# CORRESPONDENCE

*This department is for the publication of informal communications that are of interest because they are informative and stimulating, and for the discussion of controversial matters. The mandate of this JOURNAL is to disseminate information relating to leprosy in particular and also other mycobacterial diseases. Dissident comment or interpretation on published research is of course valid, but personality attacks on individuals would seem unnecessary. Political comments, valid or not, also are unwelcome. They might result in interference with the distribution of the JOURNAL and thus interfere with its prime purpose.*

## Leprosy Vaccines

TO THE EDITOR:

In the June issue (1982) of this JOURNAL there appeared an erudite editorial: "BCG Vaccination in Leprosy" by Janet Price. In her concluding paragraph Dr. Price points out: "At present, research is being conducted with killed *M. leprae,* closely related atypical mycobacteria, and BCG in combination with killed *M. leprae* or atypical mycobacteria ([25]). Few conclusive results are yet available and time consuming controlled trials will be required before respective efficacy of vaccines can truly be assessed" ([18]). I would like here to consider the above conclusions in relation to a letter from M. G. Deo (Indian Cancer Research Institute) appearing in *Science* ([12]) in response to the article, "Leprosy Vaccine Trials Begin Soon," Research News, *Science* **26** (February, 1982) ([16]), and in relation to the vaccination efforts of the Caracas group discussed in the editorial columns of this JOURNAL in 1980 ([10]). Certain well-known immunological interactions which can occur between human subjects and mycobacteria, although apparently not considered in assessments of the efforts of the Caracas investigators, may well be the basis for the promising results reported by Dr. Convit and his associates.

Dr. Deo in his letter suggested that vaccination with by-gamma-rays-irradiated *Mycobacterium* strain ICRC served to improve the immune status of 70% of a group of cases of lepromatous leprosy in India. *Mycobacterium* ICRC was originally isolated from fresh lepromatous tissue inoculated into complex media on 7 May 1957 in experiments designed by Kamal J. Ranadive, C. V. Bapat, and V. R. Kanolkar ([19]). Since early on in our work we had not isolated a *Mycobacterium* from cases of leprosy, we became interested in the specific properties of such leprosy-derived mycobacteria (LDM) and, through the kindness of K. J. Ranadive and C. V. Bapat, cultures of strain ICRC were obtained and examined for the chemistry of its structural components in relation to the structure of five other LDMs ([4]). Later, by arrangement with Mayer B. Goren and Magdalena Souhrada, certain of these strains which had been examined for their chemical components were submitted for serologic identification under a service contract then existing between NIAID and the National Jewish Hospital in Denver. The serological identities of the strains and a key to their code numbers are published here for the first time: (8393) ICRC agglutinates with *M. avium,* Type 1, antisera; (7519) HI75 ([21]) agglutinates with antisera vs *M. intracellulare,* Type 7; strain NQ ([5]) was diagnosed as "*M. avium-intracellulare* Type 20"; strains 316, 1081 and 917 from Convit ([8]) each agglutinates with *M. simiae,* Type I antisera. What seems worthy of note here is that a *Mycobacterium* (the ICRC bacillus) of the *avium-intracellulare* group, species reported to be genetically unrelated to leprosy bacilli ([14, 15]), served to enhance the immune response in patients with the lepromatous form of leprosy.

In 1972, Convit ([7]) reported a very fundamental observation with regard to leprosy bacilli and the macrophages of lepro-

matous patients. He noted that autoclaved, purified human leprosy bacilli injected into lepromatous individuals elicited the formation of a foreign body type of granuloma (FBG) ([23]), i.e., macrophages gathered at the injection site, engulfed leprosy bacilli and there the process appeared to stop [also termed "pure macrophage anergic type" granuloma ([20]); also discussed in ([17])]. On the other hand, autoclaved, cultivated mycobacteria injected at nearby sites elicited granulomatous responses histologically showing the cellular reactions common to the successful elimination of mycobacteria [hypersensitivity granulomas (HG) ([23]); termed "epithelioid high resistant" granulomas ([20]); also discussed in ([17])]. In 1974 ([9]) Convit and his associates further showed that when autoclaved, purified leprosy bacilli and mycobacteria were injected into the skin of lepromatous subjects as mixtures, the results were very different from the injection of leprosy bacilli alone. The histological examinations of biopsies of the mixtures taken at 30 days revealed competent clearing of both the leprosy bacilli and the mycobacteria, i.e., the mixtures had elicited a satisfactory HG response. This successful exploitation of an "outpost of the reticuloendothelial system" ([23]) for gaining insights into cell-mediated interactions in leprosy has more recently led to the possibility of very real benefits for significant numbers of leprosy patients. In 1979, Convit and his associates reported the effects of administering a combination of living BCG [*Mycobacterium tuberculosis bovis* strain of Calmette and Guerin ([2])] and autoclaved, purified leprosy bacilli (produced in the nine-banded armadillo) upon six patients classed as indeterminate leprosy; six with lepromatous leprosy and two subjects who had been in contact with the disease and who failed to clear injected autoclaved leprosy bacilli. In the authors' own words, "A radical change was observed in the specific immunological activity of the indeterminate group, all of whom initially had occasional bacilli in cutaneous nerves in biopsies taken from hypopigmented areas" ([6]). This group and those contacts previously unable to clear leprosy bacilli underwent a change from negative to positive responses to injected leprosy bacilli. Although the response of lepromatous patients to the combination vaccine was not encouraging, overall the effects were positive and offer hope for immunotherapy in a fraction of leprosy patients. Further discussion of the significance of these results is to be found in an editorial in the INTERNATIONAL JOURNAL OF LEPROSY ([10]).

Returning to Dr. Deo's reported success in vaccinating cases of lepromatous leprosy with the ICRC bacillus, one would like to have more details regarding the criteria he used for evaluating improvement. Perhaps he and his coworkers could introduce into their program some of the methodology worked out in Caracas and discussed above.

The general principle underlying the promising results of Convit and the Caracas group (and presumably the workers in Bombay), while new to leprology, is not new to immunology: when living mycobacteria are injected (into an animal) along with an antigen X, there follows an enhanced cell-mediated immune (CMI) response to antigen X and an enhanced humoral immune response (HI, immunoglobulin synthesis) to antigen X [Dienes, 1928, discussed in ([3])]. If the vaccinated subject has previously been sensitized to a mycobacterial antigen, Y, then the newly initiated CMI may manifest itself with greater delayed hypersensitivity to Y than to X ([1, 13, 22]). Abrahams has dubbed this phenomenon "original mycobacterial sin." While there seems to be a wide-spread view that the effect of BCG on mycobacterial infections has to do with unknown antigen(s) shared between BCG and the *Mycobacterium* under study, in the case of *M. tuberculosis,* at least, this interpretation does not agree with the facts. Facts from the analyses of Sutherland say that when the amount of *M. tuberculosis* in the population is high (15.6 cases/1000/year) "% protection" from BCG can be as high as 80%; when the amount of *M. tuberculosis* in the population is low (0.11 cases/1000/year) the percentage protection from vaccination will be nil ([24, 25]); also discussed in ([3]). So, until very differing statistics are available, one has to regard *M. tuberculosis,* itself, as antigen X in BCG trials "against tuberculosis." The findings of Abrahams ([1]) bolstered by the work of Edwards ([13]) and Smith ([22]) indicate that BCG vaccination (or other my-

cobacterial infection) can stimulate an existing CMI as well as inducing CMI towards co-introduced antigen X.

In the CMI response involving lymphocyte mediators, two general classes of cells participate: sensitized lymphocytes and macrophages. Activated macrophages exhibit an enhanced ability to effect non-specific killing of a variety of bacteria and, even, tumor cells. In fact, that is the basis for courses of immunotherapy aimed at killing tumor cells by the adjuvant action of living BCG [for general discussion see ([11])]. The Convit approach to immunotherapy, then, has in its favor a well-established event: the adjuvant effect of living mycobacteria (BCG vaccine) where the specificity for leprosy bacilli comes from the leprosy bacilli, themselves present to function as antigen(s) X in the adjuvant event(s).

From what we know today about the capacities of certain bacteria and/or their derivatives to enhance CMI and HI ([11]), it would seem that the work of the Caracas group should be followed with profound interest and encouragement. Despite the fact that there are immunologic complexities associated with the "leprosy spectrum" and despite the fact that detailed interactions that lead to immunosuppression and immunopotentiation are far from understood at the level of molecular signals and their cellular receptors, the well-established effects of adjuvants on human population are real enough to justify efforts at immunotherapy in leprosy. In public health in particular and in science in general there are often times when half a loaf is better than none.

—Lane Barksdale, Ph.D.

*Leprosy Research Group*
*Department of Microbiology*
*New York University School of Medicine and Medical Center*
*550 First Avenue*
*New York, NY 10016, U.S.A.*

## REFERENCES

1. Abrahams, E. W. Original mycobacterial sin. Tubercle **51** (1970) 316–321.
2. Baess, I. Deoxyribonucleic acid relatedness among species of slowly growing mycobacteria. Acta Pathol. Microbiol. Scand. [B] **87** (1979) 221–226.
3. Barksdale, L. and Kim, K.-S. *Mycobacterium.* Bacteriol. Rev. **41** (1977) 217–372.
4. Beaman, B. L., Kim, K.-S., Laneelle, M.-A. and Barksdale, L. Chemical characterization of organisms isolated from leprosy patients. J. Bacteriol. **117** (1974) 1320–1329.
5. Binford, C. H. Studies on a mycobacterium obtained from the golden hamster (*Cricetus auratus*) after inoculation with lepromatous tissue. Lab. Invest. **11** (1962) 942–955.
6. Convit, J., Aranzazu, N., Pinardi, M. and Ulrich, M. Immunological changes observed in indeterminate and lepromatous leprosy patients and Mitsuda-negative contacts after inoculation of a mixture of *Mycobacterium leprae* and BCG. Clin. Exp. Immunol. **36** (1979) 214–220.
7. Convit, J., Avila, J. L., Goihman, M. and Pinardi, M. E. A test for competency in clearing bacilli in leprosy patients. WHO Bull. **46** (1972) 821–826.
8. Convit, J., Lapenta, P., Ilukevich, A. and Imaeda, T. Experimental inoculation of human leprosy in laboratory animals. I. Clinical bacteriologic and histopathologic study. Int. J. Lepr. **30** (1962) 239–253.
9. Convit, J., Pinardi, M. E., Rodriguez-Ochoa, G., Ulrich, M., Avila, J. L. and Goihman, 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.
10. Convit, J., Ulrich, M. and Aranzazu, W. Vaccination in leprosy—Observations and interpretations. (Editorial) Int. J. Lepr. **48** (1980) 62–65.
11. David, J. R., Goetzl, E. J. and Austen, K. F. Immunology. In: *Pathophysiology, the Biological Principles of Disease.* Smith, L. H. and Thier, S. O., eds. Philadelphia, Pennsylvania, U.S.A.: W. E. Saunders Company, 1981, pp. 165–266.
12. Deo, M. G. Letter to the Editor. Science **216** (1982) 1172.
13. Edwards, L. B. Current status of the tuberculin test. Ann. N.Y. Acad. Sci. **106** (1963) 32–42.
14. Imaeda, T., Barksdale, L. and Kirchheimer, W. F. Deoxyribonucleic acid of *Mycobacterium lepraemurium*: Its genome size, base ratio and homology with those of other mycobacteria. Int. J. Syst. Bacteriol. **32** (1982) 456–458.
15. Imaeda, T., Kirchheimer, W. F. and Barksdale, L. DNA isolated from *Mycobacterium leprae*: Genome size, base ratio and homology with other bacteria as determined by optical DNA-DNA reassociation. J. Bacteriol. **150** (1982) 414–417.
16. Maugh, T. H., II. Leprosy vaccine trails to begin soon. (Research News) Science **215** (1982) 1083–1086.
17. Narayanan, R. B., Jones, B. and Turk, J. L. Experimental mycobacterial granulomas in guinea pig lymph nodes: Ultrastructural observations. J. Pathol. **134** (1981) 253–265.

18. PRICE, J. E. BCG vaccination and leprosy. [Editorial] Int. J. Lepr. **50** (1982) 205–212.
19. RANADIVE, K. J. Experimental studies on human leprosy. In: *The Pathogenesis of Leprosy.* Wolstenholme, G. E. W. and O'Connor, M., eds. Boston: Little, Brown and Company, 1963, pp. 61–79.
20. RIDLEY, M. J. and RUSSELL, D. An immunoperoxidase study of immunological factors in high immune and low resistance granulomas in leprosy. J. Pathol. **137** (1982) 149–157.
21. SKINSNES, O. K., MATSUO, E., CHANG, P. H. C. and ANDERSSON, B. *In vitro* cultivation of leprosy bacilli on hyaluronic acid based medium. 1. Preliminary report. Int. J. Lepr. **43** (1975) 193–203.
22. SMITH, D. T. Diagnostic and prognostic significance of the quantitative tuberculin tests. The influence of subclinical infections with atypical mycobacteria. Ann. Intern. Med. **67** (1967) 919–946.
23. SPECTOR, W. G. The granulomatous inflammatory exudate. Int. Rev. Exp. Pathol. **8** (1969) 1–55.
24. SUTHERLAND, I. Fifty years of BCG. Tubercle **53** (1972) 150–151.
25. SUTHERLAND, I. Future policy for BCG vaccination in Britain. Postgrad. Med. J. **47** (1971) 756–758.

# *In Vitro* Lymphocyte Stimulation in an Approach to Study the Expression of HLA-encoded Genes Predisposing to Tuberculoid Leprosy

TO THE EDITOR:

Since the original observations that murine major histocompatibility complex located genes control susceptibility to Gross leukemia virus ([5]) and regulate—Ir genes—the immune response to synthetic polypeptides ([6]), a search for relationships between HLA and infectious diseases in man was made.

Especially in leprosy such a search appeared to be rewarding. The existence of HLA-encoded genes, contributing to the susceptibility to tuberculoid leprosy, was shown as well by means of population studies, as by means of family studies ([3]). The latter studies showed a recessive mode of inheritance of the susceptibility trait. Although the involvement of HLA-encoded Ir genes is supposed to underlie the observations made, no formal proof of this supposition has been obtained as yet.

One of the most reliable test systems currently used to study the expression of Ir genes *in vitro* is the lymphocyte transformation test (LTT) ([1, 4, 8]). The LTT measures the proliferative response of mononuclear cells, obtained from peripheral blood, in the presence of antigens. Healthy contacts of leprosy patients are known to show variability in this proliferative response after stimulation with *Mycobacterium leprae* antigens ([7]). Since clinical observations indicate that an *in vitro* proliferative response to *M. leprae* correlates with a state of hypersensitivity to *M. leprae,* it could well be that the individual variability in the LTT reflects the differential expression of HLA-encoded genetic factors, previously described to predispose to tuberculoid leprosy.

To test the above-mentioned hypothesis, we tested in the LTT healthy siblings (sibs) of tuberculoid leprosy patients who were either fully HLA-identical with their tuberculoid leprosy-affected sibs or HLA nonidentical with their tuberculoid sibs. This grouping was based on the recessive mode of inheritance, reasoning that only the fully HLA-identical sibs would carry the genetic make-up predisposing to tuberculoid leprosy. All individuals tested were derived from multicase tuberculoid leprosy families which were collected in Whardha District, Maharashtra, India, in collaboration with Dr. N. K. Mehra (AIIMS, New Delhi).

The segregation pattern of parental HLA-haplotypes has been reported previously ([9]). The mononuclear cells used for LTT were isolated on a Ficoll-Isopaque density gradient and successfully frozen in a freezing medium containing dimethylsulfoxide (10% in culture medium). For the freezing procedure a polystyrene box, containing 1 ml plastic ampules filled with the cells in freez-