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EDITORIALS

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Leprosy Vaccine—a Reappraisal

The rationale for an appropriate vaccine for leprosy has been to seek an organism or antigen which crossreacts with *Mycobacterium leprae* and activates T cells but lacks the suppressor epitope(s) which is considered to be responsible for the anergy in lepromatous leprosy. As a continuation of our previous overview,¹ we submit that even if such an antigen were available there may be numerous reasons why it may be ineffectual when finally tested *in vivo*.

Entry of *M. leprae* into host cells. It is generally assumed that degradation of complex molecules following phagocytosis is a prerequisite for major histocompatibility complex (MHC)-restricted antigen presentation. With respect to *M. leprae*, studies by Mukherjee, *et al.*,² Poulter, *et al.*,³ and Mistry, *et al.*⁴ have shown that viable *M. leprae*

are capable of facilitative entry into cells since they are detected in otherwise non-phagocytic cells, such as dendritic cells and Schwann cells, or in macrophages made non-phagocytic by treatment with cytochalasin B. Similar observations have been made in leishmaniasis.⁵ Such aberrant entry would result in the viable bacilli escaping lysosomal degradation and lying free in the cytoplasm. Job and Verghese⁶ and Shetty, *et al.*⁷ have documented the presence of bacilli lying free in the cytoplasm of Schwann cells from lepromatous patients. Such bacilli, although not significant in terms of numbers, could have far-reaching consequences on the final pathology of the disease.^{8,9} In vacci-

⁵ Chang, K. P. *L. donovani*: promastigote-macrophage surface interaction *in vitro*. *Exp. Parasitol.* **48** (1979) 175-189.

⁶ Job, C. and Verghese, R. Schwann cell changes in lepromatous leprosy. *Indian J. Med. Res.* **63** (1975) 897-901.

⁷ Shetty, V. P., Mehta, L. N., Irani, P. F. and Antia, N. H. Study of the evolution of nerve damage in leprosy: Part I. Lesion of the index branch of the radial cutaneous nerve in leprosy. *Lepr. India* **52** (1980) 5-18.

⁸ Birdi, T. J., Mistry, N. F., Mahadevan, P. R. and Antia, N. H. Alterations in the membrane of macrophages from leprosy patients. *Infect. Immun.* **41** (1983) 121-127.

⁹ Salgame, P., Mahadevan, P. R. and Antia, N. H. Mechanism of immunosuppression in leprosy: presence of suppressor factor(s) from macrophages of lep-

¹ Antia, N. H. and Birdi, T. J. An overview of the leprosy vaccine. *Indian J. Lepr.* **56** (1984) 301-306.

² Mukherjee, R., Mahadevan, P. R. and Antia, N. H. Organized nerve culture I. A technique to study the effect of *M. leprae* infection. *Int. J. Lepr.* **48** (1980) 183-188.

³ Poulter, L. A., Collings, L., Tung, K. and Waters, M. F. R. Parasitism of antigen presenting cells in hyperbacillary leprosy. *Clin. Exp. Immunol.* **55** (1984) 611-617.

⁴ Mistry, N. F., Birdi, T. J. and Antia, N. H. *M. leprae* phagocytosis and its association with membrane changes in macrophages from leprosy patients. *Parasite Immunol.* **8** (1986) 129-138.

nated individuals, the antigen(s) of *M. leprae* entering by this means would be unavailable for degradation and presentation by the antigen-presenting cells (APC). Thus, even the presence of antigen-specific lymphocytes would be ineffectual. The other consequence of this would be that macrophages activated by agents such as interferon (IFN) would not be able to kill these organisms lying free in the cytoplasm.

In the event that the individual is infected by *M. leprae* which are phagocytosed, effective handling of the bacilli is still not guaranteed. The defective regulation of *M. leprae* phagocytosis by macrophages from susceptible individuals would result in excessive phagocytosis.⁴ This could neutralize the bactericidal mechanisms available to the host cell, thus allowing a proportion of the *M. leprae* to remain viable.

Antigen degradation. Let us assume that the vaccinated "susceptible" individual does not encounter these initial problems due to an initial small dose of infection and/or by an initial exposure to nonviable bacilli. The latter possibility is not so remote, since it is believed that the majority of bacilli in a lepromatous patient are dead. The phagocytic APC is then allowed to function normally.

Various workers¹⁰⁻¹² have documented that the outer coat of *M. leprae* is rich in lipids and carbohydrates. The studies of Sriharan, *et al.*¹³ also suggest that serum lipase activity is significantly reduced in lepromatous patients. This is further substantiated by the observations of Sibley, *et al.*¹⁴

romatous patients. *Infect. Immun.* **40** (1983) 1119-1126.

¹⁰ Lederer, E., Adam, A., Ciorbaru, R., Petit, J. and Wietzerbin, J. Cell walls of mycobacteria and related organisms; chemistry and immunostimulant properties. *Mol. Cell. Biochem.* **7** (1975) 87-104.

¹¹ Stewart-Tull, D. E. S. Immunologically important constituents of mycobacteria: adjuvants. In: *The Biology of the Mycobacteria. Volume 2: Immunological and Environmental Aspects*. Ratledge, C. and Stanford, J., eds. London: Academic Press, 1983, pp. 3-84.

¹² Gaylord, H. and Brennan, P. J. Leprosy and the leprosy bacillus; recent developments in characterization of antigens and immunology of the disease. *Ann. Rev. Microbiol.* **41** (1987) 645-675.

¹³ Sriharan, V., Venkatesan, K., Bharadwaj, V. P. and Ramu, G. Serum lipid profile in leprosy. *Lepr. India* **51** (1979) 515-520.

¹⁴ Sibley, D. L., Franzblau, S. G. and Krahenbuhl, J. L. Intracellular fate of *M. leprae* in normal and ac-

that lysosomal contents stained with Thoria Sol[®] were often excluded from contact with the bacteria by the electron transparent zone of *M. leprae*. How then is a cell, low in lipase content, to degrade the outer coat to reach the antigenic components within? This question has been answered partly by observations that antibodies to the delipidified cell wall of *M. leprae* are present in sera of lepromatous patients (Mahadevan, *et al.*, submitted for publication). This could be due to the enhancement of lipase activity by the action of nonspecifically induced activating agents. Alternatively, the individual may have been exposed to already delipidified antigens from individuals with normal lipase activity, i.e., tuberculoid patients. Numerous studies have indicated that tuberculoid patients are also infectious.^{15, 16} Thirdly, the possibility of crossreactivity between *M. leprae* cell-wall proteins and those of environmental mycobacteria also exists.¹⁷

Antigen presentation. Substantial evidence has accumulated to support the "determinant selection hypothesis" of antigen presentation which states that the antigenic determinants are selected by Ia molecules which interact specifically with unique sequences of the antigen and bring about their presentation.¹⁸ In macrophages from susceptible individuals, the Ia molecule may not be able to combine with the relevant antigenic determinant necessary for protection. These individuals would then be unable to benefit from any vaccine regimen and may correspond approximately to the 40% who do not show conversion in the present *in vivo* vaccine trials or *in vitro* in the presence of exogenous activating agents.

tivated mouse macrophages. *Infect. Immun.* **55** (1987) 680-685.

¹⁵ Brown, J. Factors influencing the transmission of leprosy. *Trans. R. Soc. Trop. Med. Hyg.* **53** (1959) 179-189.

¹⁶ Rao, P., Karat, A., Kaliaperumal, V. and Karat, S. Transmission of leprosy within households. *Int. J. Lepr.* **43** (1975) 45-54.

¹⁷ Stanford, J. L., Rook, G., Convit, J., Godal, T., Kronvall, G., Rees, R. J. W. and Walsh, G. P. Preliminary taxonomic studies on the leprosy bacillus. *Br. J. Exp. Pathol.* **56** (1976) 579-585.

¹⁸ Werdelin, O. Determinant protection: a hypothesis for the activity of immune response genes in the processing and presentation of antigens by macrophages. *Scand. J. Immunol.* **24** (1986) 625-636.

Alternatively, the two antigens (native *M. leprae* antigen and the vaccine candidate), to which immune responsiveness is regulated by the same immune response genes, may compete for binding to the Ia molecule and, hence, for presentation by the macrophage. Such competition has been shown to occur for various antigens.¹⁹ The outcome of this competition might determine whether or not the individual's immune system on challenge with *M. leprae* sees the epitope which is crossreactive with the sensitizing antigen. This may provide an explanation for the differing regional response to BCG vaccination. A protocol for testing a potential vaccine must necessarily, by this reasoning, demonstrate that the vaccinating antigen has a higher affinity for the Ia molecule than the native *M. leprae* antigens.

Depending upon the route of infection, *M. leprae* may encounter a variety of accessory cells with recurring capacities for processing and presentation of mycobacterial antigen, such as Langerhans' cells of the skin,³ dendritic cells of the spleen,²⁰ Schwann cells of the peripheral nerve,²¹ and B cells of the lymph node.²² Examples may be cited in the case of splenic cells, where splenectomy in mice resulted in increased resistance to mycobacterial infection due to a reduced generation of T-suppressor cells.²³ Dendritic cells, on the other hand, are better presentors of soluble versus particulate matter and would be ineffectual in the handling of integral *M. leprae*. A faulty combination between "inappropriately" processed antigen and class II molecules in light of recent knowledge may well serve to ini-

tiate the deletion of clones of T cells responsive to *M. leprae*.²⁴

Schwann cells, on the other hand, are the prime hosts for *M. leprae* in an environment which is innately secured against immunological attack. It is probable that fresh infection of *M. leprae* into nerves may escape vaccine-dependent surveillance processes.²⁵ Alternatively, if inflammatory processes are initiated within the nerve, they would probably give rise to nerve damage.

Development and screening of vaccines. The 65 kDa protein is one of the immunodominant antigens of *M. leprae* and is also present in a diverse range of bacteria.²⁶ Priming of an individual by vaccination to *M. leprae* may affect the balance of their responses to pathogenic and nonpathogenic environmental mycobacteria. This may result in a potentially harmful response in the vaccinated individual. Thorns and Morris²⁷ have demonstrated crossreactivity between the antigens of *M. leprae* and host tissue. It is likely, by this line of reasoning, that vaccination procedures carry a risk of inducing some form of autoimmunity. In view of these possibilities, it is imperative that vaccines should initially be tested not only for efficacy, but more importantly, for potentially harmful long-term side effects.

A vaccine would be a welcome additional tool for the control of leprosy even if it were active only in a proportion of the individuals at risk, provided one accepted this limitation and incorporated vaccinations into the overall study of the control program of this disease. With our increasing knowledge of the immune system, we have still failed to employ suitable methods to determine the efficacy of various vaccine preparations for leprosy. We continue to employ skin-

¹⁹ Guillet, J.-G., Lai, M.-Z., Briner, T. J., Smith, J. A. and Geffer, M. L. Interaction of peptide antigens and class-II major histocompatibility complex antigens. *Nature* **324** (1986) 260–262.

²⁰ Pietrangeli, C., Pang, K. C., Skamene, E. and Kongshavn, P. Characteristics of mononuclear phagocytes mediating anti-Listerial resistance in splenectomized mice. *Infect. Immun.* **39** (1983) 742–749.

²¹ Nilsen, R., Mshana, R., Negesse, Y., Menigistu, G. and Kana, B. Immunohistochemical studies of leprosy neuritis. *Lepr. Rev.* **57** Suppl. 2 (1986) 177–187.

²² Janeway, C. A., Ron, J. and Katz, M. The B-cell is the initiating antigen-presenting cell in peripheral lymph nodes. *J. Immunol.* **138** (1987) 1051–1055.

²³ Turcotte, R., Chaput, J. and Lemieux, S. Enhancement of resistance in *M. lepraemurium* infected C3H mice by treatment with sonicated *M. lepraemurium* or splenectomy. *Clin. Exp. Immunol.* **56** (1984) 97–104.

²⁴ Jenkins, M. and Schwartz, R. Antigen presentation by chemically modified splenocytes induces antigen-specific T-cell unresponsiveness *in vitro* and *in vivo*. *J. Exp. Med.* **165** (1987) 302–319.

²⁵ Convit, J., Ananzazu, N., Ulrich, M., Pinardi, M., Reyes, O. and Alvarado, J. Immunotherapy with a mixture of *M. leprae* and BCG in different forms of leprosy and in Mitsuda-negative contacts. *Int. J. Lepr.* **50** (1982) 415–424.

²⁶ Young, D. B., Ivanyi, J., Cox, J. H. and Lamb, J. R. The 65KDa antigen of mycobacteria—a common bacterial protein? *Immunol. Today* **8** (1987) 215–219.

²⁷ Thorns, C. J. and Morris, J. A. Common epitopes between mycobacterial and certain host tissue antigen. *Clin. Exp. Immunol.* **61** (1985) 323–328.

test conversion as the only indicator, arguing that no other method is available despite numerous reports stating that delayed-type hypersensitivity and protection are not synonymous.^{28, 29} If screening is continued using this parameter, the selection bias will be toward antigens generating hypersensitivity and protective antigens may go undetected. Vaccines selected under these conditions may fail to provide any protection or may exacerbate an even stronger hypersensitivity reaction in tuberculoid patients.

We propose, therefore, that basic information should first be obtained on suitable methods to determine protection before large-scale field trials of various vaccines are initiated. These trials require a waiting period of 5–10 years and enormous expenditure. At the end of 10 years, the leprosy scientist may again be faced with a similar situation as that experienced after the BCG trials in Uganda and Burma. All vaccine trials have reported the inability of 40% of lepromatous patients to give a positive lepromin reaction. An important question which has remained unanswered is whether

an individual not responding to a vaccination with one mycobacterial species would respond to another.

Meanwhile, the enthusiasm of the scientist and the associated wide publicity given to the vaccine as the final solution for leprosy should be tempered with caution since, unfortunately, it has tended to divert and dilute the attention from other priority areas such as early detection, drug research, and ensuring regularity of treatment. It should also be remembered that even if a vaccine was available, its delivery, especially if it requires repeated injections, may prove an insurmountable problem with the existing machinery for delivery of health services. This has been well demonstrated in the case of tetanus,³⁰ where an effective vaccine has been available for over half a century yet tetanus continues to be the second largest killer in India today.

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²⁸ Ridley, D. S. The dissociation of hypersensitivity and immunity in the spectrum of leprosy. *Int. J. Lepr.* **50** (1982) 363–364.

²⁹ Lovik, M. and Closs, O. Induction of DTH against ultrasonicated MLM bacilli without simultaneous local reactivity against live bacilli or protective immunity. *Clin. Exp. Immunol.* **53** (1983) 319–327.

³⁰ Dastur, F. D. Response to single dose of tetanus vaccine in subjects with naturally acquired tetanus antitoxin. *Lancet* **2** (1981) 219–221.