Immunology, Epidemiology and Social Aspects of Leprosy
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IMMUNOLOGICAL AND EPIDEMIOLOGICAL SPECULATIONS ON LEPROSY

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The infection of man by *Mycobacterium leprae* (ML) leads to a chronic granulomatous infection with a wide spectrum of clinical manifestations, from lepromatous to tuberculoid leprosy.

Refined histopathologic studies combined with bacterioscopy and with the lepromin test led to the amplification of Rabello's classical polar concept of leprosy (LL and TT), by recognizing three intermediates in the borderline group (BL, BB, and BT), thus providing objective criteria for comparing the evolution of the disease in patients subject to different treatments [1].

As an obligate intracellular parasite, *M. leprae* cannot be cultivated *in vitro*, but this was somewhat circumvented by Storrs and Kirchheimer's discovery of the high susceptibility of the nine-band armadillo of the South of the United States (*Dasypus novemcinctus*), a "quantum jump" in the study of leprosy, because it provided a means for obtaining large amounts of bacilli for the preparation of antigens [2]. Up to 10^{10} ML/g of infected tissue can be obtained from infected armadillos and as much as 12.5 kg of infected tissues were held by the IMMLEP tissue bank in May 1981 [3].

As in other diseases caused by intracellular parasites, immunity in leprosy is mainly, if not exclusively, cell mediated (CMI) and involves two kinds of effector cells: macrophages and T lymphocytes. Humoral antibodies may be important in pathogenesis (as in *erythema nodosum leprosum*, ENL), but do not seem to play any direct role in the mechanism of immunity. As a matter of fact, there is a striking contrast between the deficiency of CMI and the high antibody response in the most susceptible multibacillary forms of leprosy (LL and BL). On the contrary, in the paucubacillary forms (TT and BT), antibody formation is poor and CMI is normal.

As a basic immunologist who is not working in the field, I am afraid I cannot contribute much to the critical evaluation of specific data, but I hope to bring some contribution in discussing mechanisms by which immunity in leprosy is established, by focusing especially on the key role of macrophages.

Macrophages act in two different ways: (a) by presenting the antigen at its surface in close proximity of, or complexed to, class II molecules (Ia in the mouse, HLA-D in man) as a modified-self; (b) by destroying intracellular bacteria which in the non-immune host are able not only to survive, but also to multiply inside the phagocyte.

As to T lymphocytes, at least three sets are presently identified: TH (Helper), TC (Cytotoxic), and TS (Suppressor). Monoclonal antibodies are available to distinguish TH (phenotype Lyl+2–) from sets TC and TS of antithetical phenotype (Lyl—2+).

The T-unresponsiveness to ML antigen(s) [4], early attributed to a blocking effect of antibodies or to a deletion of specific clones, is now better interpreted by the antagonistic action of TS, i.e., by a decreased TH/TS ratio, as found in lepromatous patients, except in cases of recent ENL [5].

Macrophage capacity to digest intracellular M. leprae. Some twenty years ago, Beiguelman and Barbieri [6] reported *in vitro* experiments showing that peripheral blood monocytes (PBM) from lepromatous subjects, in contrast with those of tuberculoid cases, were unable to lyse autoclaved ML. It was then hypothesized that the capacity to be "Lyser" was codified by gene(s) phenotypically expressed at the macrophage level by the presence of effective lysosomal enzymes, under the influence of environmental factors, particularly of antigenic stimuli from ML or from cross-reacting mycobacteria. Only a small percentage of the population lacked the gene(s) and remained "Non-Lyser" throughout their life.

Beiguelman's view was supported by Skinsnes [7], who showed that macrophages from lepromatous cases were deficient in β-glucuronidase, whereas those of tuberculoid subjects had a normal content of the enzyme. These findings were, however, contradicted by Avila & Convit [8]. Furthermore, the studies of Drutz *et al.* [9], among others, failed to show any difference in the ability of macrophages from tuberculoid and lepromatous patients to lyse heat-killed ML.

Undoubtedly, as shown by Convit *et al.* [10] in Mitsuda-positive subjects, macrophages accumulated at skin sites injected with concentrated lepromin behave like lysers and transform themselves into

epithelioid cells, but obviously in this case a local activation by lymphokines cannot be excluded. The same applies to the experimental conditions of Beiguelman *in vitro*.

It has been postulated that lepromatous macrophages would not be "activable" by lymphokines, but this possibility was ruled out by Convit *et al.* [11] by elimination of ML subsequent to local *in vivo* activation of lepromatous macrophages by BCG in BCG-sensitive patients.

The body of evidence suggests that the failure of lepromatous macrophages to digest intracellular ML lies in a defective T cell function, albeit an intrinsic defect of the macrophage is probably the primary cause for the incapacity of Mitsuda-negative subjects to mount an effective CMI. It may be postulated that a defect in the presentation and processing of the antigen accounts for a deficient proliferation of TH in favor of TS. In fact, the increased antibody responsiveness in LL patients could imply that the presentation and processing of the antigen must be different, as suggested in [12].

Leprosy and Biozzi mice. In the context of the primary role of the macrophage in the mechanism of immunity in leprosy, it is interesting to compare the immunological behavior of LL and TT patients with that of the two lines of mice obtained by Biozzi *et al.* [14, 15] by selective breeding for the amplitude of antibody response in an outbred population.

The investigation of the genes controlling the immune response has been approached from two different angles. McDevitt and Benacerraf [13] studied the antibody production in relation to specificity, by using inbred animals responders or non-responders to antigens of restricted specificity. This was found to be controlled by one single gene located in the I region of the major histocompatibility complex (MHC) – the Immune Response Gene, Ir.

Biozzi's approach was radically different and consisted in selective breeding for the amplitude of antibody response to polyepitopic antigens, such as sheep erythrocytes or salmonella antigens. The selective experiments led to the development of two lines of mice: High(H) and Low(L) Responders. Five selective breedings have been carried out so far in France and in Brazil. In the most studied Selection I, the selection limit was attained after 16 generations and the interline difference corresponded to 220-fold. Variance analysis of the distribution of responsiveness in interstrain F1 and F2 hybrids and their back-crosses (the homozygous parentals being represented by

animals after the 16th generation) indicated that the difference in antibody response was under the control of 8-10 genes (polygenic regulation).

The phenotypic expression of the genes controlling the amplitude of antibody responsiveness involves a difference in macrophage function, as well as in the differentiation and multiplication of B lymphocytes.

As shown by Weiner & Bandieri [16], splenic macrophages of L mice degrade the antigen more readily than H macrophages. The greater ability of L macrophages to reduce the immunogenicity of the antigen accounts for low antibody production, as well as for increased resistance to infection, and correlates with better capacity to be protected by vaccination (Tables I and II).

TABLE I — *Immunity functions in H and L mice.*

Line	Antibody response	Macrophage function			
		Antigen uptake	Lysosomal enzymes	Intracellular degradation	Surface presentation
H	+ + +	+	+	+	+ + +
L	+	+ + +	+ + +	+ + +	+

TABLE II — *Natural resistance and protective effect of vaccination against* S. typhimurium *in H and L mice.*

Treatment	H Line		L Line	
	Mortality %	MST	Mortality %	MST*
None	100	5.4	100	8.7
Vaccinated	100	8.6	10	

* Mean survival time, days.

As to B lymphocytes, their involvement in the interline difference is indicated by: (a) better antibody response in radiosuppressed recipients when restored with H line than with L line lymphocytes; (b) stronger antibody response in H than in L mice to T-independent antigens like pneumococcus polysaccharide SIII. The possible regulatory role of TH and TS in the two lines of mice is still open to investigation.

At first sight, H mice (high antibody response, hypoactive macrophages) are comparable to lepromatous patients and L mice (low antibody response, hyperactive macrophages) to tuberculoid subjects. However, this statement needs to be qualified: the comparison is only valid for the activated macrophages, after CMI has been established.

Experimental data show that the natural bactericidal activity of lepromatous macrophages is higher than that of tuberculoid cases.

1) By infecting mice with BSG, Lagrange *et al.* [17] found, by viable counting of the mycobacteria in spleen and liver, that there were more bacteria in H mice on days 2 and 21, but in later stages of the infectious process (on day 35) the situation was reversed and countings were higher in the L mice. Similar results were found in the experimental infection of mice with *M. lepraemurium*, in comparative studies with BCG-resistant and sensitive strains of mice (C^3H vs C57BL/6) [18].

It would appear that in the case of replicate antigens the presence of adequate amounts of viable organisms is a prerequisite for the elaboration of CMI. Initial inhibition of intramacrophagic multiplication would then result in a weak cell mediated response, so that in late stages of the infection the macrophage activity remains low, as compared to the case in which the initially lower bactericidal activity of the macrophage is highly intensified by appropriate lymphokine stimulation.

2) Another line of evidence is provided by recent studies [19, 20] on the interaction BCG-macrophage in congenic strains of mice, by using a radiometric test with ^3H-Uracil, an RNA precursor readily incorporated by multiplying mycobacteria but not by macrophages.

Non-stimulated resident peritoneal macrophages from BCG-resistant mice (DBA/2, A/J) are more able to inhibit the growth of BCG than those derived from sensitive mice strains (C57BL/6, BALB/c). On the other hand, PBM cultures, free of T lymphocytes, from lepromatous patients, exhibited a higher natural bactericidal activity as compared to those of tuberculoid patients. This finding

suggests that genes analogous to Bcg^r/Bcg^- control the evolution of leprosy in man towards the lepromatous and tuberculoid forms, respectively.

Mechanisms of T-unresponsiveness in lepromatous leprosy. The immunodeficiency of Mitsuda-negative LL and BL patients lies in a defect in antigen(s) recognition on the level of T lymphocytes.

Three main hypotheses are offered for discussion in relation to this defect:

1) Lepromatous macrophages produce a particular type of immunogen that is appropriate, by interacting with TH and B cells, to elicit antibody formation, but is inappropriate for mounting an effective cell mediated response.

2) T-unresponsiveness in LL and BL subjects is a consequence of the natural bactericidal activity of their macrophages, as explained in the preceding section. The reason why the intensity of CMI varies as a function of the bacterial load of the macrophage is unknown.

3) The lack of T response in lepromatous subjects is due to the antagonistic effect of TS. In this context it is pertinent to consider that the restoration of T-responsiveness by Transfer Factor (TF) may be ascribed to a shift in the balance of TS and TH in favor of the latter. The same may be said in relation to other lymphokines such as α--Interferon and Interleukin 2, whose effectiveness as immunotherapic agents in leprosy is presently under investigation.

An epidemiological speculation. If the degree of macrophage activity and its inversely related antibody responsiveness are polygenic regulated, the individual phenotypic variability in a genetically heterogeneous population may follow a normal distribution curve (Fig. 1)

The bulk of the population is distributed around the median and comprises the individuals that under mild endemic conditions are not subject to infection, whilst the tails of the curve correspond to extreme phenotypes subject to high risk, according to the nature of the infection (macrophage or antibody dependent).

A theory has been proposed by Biozzi *et al.* that postulates that under mild endemic conditions only the individuals situated in the sensitive tails are affected, determining by the continuous loss of one of the two extreme phenotypes the stabilization of the genetic

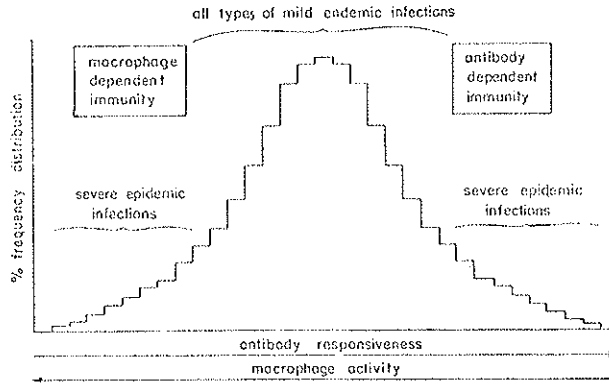

FIG. 1. Hypothetical regulation of genetic heterogeneity to infection according to Biozzi *et al.* (cf. ref. 15).

heterogeneity of the population. As a counterpart, during severe epidemics the individuals situated in one or the other distribution tails would be apt to resist and would ensure the survival of the population.

One might extend Biozzi's epidemiological hypothesis to leprosy, by substituting in Figure 1 the capacity of mounting CMI for macrophage activity, as indicated by the vector pointing to the left. A tail at the extreme right of the curve representing, say 10% of the population, would correspond to the individuals at risk of contracting lepromatous leprosy, whereas a contiguous area of another 10% would comprise those at risk of contracting the tuberculoid form of the disease. The remaining area under the curve would represent 80% of the population that ignores the infection. The percentages given may of course vary under the influence of genetical and environmental factors.

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