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EDITORIALS

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Immunologic Suppression in Leprosy and its Relation to Lepromatous Disease

Research on the suppression of immunity in leprosy has recently led to several interesting developments. Two general areas are involved: (a) attempts to produce an experimental disease that is more extensive and severe than the usual infection with *Mycobacterium leprae* in the foot pads of normal mice, and (b) attempts to explore the immunologic phenomena in lepromatous patients that lead to the absence of lepromin reactivity.

Earlier attempts to enhance the foot pad infections in the mouse[1] were largely unsuccessful. Suramin, a compound that enhances growth of tubercle bacilli in the mouse, had no effect on *M. leprae* infections in the mouse.[1] Cortisone, which also promotes the multiplication of *M. tuberculosis* in the mouse, had only a limited effect on the growth of *M. leprae*; it prevented some of the loss of bacterial viability that normally occurs in the plateau phase, but it did not increase the numbers of bacteria significantly.[2] Some other procedures that are capable of producing more pronounced immunologic deficit do not allow the mice to survive long enough for decisive experiments with *M. leprae*. Thus the radiomimetic drug cyclophosphamide (cytoxan), when used in doses needed to enhance other mycobacterial infections, killed the mice in about one month.[3] Also thymectomy of new-born mice usually causes "runting" and death in two to three months. The thymus is necessary for the normal development of the lymphoid tissues in neonatal mice.

In adult mice, thymectomy by itself does not have such a profound effect, but if the

[1]REES, R. J. W. Limited multiplication of acid-fast bacilli in the foot-pads of mice inoculated with *Mycobacterium leprae*. British J. Exper. Path. **45** (1964) 207-218.

[2]SHEPARD, C. C. and MCRAE, D. H. *Mycobacterium leprae* in mice: Minimal infectious dose, relationship between staining quality and infectivity, and effect of cortisone. J. Bact. **89** (1965) 365-372.

[3]SHEPARD, C. C. and REDUS, M. A. Effect of immunosuppressive drugs on infection in mice by *M. marinum* (*balnei*), *M. tuberculosis* and *M. leprae*. Internat. J. Leprosy **35** (1967) 348-354.

thymectomy is followed by heavy x-irradiation lymphoid tissues do not regenerate and a profound immunologic depression develops.[4] In this procedure transfusion of syngeneic bone marrow cells is given to protect the mice against the otherwise fatal effects of irradiation on the myeloid system; lymphocytic elements are present in the bone marrow transfusion, but they are apparently unable to develop properly in the absence of a thymus. Some of these thymectomized-irradiated mice die in the first few weeks, but the survivors live for approximately normal life spans. The immunologic depression is manifested by poor antibody response to many antigens and markedly delayed rejection of skin grafts from foreign lines of mice.

Thymectomized-irradiated mice have now been employed to excellent advantage by Rees [5, 6] to study foot pad infections with *M. leprae*, and by Rees and coworkers[7] to study infections by other routes as well. Their findings after foot pad inoculations have been confirmed.[8, 9] In thymectomized-irradiated mice inoculated in the foot pad, bacillary multiplication occurs at first at approximately the same rate as that in normal mice. However, at the time when the logarithmic phase of growth is terminated, at about 1×10^6 bacilli per foot pad in normal mice, bacillary multiplication continues in thymectomized-irradiated mice. Although multiplication gradually slows, the population eventually reaches a level of 10^7 to 10^8 bacilli, or about 10 to 100 times that in normal mice. Furthermore, solidly staining, presumably viable, bacilli continue to be present. Late in the course of these infections bacillary invasion of nerves becomes frequent, and there is an interesting spread to the other peripheral sites, that is, uninoculated feet, ears, and nose, and invasion of lymph nodes and bone marrow as well.

The immunologic story seems to be that the normal mouse reacts to the presence of *M. leprae* in the foot pad when it reaches a level of about 1×10^6 bacilli, and most of the bacilli are then killed. Presumably the reaction is mediated by the infiltrate of lymphocytes and macrophages that appears at about this time. (Mice that have been well vaccinated with BCG are able to react to a lower number of *M. leprae* and thereby impose a lower plateau.[10]) Thymectomized-irradiated mice respond only imperfectly at this time, merely slowing the multiplication rather than stopping it. The continued multiplication of bacteria leads to invasion of nerves, and eventually enough bacteria enter the local lymphatic and vascular pathways to seed the other peripheral sites effectively.

When thymectomized-irradiated mice are inoculated intravenously with large numbers of *M. leprae*, the infection generalizes much more quickly.[7] Approximately 10^{10} *M. leprae* can be harvested from one mouse 19 months after infection, about 95 per cent of the yield coming from ears, nose, and foot pads. Significantly, the histopathologic findings in these intravenously inoculated mice resemble very much those seen in human lepromatous disease.[2] In the heavily infected foot pads foam (Virchow) cells are frequent, and bacilli are often found in perineurial and Schwann cells of nerves. The involvement of skin may be comparable in all respects to that in human disease, and there may even be marked epidermal flattening and a "clear zone" where bacilli are

[4] Miller, J. F. A. P., Doak, S. M. A. and Cross, A. M. Role of the thymus in recovery of the immune mechanism in the irradiated adult mouse. Proc. Soc. Exper. Biol. & Med. **112** (1963) 785-792.

[5] Rees, R. J. W. Recent bacteriologic, immunologic and pathologic studies on experimental human leprosy in the mouse foot pad. Internat. J. Leprosy **33** (1965) 646-655.

[6] Rees, R. J. W. Enhanced susceptibility of thymectomized and irradiated mice to infection with *Mycobacterium leprae*. Nature (London) **211** (1966) 657-658.

[7] Rees, R. J. W., Waters, M. F. R., Weddell, A. G. M. and Palmer, E. Experimental lepromatous leprosy. Nature (London) **215** (1967) 599-602.

[8] Gaugas, J. M. Effect of X-irradiation and thymectomy on the development of *Mycobacterium leprae* infection in mice. British J. Exper. Path. **48** (1967) 417-422.

[9] Shepard, C. C. and Congdon, C. C. Increased growth of *Mycobacterium leprae* in thymectomized-irradiated mice after foot pad inoculation. *To be published.*

[10] Shepard, C. C. Vaccination against human leprosy bacillus infections of mice: Protection by BCG given during the incubation period. J. Immunol. **96** (1966) 279-283.

very few. Micro-colonies of bacilli in the voluntary muscle fibers are a prominent feature. The muco-perichondrium of the nose may be heavily infected and bacilli shed into the mucus.

The other area of research has involved tests of the lepromatous patient's immunologic capacity. As is well known, lepromatous patients fail to react to lepromin, but they react normally to suspensions of many other mycobacteria, including tubercle bacilli (reviewed in [11]). However, carefully controlled studies indicate that lepromatous patients have decreased sensitivity to tuberculin.[12] The tuberculin reaction is, of course, the classic example of delayed-type hypersensitivity, and two studies have now been made of the lepromatous patient's ability to develop delayed-type sensitivity to chemicals. Normal persons are not sensitive to these substances, but they may be sensitized easily by exposure through the skin. It was found that lepromatous patients were markedly deficient in their ability to develop sensitivity to picric acid[13] and dinitrochlorobenzene.[14] Apparently the situation is similar to that seen in other diseases with immunologic deficits; the ability to develop a new sensitivity is definitely impaired, even though the ability to manifest a preexisting sensitivity may persist.

Lymphocyte transformation in cultures *in vitro* from peripheral blood is a closely related phenomenon. When antigens to which the patients are sensitive (e.g., tuberculin) are added to cultures of white cells, a limited percentage of the small lymphocytes transform into large blast-like cells that are capable of mitosis. Another class of substances, such as phytohemagglutinin (PHA) and streptolysin O (SLO), causes a large fraction of the cells of normal persons to transform in a similar process. It has now been reported that most patients with lepromatous leprosy have markedly impaired lymphocyte transformation to PHA[15] and to SLO.[16] Defective lymphocyte transformation has previously been observed in chronic lymphatic leukemia, Hodgkin's disease, primary acquired agammaglobulinemia, and in ataxia telangectasia (see [15] and [17] for references). These conditions are also marked by other immunologic deficiences, including impaired ability to develop delayed sensitivity. More recently depressed lymphocyte transformation to PHA has been observed in infants with congenital rubella.[17] At first sight congenital rubella appears to be a more obvious parallel to lepromatous leprosy, because it also is an infectious disease characterized by some of the features of immunologic tolerance. However, it is now suggested that the action of rubella virus is a direct one, since it can cause impaired lymphocyte transformation when added directly to cultures of normal cells.[18] Lymphocyte transformation in leprosy continues under investigation and a better notion of the mechanism involved should be gained soon, since the procedure is relatively simple and rapid.

Clarification of these immunologic phenomena is obviously important for the understanding of lepromatous disease. The very long persistence of leprosy bacilli after they have been killed by effective drugs seems to be a related phenomenon. Ridley suggests that in treated lepromatous patients those with borderline features rid

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[11] SHEPARD, C. C. and GUINTO, R. S. Immunological identification of foot-pad isolates as *Mycobacterium leprae* by lepromin reactivity in leprosy patients. J. Exper. Med. **118** (1963) 195-204.

[12] GUINTO, R. S. and MABALAY, M. C. A note on the tuberculin reaction in leprosy. Internat. J. Leprosy **30** (1962) 278-283.

[13] BULLOCK, W. E. Depression of the delayed-type allergic response by leprosy. Clin. Res. **14** (1966) 337 (only).

[14] WALDORF, D. S., SHEAGREN, J. N., TRAUTMAN, J. R. and BLOCK, J. B. Impaired delayed hypersensitivity in patients with lepromatous leprosy. Lancet **2** (1966) 773-775.

[15] DIERKS, R. E. and SHEPARD, C. C. Effect of phytohemagglutinin and various mycobacterial antigens on lymphocyte cultures from leprosy patients. Proc. Soc. Exper. Biol. & Med. *In press.*

[16] SHEAGREN, J. N., BLOCK, J. B., TRAUTMAN, J. R. and WOLFF, S. M. Immunologic reactivity in leprosy. Clin. Res. **15** (1967) 300.

[17] OLSON, G. B., SOUTH, M. A. and GOOD, R. A. Phytohaemagglutinin unresponsiveness of lymphocytes from babies with congenital rubella. Nature (London) **214** (1967) 695-696.

[18] MONTGOMERY, J. R., SOUTH, M. A., RAWLS, W. E., MELNICK, J. L., OLSON, G. B., DENT, P. B. and GOOD, R. A. Viral inhibition of lymphocyte response to phytohemagglutinin. Science **157** (1967) 1068-1070.

themselves of bacilli more rapidly than do those with pure lepromatous disease.[19] Perhaps unrelated is the well-known observation that erythema nodosum leprosum is much more frequent in patients with pure lepromatous disease.

These two areas of immunologic research in leprosy[20] are, of course, related. The mice have been shown to develop lepromatous-type infections when they are treated by a procedure (thymectomy and irradiation) that produces a profound and long lasting immunologic depression, and the immunologic depression in lepromatous patients has been more clearly delineated by well-known immunologic procedures.

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[19] RIDLEY, D. S. A logarithmic index of bacilli in biopsies. 2. Evaluation. Internat. J. Leprosy **35** (1967) 187-193.

[20] The two areas were reviewed and discussed in Silver Spring, Maryland on 19 October 1967, in a Workshop on Immunology of Leprosy sponsored by the U. S. Leprosy Panel of the U. S.-Japan Cooperative Medical Science Program. Copies of the abstracts and bibliographies from the Workshop are available from the Office of International Research, National Institutes of Health, Bethesda, Maryland, U.S.A. 20014.