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EDITORIALS

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Vaccination in Leprosy—Observations and Interpretations

During the last decade we have developed a research program oriented towards clarifying some of the questions which arise in the observation of the reactions of leprosy patients across the clinical spectrum toward *Mycobacterium leprae* and other mycobacteria *in vivo*. This program has culminated in the development of a vaccination procedure which induces profound, persistent changes in immunological reactivity of the treated individuals. The results of this experience seem to merit some commentary and interpretation, apart from the formal presentation of the relevant data in other publications[1,2].

Briefly, in 1972, we reported the results of the injection of a concentrated suspension of lepromin (6.4×10^7 autoclaved acid-fast bacilli) in patients with lepromatous and other forms of leprosy and in persistently Mitsuda-negative contacts[3]. Lepromatous individuals were unable to eliminate these bacilli from the injection site in one month while patients with tuberculoid leprosy developed a typical immune granuloma with essentially complete bacillary elimination in the same period of time. This defect in the lepromatous patients was specific for *M. leprae* since they developed immune granulomata and readily eliminated other mycobacteria, including *M. lepraemurium* and BCG. This test for the capacity to eliminate *M. leprae* was called the CCB test (competency in clearing bacilli).

Subsequently, lepromatous patients were injected intracutaneously with a mixture of viable BCG and heat-killed *M. leprae*[4]. Biopsies taken after one month demonstrated the formation of an immune granuloma

[1] Convit, J. The development of an active vaccine against leprosy. Acta Cient. Venez. (in press)

[2] Convit, J., Aranzazu, N., Pinardi, M. E. and Ulrich, M. Immunological changes observed in indeterminate and lepromatous patients and Mitsuda-negative contacts after inoculation of a mixture of *Mycobacterium leprae* and BCG. Clin. Exp. Immunol. **36** (1979) 214–220.

[3] Convit, J., Avila, J. L., Goihman-Yahr, M. and Pinardi, M. E. A test for the determination of competency in clearing bacilli in leprosy patients. Bull. WHO **46** (1972) 821–826.

[4] Convit, J., Pinardi, M. E., Rodriguez Ochoa, G., Ulrich, M., Avila, J. L. and Goihman-Yahr, M. Elimination of *Mycobacterium leprae* subsequent to local *in vivo* activation of macrophages in lepromatous leprosy by other mycobacteria. Clin. Exp. Immunol. **17** (1974) 261–265.

at the injection site and the elimination of all acid-fast bacilli. Since the concentration of *M. leprae* in this test was roughly equivalent to the concentration in the CCB test, there seemed to be no doubt that both mycobacterial species had been digested although these patients were unable to digest *M. leprae* when injected by itself. The experimental design in this study did not contemplate the study of any aspect other than the local phenomenon.

During the past year, we reported on the immunological changes induced in Mitsuda-negative contacts and patients with Mitsuda-negative indeterminate leprosy and bacteriologically negative lepromatous patients when injected in several intracutaneous sites with a mixture of BCG and heat-killed *M. leprae*[2]. These patients and contacts had been injected with *M. leprae* and BCG separately on several occasions without producing any clinical modifications. After injection with the mixture, the Mitsuda-negative contacts and indeterminate patients became immunologically reactive by all the criteria employed—positive Fernández, Mitsuda, and CCB tests; positive reactions at 48 hours to a soluble antigenic extract of *M. leprae* which is rather highly specific[5]; and positive lymphocyte transformation with *M. leprae in vitro*. Changes were less dramatic in the lepromatous group; they developed positive Fernández and Mitsuda reactions but did not develop the capacity to eliminate bacilli in the clearance test nor to respond to the soluble protein antigen. Clinical changes were also observed; some of the indeterminate patients developed inflammatory activity at the sites of hypopigmented lesions, and these lesions then regressed with no appearance of new lesions. Also, papular lesions developed, with the clinical appearance and structure similar to the early tuberculoid lesions described in young children contacts. These lesions regressed in three or four months. The clinical and immuological changes reported in this study had persisted for five years at the time of publication.

In a recent group of thirty Mitsuda-negative patients with indeterminate leprosy, whom we consider pre-lepromatous because they have relatively large numbers of bacilli in their lesions and bacilli at distant sites from the lesions, the injection of BCG and autoclaved *M. leprae,* which had been purified from unirradiated armadillo liver by the recently developed Draper protocol[6], produced even more striking clinical results. Inflammatory activity in the hypochromic lesions was evident within three to six weeks; a papular rash was often observed which had a tuberculoid structure and may reflect reactivity at the sites where bacilli were present, sometimes at some distance from the hypopigmented lesions. The 48-hour skin test became strongly positive after a variable period of time; Mitsuda tests applied several months earlier, which were negative, have become activated and are clearly positive now by clinical and histological criteria.

Various points observed in these studies merit further comment. First, the lack of reactivity in potentially or pre-lepromatous patients to *M. leprae* would appear to be due to a defect in an early step in the primary immune response, reflected visually in a lack of adequate digestion by the macrophage. The vaccination procedure used would stimulate primary macrophage digestion of *M. leprae* in one of two ways. In individuals previously sensitized to BCG, sensitized lymphocytes reacting to BCG would induce macrophage activation, resulting in the digestion of *M. leprae* so that adequate immunogenic components are produced. In individuals not sensitized to BCG, presumably a primary response to this antigen would be necessary before macrophages become activated sufficiently to produce the digestion of *M. leprae* and a subsequent immune response to the latter microorganism. These observations are supported by two observations: a) reactivity to soluble protein antigen of *M. leprae* and appearance of the papular lesions previously described developing within two to three weeks in individuals with positive

[5] Convit, J., Pinardi, M. E., Avila, J. L. and Aranzazu, N. Specificity of the 48-hour reaction to Mitsuda antigen. Use of a soluble antigen from human and armadillo lepromin. Bull. WHO **52** (1975) 187–191.

[6] Draper, P. Annex 1, Protocol 1/79, In: Problems related to purification of *M. leprae* from armadillo tissues and standardization of *M. leprae* preparations. Report of IMMLEP meeting, Geneva, February 1979.

tuberculin reactions at the time of vaccination; they do not develop until four or five weeks after vaccination in tuberculin-negative individuals. b) It has not been possible to sensitize one of the patients in the recent group to BCG in spite of repeated injections, and she has not responded by any criterion to *M. leprae*. In this patient, *M. vaccae* has recently been used instead of BCG because she is sensitized to the former.

The results suggest that the primary defect may originate in the macrophage, which is not effective in producing the partial digestion or orientation of the appropriate antigens, which is a prerequisite for initiating the primary response for protective immunity. Nevertheless, the possibility exists that primary lymphocyte responses to certain antigens are absent or rapidly suppressed in which case a secondarily induced digestion by BCG might unmask other antigens which are adequate to induce protective immunity. Until some information is available about the specific antigen or antigens which induce protection to *M. leprae*, this question will remain unanswered.

The use of the mixture of *M. leprae* and BCG as a vaccine against leprosy creates a new model of vaccination that is distinct from the conventional vaccines used against infectious diseases. This is because conventional vaccines protect a virgin population which is able to develop a normal immunological response to the non-pathogenic specific antigen. The system described above employs two microorganisms: a) the specific microorganism, *M. leprae*, killed by heat in this case, and b) a second living non-pathogenic microorganism, BCG, which serves as a macrophage activator to correct the defect in primary presentation of the specific antigen. This type of vaccine would be applied only to the persons susceptible to develop the disease who have the described defect. In leprosy, the previous screening of the population in endemic areas for reactivity against the soluble protein antigen of *M. leprae* permits the identification of this susceptible population with some precision. Epidemiologic studies suggest that most of the population in leprosy endemic areas have been sufficiently exposed to the infection to have developed a primary response if they are capable of reacting normally. Therefore, the vaccine described above is really not a preventive vaccine in the strictest sense but rather a curative vaccine for certain forms of the disease or for subclinical, latent pre-lepromatous infections. This vaccination system revives a somewhat abandoned chapter of vaccine therapy.

The clinical and immunological changes observed in the indeterminate cases suggest that the vaccination has produced not only a positivization of selected immunological parameters in the sense that we might have expected a shift toward borderline tuberculoid or tuberculoid leprosy; what we observed was the appearance of lesions with the characteristics of early tuberculoid leprosy which regressed and disappeared in a few months. There did not appear to be any exaggeration of the *in vivo* or *in vitro* responses suggesting the mere superimposition of hypersensitivity phenomena on existing quiescent lesions. The analysis of these observations leads us to believe that the changes induced by the vaccine caused the patients to regress to a state of immunologic normality characterized by the development of true protective immunity such as that present in naturally resistant individuals.

The principles described above may be applicable to other diseases caused by intracellular parasites in which primary digestion by macrophages may be an important factor in the development of a satisfactory immune response, and a similar system of vaccination may be beneficial. Perhaps one of the factors responsible for the success of vaccination against *M. leprae* is related to the fact that this microorganism appears to be highly resistant to the effects of the enzymatic arsenal of polymorphonuclear leukocytes, which are the first cells which accumulate at the injection site. Preliminary results suggest that *Leishmania*, for example, are highly fragile and susceptible to rapid digestion by leukocytic enzymes. Effective contact with macrophages will apparently require some additional manipulation. The fortuitous circumstance that the essential immunogenicity of *M. leprae* resists autoclaving may also be a rather

unique characteristic not applicable to protozoa or even to all other mycobacteria[7].

In view of the clear immunological changes produced in pre-lepromatous indeterminate patients and persistently Mitsuda-negative contacts, we consider that this vaccination procedure would be very effective for application to susceptible persons in endemic areas for leprosy.

—Jacinto Convit, M.D.
—Marian Ulrich, Ph.D.
—Nacarid Aranzazu, M.D.

Instituto Nacional de Dermatología
Apartado Postal 4043
Caracas, Venezuela

[7] Shepard, C. C., Walker, L. L. and Van Landingham, R. Heat stability of *Mycobacterium leprae* immunogenicity. Infect. Immun. **22** (1978) 87–93.

Mechanism of Action of DDS

We are fortunate to begin the decade of the 1980s with a group of excellent original articles in this issue among which is the authoritative article by Professor Seydel, *et al.* concerning the mechanism of action of dapsone (DDS). Molecular mechanism of action studies are, by their very nature, complex biochemical puzzles, frequently seeming beyond the grasp of those of us engaged in more pedestrian efforts such as patient care, leprosy control, rehabilitation, and editing. This work stands on its own merit, of course, as meticulous basic science, but we would like to point out and emphasize that, additionally, work of this sort has profound implications for all leprosy workers.

The fundamental question being addressed by Professor Seydel, *et al.* is why is dapsone so uniquely useful in leprosy? If it were an "ordinary" sulfonamide and *M. leprae* were an "ordinary" microorganism, we would undoubtedly have been deluged with dapsone resistant cases within a few years after the introduction of the sulfones in 1941. We were not, and, indeed, it was not until after almost a quarter century of use that the first cases of sulfone resistance were documented in leprosy. Providence looked over us in our ignorance as we empirically dispensed this cheap and relatively innocuous chemical to our patients. As more and more cases of secondary sulfone resistant leprosy accumulate, and now that patients with primary resistant disease are appearing, it is clear that we can no longer comfortably rest on our empirical good fortune. If the almost unique efficacy of dapsone against *M. leprae* is due to a unique mechanism of action, it is indeed imperative that this mechanism of action be elucidated, for only in so doing can we hope to develop rational alternatives to, or rational companion drugs for, the sulfones.

In *E. coli* model systems, necessary because of *M. leprae*'s reluctance to propagate *in vitro*, Professor Seydel, *et al.* have shown systematically that, by and large, dapsone behaves like a sulfonamide in its mechanism of action, i.e., it inhibits the enzyme dihydropteroic acid synthetase. There are some clues, however, (e.g., the two phases of inhibition of growth of *E. coli* caused by dapsone) that there may be something different about the way dapsone works. The likely possibilities are outlined, and some sound familiar to leprologists, e.g., the ideas that dapsone may act in some fashion in leprosy completely unconnected with the bacterial synthesis of folic acid or that it may perhaps uniquely accumulate in leprosy bacilli. The other likely possiblities involve dapsone, either directly or through a "false" folic acid precursor acting to inhibit the other enzyme (dihydrofolate reductase) involved in manufacturing the useable form of folic acid (tetrahydrofolate). Although Professor Seydel, *et al.* show that dapsone does not seem to work that way in *E. coli*, the possibility remains that dapsone may work that way in *M. leprae*. The prospect that answers may be forthcoming to these questions is exciting not only from a basic biochemical-microbiologic-pharmacologic standpoint but from the standpoint of every frustrated clinician and paramedical worker who has longed for more effective drugs for his leprosy victims.

—RCH