is 25% that any two children are HLA identical, by chance alone. Nijenhuis has provided an elegant method for calculating the statistical significance of any departure from this prediction, allowing for pooling of families of different sizes[57]. This study design has been applied in studies of leprosy in several populations in Surinam and in India, as summarized in Table 4[57, 58, 59, 60]. With one exception[60], these studies have

[54] McDevitt, H. O. and Bodmer, W. F. HLA, immune response genes and disease. Lancet **1** (1974) 1269–1275.

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[59] DeVries, R. R. P., Van Rood, J. J., Lai A Fat, R. F. M., Mehra, N. H. and Vaidya, M. C. Is susceptibility to tuberculoid leprosy due to a recessive HLA-linked gene? In: *Interface Between Immune Mechanisms and Diesase*. Proceedings of the Brooklodge Conference, 1977. New York: Academic Press, 1980.

[60] Van Eden, W. DeVries, R. R. P., Mehra, N. H., Vaidya, M. C., D'Amaro, J. and Van Rood, J. J. HLA segregation of tuberculoid leprosy: confirmation of the DR2 marker. J. Infect. Dis. **141** (1980) 693–701.

TABLE 4. *Summary of family segregation analyses for children with tuberculoid leprosy. Statistical significance calculated according to method described by de Vries,* et al. [57].

Parental leprosy distribution	Segregation from	Location of study (ref.)			
		Surinam[57]	Wardha[59]	Wardha[60]	Karigiri[58]
Neither affected	Both parents	(5)[a] $\chi^2 = 7.61$[b]	(3) $\chi^2 = 2.04$	(3) $\chi \approx 0.0$	(27) $\chi^2 = 4.76$[c]
One parent affected	Affected parent	(6) $\chi^2 = 1.14$	(4) $\chi^2 = 0.07$	(4) $\chi^2 \approx 0.0$	(17) $\chi^2 = 0.65$
	Unaffected parent	(6) $\chi^2 = 0.05$	(4) $\chi^2 = 4.26$[c]	(3) $\chi^2 \approx 0.08$	(17) $\chi^2 = 2.18$
Both affected	Both parents	—	—	(1) $\chi^2 \approx 0.0$	(1) $\chi^2 = 1.12$

[a] Parenthesized numbers before χ^2 refer to number of families involved in analysis.
[b] $p < 0.01$.
[c] $p < 0.05$.

revealed evidence for a nonrandom allocation of HLA haplotypes, but only among tuberculoid case children of nonaffected parents. On the face of it, this would appear to suggest that a recessive factor sited on human chromosome 6 is somehow associated with tuberculoid leprosy. This evidence for a genetic factor associated with tuberculoid leprosy, now upheld in three out of four studies, represents the most rigorous argument for genetic factors in leprosy which has yet been produced. It needs further comment.

First, it should be noted that the observed association is not a very powerful one. There are many inconsistencies in the published data, such as the fact that neither the Surinam[57] nor the Karigiri[58] series show evidence of nonrandom segregation from the unaffected member of one-parent-affected families, though this is to be expected on the recessive gene hypothesis. Only two of the four studies showed a significant association for the two-unaffected-parent families. The significance is not huge, even in the large number of families included in the Karigiri series. These aspects of the results may suggest a low penetrance and low relative risk for the putative genetic factor, or may reflect weak linkage of a factor distant from the HLA region. Another explanation for the weakness of the association is that some of the parents classified as free from leprosy were in fact recovered tuberculoid cases. Such classification errors would have the effect of diluting the observable association.

Second, it should be noted that none of these family studies has shown an association with lepromatous leprosy. The possibility of such an association has not really been examined, however, because of a lack of families with at least two lepromatous children in the populations examined. This is unfortunate because of the considerable practical interest in the possibility of genetical factors in lepromatous disease.

It has been suggested that the chromosome 6 gene associated with tuberculoid leprosy might be an "immune response" gene either identical with, or very tightly linked to, the HLA-D locus. De Vries, *et al.* thus found an association between DR-2 and familial tuberculoid leprosy in two studies in India[59]. Van Eden, *et al.* have recently confirmed the association of DR-2 with familial tuberculoid leprosy in a small series in India; but they found no evidence for an association between this antigen and unrelated tuberculoid cases[60]. They make an unorthodox suggestion that familial tuberculoid leprosy might have one genetic mechanism, involving DR-2, but that "sporadic" tuberculoid leprosy may involve another genetic background. In this context it is of interest that Rea and Terasaki have reported only a slight (not statistically significant) association between tuberculoid leprosy and DRW2, in a case-control study carried out in a Mexican population[49]. More importantly, Sugiyama, *et al.* have recently reported a strong association between DR-2 and both lepromatous and tuberculoid leprosy in Japanese[50]. DR-2 thus becomes the first specific allele to be associated with leprosy in several different studies, on both a case/control and family segregation basis. This cumulative evidence is impressive.

7) **Gene identification.** The bottom line of

Table 1, as of all genetic studies, is the precise identification of putative genes. This has not yet been achieved for any of the systems discussed here. It has been fashionable to suggest that an Ir gene linked to the major histocompatibility locus might be an important controlling factor, but speculation still runs in advance of evidence[13,61,62].

The evidence in animals. It is much easier to study genetics in experimental animals than in man. The use of animals avoids ethical problems, allows programmed mating of selected parents, and provides rapid generation times. There may be difficulties in extrapolating genetical results directly from animals to man, but this qualification does not negate the considerable practical advantages and appeal of this approach.

Several workers have reported genetically determined differences in susceptibility to mycobacterial infections between different strains of experimental animals. Shepard and Habas found evidence for differences in numbers of *M. leprae* harvested in different inbred mouse strains, and they suggested this might be associated with strain differences in foot pad temperature[63]. Other workers have found differences between inbred mouse strains in their response to *M. lepraemurium* infection[64]. The classical work of Lurie on *M. bovis* in rabbits identified families with greater and lesser degrees of resistance to the experimental infection[65]. Unfortunately, none of these studies has succeeded in defining the genetical mechanism, in assessing its contribution, or in mapping the genes involved.

The situation with experimental *Leishmania* infections is different. Over the past several years, Bradley and his co-workers have succeeded in defining two genetic loci which exert major control over the course of *Leishmania donovani* infections in mice[66,67,68,69]. This elegant work has provided a model for studies of the genetics of responses to infection and is of particular relevance to the subject of this review.

Bradley early noted that the course of *L. donovani* infections varied markedly between different mouse strains in terms of time trends in the density of parasites in the liver. Careful investigation of approximately 25 inbred strains revealed three distinct patterns of response to a standard challenge with (generally 10^7) amastigotes, as shown in Figure 2. In one group (*X* in Figure 2, typified by strains A, C3H, C57L, NZB), the parasite density rarely rose above 100 LDU ("Leishman Donovan units," calculated as the average number of parasites per liver cell nucleus multiplied by the organ weight in milligrams) per liver. In a second group (*Y* in Figure 2, e.g., strain B10.D2) the average parasite concentration rose above 1000 LDU by day 15 and remained at this level for several months. In the third group (*Z* in Figure 2, e.g., C57BL/10ScSn), the level rose above 1000 LDU by day 15, but then gradually fell to below 100 LDU over the next three to four months.

Backcross experiments in conjunction with known markers revealed that these three course-of-infection patterns reflected the action of two independent loci, as set out in Table 5. One locus, called *Lsh* and mapped on chromosome 1, regulates the

[61] Hastings, R. C. Transfer factor as a probe of the immune defect in lepromatous leprosy. Int. J. Lepr. **45** (1977) 281–291.

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[67] Bradley, D. J. Regulation of *Leishmania* populations within the host. II. Genetic control of acute susceptibility of mice to *Leishmania donovani* infection. Clin. Exp. Immunol. **30** (1977) 130–140.

[68] Bradley, D. J., Taylor, B. A., Blackwell, J., Evans, E. P. and Freeman, J. Regulation of *Leishmania* populations within the host. III. Mapping of the locus controlling susceptibility to visceral leishmaniasis in the mouse. Clin. Exp. Immunol. **37** (1974) 7–14.

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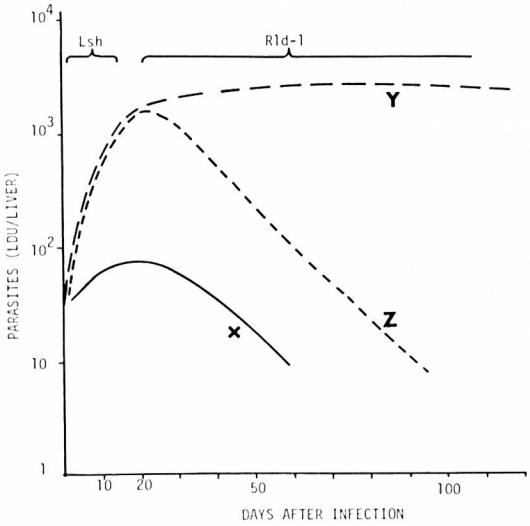

FIG. 2. Patterns of *Leishmania donovani* infections observed in inbred mouse strains. The vertical axis represents a measure of parasite numbers in terms of "Leishman Donovan Units," as explained in the text. The *Lsh* gene acts during the first 15 days of infection, and the *Rld-1* gene has an important effect thereafter. Parasite density readings below 10 LDU are unreliable.

early response to infection, as measured in the parasite density 2 weeks after challenge. It is not lymphocyte dependent and is incompletely dominant for resistance. Mice carrying at least one dominant resistance allele at this locus fall into the *X* pattern of Figure 2. Homozygous recessives fall into groups *Y* or *Z*. The second locus has been named *Rld-1* and is linked to the H-2 region on chromosome 17. It is T cell dependent. It controls the longer term (over 3–4 months) response in those animals which build up initial heavy parasite burdens. Animals with at least one dominant allele at this locus respond as group *Y*, maintaining large numbers of parasites for periods of several months, whereas homozygous recessives undergo a cure reaction, as illustrated by *Z* in Figure 2. The action of the *Rld-1* gene in animals who are homozygous for the recessive resistance allele at the *Lsh* locus (group *X*) has not yet been defined but is under investigation (D. J. Bradley, personal communication, 1980).

Other workers have recently explored the genetics of response to *Leishmania tropica* infections in mice and have found evidence for single gene control of extreme susceptibility in BALB/c mice, which is associated with a high level of suppressor T cells[70, 71]. This susceptibility mechanism in BALB/c mice may extend to *Leishmania mexicana* infections as well[72].

DISCUSSION

This review of the literature on the genetics of host responses in leprosy, tuberculosis, and leishmaniasis reveals a marked asymmetry in the summary matrix, Table 1. On the human side there has been most interest in leprosy whereas in experimental animals the concentration has been on leishmanial infections. The first can be explained on grounds of deep traditional be-

[70] Howard, J. G., Hale, C. and Chan-Liew, W. L. Immunological regulation of experimental cutaneous leishmaniasis. I. Immunogenetic aspects of susceptibility to *Leishmania tropica* in mice. Parasite Immunol. **2** (1980) 303–314.

[71] Howard, J. G., Hale, C. and Liew, F. Y. Immunological regulation of experimental cutaneous leishmaniasis. III. The nature and significance of specific suppression of cell mediated immunity in mice highly susceptible to *Leishmania tropica*. J. Exp. Med. **153** (1981) 557–568.

[72] Perez, H., Arrendondo, B. and Gonzalez, M. Comparative study of American cutaneous leishmaniasis and diffuse cutaneous leishmaniasis in two strains of inbred mice. Infect. Immun. **22** (1978) 301–307.

TABLE 5. *Description of gene loci exerting major control over the course of* Leishmania donovani *infections in mice* [66–69].

Description of gene	Locus name	Action	Mechanism	Linkage
"acute susceptibility"	*Lsh*	Resistance = incompletely dominant	Not lymphocyte dependent	Chromosome 1
"cure/non-cure"	*Rld-1*	Resistance = recessive	T-lymphocyte dependent	Linked to H-2 (Chromosome 17)

liefs in familial leprosy and in the persisting riddles of its natural history. The second can be explained in terms of serendipity and feasibility. The fact that easily quantifiable phenotypes are distinguishable in mice within a few weeks after experimental challenge with *Leishmania* has attracted laboratory workers to this model. In contrast, the considerable practical difficulties in obtaining suitable experimental material and in the very long latent or incubation periods have deterred such workers from studies of *Mycobacterium leprae*. In another vein, the relative absence of genetical and experimental work on tuberculosis in recent years may reflect a naive belief in some circles that tuberculosis was no longer a problem. This is perhaps ironic as tuberculosis undoubtedly remains the more important of the three diseases from a worldwide public health standpoint. History may show us that BCG has contributed more to the eradication of phthisiology than it has to the eradication of tuberculosis. The recent failure of BCG in South India might at least rekindle interest in basic studies of this important disease[73].

What have we learned? With regard to the human infections we now have a considerable pile of literature arguing for some role of genetic factors in determining responses in leprosy and in tuberculosis. Much of the evidence is open to biases which have been inadequately assessed. The combination of obvious methodological flaws, coupled with the fact that the putative genetical factors do not appear to have either very high penetrance or very high relative risks, do not always make for convincing reading. Of all the evidence thus far available, that drawn from the family studies of leprosy, and using HLA markers, is the most rigorous. The cumulative evidence for an association between the HLA-DR2 allele and clinical leprosy is now quite strong. But the relative risks involved are not huge. It may be that we have thus far been studying the wrong loci or the wrong chromosomes, but we should recognize that the hard evidence to date need not convince the critical observer of a major role of genetical polymorphisms in determining epidemiological patterns of either leprosy, tuberculosis, or leishmaniasis in man.

With regard to animal infections, we have evidence for some strain differences in susceptibility to several mycobacterial agents, including *M. leprae*, *M. lepraemurium*, and *M. bovis*. Few biologists will be surprised by this. Only with reference to the leishmanial infections in mice is the evidence of sufficient detail and rigor to give some insight into the mechanism of the interaction between animal host and infectious agent. So what? A critical look at the reasons typically given for these genetical studies may not be inappropriate.

A first reason widely cited for studying genetic factors is that the identification of disease susceptibility genes might allow the recognition of high risk individuals and hence be of practical use in field control programs. This author finds such a rationale unconvincing and doubts that it would prove either technically feasible or ethically acceptable. If we had the technical, logistic, financial, and personnel capacity to do this sort of thing—and think, for example, of the compliance problems in those areas where leprosy and leishmania occur—we would have little need to do so, for the elimination of these diseases would be relatively easily performed by other and simpler means. Quite apart from technical capacity, any of us would question the ethics of widespread screening for genetical risk factors.

A second reason given for human genetical studies, especially with reference to leprosy, is that the confirmation of strong genetic determination of certain responses might invalidate the potential usefulness of some future vaccine. Implicit in this argument is the assumption that a useful vaccine should prevent lepromatous disease and that a genetically controlled immune "defect" underlying the lepromatous response might render such individuals incapable of responding to the vaccine. My personal sympathies are partially in agreement here. One of the problems with many discussions of leprosy vaccines is the vagueness of the notion of what such a vaccine might be meant to do. On the other hand, if genetical factors were in any way respon-

[73] Tuberculosis Prevention Trial, Madras. Trial of BCG vaccines in South India for tuberculosis prevention. Indian J. Med. Res. **70** (1979) 349–363.

sible for the notorious variation in the efficacy of BCG against tuberculosis and leprosy in different populations, then it would certainly be useful to know of this before embarking on further studies or campaigns with mycobacterial vaccines.

A third reason frequently cited for carrying out genetic studies is that they may provide an insight into the detailed biochemical mechanism underlying the host response to infection. The leishmanial studies reviewed above begin to provide a justification of this motive in their revelation of important mechanisms acting at different stages of the infection. Certainly, this work further demonstrates the inadequacy of simple concepts of susceptibility to infection and to disease. Such mechanism orientated genetical studies are most efficiently carried out in animals, however, and this generally means animals which are not natural hosts of the infections concerned. The step from mouse to man is a large one, and, as an epidemiologist, I am perhaps less sanguine over this leap than are some laboratory scientists.

Rather than close on so pessimistic a note, I would conclude with suggestions of certain genetical studies which appear most likely to advance our understanding of leprosy, tuberculosis, and leishmaniasis:

1) Animal studies such as those on *Leishmania* infections in mice should be pursued with a goal to sort out the biochemistry, and not just the genetics, involved. The work of Bradley and his colleagues provides important guidelines on methods, variables, analysis, and interpretation of such studies[66, 67, 68, 69].

2) Family studies using the methods of Stoner, *et al.*[56] or of De Vries, *et al.*[57] seem most likely to succeed in revealing the human genetic mechanisms involved. The methods of Day and Simons[74] and of Thomson and Bodmer[75] are also applicable. These approaches should be applied with reference to as many gene markers as possible, with due respect for the statistical problems this raises. Special consideration should be given to careful diagnoses and classification of human cases. Whenever possible, case history information should be published in detail.

3) Greater attention should be given to the distinction between subclinical infection and clinical disease, as this has implications for classification of "cases" in genetical studies and may provide clues to genetically determined mechanisms.

4) A special effort should be made to check the zygosity and disease classification of all monozygous twin pairs reported as discordant for disease type in leprosy[39, 40]. If any of these pairs could be confirmed beyond reasonable doubt, the implications for genetics would be considerable.

Genetics may yet make a contribution to the control of leprosy, tuberculosis, and leishmaniasis.

SUMMARY

The literature on the genetic regulation of susceptibility in leprosy, tuberculosis, and leishmaniasis is critically reviewed. Of the three groups of diseases, leprosy has received the most attention from the standpoint of human genetics. There is now evidence that genetic factors, some of them HLA-linked, play a role in tuberculoid leprosy. However, the evidence leaves considerable room for environmental determinants in addition to genetic background. Several twin studies of tuberculosis have favored some genetic factors in clinical tuberculosis, but their evidence is mitigated by the many biases underlying such studies. Though very little work has been done on the genetics of leishmaniasis in man, experimental studies in mice have begun to unravel mechanisms controlling successive steps in the course of both *L. donovani* and *L. tropica* infections. It is suggested that future work should concentrate on moving from genetics to biochemical genetics in the mouse, should extend family studies in conjunction with markers in man, and should place high priority on confirmation of reported leprosy type discordance among monozygous twins.

[74] Day, N. E. and Simons, M. J. Disease susceptibility genes—their identification by multiple case family studies. Tissue Antigens **8** (1976) 109–119.

[75] Thomson, G. and Bodmer, W. F. The genetic analysis of HLA and disease associations. In: *HLA and Disease*, Dausset, J. and Svejgaard, A., eds. Copenhagen: Munksgaard, 1977.

RESUMEN

Se hace una revisión crítica de la literatura sobre la regulación genética de la susceptibilidad a la lepra, a la tuberculosis ya a la leishmaniasis. De las 3 enfermedades, le lepra he recibido la mayor atención desde el punto de vista de la genética humana. Hay evidencias de que ciertos factores genéticos, algunos de ellos relacionados con el sistema HLA, juegan un papel en la lepra tuberculoide. Sin embargo, las evidencias sugieren que además de los aspectos genéticos, algunos factores ambientales pueden también participar. En tuberculosis, varios estudios can gemelos han apoyado la participación de factores genéticos en la tuberculosis clínica pero esta evidencia es minimizada por las múltiples variables no controladas en tales estudios. Aunque se ha trabajado muy poco en la genética de la leishmaniasis humana, los estudios experimentales en ratones han comenzado o revelar los mecanismos que controlan el curso de la infección por *L. donovani* y por *L. tropica*. Se sugiere concentrar el trabajo futuro en el estudio de la genética bioquímica en el ratón, en la expansión de estudios familiares y de marcadores en el humano, y en la confirmación de publicaciones que señalan discordancia en cuanto al tipo de lepra entre gemelos homocigóticos.

RÉSUMÉ

On passe en revue dans cet article la littérature concernant les mécanismes génétiques qui règlent la susceptibilité à la lèpre, à la tuberculose et à la leishmaniose. Parmi ces trois groupes de maladies, c'est la lèpre qui a reçu le plus d'attention sur le plan de la génétique humaine. Il existe à présent une série d'éléments qui permettent d'admettre que des facteurs génétique, dont certains sont liés au système HLA, jouent un rôle dans la lèpre tuberculoïde. Néanmoins, ces éléments laissent encore une place considérable à l'intervention de facteurs environnement, s'ajoutant aux facteurs génétiques de base. Plusieurs études sur les jumeaux dans la tuberculose militent en faveur de facteurs génétiques dans le développement de la tuberculose clinique; toutefois, la portée de ces études est diminuée par une série de biais. Quoique très peu de travaux aient traité de la génétique dans la leishmaniose humaine, les études expérimentales chez la souris ont fourni des données permettant de fournir un début d'explication quant aux mécanismes qui interviennent pour déterminer les étapes successives de l'évolution des infections par *L. donovani* et par *L. tropica*. On propose que le travail futur porte dorénavant sur la génétique biochimique chez la souris, que l'on étende les études familiales en utilisant des indicateurs génétiques chez l'homme, et que l'on réserve une priorité élevée à la confirmation de la discordance qui a été signalée quant au type de lèpre notée chez des jumeaux monozygotes.

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