IMMUNOLOGY AND SEROLOGY. IMPLICATIONS OF CUTANEOUS AND SEROLOGIC REACTIVITY[1]

JOHN H. HANKS, PH.D

Johns Hopkins-Leonard Wood Memorial Leprosy Research Laboratory
Johns Hopkins School of Hygiene
Baltimore 5, Maryland

This discussion is undertaken in order to examine the potentialities and limitations of immunologic and serologic studies for explaining the events which occur during mycobacterial disease.

In trying to examine these questions, it is necessary to admit that resistance—or susceptibility—is compounded of many factors (nutrition of microbes and host, modified biochemical environments during infection, and the capacity for and character of immune response), also to emphasize that the classical views and tools of immunology and serology fail to deal with many facets of the host-parasite relationship.

Since infection—or resistance—depends upon the interplay of many genetically-determined properties in a microbe and a host ([23]), I have in Table 1 undertaken to indicate a few of the considerations which seem applicable to mycobacterial disease, and to suggest how they might pertain in leprosy of the lepromatous type. Crucial segments in this rough outline have been clarified but slowly over the years, often by ingenious experiments or by the fortunate new insights permitted when chemical components (and corresponding synthetic systems) have been deleted from a microbe or host by mutations or through selection within heterozygous populations. Three reviews ([39, 47, 55]) are recommended to those who wish to examine the background of evidence, analysis and speculation in recent years.

Cellular allergy and antibody production may seem to be too widely separated in the table. This occurs in part because of proceeding from the outer toward the inner structures and systems of microbes. It may in fact provide a more realistic framework for cogitations on the nature of host response.

THE MODES OF HOST RESPONSE

I. *Host metabolism.*—The host metabolism of capsular lipids probably proceeds at various speeds in different circumstances. This process may be important in modulating the impenetrabilities of host-adapted species during the less-restrained phases of their growth, in causing metabolic impairment of host systems early during infection ([4]), and, later, in exposing bacterial cell walls during the host's recovery from infection. Nothing is known concerning an adaptive character of host response to these materials.

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Middlebrook ([44]) has emphasized the major reason for inefficient performance of hosts during mycobacterial infection, namely: that the inert or toxic lipids on the surfaces of mycobacteria are not antigenic; that mechanisms which cannot decapsulate and/or disintegrate mycobacteria are likely to be of secondary importance to resistance; and that studies which fail to take this situation into account are likely to lead to unrealistic conclusions. The tubercle bacilli carry less of these lipids when grown *in vivo* than when grown *in vitro* ([62]). Hanks ([39]) has shown by simple cytologic methods that the lipids outside cell walls of the noncultivated species are more impenetrable or voluminous than on tubercle bacilli. He has also found ([31]) that the quality and abundance of such materials are related to available substrate and physiologic age of the bacterial cells. The accumulation of undigested droplets of electron-transparent lipids in Virchow and lepra cells in lepromatous leprosy has been demonstrated by Brieger ([5]) and others.

II. *Cellular allergy.*[1]—The allergic aspects of host response have attracted major interest since the earliest days of studies of the mycobacteria. The beautiful demonstration by Kanai and the Youmans ([35, 36]) of the role of cell walls in inciting cellular allergy is consistent with evidence from many sources. Elberg ([16]) has summarized a portion of the evidence while emphasizing the fact that many issues in bacterial disease rest upon abilities of microbes to maintain all walls and of host cells to maintain metabolizing membranes. He also has summarized the ways in which appropriate impairment of microbial cell walls opens the door for the action of host enzymes such as lysozyme.

A few well-known examples of divergent individual efficiencies of hosts include: relationships between natural or stimulated resistance and good or enhanced capacities to disintegrate the cell walls of Salmonellae, as observed in different strains of mice ([23]); between allergic capabilities and natural or stimulated resistance in several families of

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[1]"Cellular allergy" is intended to imply for mesenchymal cells a relatively specific, adaptive alteration of metabolism. This physiologic activation ([21]), which improves the capacity of cells to hydrolyze bacterial cell walls ([23]), is associated with the synthesis of factors which create and transfer tuberculin-type hypersensitivity ([39]). It develops in highest degree under the influence of lipopolysaccharides ([34, 50]) and when the maturation of plasma cells is blocked ([21]). It, therefore, can develop strongly in the absence of antibody production, e.g., when vaccinia virus or BCG is administered during agammaglobulinemia ([22]). Since cellular allergy is generated most strikingly by viral agents and by bacteria which grow within cells, and since it involves an element of autoimmunization ([19]), an important factor may be primary interactions of heterologous proteins with internal systems of host cells rather than with their surface membranes. Here the term usually will be employed without distinction between the importance of hypersensitive (damaging) effects and protective or curative effects, since these so often are operational or circumstantial.

Strong associations between high degrees of cellular allergy and subsequent maximal production of antibody to proteins, polysaccharides or other haptenes have been demonstrated during many studies of adjuvant phenomena ([19, 29]). The fact that both types of immune response are exaggerated by lipopolysaccharides is small reason for a persistent faith that cellular allergy must be explained in terms of "antibody precursor" or "intracellular antibody." Such conviction implies that the varied systems in tissue cells exist solely in order to embroider the hems of globulin molecules.

TABLE 1.—*The ways in which host systems and mycobacteria engage in competitions; an over-simplification.*

Modes of host response	The anatomy and chemistry of mycobacterial components	Problems arising from genetic limitations of host response	Identifiable defects in lepromatous leprosy
Parenteral digestion: I. Slow	Capsular lipids: inert, toxic, nonantigenic.	Nutritional backgrounds which permit steady synthesis of bacterial cells and capsular lipids.	Electron transparent droplets accumulate in lepra cells.
II. Variable (Cellular allergy)	Cell walls: lipoproteins lipopolysaccharides.	Difficulties in disintegrating cell walls to expose the labile systems noted in III and IV.	Mitsuda negativity.
III. Rapid	Bacterial proteins, type unspecified.	?	?
IV. Metabolic inhibition	Particles from plasma membranes,[a] chiefly enzyme proteins and lipoproteins.	?	?
V. Antibody production	Miscellaneous proteins and polysaccharides.	Bacilli, being intracellular and encapsulated, are not susceptible to the classical effects.	? vs proteins + vs polysaccharides.

[a]Major sites of oxidation-reduction.

rabbits ([49]); and the influence of familial factors on human capacities for tuberculin sensitization following BCG ([49]).

In a later section of this paper, the Koch and Mitsuda reactions to intact mycobacteria will be interpreted in terms of the *rates* at which animals and humans can disintegrate mycobacterial cell walls. This will be done largely irrespective of the manifest degrees of sensitivity, since it will be seen that parenteral hydrolysis of liberated proteins may proceed so rapidly as to minimize this feature of a more fundamental process. It appears, therefore, that one of the definable defects in persons with lepromatous leprosy is poor capacity to act on cell-wall components of *M. leprae*.

III. *Parenteral utilization of heterologous proteins.*—Given conditions which do not excite cellular allergy, or which delete low capacities for allergy and antibody production, the readiness with which animals utilize heterologous proteins parenterally is readily demonstrated. Such deletions can be induced in young or adult animals, particularly when the genetic basis of allergic response is weak. One example is the induction of immune tolerance in mice by methods which ensure adequate and continuous flooding with extraneous protein, for instance by parabiosis ([53]). Tolerant surviving pairs (41% among closely related hybrids and parents and 5% among more divergent strains) accept and

retain grafts for long periods after their separation, some individuals throughout life. Reestablishment of graft-rejecting capacity is highly individual. It may be promoted by successive application of new grafts and, more decisively, by repopulating the mice with competent lymphocytes. As an explanation of these tolerant states, the theory of persistent antigen excess seemed to Rubin [54] less realistic than the premise that all "recognizing" clones of host cells are deleted [6] and that they reappear again only through the operation of time and chance.

There is abundant evidence that, in spite of induced losses of immunologic reactivity, a normal or enhanced biochemical basis for the utilization of heterologous protein operates continuously. Efforts to sustain animals in nitrogen balance solely by parenteral administration of heterologous proteins require that the latter be given in large amounts. Failures to succeed in dogs and humans emphasize the hazards encountered in animals which readily develop cellular allergy and antibody. Martin *et al.* [43], however, showed that within one or two weeks rats adapt to parenteral utilization of heterologous proteins (raw egg white and bovine plasma), maintain a positive N balance, and grow normally.

Enhanced capacities to hydrolyze solubilized mycobacterial proteins, even to the extent that cellular allergy is minimized or obliterated, is suggested in several sources. The first of these is the frank dichotomy between reactivity to tuberculin and the elevated rates of disintegrating killed tubercle bacilli. This has been brought to light by persistent efforts to maintain tuberculin positivity in France. BCG incites tuberculin reactivity in nearly 100 per cent of infants and young children, but reactivities to 50 TU decline slowly with age and dramatically in the period between 15 years and early adulthood [3, 10, 58]. Reactions to 50 TU have been reported to occur in only 50 per cent of nurses [58] and in 81 per cent of students at the Academy of Paris [3]. Intradermal administration of BCG improves tuberculin reactivity in only 10 to 50 per cent of these nonreactors [3, 10, 58]. Multiple injections of BCG do not change the situation. The Koch phenomenon, meanwhile, is elicited in nearly 100 per cent of these individuals by scarification with killed, concentrated BCG or intradermal injection of diluted BCG. Improved capacity to disintegrate tubercle bacilli is indicated by the fact that during efforts to restimulate these persons, the day of maximal reaction to BCG is shifted from 5-6 days to 2 days. The experimental basis for inducing these states is illustrated by the work of Kraus and Dvorak [37], followers of de Assis. They showed in infants that monthly ingestion of 100 mgm. of BCG for a time increased the proportion of tuberculin reactors. However, by the end of six months, the proportion of tuberculin reactors fell to 30 per cent while the reactors to intradermal BCG had increased to 100 per cent.

Some hold that tuberculin negativity plus Koch positivity is the

ideal basis for resistance; others regard it as a liability. Humphrey's studies ([33]) on the metabolic utilization of foreign proteins during immunologic tolerance led him to conclude that foreign proteins may be hydrolyzed by parenteral mechanisms so promptly that they provide no templates which could stimulate or direct an immune response.

Studies on host response to large doses of the endotoxic lipoprotein-polysaccharides from the cell walls of gram-negative bacteria provide further evidence that apparent deletions of immunologic reactivity reveal underlying, highly active biochemical mechanisms for dealing with cell wall components. Similarities between the walls of mycobacteria and of gram-negative organisms have been summarized by Tepper ([62]). Parallelisms between cutaneous and systemic reactions to tuberculin and to endotoxins have been emphasized by Stetson ([57]). The induced tolerance to endotoxin is nonspecific in respect to gram-negative species, and appears within 24 hours. The onset of tolerance coincides with improved phagocytic capacities, greatly increased rates of clearance of endotoxin (i.e., cell-wall components), and remarkable resistance to infection by a variety of gram-negative bacteria ([63]). Thus, while small amounts of these cell-wall complexes enhance immune response by adjuvant effects, over-titration of this aspect of host capacity reveals increased potentiality for microbial destruction by highly adaptive host systems which minimize rather than promote host sensitization.

It will be noted that many of the foregoing observations have been made during studies of the Shwartzman phenomenon ([63]). It is not yet clear whether a counterpart is seen in the Lucio phenomenon in lepromatous patients.

Thus, there remains from our examination of Items II and III the clear possibility that the lepromatous patient may retain capacities for deriving a part of his N supply from the membranes and other proteins of *M. leprae*. Nevertheless, he is handicapped in controlling the growth of the bacilli and is defective in capacity for distintegrating their walls by means of cellular allergy and underlying biochemical mechanisms.

IV. *Metabolic inhibitions.*—It is becoming increasingly clear that, during the course of mycobacterial infections, the microorganisms may be repressed by metabolic inhibitions. These may be due in part to small molecular metabolites ([13]) and in part to nondialyzable inhibitors ([67]), possibly to DPNases which repress the metabolism of both the bacilli and the host cells ([2]). The extent to which these modifications rest upon direct biochemical interactions or upon more immunologic mechanisms cannot now be said. In any case, it is evident that synthesis and maintenance of protein carriers for coenzymes are crucial for the microbe. The evidence from Kanai and the Youmans ([35, 36]) that an immunologic (or adaptive?) response can be induced in an animal host

without appreciable allergy, and can be directed against a metabolic system in mycobacteria, is exciting indeed.

V. *Antibody production.*—The benefits and sensitivities arising from the production of antibody has been a central point of interest in immunology and serology, in part because antibodies against a series of microbial components have been shown to be crucial to enhanced resistance. That the role of antibodies during mycobacterial infections is modest or obscure has been revealed in many ways. Most significant would seem to be the tendency of mycobacteria to enhance cellular allergy while blocking the maturation of plasma cells, and the fact that persons with dysproteinemias exhibit normal sensitization and resistance without producing antibody.

Although existing evidence indicates that cellular allergy rather than antibody is coupled with certain of the mechanisms which damage mycobacteria, it is a misfortune that allergy tends to damage the host in almost equal degree. Antibodies which combine with solubilized bacterial protein may reduce the price the host must pay while developing resistance and accomplishing recovery. Evidence of such a protective role may be found in three sources. In Dienes-type guinea-pigs exhibiting severe tuberculin-type reactions to horse serum, Hanks ([26]) observed a marked decrease in tuberculin-type reactivity following passive transfer of antibody against horse serum. Moen observed progressive loss of weight and severe skin reactions to streptococcal proteins early in the course of type C streptococcus infections in guinea-pigs, and milder skin reactions plus antibody later in the course of infection. Parallel studies *in vitro* showed that monocytic cells in the presence of serum drawn early during infections were damaged by streptococcal proteins, but that cells were protected by antibody-containing convalescent serums ([46]). Elberg ([16]), while studying the toxic effect of tubercle bacilli on guinea-pig monocytes *in vitro,* demonstrated that relatively nonspecific factors in immune serums greatly decrease the damage which occurs in normal serum.

It must be emphasized that such observations have been made in the presence of allergically competent cells. Whether suitable antibodies are produced during leprosy infections and whether they could spare host tissues is not known. Antibodies against mycobacterial polysaccharides seem to be indicative of serious infections; they are not present at significant levels when resistance to leprosy is greatest.

THE BASIS OF MYCOBACTERIAL RESISTANCE TO HOST RESPONSE

Relationships between the different modes of host response and the metabolic and structural components of mycobacteria have been outlined by generalization in the foregoing section. A point which remains to be emphasized is the relative constancy of internal constituents and cell wall components in the mycobacteria. This stands in sharp contrast

with the extreme variability of the extracellular capsular lipids. These variabilities include: the readily penetrable surfaces of saprophytic as compared with pathogenic species; the smaller amounts and toxicity of lipids on attenuated mutants as compared with virulent strains of tubercle bacilli ([4, 45]); the greater impenetrability or volume of lipids on the "host-dependent" species ([30]); and the fact that synthesis of these materials is dependent upon success in utilizing substrate ([31]).

It is probably for these reasons that such fantastic alterations of host metabolism and immune response ([28]) are required to produce a significant effect upon resistance.

CUTANEOUS RESPONSES TO MYCOBACTERIAL PROTEINS AND CELLS

It is evident that skin reactions to soluble proteins, or to structurally bound proteins in killed mycobacterial cells, cannot assay all the factors controlling mycobacterial disease. There will be both associations and discrepancies between skin reactions and resistance. However, skin tests are applied so readily and universally that they will continue to be useful if we can improve our interpretation of their significance.

Reactions to soluble proteins.—While we may agree that skin reactions to soluble proteins are particularly useful to the epidemiologist, we must reject the idea that the present protein derivatives are worthy tools in immunology and question whether they are ideal for the epidemiologist.

Firstly, an important possible cause of low specificities has been overlooked in spite of ancient, clearly-defined warning. All immunochemical studies have demonstrated that heat-denaturation of proteins has several effects: to destroy the majority of the original specific configurations; to broaden specificity; and to alter original configurations so greatly that derivatives of animal proteins become antigenic in the species of origin ([64]). It is impossible for me to suppose that such treatment does not likewise decrease and broaden specificities in mycobacterial proteins. Thus, while some part of the original specificities remain in test reagents, present information on cross-reactivities has been derived from heat-distorted molecules and may not reveal the original differences in specificities. Investigation of other methods of "cracking" proteins or of inactivating mycobacterial cells for skin test purposes would seem to be in order.

Secondly, while it seems probable that the skin-reactive tuberculoproteins are derived in large part from autolysis of lipoproteins from the cell-walls, they are accompanied by nucleoproteins, polysaccharides, etc. It is not known whether elicited reactions are directed against structural derivatives alone, or at times against more vulnerable components involved in mycobacterial metabolism.

Thirdly, as we have seen, tuberculin negativity may occur in per-

sons who disintegrate the cell walls of BCG rapidly but dispose of the soluble proteins without manifest hypersensitiveness.

Skin reactions to soluble proteins are regarded as assays of preexisting allergic hypersensitivity. They have not been found helpful in *prognosis*. The *diagnostic* significance of the tuberculin reaction is dependent upon knowledge of the age, physiologic state and epidemiologic background of the individual. In *epidemiologic* studies, positive reactions to 5 TU are indicative of recent or active infection with mammalian type tubercle bacilli ([15]). Reactions to 100-250 TU may reveal more remote experience with tubercle bacilli ([56]), or encounters with other mycobacteria, or cross-reacting proteins of unknown origin ([15]). In view of the mild sensitization induced by *M. leprae*, it seems that cross-stimulations are a frequent cause of Fernandez reactions ([24]). Quantitative comparisons of reactions to tuberculins from a variety of mycobacteria indicate a marked tendency for cross-stimulations and the necessity of titrating relative specificities of response.

Reactions to killed mycobacteria.—While skin tests with killed mycobacterial cells are also highly artificial (and involve the same questions regarding specificity), they at least permit assay of natural or stimulated capacities to disintegrate mycobacterial structures and cell walls. In suspensions of tubercle bacilli, accompanying soluble proteins induce reactions equivalent to tuberculin reactions, while the soluble proteins in lepromin[2] elicit the corresponding Fernandez reaction ([17]). The reactions to mycobacteria, therefore, are biphasic: (*a*) to soluble proteins, and (*b*) to proteins which can be liberated from the bacilli. Since the 48-hour reactions confuse interpretation of the respective Koch and Mitsuda reactions to structurally bound proteins, a study with freshly washed suspensions might help to clarify certain of the present arguments. For example, those who hold that Koch and Mitsuda reactions indicate "immunity" are challenged by those who argue that they are merely interactions between previously sensitized tissues and injected bacteria. Others ([24]) present evidence that, since killed mycobacteria are antigenic, these tests assay also capacities to enhance response during the persistence of a local antigen depot.

Speaking for myself, it has been helpful to compare the kinetics of mycobacterial destruction in four circumstances. 1. A large proportion of the protein antigens of microbes is retained in heated bacterial bodies during prolonged storage of suspensions of lepromin; cells remain intact and stainable. 2. The major part of such antigen cannot be released from heated (permeable) leprosy bacilli by digestion for three

[2]Lepromin, the principal skin-test reagent of the leprologist, is a 3 or 5 per cent suspension of pooled, autoclaved human lepromas, filtered through fabric and preserved with 0.5 per cent phenol. Though heat-denatured tissue components are antigenic in the homologous species ([60, 64]), lepromin has been regarded by leprologists primarily as a heat-killed suspension of *M. leprae*.

days in trypsin or by briefer exposures to bile plus creatinine ([61]). 3. In some Mitsuda-negative children, antigen is stored in skin sites for periods up to three years, and is demonstrable by local flares (remote positivization) following stimulus by BCG administration([51]). 4. In reactive persons, the early termination of strong reactions to tubercle bacilli (BCG maxima in 48 hours, see earlier section) and the slower waning of reactions to lepromin (after the 3rd or 4th week) indicates mechanisms for the liberation and destruction of structural proteins at *rates* totally different from those cited in item 3 above.

A conclusion that the *rates* at which Koch and Mitsuda reactions can be terminated is a measure of capacity to disintegrate mycobacteria is consistent with the known destructive effects of competent tissue cells on bacterial cell walls ([16, 23]), and with existing data on the disappearance of stainable acid-fast organisms during Koch and Mitsuda reactions. Parallelisms between Koch and Mitsuda reactions have been demonstrated by Cummins and Williams ([9]), Fernandez ([17]), Floch ([18]) and Hadler ([25]). Since the Koch phenomenon usually is elicited after strong sensitization, reactions to soluble and to structurally bound proteins tend to merge, and the reactions to subside early. Reactions to available strengths of soluble proteins of *M. leprae* are milder and the response to bacillus-bound proteins slower, with the result that bimodal curves often are seen. The tubercle bacilli are destroyed more rapidly than *M. leprae* ([18, 25]).

I would suggest, therefore, that interpretation of Koch and Mitsuda reactions does not depend upon the size of the reactions on a specified day, nor necessarily upon the violence of the intervening inflammation, but upon the spacing of readings in such a way that the dynamics of mycobacterial destruction can be plotted.

Evidence of prognosis.—In spite of the fact that Mitsuda reactions have not been read as suggested above, leprologists (being blessed with nothing more elegant than autoclaved suspensions of bacillus-rich tissues) have learned that reactions to intact mycobacteria are useful in classification of patients and in prognosis. Table 2 illustrates relationships between strengths of Mitsuda reactions and the clinical status of patients five years later ([55]). These data are of particular interest for two reasons: (*a*) they are compiled from a paper on anomalous immunobiologic situations in leprosy, and (*b*) they reflect results in patients whose response to active infection and to lepromin had followed an atypical or erratic course. Nevertheless, even in individuals with indeterminate lesions, 3+ or 2+ Mitsuda reactions predicted an evolution toward self-healing tuberculoid lesions, while weak or negative reactions foretold lepromatous tendencies. If account is taken of the interim alterations of the Mitsuda reactions in some patients, the results are even more interesting. In all cases but one, patients who transformed from positive to negative Mitsuda reactions became leproma-

tous. Among 12 who transformed from negative to positive, only three eventually developed lepromatous leprosy.

It is for such reasons that many leprologists believe the Mitsuda reaction to be of value in assessing the potential for resistance in relation to age or in populations at large. Present evidence is consistent with this view. If sound conclusions are to be reached in contacts of leprosy patients, two provisos must be noted: (1) Some persons, particularly children, may give negative reactions, not from want of capacity to make an immune response but for lack of prior adequate stimulation. Concern would be focused on those who remain Mitsuda negative after attempted immunizations with BCG and/or other antigen. (2) While 3+ and 2+ Mitsuda reactions indicate a high probability of adequate resistance, (see Table 2) the specificity and significance of 1+ reactions require further study.

TABLE 2.—*Prognostic value of the Mitsuda response in leprosy patients exhibiting anomalies* ([57]).

Classified in 1941			Condition in 1946		
Type	No. of cases	Mitsuda reactions	Lesions healed	Tuberculoid	Lepromatous
Undifferentiated[a]	32	3+	84%	7%.	6%
	63	2+	78%	2%	12%
	61	1+[b]	36%	0%	56%
	60	—	18%	2%	68%
Tuberculoid	193	3+	Majority		0%
	266	2+			2%
	147	1+[b]			10%
	79	—			37%

[a]Officially the "intermediate" form, which name fails to convey to persons outside the field the tendency of these lesions to evolve into one or the other of the maturated types.
[b]Modified from the original by combining 1+ and ± reactions.

The possibility that dilution of the dose would make 1+ Mitsuda reactions a more significant threshold is illustrated in an interesting study by Guinto and Wade ([24]), who reviewed pertinent literature. Among children 6-9 years of age living in a town (Opon, Philippines), 12 per cent gave 3+ and 2+ reactions to undiluted lepromin, while 11 per cent gave 2+ and 1+ reactions to lepromin diluted 1:20. Among rural children living near Opon, 21 per cent gave 3+ and 2+ reaction to lepromin, while 28 per cent gave 3+ to 1+ reactions to the 1:20 dilution. Happily, dilutions which would increase specificity and significance would also result in conservation of available supplies of lepromin.

The response to attenuated mycobacteria.—More satisfactory evaluations of prognosis could be based on capacities to heal benign cutaneous infections caused by attenuated derivatives of virulent myco-

bacteria. Although attenuated strains of *M. leprae* are not available, this topic is pertinent because of the use of BCG as a potential immunizing agent, and particularly because it will again be seen that the significance of BCG lesions does not depend on size of reactions or tuberculin reactivities but upon the *rates of their evolution.* The poor association between tuberculin reactivity and average diameters of BCG lesions after 3 to 17 weeks have been demonstrated by Edwards *et al.* ([14]). These discrepancies were explained in part by the influence of the depth of BCG deposits in the skin, by dilution of the vaccine, and by the ratio of living to dead organisms. Most importantly, this group of investigators observed strong familial differences in response ([49]).

The *rates* of onset of response and of healing have been recognized to measure immunologic experience and capacities to respond to BCG. The appearance of papular reaction nodules after 12-20 days denotes primary response in children of ages 4-12 years, while earlier onsets denote prior experience with mycobacterial antigens ([20]). The most significant observations were those of Aronson, Parr and Saylor ([1]), since they recognized clearly the implications of the evolution and healing of BCG reaction sites. Within age groups 1-19 years the 48-hour lesions were analogues of the deeper tuberculous lesions described by pathologists and of cutaneous lesions seen in leprosy. That is, they were of two types: (*a*) with sharply-defined borders, soon becoming hard or shotty to palpation, and (*b*) firm but with diffuse borders which merged gradually into the surrounding tissue. After 6 weeks the lesions were clearly of two types: (*a*) in 40 per cent the base of ulcers was covered with granulation tissue and rapid healing ensued, (*b*) in 60 per cent the lesions were larger, with barely perceptible ulceration and with more diffuse margins. On pressure the latter yielded gray, white or blood-streaked pus containing acid-fast organisms. If undisturbed, these ulcers continued slowly to enlarge and persisted for a long time before healing was complete. Aronson recognized these two types of lesions as counterparts of the two types of cutaneous lesions caused by infection with virulent tubercle bacilli in the more resistant and the more susceptible families of guinea-pigs studied by Lewis and Loomis, and of the dermal lesions in similarly differentiated rabbits which he had studied. As he stated in 1940,

> Further observations . . . . will be necessary to determine whether or not in man the local response to the injection of BCG vaccine possesses prognostic value in determining resistance to tuberculosis.

In the intervening 21 years, I find only two papers on the proposition that *slow* healing of benign cutaneous lesions suggests poor capacity to destroy more virulent strains in naturally-infected tissues. Lurie and associates ([40]) studied the question in families of rabbits in which the genetic basis of susceptibility and resistance had been in part segre-

gated by selective inbreeding. In the more resistant family, the BCG lesions developed promptly and healed within 60 days. In the susceptible animals, the BCG lesions developed slowly, lasted for a longer time, and healed slowly. The same animals, whether infected previously, simultaneously, or later with virulent bovine-type tubercle bacilli, exhibited resistances which were strongly associated with their capacities to heal cutaneous lesions incited by BCG. This relationship held true even within individual rabbits in the same families.

While the situation in patients having both tuberculosis and leprosy may not be typical, it is interesting that Magarão and Lima ([42]) classified 44 such patients on the basis of capacity to heal BCG lesions within 6 weeks. The tuberculosis followed a favorable course in 29 out of 32 patients (90%) who had healed their BCG lesions within 6 weeks. Among the 12 who had not healed BCG lesions at that rate, tuberculosis remained chronic in 5, became worse in 4, and was fatal in 3.

DEFICIENCIES IN IMMUNE RESPONSE

It is known from many lines of investigation that genetic constitution and capacities for sensitization ([40, 49]) and resistance ([41]) are interrelated, and due to several causes rather than a single attribute ([23]). We must not presume that the normal or "average" development of allergy and resistance will be seen in clinically recognized leprosy. We must, on the contrary, recognize that individual capacities for immune response differ remarkably, and that they determine many of the events seen in natural and experimental disease.

Fig. 1 reminds us that on the basis of incubation period we can divide infectious diseases into very different epidemiologic and immunologic patterns.

The shaded area, A, represents a pattern seen in diseases with short incubation periods. Infectious agents which proliferate rapidly can cause disease in a considerable proportion of individuals before there is time for significant enhancement of resistance. Previous immunizations against such diseases produce dramatic reductions in incidence.

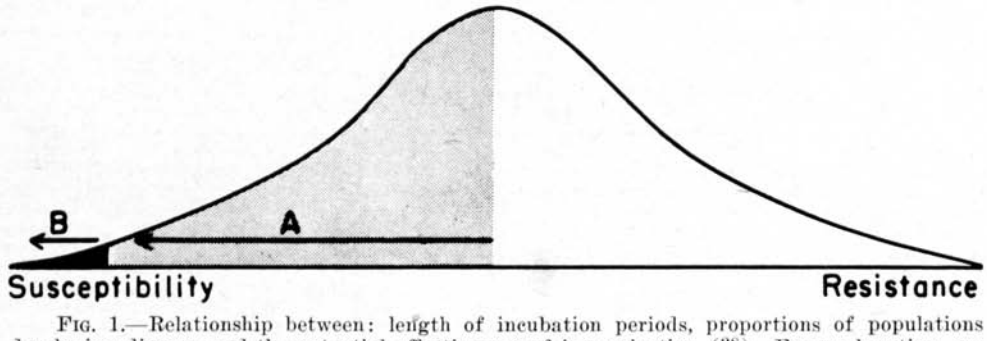

FIG. 1.—Relationship between: length of incubation periods, proportions of populations developing disease, and the potential effectiveness of immunization ([28]). For explanation, see text.

The blackened area, B, represents the situation for agents which proliferate slowly. These agents are handicapped by longer incubation periods, which permit significant elevation of resistance in persons capable of effective response. While a considerable proportion of a population may be hosts for the infectious agent (e.g., tuberculin reactors), the majority tend to heal incipient lesions. As incubation periods are more prolonged, recognized disease tends to be restricted to a smaller proportion of individuals exposed; in short, to those who are innately least capable of being immunized.

The results observed in mycobacteria disease may be illustrated as follows: (1) A rapid or average immune response averts disease. The numerous contacts between lepromatous patients and family or community result in low attack rates. (2) A slow but gradually effective response permits the onset of disease, with subsequent slow healing. Before the era of chemotherapy, this was the history of many tuberculosis patients. In leprosy, the majority of early and benign lesions heal spontaneously (e.g., in 75% of children observed by Lara and Nolasco [38] at Culion, where susceptibility factors may have been transmitted by both parents). (3) It is only in individuals with exceptionally poor response that the infectious agent gains the upper hand. In tuberculosis, deaths occur from fulminating disease and/or pathology which arises due to immune response during serious infection. In leprosy, elevation of immune response gives rise to tuberculoid lesions, to nerve damage, and to deformities. More belated response permits bacteriologic positiveness with seemingly helpless immune mechanisms and severe reactional states. Nevertheless, in all forms of the disease there is a tendency toward eventual recovery at the expense of serious tissue damage.[3]

The background which supports this view of leprosy is familiar to those who have used experimental infection models to explore the genetic basis of susceptibility, or to investigate the immunization of genetically heterogeneous animals against mycobacterial infection ([28, 32, 65, 66]). This background must be examined in order to obtain a clearer view of the two totally different questions which must be answered by studies on immunization.

The problem of the apparent inefficiency of immune response can be examined in terms of the effectiveness of available antigens, i.e., by estimating the degree to which resistance is enhanced in the "average" animal ([28]). The immunogenicity of BCG in mice subsequently challenged with tubercle bacilli has been assessed by plotting the relative extensions of survival time of 50 per cent of the animals. In respect

[3]The reader is warned of a logic-tight situation. In descriptions of the natural course of lepromatous leprosy or of the controls required in chemotherapeutic trials, the disease tends toward self-healing. In discussions of bacteriology and immunology, the resistance of the lepromatous patient has "collapsed."

to murine leprosy, the effectiveness of BCG, killed *M. leprae murium*, and combinations of the two in rats later challenged with *M. leprae murium* was judged by calculating relationships between log dilutions of challenge dosages and the delayed development of lepromas in 50 per cent of the animals. This work permitted three conclusions: (1) that the protection afforded by BCG against tubercle bacilli was greater than that induced against rat leprosy by BCG or BCG plus killed *M. leprae murium;* (2) that all these antigens were remarkably effective in inciting immune response in the "average" animal; and (3) that the problem of achieving protection is due to the extraordinary levels of resistance required to restrain or destroy virulent mycobacteria.

When the necessity of achieving exceptional levels of resistance is coupled with the stark fact that the required genetic elements are lacking in some individuals in all heterozygous stocks ([23]), optimism concerning miracles in chemotherapy and prophylactic immunization must be tempered by recognition of the dilemma involved.

Quantitative depiction of the unexpected differences which arise in

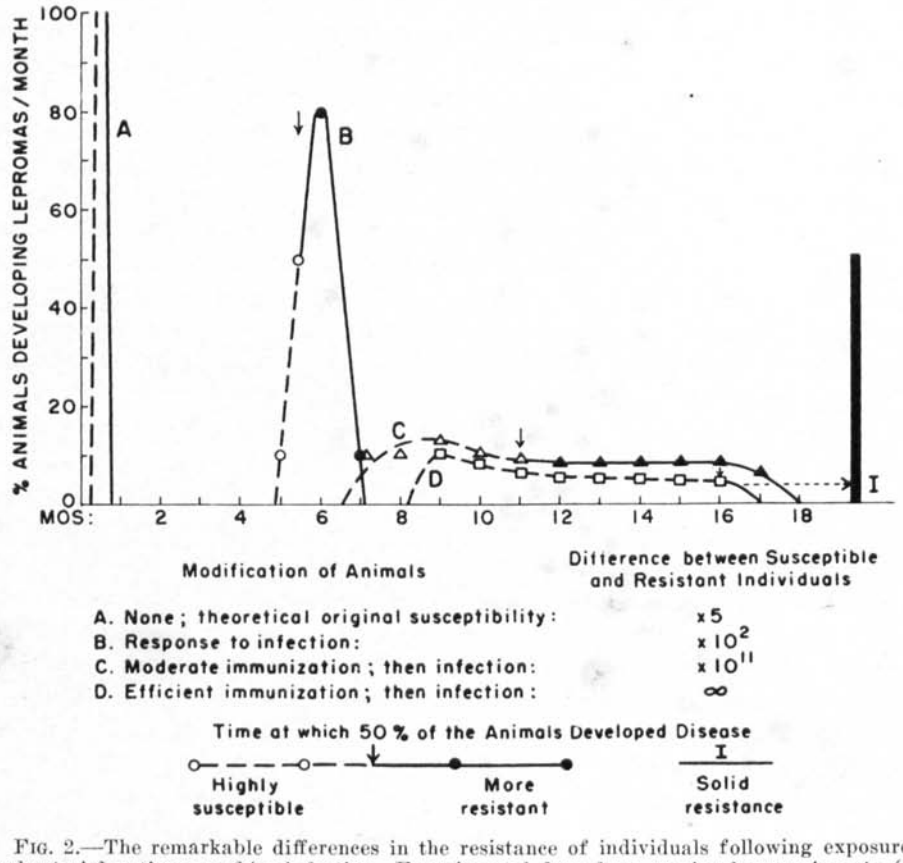

FIG. 2.—The remarkable differences in the resistance of individuals following exposure to mycobacterial antigens and/or infection. Experimental data from murine leprosy in rats ([33]).

individuals that are constantly stimulated by mycobacterial agents has been attempted in Fig. 2, a further analysis of data from the immunization studies of Hanks and Fernandez ([32]).

Curve A is theoretical, to remind us that following challenge with agents causing acute disease a narrow range of incubation periods encompasses the differing susceptibilities of individuals. This curve is based on assumption that some animals might initially be 5 times more resistant than the most susceptible. Curve B shows the results observed in control rats challenged with *M. leprae murium*. By the time disease develops, these infected animals stand far to the right of groups of animals challenged with more acute infections. They have been responding to excellent antigens for some months. Incubation periods in the most susceptible and the most resistant individuals are now seen to differ by two months. Since 100X dilutions of the challenge dose are required to prolong incubation periods in "average" rats by two months, the most susceptible behave as though they had received 100X the challenge dose administered to the most resistant.

Among animals immunized before infection, differences between individuals are further magnified. In Curve C the spread between individuals in a group which had been immunized with intermediate success is 11 months, which corresponds to 11 log dilutions of challenge dose. Between animals which had received a more effective antigen (Curve D), the difference between the most susceptible and the more resistant is infinite, since 50 per cent of the animals developed no disease.

The most significant point in regard to immunization against murine leprosy is the fact that, even after using an efficient method for elevating immune response, the most susceptible individuals remained so incompetent. Once disease was established, these individuals developed fulminating infections. Despite some delay in onset of disease, they did not perform as well as the more capable individuals which had received no prior immunization.

Gowen ([23]) has summarized his experience with Salmonella infections by stating:

> The host genotypes varied in their capacity to develop and utilize the immunity generated by the introduction of the killed vaccine. The naturally most susceptible strains remained susceptible after vaccination .

Similar results are seen in all chronic infections. Consider, for example, the extreme rarity of genetic susceptibilities which permit systemic fungal infections, the severity and/or chronicity of cases which occur, and the parallelisms between immunologic states in fungal infections and leprosy. Favorable prognosis: positive skin reactions to proteins of the infecting agent; serologic tests weak or negative. Unfavorable prognosis: negative skin reactions to proteins, positive or high titers of antibody against polysaccharides. Thus, whether we deal with mice, men or mycology, we see immunization failures or disastrous infections

confined to the minority which is innately least capable of an effective immune response.

In immunization campaigns we must not celebrate victories in honor of those who achieve the selected index of successful response (e.g., strong Mitsuda reactions). We were concerned in the first place with those who achieve reactivity slowly or ineffectually.

An indication of the efficiency with which BCG can induce the capacity for positive reactions in Mitsuda-negative persons may be found in a study by Convit and Rassi ([8]), as shown in Table 3. It must be emphasized that the procedure involved the immunologic stimulus of

TABLE 3.—*Attempt to induce Mitsuda reactivity by means of oral BCG* ([8]).[a]

Age groups (years)	Mitsuda negative[b]		Conversion rates[c]	Mitsuda negatives not converted[c]
	Before BCG	After BCG		
0- 4	43%	19%	56/100	44%
5-14	24%	6%	75/100	25%
15-24	12%	2%	83/100	17%
25-44	6%	1%	83/100	17%
45-	5%	0%	100/100	0%

[a]Sequence of antigens used in testing: (1) lepromin; (2) BCG, 200 mgm. by mouth, twice; lepromin.

[b]Proportions based on the total number of persons in each age group.

[c]Proportions based on the Mitsuda negatives who received BCG.

the preliminary lepromin dose, BCG twice by mouth, and a final test with lepromin for reading the results. Nevertheless, 44 per cent of the Mitsuda-negative preschool children (the age group thought to be most susceptible) failed to attain a 1+ Mitsuda reaction.

The influence of the several stimulating factors involved during these three-step programs has been analyzed by Doull *et al.* ([12]). In an unselected population of children aged 6-35 months and tested for the first time with PPD and lepromin, 23 per cent were Mitsuda positive. Following 1 tuberculin test and a lapse of 143 days, 35 per cent of an originally comparable group were Mitsuda positive, an increase of 12 per cent due to tuberculin, time and unknown causes. The remaining children (Mitsuda and tuberculin negative) represented a population selected on the basis of inadequate prior exposure to antigen and/or low capacities for response. Final retests in a portion of these after 143 days showed that 27 per cent had become Mitsuda positive due to tuberculin, lepromin, time and unknown causes. A further group, given the complete battery of tests plus BCG, yielded 71 per cent positive reactors. If summaries are based on 100 unselected children, 23 were Mitsuda positive at the outset, 20 positives were added by the skin tests plus time, and 55 by these factors plus BCG. Since the total

is 78, it follows that 22 per cent of the children remained nonreactive after this extensive program. The difference in reactivity attributed to the second lepromin test was only 7.2 per cent. A later report by Guinto and Wade ([24]) contains an excellent discussion of evidence that Mitsuda reactions are due in part to antigens liberated from the test dose.

The picture which emerges is one of consistent failure to induce conversions in the order of 98 per cent of supposed susceptibles. It is only when this has been accomplished that identification of the innately poor responders ([27]) and accessory surveillance or protection can become an adjunct to prophylactic immunization.

Major differences between tuberculosis and leprosy are: (*a*) the greater ease of inducing cellular allergy with tubercle bacilli, and (*b*) the more universal occurrence of tuberculoid histology in tuberculous infections. These differences are thought to depend primarily on two factors: (*a*) the greater antigenicity of tubercle bacilli and (*b*) the fact that leprosy is restricted more narrowly to the immunologically poor responders. It is fortunate for the leprologist concerned with classification or prognosis that this disease exhibits a meaningful balance between resistance, tuberculoid histology and cutaneous reactions to killed leprosy bacilli.

A collapse of resistance?—Immune paralysis due to mycobacterial polysaccharides does not seem pertinent to lepromatous leprosy, since plasma cells sometimes are observed in mildly reactive lepromas ([7]) and antibody production proceeds against polysaccharides of *M. leprae* and tuberculin. As indicated in an early section of this paper, these antibodies seem more symptomatic of heavy infection than of resistance. Perhaps their deeper significance remains to be determined.

Mitsuda negativity in lepromatous leprosy remains a challenge to scientific curiosity. The probability of a genetic basis of the type which facilitates induction of immune tolerance to proteins is suggested by the fact that the bacilli which occur in the majority of the earliest lesions in infancy incite tuberculoid histology ([48]). These bacilli do not prevent spontaneous healing in 75 per cent of children studied ([38]). In more persistent cases, however, two observations suggest an obliteration of inherently low capacities for response. These are: the losses of Mitsuda reactivity during transformations from tuberculoid to borderline and from that to lepromatous leprosy, and the very slow reappearance of mild Mitsuda reactivity following spontaneous recoveries ([11]).

Nevertheless, the fact that recoveries occur during Mitsuda negativity makes it necessary to reject the view that this state can be equated with lack of resistance.

On some points the curiosity of the immunologist can be satisfied by challenge with viable microorganisms or by skin tests with their deriva-

tives. Proper interpretation of cutaneous response to derivatives requires fractionations by the carefully devised methods of the physio- and immuno-chemist. An understanding of situations such as lepromatous leprosy requires analytical study by students of metabolism and by serologists.

SEROLOGY

When a person first examines existing information on the serology of leprosy, he is struck by a baffling series of observations. He notes that no significant levels of antibody have been demonstrated in those forms of the disease which are characterized by very small numbers of bacilli, strong Mitsuda reactions and a frank tendency toward self-healing. In lepromatous leprosy, with exceptional concentrations of antigen, he will encounter the absence of cutaneous response despite abundant antibody. If the unwary did not look beneath these superficialities, he could conclude that resistance depends upon inability to make antibody and that formation of antibody is a "cause" of susceptibility.

A remark by H. G. Wells during the annual meeting of the American Association of Immunologists in 1935 terminated a lengthy discussion. He said: "Gentlemen, do you have to continue? Or could we agree that horse serum will never be anything less than an aqueous extract of a whole horse?" In brief, the serologist must be prepared to answer the inevitable question: "*Which* antigen?"

The apparent dichotomy between skin reactivity and serologic reactivity in leprosy disappears the moment it is recognized that skin reactions titrate response to proteins of the microbe, whereas the serologic reactions studied to date seem to be titrations of antibodies against polysaccharides. The failure in tuberculoid leprosy to demonstrate antibodies against polysaccharides excites speculation. Could it be that cellular allergy and underlying mechanisms hydrolyze the protein moities in protein-polysaccharide polymers promptly, and that the polysaccharide haptenes alone induce no appreciable response? In lepromatous leprosy, on the other hand, are the protein-polysaccharide polymers less readily dissociated, with the result that antipolysaccharide antibodies are produced? Must we assume that, meanwhile, the specific configurations in the proteins remain unrecognized?

Given an answer to such questions, there might be a basis for deciding whether measurement of antibody levels against the proteins or polysaccharides of *M. leprae* could make a contribution to serologic epidemiology. Present limited knowledge does not excite optimism, since *M. leprae* has not been shown to be an important incitant of immunologic activations within human populations. If significant levels of antibody occur only against polysaccharides and only during severe infections, suitable serologic procedures may prove more time-consum-

ing and more presumptive than direct microscopic search for the bacilli. Nevertheless, fascinating and important questions are involved. It seems a pity that almost universal preoccupation with other problems has prevented strategists in the field of leprosy from enlisting the full-time participation of professional immunologists and serologists.

Diagnosis.—A search for mycobacterial antigen could be of particular value where questionable, undifferentiated and "bacteriologically negative" lesions present problems in differential diagnosis. The persistence of such lesions indicates continuous elaboration of antigens. Taking tuberculoid lesions as a starting point, we know that stainable bacilli are very rare and suspect that liberated proteins may be hydrolyzed rapidly. The polysaccharides, however, may be present in amounts which could be identified by fluorescent antibody. Eventually it might become possible to use these techniques to study contacts of lepromatous patients for inapparent infections.

Prognosis.—Clinical lesions are taken to denote a reactive combination between degree of host response and local concentrations of *M. leprae*. Adequate measurements of circulating antibody might answer at least three questions: (*a*) In nonlepromatous leprosy, is there *any* antibody against proteins or polysaccharides? (*b*) In lepromatous leprosy, does the circulation contain an excess of proteins from *M. leprae*, protein-antibody complexes, or excess of antibody against the proteins of the bacillus? (*c*) Are our present concepts so inadequate that other questions are the pertinent ones?

An excellent review of serologic work through 1954 has been prepared by Ross ([52]). Further papers in the program of this Symposium will throw light on certain of the questions which here have been merely raised.

SUMMARY

As a basis for considering the immunology and serology of leprosy, factors involved in the competitions between hosts and mycobacteria have been examined. The most significant properties of *M. leprae* seem to have arisen from the selection of microbial genotypes which do not readily excite immune response, and which during growth in a favorable nutritional background synthesize large amounts of capsular lipids. The poor performance of susceptible persons seems in major part to be due to the problem of dealing with the outer structures of *M. leprae*. These difficulties include: (*a*) possibly low capacities to metabolize capsular lipids, and definitely inferior abilities to disintegrate cell walls, and (*b*) inability to achieve the high levels of adaptive host response required to deal with intracellular mycobacteria. The intracellular locus of infection and the nonantigenic surface of mycobacteria are thought to explain the minimal or obscure effects of antibody.

A review of mechanisms whereby animals metabolize parentally-

introduced heterologous proteins indicates that skin reactivity to soluble proteins may be enhanced by adaptive (immunologic) experience, and that protein hydrolysis finally may become so efficient as to minimize the capacity of proteins to induce skin reactions. It is apparently for this reason that reactions to soluble proteins of mycobacteria lack prognostic significance. By the same token, the significance of Koch and Mitsuda reactions to cell walls of tubercle and leprosy bacilli does not depend upon the intensity of inflammatory reactions, nor upon size at a single selected interval, but upon what they reveal concerning the rates at which mycobacterial cell walls can be disintegrated.

Since exceptionally slow bacterial growth and long incubation periods in leprosy afford opportunity for enhancement of response in persons with average or moderate capabilities for adaptive physiologic and immunologic response, leprosy is a disease of very slow or poor responders. Nevertheless, even when cutaneous reactivity has been lacking or suppressed for long periods of time, other factors produce a trend toward self-healing.

The view that leprosy is restricted to a highly selected segment of human populations makes it unnecessary to accept the idea that there is otherwise anything "peculiar" about the immunology of leprosy. The corresponding segment of poor responders among heterozygous animals has been ignored rather than selected for experimental study.

In view of the limitations of studying responses to infection or to crude extractives of mycobacteria, the need for investigations with definable anatomic fractions of *M. leprae* and for serologic analysis of the antigen-antibody balances with respect to microbial proteins and polysaccharides is evident. A few questions which invite serologic study have been outlined.

RESUMEN

Como base para considerar la inmunología y serología de la lepra, se estudian los factores que intervienen en las rivalidades entre huéspedes y micobacterias. Al parecer, las propiedades más importantes del *M. leprae* han surgido de la selección de genotipos microbianos que no excitan fácilmente inmunirreacciones, y que durante su proliferación en un medio nutritivo favorable sintetizan grandes cantidades de lípidos capsulares. La deficiente ejecución ofrecida por las personas susceptibles parece en su mayor parte deberse al problema creado por los accesorios externos del *M. leprae*. Comprenden estas dificultades: (*a*) capacidades posiblemente bajas para metabolizar los lípidos capsulares y destrezas decididamente inferiores para desintegrar las paredes celulares; y (*b*) incapacidad para alcanzar los altos niveles que se requieren en la respuesta del huésped adaptivo para atender a las micobacterias intracelulares. El sitio intracelular de la infección y las superficies anantigénicas de las micobacterias parecen explicar los mínimos y oscuros efectos de los anticuerpos.

Un repaso de los mecanismos por los que los animales metabolizan las heteroproteínas introducidas parentéricamente indica que, por la experiencia adaptiva (inmunológica), cabe realzar la reactividad cutánea a las proteínas solubles y que la hidrólisis de las proteínas puede volverse tan eficaz que reduzca al mínimo la capacidad de las proteínas para inducir cutirreacciones. Es por esta razón aparentemente que las reacciones

a las proteínas solubles de las micobacterias carecen de valor pronóstico. Por el mismo motivo, la importancia de las reacciones de Koch y Mitsuda a las paredes celulares de los bacilos tuberculoso y leproso no procede de la intensidad de las reacciones inflamatorias ni del tamaño alcanzado a un solo plazo escogido, sino de lo que revelan acerca de las velocidades con que pueden desintegrarse las paredes de las células micobacterianas.

Visto que la proliferacion bacteriana excepcionalmente lenta y los largos períodos de incubación en la lepra brindan ocasión para realzar la respuesta en personas de capacidades medianas o moderadas para dar una reacción fisiológica e inmunológica adaptiva, la lepra es una enfermedad de reactores muy lentos o malos. No obstante, aun cuando falta o está suprimida por largos períodos de tiempo la reactividad cutánea, otros factores producen una tendencia hacia la autocuración.

La opinión de que la lepra está limitada a un segmento muy seleccionado de las poblaciones humanas elimina la necesidad de aceptar la idea de que haya, aparte de eso, algo "peculiar" acerca de la inmunología de la lepra. Se suscitó esta último opinión porque se ha pasado por alto más bien que escogido para estudio experimental el segmento correspondiente de malos reactores entre los animales heterócigos.

A la luz de las limitaciones impuestas por el estudio de las respuestas a la infección o a extractos no muy refinados de micobacterias, resulta evidente la necesidad de realizar investigaciones con fracciones anatómicas definibles del *M. leprae* y análisis serológicos de los equilibrios de antígenos-anticuerpos con respecto a las proteínas y los polisacáridos microbianos. Se han delineado algunos puntos que invitan estudio serológico.

RESUMÉ

Comme point de départ pour considérer l'immunologie et la sérologie de la lèpre, on a examiné les facteurs mis en jeu dans la compétition entre hôtes et mycobactéries. Les propriétés les plus significatives de *M. leprae* semblent découler de la sélection de génotypes microbiens n'entraînant pas une prompte réponse immunologique, et synthétisant, lors de la croissance dans un environnement nutritionnel favorable, des quantités importantes de lipides capsulaires. La faible défense des individus susceptibles semble en majeure partie devoir être attribuée à la difficulté que se présente lorsque l'on a à faire avec les structures exterieures de *M. leprae*. Ces difficultès comprennent: a) éventuellement un faible pouvoir de métaboliser les lipides capsulaires, et une déficience sans contredit plus marquée à désintégrer les parois cellulaires, et b) une inaptitude à atteindre les hauts niveaux de réponse et d'adaptation requis chez l'hôte pour affronter les mycobactéries intra-cellulaires. Il est concevable que la situation intra-cellulaire de l'infection, ainsi que le caractère non-antigénique de la surface des mycobactéries, peuvent rende compte des effets peu marquès, ou peu clairs, d'un anticorps.

Une revue des mécanismes par lesquels les animaux métabolisent les protéines hétérologues introduites par voie parentérale met en évidence le fait que l'aptitude de la peau à réagir à des protéines solubles peut être exaltée par une experience d'adaptation (immunologique), et que l'hydrolyse des protéines peut à la longue devenir suffisament efficace que pour minimiser le pouvoir qu'ont ces protéines d'entraîner des réactions cutanées. C'est apparement pour cette raison que les réactions aux protéines solubles des mycobactéries sont dénuées de signification pronostique. Du même coup, la signification des réactions de Koch et de Mitsuda, réactions aux parois cellulaires des bacilles de la tuberculose et de la lèpre, ne repose pas sur l'intensité de la réaction inflammatoire, ni sur la dimension à un intervalle de temps déterminé. Leur signification réside en ce qu'elles revèlent de la vitesse à laquelle les parois cellulaires des mycobactéries peuvent être détruites.

Puisque, dans la lèpre, une croissance microbienne exceptionnellement lente, ainsi qu'une période d'incubation prolongée, offrent, chez des individus doués d'une aptitude moyenne ou modérée à developper une réponse immunologique et physiologique d'adaptation, la possibilité d'une exaltation de cette réponse, cette maladie est une affection qui

touche les individus à réponse faible ou lente. Toutefois, lorsque cette réactivité cutanée a fait défaut, ou a été supprimée, pendant longtemps, d'autres facteurs tendent à mener vers la guérison spontanée.

L'opinion que la lèpre est limitée à une portion hautement selectionnée de la population humaine rend superflue l'idée qu'il existe en outre quelque chose de "spécial" dans l'immunologie de cette maladie. Cette opinion provient du fait que, dans les études experimentales, les catégories correspondantes de réacteurs faibles parmi les animaux héterozygotes, au lieu d'être choisies comme sujet d'études, ont bien plutôt été negligées.

Si l'on considère les limitations imposées à l'étude des réponses aux infections mycobacteriennes, ainsi qu'aux extraits bruts de mycobactéries, il est évident qu'il est indispensable de recourir à des recherches menées avec des fractions anatomiques définies de *M. leprae*, et de procéder à une analyse sérologique des rapports antigénes-anticorps en tenant compte des protéines et des polysaccharides microbiens. Quelques problémes ont été soulignés, qui réclament une étude sérologique.

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