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EDITORIALS

Editorial opinions expressed are those of the writers.

The Relation Between Allergy and Immunity in Leprosy*

Leprosy is a chronic infectious disease of man caused by the intracellular *Mycobacterium leprae* and affects chiefly the cooler parts of the body, especially the peripheral nerves, skin, upper respiratory tract, anterior part of the eyes, and the testes.

Human leprosy has been known for many centuries and although its causative organism, first described by Hansen in 1874,[1] was one of the first to be identified as an infectious agent, it remains a significant medical and social problem in many developing countries. It is the only bacterial disease included among the six selected for the United Nations Development Programme/World Bank/World Health Organization's Special Programme for Research and Training in Tropical Diseases, and it alone has neither intermediate host nor significant animal reservoir or insect vector.

The earliest records which give accurate descriptions of the disease come from India, and may have been written as early as 600 B.C. The earliest absolute evidence of leprosy is seen in a Coptic mummy buried in the 5th century A.D., and it seems likely that the disease spread into Europe in the Middle Ages reaching epidemic proportions in the 12th century and then slowly declining.

Today, leprosy is most common in the tropics and the subtropics and probably affects in excess of 12 million people throughout the world.[2] This figure may be lower than the actual incidence because people may not come forward to be identified, and there is no simple clinical test to determine whether a person has been infected or not. People are also reluctant to report leprosy due to the stigma the disease still carries and the fear of social ostracism. It has been said that "leprosy is a disease which affects the body of the patient and the mind of the public."[3] Leprosy has also been known to

* This review was written by Michael Maier, D.Phil., while a first-year clinical student at the Charing Cross and Westminster Medical School, University of London. It was awarded first prize in the annual competition set up by the British Leprosy Relief Association (LEPRA) for essays on various aspects of leprosy. We take pleasure in publishing this review. Dr. Maier's present address is 197A Fulham Palace Road, London W6 8QX, England.

[1] Hansen, G. A. Spedalskhedens arsager. Norsk Mag. Laegevidensk **4** (1874) 76–79. Reprinted in part, in English translation, as Causes of leprosy. Int. J. Lepr. **23** (1955) 307–309.

[2] World Health Organization Wkly. Epidem. Rep. **3** (1979) 17–19.

[3] Antia N. H. The people we fail to reach. Lep. Rev. **48** (1977) 155–157.

occur in more temperate climates, is an endemic disease in the islands of the Mediterranean Sea and the countries of southern and eastern Europe and, until the early part of this century, flourished in Norway. In western Europe and in Britain, the disease is now confined to immigrants and to persons who have lived abroad.

Leprosy and tuberculosis

M. leprae shares many similarities with *M. tuberculosis,* the causative infectious agent of tuberculosis. The genus *Mycobacterium* contains a large number of species which are widely distributed in man's environment. The different members of the genus share in common many antigens, although in different combinations, and because of this there exists a complicated pattern of interaction between concomitant infections with different mycobacteria. The importance of such interactions in the epidemiologies of tuberculosis and leprosy is illustrated by the effect on infection with BCG (bacille Calmette-Guérin) vaccine. At least eight randomized controlled trials have been carried out to measure the efficacy of BCG against tuberculosis,[4] and four such trials have investigated the effect of BCG against leprosy.[4] Both sets of trials are similar in showing a wide range of protective efficacies against the two diseases, although clear trends in some trials appear distinctly absent from others, and no one has yet provided a convincing explanation for all the data now at hand. Nevertheless the hypothesis that tuberculosis provides cross-immunity to leprosy has often been entertained and was used some 40 years ago by Chaussinand[5] to suggest that the disappearance of leprosy from Europe in recent centuries was attributable to an increased prevalence of *M. tuberculosis* infections providing immunity against infections with *M. leprae.*[5] It should be noted that research lends support to the theory of cross-immunity between tuberculosis and leprosy, because BCG vaccination of mice inhibits subsequent multiplication of *M. leprae* in the mouse foot pad test.[6]

A less-rigorous series of experiments was conducted by Hansen in the years following his discovery of the leprosy bacillus. He made repeated and unsuccessful attempts to infect himself with material obtained from his patients' nodules. Hansen also persuaded his father-in-law, Dr. Danielssen, to be inoculated with leprosy bacilli, but the result was equally negative. This failure was perhaps attributable to an immunity to tuberculosis, for a few years previously, Hansen's wife had died of pulmonary tuberculosis and yet he remained healthy, and his father-in-law had had an attack of pulmonary tuberculosis at the age of 17.

M. leprae and *M. tuberculosis* are both obligate intracellular parasites, and cause slow diseases with long incubation periods, relatively chronic clinical courses, and spread slowly through communities. The incubation periods of both diseases may be as short as a few months, but are generally measured in years. Research on tuberculosis has always been more advanced than that on leprosy, chiefly because it has been impossible to grow *M. leprae in vitro* and because of the lack of an animal model in leprosy research. The earliest successful studies on the transmission of *M. leprae* to experimental animals were in mice, when in 1960 Shepard[7] produced a limited proliferation of the organism in their foot pads. Rees and his colleagues[8] were able to overcome the resistance in mice by injecting *M. leprae* into the foot pads and ears of mice which had been thymectomized, irradiated, and transfused with syngeneic bone marrow. Unfortunately, this animal model is far from satisfactory because the natural susceptibility and resistance of the mouse is hindered. Therefore, many workers have looked at the infection with *M. lepraemurium* that develops in unmodified mice or

[4] Fine, P. Leprosy and tuberculosis—an epidemiological comparison. Tubercle **65** (1984) 137–153.

[5] Chaussinand, R. Tuberculose et lèpre; maladies antagoniques. Eviction de la lepre par la tuberculose. Int. J. Lepr. **16** (1948) 431–438.

[6] Rees, R. J. W. Recent bacteriologic, immunologic and pathologic studies on experimental human leprosy in the mouse foot pad. Int. J. Lepr. **33** (1965) 646–657.

[7] Shepard, C. C. The experimental disease that follows the injection of human leprosy bacilli into foot pads of mice. J. Exp. Med. **112** (1960) 445–454.

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

rats.[9, 10] *M. tuberculosis,* on the other hand, infects humans, other primates, and mammalian species in close contact with humans, especially domestic dogs and cats. It is also possible to grow the organism *in vitro* with relative ease.

The situation in leprosy research changed in 1971 when Kirchheimer and Storrs found that the nine-banded armadillo, whose relatively low body temperature of 32–35°C is ideally suited for infection with *M. leprae,*[11] produces a widespread disseminated disease with enormous numbers of bacilli in the lesions. Indeed, a disease closely resembling human leprosy has recently been identified in wild armadillos killed in Louisiana, U.S.A., giving rise to the suspicion that leprosy is a disease of animals other than just man.[12] This animal has provided, for the first time, a good animal model suitable for studying the disease and producing sufficiently large amounts of *M. leprae* antigen for basic research: lepromatous armadillo tissue may contain 1000 times more organisms than the equivalent human tissue. This development led in 1975 to the establishment of the Immunology of Leprosy (IMMLEP) Scientific Working Group acting as a model for the WHO special program for tropical diseases. Two primates from Africa used in laboratories in the United States, a chimpanzee and a mangabey monkey, were found to have leprosy, possibly from exposure to untreated leprosy patients before being sold to dealers.[13, 14] In 1978 Waters, *et al.* reported the successful infection of a primate, the white-handed gibbon, with *M. leprae.*[15] In spite of these reports, there is insufficient information on leprosy in wild primates to consider them as a reservoir for leprosy at this time.

The host response to both tuberculosis and leprosy depends on a cell-mediated pathway, yet the pathogenesis of the two diseases is believed to be dramatically different. The answer to the difference in pathologies of these two diseases lies in the complexity of cell-mediated immunity (CMI) and in the regulatory mechanisms which control it. Many excellent reviews and monographs exist on this topic.[16–20]

The leprosy spectrum

Leprosy is a polymorphic disease, manifesting itself through a wide clinical spectrum of severity according to the immune status of the host. When leprosy bacilli gain access to the host's tissues, either through the skin or via the respiratory tract, they may be quickly destroyed by the protective phagocytes of the host. If the bacilli are not vanquished in this way, the defense mechanisms of the host create a reaction which at first is called "indeterminate" because the lesion is too immature to be classified. This lesion can either persist for months or years, or go on to complete healing, or develop into one of the overt forms of clinical leprosy.

At one pole, tuberculoid leprosy, patients develop high levels of CMI which results in

[9] Alexander, J. and Curtis, J. Development of delayed hypersensitivity responses in *M. lepraemurium* infections in resistant and susceptible strains of mice. Immunology **36** (1978) 563–567.

[10] Lefford, M. J., Patel, P. J., Poulter, L. N. and Mackaness, G. B. Induction of cell-mediated immunity to *M. lepraemurium* in susceptible mice. Infect. Immun. **18** (1977) 654–659.

[11] Kirchheimer, W. F., Storrs, E. E. and Binford, C. H. Attempts to establish the armadillo (*Dasypus novemcinctus* Linn.) as a model for the study of leprosy. II. Histopathologic and bacteriologic post-mortem findings in lepramatoid leprosy in the armadillo. Int. J. Lepr. **40** (1972) 229–242.

[12] Marchiondo, A. A., Smith, J. H. and File, S. K. Naturally occurring leprosy-like disease of wild armadillo. Ultrastructure of lepromatous lesions. J. Reticuloendothel. Soc. **27** (1980) 311–325.

[13] Leninger, J. R., Donham, K. J. and Meyers, W. M. Leprosy in a chimpanzee: postmortem lesions. Int. J. Lepr. **48** (1980) 414–421.

[14] Meyers, W. M., Walsh, G. P., Brown, H. L., Fukunishi, Y., Binford, C. H., Gerone, P. J. and Wolf, R. H. Naturally acquired leprosy in a mangabey monkey (*Cercocebus* sp.) Int. J. Lepr. **48** (1980) 495–498.

[15] Waters, M. F., Bakri, I. B., Isa, H. J., Rees, R. J. and McDougall, A. C. Experimental lepromatous leprosy in the white-handed gibbon (*Hylobatus lar*): successful inoculation with leprosy bacilli of human origin. Br. J. Exp. Pathol. **59** (1978) 551–557.

[16] Stanford, J. L. Skin testing with mycobacterial reagents in leprosy. Tubercle **65** (1984) 63–74 and references therein.

[17] Sansonetti, P. and Lagrange, P. H. The immunology of leprosy: speculations on the leprosy spectrum. Rev. Infect. Dis. **3** (1981) 422–469.

[18] Turk, J. L. and Bryceson, A. D. M. Immunological phenomena in leprosy and related diseases. Adv. Immunol. **3** (1971) 209–266.

[19] Jopling, W. H. *Handbook of Leprosy.* 2nd ed. London: Heinemann Medical Books, 1978.

[20] Rook, G. A. W. The immunology of leprosy. Tubercle **64** (1983) 297–312.

the killing and clearing of bacilli in the tissues and the localized signs are restricted to skin and nerves. At the lepromatous pole, patients exhibit a selective immunological unresponsiveness to antigens of *M. leprae,* leading to widespread lesions of skin, peripheral nerves, upper respiratory tract, eyes, testes, and the reticuloendothelial system. The lepromatous pole of leprosy is characterized by a massive bacillary infiltration of the tissues and continuous bacteremia, but tissue destruction is minimal and appears late.

Ridley and Jopling[21] described the clinical and histopathological spectrum of leprosy according to five categories. The polar forms, tuberculoid (TT) and lepromatous (LL), are relatively stable; whereas the borderline forms are unstable and, without treatment, tend to deteriorate toward the more stable polar forms. The borderline forms are borderline tuberculoid (BT), borderline (BB), and borderline lepromatous (BL), going from the tuberculoid to the lepromatous pole of the spectrum. Sometimes a further category is included, subpolar lepromatous (LLs), and placed between the borderline lepromatous and the polar lepromatous. Borderline leprosy is an intermediate state, unstable and temporary, between the lepromatous and tuberculoid forms of the disease. Without treatment the disease progresses, sometimes suddenly, toward the lepromatous form; this progression is known as the downgrading reaction. With treatment the disease regresses, sometimes suddenly, toward the tuberculoid form; this is known as the reversal reaction. The reversal reaction affects those subjects with subpolar and borderline lepromatous and borderline leprosy typically during the first year of treatment marking a sudden change in their immune status and a rapid increase in CMI to *M. leprae.*

Tuberculoid leprosy can be self-healing, and typically has low levels of antibodies and good CMI. In contrast to this, the disseminated lepromatous form of the disease is associated with high levels of relevant and irrelevant antibodies and poor cellular immune function.[18, 22] It has therefore been suggested that cell-mediated immune responses are linked to protective immunity in leprosy, and that the quality of this CMI determines the position the host finds on the disease spectrum.

The spectrum model provides an important conceptual framework in the study of leprosy and may be summarized as follows: a) Clinical manifestations of established leprosy form a spectrum ranging from the highly focal tuberculoid leprosy to the globally diffuse lepromatous leprosy. b) The type of leprosy depends on and is a faithful reflection of the degree of specific CMI the patient has against *M. leprae,* tuberculoid and lepromatous types being states of high and absent specific CMI, respectively. c) These two types form two poles of the spectrum of the degree of immunity against *M. leprae.* In between the poles is a continuum of intermediate states of "borderline" leprosies in which the CMI may be at a relatively high (borderline tuberculoid, BT), low (borderline lepromatous, BL), or intermediate (mid-borderline, BB) level. d) Polar leprosies are stable forms; the nonpolar borderline types are unstable forms.

In place of this line model for the spectrum of leprosy, Srinivasan[23] has proposed a model which is a considerable conceptual improvement. It uses Catastrophe Theory,[24] a branch of topology in mathematics which provides descriptions of how continuous causes can produce sudden or discontinuous changes. The model uses the number of living and the number of dead bacilli as the control factors, and is able to describe the discontinuous behavior of leprosy as demonstrated by skip sequence changes that some patients go through. It can show that, for certain values of control factors, the disease may manifest in one of two forms of borderline leprosy, and how lesions very similar to start with can progress to quite different states. This model is also able to

[21] Ridley, D. S. and Jopling, W. H. Classification of leprosy according to immunity: a five-group system. **34** (1966) 255–273.

[22] Nath, I. Immunology of human leprosy—current status. Lepr. Rev. June (Spec. No.) (1983) 31S–45S.

[23] Srinivasan, H. Models for leprosy. An appraisal of graphic representations of the "spectrum" concept as models and a suggestion for a catastrophe theory model for leprosy. Int. J. Lepr. **52** (1984) 402–413.

[24] Thom, R. *Structural Stability and Morphogenesis; an Outline of a General Theory of Models.* Menlo Park, California: Benjamin, Inc., 1975.

locate the normal (nonleprous) state as well as the early (indeterminate) type, something not possible with the line model.

Allergy

The allergy seen in response to *M. tuberculosis* and *M. leprae,* known to be T-cell mediated, is named delayed-type hypersensitivity (DTH) because of its relatively slow onset of action. It is not transferable from one animal to another by serum but can be transferred by T lymphocytes, and is believed to be associated with T-cell-mediated immunity but does not necessarily run parallel to it. The similarities and the differences between DTH and CMI have been discussed by Youmans[25] and by Salvin and Neta,[26] and it seems that the development of DTH is not essential to acquired cellular resistance. This may not be as contradictory as it appears, because the specific antigens that participate in recognition in the DTH reaction and in the induction of the acquired cellular resistance may not necessarily be the same. Similarly, attempts have been made to correlate DTH and resistance to infection with two *in vitro* tests, the leukocyte migration inhibition test (LMIT) and the lymphocyte transformation test (LTT).[27] These tests measure the T-cell response to foreign antigen, both in terms of ability to respond and the extent of the response.

Four types of DTH reaction are recognized and, of these, the first three (the Jones-Mote reaction, contact hypersensitivity, and tuberculin-type hypersensitivity) all occur within 72 hours of antigen challenge. By contrast, the fourth type, granulomatous reaction, develops over a period of weeks. Because all four may overlap to some extent, or occur sequentially following a single antigenic challenge, many of the hypersensitivity reactions seen in practice do not correspond to one category alone.

The first type of reaction to appear is the Jones-Mote, which is maximal at 24 hours. Contact and tuberculin hypersensitivities both peak at 48–72 hours after antigen challenge, and these may be followed, at least 14 days later, by an even more delayed response of granuloma formation characterized histologically by the aggregation and proliferation of macrophages. These four different types of hypersensitivity were originally distinguished in cutaneous lesions by measuring the thickening which occurred in the skin at the site of antigen injection.

Two phases can be identified in delayed hypersensitivity. The first is a phase of sensitization of T lymphocytes to a given antigen injected into the cutaneous tissue and migration to the T-cell dependent paracortical zones of draining lymph nodes. A population of T lymphocytes specifically sensitized with antigen is thereby created, and certain cells among them, called memory cells, can circulate for several years. The second phase is a recognition phase that takes place during the re-exposure to antigen. In this phase, blast transformation of sensitized T lymphocytes is associated with the release of mediators, the lymphokines, that are classified according to their biological activity.

Jones-Mote hypersensitivity. Jones-Mote hypersensitivity is characterized by infiltration of the area immediately under the dermis by basophils, and is therefore frequently called cutaneous basophil hypersensitivity. It is induced by soluble antigen, shows a transient sensitivity lasting 2–4 weeks, reaches a maximum 7–10 days after induction, and tends to disappear when antibody to the antigen appears. The skin swelling is maximal 24 hours after antigen challenge.

Lenzini, *et al.*[27] describe this reaction as one of the three types of skin-test reactions to tuberculin, the other two being the Mantoux reaction and a mixed reaction. The significance and relationship of the Jones-Mote reaction to the classic tuberculin-type delayed hypersensitivity remains to be clarified. However, it has been demonstrated recently that in mice these two states of hypersensitivity are mediated by different subpopulations of thymus-derived lympho-

[25] Youmans, G. P. Relation between delayed hypersensitivity and immunity in tuberculosis. Am. Rev. Respir. Dis. **111** (1975) 109–118.

[26] Salvin, S. B. and Neta, R. A possible relationship between delayed hypersensitivity and cell-mediated immunity. Am. Rev. Respir. Dis. **111** (1975) 373–377.

[27] Lenzini, L., Rotolli, P. and Rotolli, L. The spectrum of human tuberculosis. Clin. Exp. Immunol. **27** (1977) 230–237.

cytes,[28, 29] and it is suspected that the Jones-Mote reaction is strongly regulated by suppressor lymphocytes.

Contact hypersensitivity. The lesion of the contact hypersensitivity reaction shows a mononuclear cell infiltrate first appearing at 6–8 hours and peaking at 12–15 hours. It is characterized clinically in humans by eczema, and is usually maximal 48 hours after the first exposure to antigen.

The most common antigens in this reaction are haptens, such as nickel, acrylates, poison ivy or other chemicals. The low molecular weight haptens, which would not normally be antigenic, traverse the skin and bind to normal body proteins forming a conjugate which is then recognized by T cells. This form of hypersensitivity is not thought to be of importance in reactions to mycobacterial antigens.

Tuberculin-type hypersensitivity. In 1891, Koch described tuberculin-type delayed hypersensitivity in the guinea pig,[30] the reaction having the following characteristics: a) a delay in appearance of 24–48 hours after the injection of the test antigen; b) an indurated erythematous papule, which may be necrotic and vesicular in the case of a violent reaction; c) and initial perivascular dermal infiltrate of neutrophils, basophils, and mononucleated cells, followed by a progressive appearance of lymphocytes and monocytes beginning at 5–6 hours and reaching a maximum at 18–24 hours. As the lesion develops, depending on the persistence of the antigen in the tissues, there may be a progression from tuberculin-like to granulomatous reaction.

In an attempt to study the relationship between CMI and the tuberculin-type delayed hypersensitivity, Lenzini found that patients with localized lesions and a prompt response to chemotherapy showed typical Mantoux reactions (tuberculin-type hypersensitivity reaction) and consistently positive results in the LMIT. Patients with chronic disease with surrounding fibrosis showed Jones-Mote or mixed reactions and negative LMIT. Patients with rapidly disseminating lesions showed absent skin reactions and absent reaction in the LMIT. This shows that tuberculin skin tests are consistent when tested on patients who already show signs of the disease in one form or another. On the other hand, the empirical evidence of tuberculin test studies concludes, rather unhelpfully, that a high degree of hypersensitivity may be associated either with rapidly progressive tuberculosis or with lesions that are being successfully resisted, and a low degree of hypersensitivity is compatible either with lesions that are being well resisted or with devastating ones.[31] Furthermore, a 1967 Medical Research Council trial of tuberculosis vaccine has highlighted the dissociation between allergy and immunity in the case of tuberculosis in man.[32] The study showed no correlation between tuberculin sensitivity and protection from tuberculosis in subjects immunized with BCG vaccine.

Experiments on guinea pigs have shown that the tuberculin-type delayed-hypersensitivity reaction can be changed without changing the actual immunity of the animal against the bacilli.[33] In one experiment, immunized hypersensitive guinea pigs were desensitized with tuberculin so that they no longer reacted hypersensitively to the local injection of large amounts of virulent tubercle bacilli or tuberculin. These same guinea pigs remained highly resistant to the proliferation and the invasion of the bacilli. The converse was also shown, that by injecting guinea pigs with tuberculoprotein and the glycolipid Wax D, the animals were made highly tuberculin sensitive but showed no increased resistance to infection. Wax D is a constituent of the mycobacterial cell

[28] Hahn, H., Kaufman, S. H. E., Miller, T. E. and Mackaness, G. B. Peritoneal exudate T-lymphocytes with specificity to sheep red blood cells. I. Production and characterization as to function and phenotype. Immunology **36** (1979) 691–698.

[29] Lagrange, P. H. and Thickstun, P. M. *In vivo* antitumor activity of various forms of delayed-type hypersensitivity. J. Nat. Cancer Inst. **62** (1979) 429–436.

[30] Koch, R. Fortsetzung der Mittheilung uber ein Heilmittel gegen Tuberculose. Dtsch. Med. Wochensch. **17** (1891) 101–102.

[31] Rich, A. R. *The Pathogenesis of Tuberculosis.* 2nd ed. Oxford: Blackwell Scientific Publications, 1951.

[32] Hart, P. D., Sutherland, I. and Thomas, I. The immunity conferred by effective BCG and vole bacillus vaccines in relation to individual variations in induced tuberculin sensitivity and to technical variations in the vaccines. Tubercle **48** (1967) 201–210.

[33] Turk, J. L. Dissociation between allergy and immunity in mycobacterial infections. Lepr. Rev. **54:** (1983) 1–8.

wall that is thought to act as an adjuvant. It was also demonstrated that guinea pigs could be made tuberculin sensitive with an extract of BCG but this was not accomplished by any increase in resistance when the animals were challenged with virulent tubercle bacilli.[33]

The confusion that arises from the fact that guinea pigs infected with *M. tuberculosis* develop acquired resistance and tuberculin sensitivity in parallel and that in man the allergic reaction may not result in immunity has been highlighted by Youmans and Youmans.[34] In these experiments a highly immunogenic fraction was prepared from attenuated *M. tuberculosis* H37Ra by carefully disrupting the cells in the cold. Upon fractionation, it was found that the antigen that would confer protection to mice was found in a preparation that contained RNA precipitated by ethanol from a ribosomal fraction. No DNA or polysaccharide could be detected, but there were significant amounts of protein. Despite producing immunity, neither the bacterial ribosomal fraction nor the RNA produced tuberculin hypersensitivity. Thus, one can conclude that immunity to tuberculous infection and tuberculin hypersensitivity are separate responses of the host to different components of the bacterial cell. Both are mediated by a specific T-lymphocyte response but probably by different subpopulations, and both involve macrophage activation. Because different antigens may be responsible for the two reactions, the responses can be dissociated although they may run in parallel. It was therefore concluded that the granulomatous response is a response due to the antigen which produces the allergic reaction (DTH) rather than being associated with the basic mechanism involved in host resistance to infection.

Myrvang, *et al.* have found that circulating lymphocytes from patients with lepromatous leprosy were not transformed by *M. leprae in vitro* in the LTT and the LMIT.[35] This defect decreased across the leprosy spectrum and, at the tuberculoid pole, strong reactivity in both these tests was seen. Even though lymphocytes taken from lepromatous patients could not be transformed by *M. leprae,* it was found that they were transformed by BCG, implying a correlation of the LTT with a specific antigen and host resistance. It has been argued, however, that this does not necessarily imply a correlation with the host's ability to eliminate the infecting organism, but perhaps with the strength of the hypersensitivity reaction shown by the subject.[36] It was observed that in patients with BT and silent skin lesions there was a fairly low LTT whereas the LTTs were stronger in actively inflamed BT, and these were frequently stronger than LTTs found in TT. Similarly, BL patients with inflamed skin lesions reacted strongly in the LTT, and the response could be stronger than in BT patients in whom there was no evidence of inflammation. The conclusion was that the LTT response was related to the state of hypersensitivity of the patient rather than to his resistance to infection.

Granulomatous hypersensitivity. Because granulomatous hypersensitivity is the cause of most pathological effects in diseases which involve T-cell-mediated immunity, it is clinically the most important of the four types of hypersensitivity described. It results from the presence of a persistent agent within macrophages, usually microorganisms, which the cell is unable to destroy. In this way, it is quite different from the tuberculin-type reaction which is a self-limiting response to antigen, even though they both depend on DTH cells which have been sensitized to a particular antigen.

Granuloma formation appears as one of the basic characteristics of the host response to infection[37] and is found in many diseases, including tuberculosis and nonlepromatous leprosy. Its formation is initiated by a variety of infectious and noninfectious agents, and appears to need the presence of poorly digestible irritants or CMI to the irritant, or

[34] Youmans, G. P. and Youmans, A. S. Recent studies in acquired immunity and tuberculosis. Curr. Top. Microbiol. Immunol. **48** (1969) 129–178.

[35] Myrvang, B., Godal, T., Ridley, D. S., Froland, S. S. and Song, Y. K. Immune responsiveness to *M. leprae* and other mycobacterial antigens through the clinical and histological spectrum of leprosy. Clin. Exp. Immunol. **14** (1973) 541–553.

[36] Bjune, G., Barneston, R. St., Ridley, D. S. and Kronvall, G. Lymphocyte transformation test in leprosy: correlation of the response with inflammation of lesions. Clin. Exp. Immunol. **25** (1976) 85–94.

[37] Unanue, E. R. The immune granulomas. In: *Immunological Diseases.* 3rd ed. Samter, M., ed. Boston: Little Brown, 1978, pp. 297–306.

both. It is characterized by the accumulations of modified macrophages, cells of the mononuclear phagocyte system, called epithelioid cells, with or without the presence of other inflammatory cell types.[38]

In general, the characteristics of a granuloma depend on the capacity of the immune response, in particular the cellular immune response of the host, and the properties of the infectious agent, although immune complexes in antibody excess have also been shown to cause granuloma formation. The epithelioid and giant cells found in granulomas are thought to originate from activated macrophages, and it is known that macrophages activated by lymphokines also play a role in the DTH reaction. Because of this, it is believed that the responses are related, although to date it has not been possible to induce macrophages in culture to take on a typical epithelioid cell appearance. The question, therefore, remains as to whether epithelioid cells develop directly from macrophages under the influence of pharmacological agents released in a cell-mediated immune response.

No animal model exists for the study of granulomatous hypersensitivity, although granulomas with histological features of nonlepromatous leprosy have been induced in rabbits by injections of sural nerve plus Freund's adjuvant.[39] In susceptible human subjects, it is possible to produce granulomas with tubercle formation by skin testing with zirconium or beryllium,[40] and these have been shown to be a hypersensitivity phenomenon.[41] These granulomas develop just as the DTH dying down and reach a peak intensity about 7 days after skin testing.

In tuberculosis, the infection spreads until acquired cellular resistance has attained a level that allows granulomatous reactions of sufficient strength to circumscribe, block, and even eradicate the infectious focus. These infectious granulomas have the positive effect of stopping or even eradicating an infection, but they also have the negative effect of causing tissue damage at the site of infectious foci or where inactivated antigenic material persists.

The appearance of tuberculin sensitivity heralds a change in the host's response to the bacilli. On the first exposure, tubercle bacilli act as inert particulate matter, and evoke a nonspecific neutrophilic inflammatory response. During this period, bacilli enter phagocytes but multiply unchecked and can drain via lymphatics and the bloodstream to distant sites. Once sensitization appears, the inflammatory reaction becomes granulomatous.

The development of a state of cell-mediated hypersensitivity to bacterial products is probably responsible for the lesions associated with bacterial allergy, such as the cavitation, caseation, and general toxemia seen in tuberculosis and in granulomatous skin lesions found in patients with the borderline form of leprosy. Granulomatous hypersensitivity is typical of lesions in leprosy patients who have borderline reactivity. These borderline reactions occur either naturally or following drug treatment, and show as swellings and inflammations of the previously hypopigmented skin lesions. When this process occurs in the peripheral nerves, it is the major cause of nerve destruction in the disease.

If the conflict between the replicating bacteria and the body's immune system fails to be resolved in favor of the host, the constant presence of the antigen provokes a chronic, local delayed-hypersensitivity reaction. The continual release of lymphokines by the sensitized T cells causes an accumulation of macrophages, some of which give rise to arrays of epithelioid cells and some of which fuse to form giant cells. Lymphokine-activated macrophages will result in indiscriminate cytotoxicity, and this will lead to further tissue damage. This combination of cell types, together with the proliferating lymphocytes and fibroblasts, is called a chronic granuloma. In this way DTH has, as its final result, formation of immune granulomas.

Spector[42] has made a distinction between

[38] Turk, J. L. *Delayed Hypersensitivity.* Research Monographs in Immunology, vol. 1. Amsterdam: Elsevier/North-Holland, 1980.

[39] Crawford, C. L., Hardwicke, P. M. D., Evans, D. H. L. and Evans, E. M. Granulomatous hypersensitivity induced by sensory peripheral nerve. Nature **265** (1977) 457–459.

[40] Sneddon, I. B. Berylliosis: a case report. Br. Med. J. **1** (1955) 1448–1450.

[41] Black, M. M. and Epstein, W. L. Formation of multinucleate giant cells in organized epithelioid cell granulomas. Am. J. Pathol. **74** (1974) 263–274.

[42] Spector, W. G. The granulomatous inflammatory exudate. Int. Rev. Exp. Pathol. **8** (1969) 1–55.

two types of granulomas. The first type comprises granulomas with a slow turnover rate, also called the foreign body granuloma, and these are not of an immunological nature. The cells forming this granuloma are long-lived macrophages with a prolonged division time, and they can be initiated with nonimmunogenic insoluble particles, such as beryllium, zirconium, or Freund's incomplete adjuvant (in which the antigen is incorporated in the aqueous phase of a stabilized water in paraffin oil emulsion). The second type comprises granulomas with a rapid turnover rate, also called immune granulomas, which are due to the development of an immune response to certain antigens, particularly microorganisms that multiply intracellularly. These granulomas can be distinguished from nonimmunologic granulomas by the presence of lymphocytes, plasma cells, eosinophils and granulocytes surrounding the central core of mononuclear phagocytes. Many of the mononuclear phagocytes may show varying degrees of differentiation into epithelioid cells and giant cells. This is the type of granuloma formed in the Mitsuda reaction. It would seem that the two different categorizations of immune/nonimmune granulomas and slow/rapid turnover rate are not exactly synonymous, but considerable overlap exists between them.

The granulomas seen in subjects with lepromatous leprosy are similar to those seen in experimental animals at the site of injection of a nonantigenic colloidal material such as aluminium hydroxide, i.e., foreign-body granuloma. On histological inspection, the skin granuloma is seen to consist of large undifferentiated macrophages with no lymphocytes or other inflammatory cells present. In subjects with tuberculoid leprosy, pathologic examination reveals immune granulomas, which are rarely necrotic, with epithelioid and giant cells, and these lead to the destruction of nerve tissue. This immunologic granuloma is evidence of the host's violent reaction against *M. leprae.*

The existence of these two different types of granulomas has prompted Sansonetti and Lagrange to postulate that in the presence of a microorganism that develops intracellularly, an individual can develop one of the two "polar" forms or an intermediate form of granuloma.[17] Which type develops depends upon the individual's own response capabilities, perhaps genetic control, and the characteristics of the invading agent. This, they suggest, is the basic concept behind the idea of a granulomatous disease with a spectrum of forms. They also suggest that the formation of a granuloma tests not only resistance to infection but that its significance is more dynamic in that it tests the capacity of the host to develop a particular response to infection.

Skin testing in tuberculosis and leprosy

In 1906, von Pirquet[43] introduced the term "allergy" to describe the biological changes which result from adequate exposure to antigen so that subsequent exposure elicits an altered reaction. Used in this original sense to describe the "acquired, specific, altered capacity to react," the term allergy encompasses responses ranging from no reaction at one pole to accelerated, increased reactions at the other, and is now commonly applied to hypersensitivity reactions in which the features are characteristic for the types of antibody or CMI involved. There are four main types of allergic reaction that have been described,[44] but the altered biological state that they indicate, in itself, carries no implications concerning clinical hypersensitivity or immunity. The analysis of the allergic phenomena is commonly based on factors which are very different from those occurring in natural infection. That is, their presence is demonstrated by procedures in which components or products of the infecting agent are tested in amounts which are likely to be far in excess of those present in the body and in ways which are not ordinarily encountered. However, in spite of these complications, skin-test reagents have been used for a long time to investigate the disease process, immunology and epidemiology in tuberculosis and, more recently, in leprosy. The skin reactions produced may be relevant, for diagnosis in the individual subject and prog-

[43] von Pirquet, C. E. Allergy. Arch. Intern. Med. 7 (1911) 259–383.

[44] Roitt, I. *Essential Immunology.* 5th ed. London: Blackwell Scientific Publications, 1984.

nosis in specific infections, for epidemiologic and immunization studies of populations at large and also, for general purposes, as a means of assessing the immunological competence of the subject.

Tuberculin is a protein filtrate of the supernatant of a culture of *M. tuberculosis,* using either live or killed organisms, and has been in use since 1890. When tuberculin is injected into the skin of an individual, two different types of reaction may be seen. The Mantoux reaction is seen in individuals in whom previous infection with the mycobacterium has induced a state of CMI. The reaction is characterized by erythema and induration which appears only after several hours, and reaches a maximum at 24–48 hours. The second type of reaction is called the Jones-Mote type and is observed maximally only 24 hours after the skin test. The Jones-Mote reaction is highly regulated by suppressor cells, and is characterized by large numbers of basophils in the skin lesion and, consequently, is sometimes called cutaneous basophil hypersensitivity. Both of these reactions are T-lymphocyte mediated, and it is supposed that they are a measure of CMI.

In the early days of leprosy research, it was not possible to produce a reagent from *M. leprae* analogous to tuberculin because of the inability to cultivate the bacilli *in vitro,* nor was there available an animal that could be infected. In 1919, Mitsuda described his use of a reagent made from a suspension of ground up bacilliferous tissues heated to kill the organisms.[45] This reagent, which is usually contaminated with human tissue components, has been named Mitsuda lepromin or integral lepromin. Subsequently, further methods of producing tissue-free bacilli were developed, described more fully in the review by Sansonetti and Lagrange,[17] and some of these are being used in current clinical trials. The following are examples of two types of preparation.

The first uses differential centrifugation and produces a reagent called lepromin H, the H indicating that the reagent is derived from bacilli in human tissues.[46] Lepromin H is used by the World Health Organization and is prepared by the Medical Research Council's National Institute for Medical Research at Mill Hill in London. The second method used for cleaning the bacilli was developed by Dharmendra, and involves treating heat-killed bacilli with chloroform and ether into which the bacilli selectively pass.[47] This treatment not only frees the bacilli of tissue but also removes many of the bacterial lipids which, some people argue, alters the antigenicity of the preparation. Dharmendra lepromin is widely used in India. Paradoxically, many leprologists consider that the cruder preparation gives more consistent results and stronger, more interpretable reactions.

More recently a cleaner preparation was made by enzyme digestion but showed no significant difference in reactivity to the cruder forms.[18]

More rapid progress has been made possible by the large supplies of *M. leprae* which are now produced by infected armadillos. An armadillo lepromin, referred to as lepromin A, has been developed and studies show it to have the same response as lepromin H.[48] Convit and his colleagues have developed "soluble protein antigen" from lepromin A using methods that produce bacilli free of armadillo tissue.[49] Independent of this work, Rees and his colleagues have developed leprosin A from a filtered sonicate of cleaned armadillo leprosy bacilli killed by exposure to 2.5 megarads from a ^{60}Co source.[16] Leprosin A has been extensively used as an epidemiological tool and for studying immune mechanisms in leprosy patients. It is an important reagent in the assessment of immunoprophylactic and immunotherapeutic studies currently in

[45] Mitsuda, K. On the value of a skin reaction to suspension of leprous nodules. Hifaku Hinyoka Zasshi (Jap. J. Derm. Urol. **19** (1919) 697–708). Reprinted in English in Int. J. Lepr. **21** (1953) 347–358.

[46] WHO Memorandum: Recommended safety requirements for the preparation of lepromin. Bull. WHO **57** (1979) 921–923.

[47] Dharmendra. Immunological skin tests in leprosy; isolation of protein antigen of *M. leprae.* Indian J. Med. Res. **30** (1942) 1–7.

[48] Meyers, W. M., Kvernes, S. and Binford, G. H. Comparison of reactions to human and armadillo lepromins in leprosy. Int. J. Lepr. **43** (1975) 218–225.

[49] 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.

progress. All suspensions of skin test reagents used in leprosy such as lepromin are standardized to contain 1.6×10^8 bacteria/ml, in order that the various reactions can be compared with each other.

When lepromin is injected into the skin, the reaction it produces is biphasic.[16] The first phase, called the Fernandez reaction, is a typical delayed-hypersensitivity reaction and appears between 24 and 48 hours after intradermal injection. The second phase, called the Mitsuda reaction, appears between 2 and 4 weeks, and is usually read at 3 weeks. This late granulomatous reaction corresponds to the ability of the individual, sensitized or nonsensitized, to produce an immunologic granuloma in the presence of whole (even dead) bacteria, a reaction that is unique to *M. leprae* and is not observed for *M. tuberculosis.* It seems likely that in the granulomatous reaction the glycolipid bacterial cell walls act as adjuvant to the soluble protein antigens contained in the bacterial cells.

The Fernandez reaction probably represents a phenomenon analogous to a Mantoux test (the tuberculin reaction) caused by soluble extrabacillary antigenic components in the lepromin. The concentration of these antigens varies from preparation to preparation; therefore some lepromins produce a stronger Fernandez reaction than others. The presence of soluble or ultrasonicated reagents produces an enhanced Fernandez reaction but a poor Mitsuda reaction at 3 weeks. The histological appearance of this reaction is characterized by dermoepidermal inflammation, edema, hemorrhage, and infiltrate of polymorphonuclear cells followed by mononuclear cells, lymphocytes, monocytes, and macrophages. In general, positive Fernandez reactions occur only in individuals who are also Mitsuda positive, and very few healthy people in leprosy nonendemic countries produce Fernandez reactions even when they may produce Mitsuda-positive reactions. This observation is illustrated by a study of 65 students in London, where only one was Fernandez positive.[50] In leprosy-endemic countries, the frequency of positive Fernandez responses increases with the closeness of contact with leprosy patients.[49] Tuberculoid (TT or BT) leprosy patients are usually Fernandez positive, lepromatous (LL or BL) patients are always Fernandez negative, and healthy subjects may also be positive. In contrast to this, it has been found that the induction of delayed hypersensitivity to tuberculin is very effective when living microorganisms are used. It is less effective with killed *M. tuberculosis,* and nonexistent with tuberculin itself.[25]

The Mitsuda reaction, used by most leprologists as an indication of lepromin positivity, is an epithelioid cell granuloma very similar to the lesions in the skin found in TT and BT leprosy. The presence of intact bacilli in the reagent is necessary for the development of this late nodular component. The reaction is characterized by mononucleated and epithelioid cells organized in a tuberculoid nodule with fibrosis. This nodule corresponds to the formation of an immunologic granuloma. Positivity of the Mitsuda reaction correlates well with the clinical status of the individual tested. Patients with lepromatous leprosy with a specific defect in host resistance to *M. leprae* are inevitably Mitsuda negative, i.e., they are unable to show the 3-week nodular reaction. Patients at the tuberculoid pole (TT or BT) who show a high host resistance to the organism are usually Mitsuda positive. The use of fractionated or purified antigen fraction tends to produce early reactions of delayed hypersensitivity (Fernandez reaction); whereas the use of total lepromin tends to produce late reactions of the granuloma type (Mitsuda reaction).

It has been suggested that the late reaction may be a consequence of the subject developing an immune response to the lepromin antigens, or of the late release of antigen from intact bacilli, as well as a reaction to contaminating tissue components. Several reports describe the sensitization by lepromin and the induction of lepromin positivity by BCG vaccination.[51] In the experiment that has already been cited in the discussion of the Fernandez reaction, 65 people who had never been exposed to a

[50] Waters, M. F. R. Significance of the lepromin test in tuberculin-negative volunteers permanently resident in a leprosy-free area. Int. J. Lepr. **41** (1973) 563.

[51] Brown, J. A. K. and Stone, M. M. Lepromin conversion following infection with leprosy. Trans. R. Soc. Trop. Med. Hyg. **55** (1961) 443–445.

leprosy-endemic area nor received BCG and who were tuberculin negative were tested with lepromin. Only one gave a delayed-hypersensitivity reaction at 72 hours, and 38% gave Mitsuda reactions at 3 weeks.[18] This high percentage of late responders suggests primary sensitization by the lepromin itself. Lepromin positivity, consequently, does not necessarily indicate any previous contact with *M. leprae* or with any other mycobacteria.

In a similar group who were tuberculin positive, one third gave a positive Fernandez reaction to lepromin, and all gave positive Mitsuda reactions at 3 weeks. This would indicate that the antigens in lepromin were common to other bacterial species and not specific to *M. leprae.* This has been a consistent conclusion of many studies on the antigenic structure of leprosy bacilli.[16] Numerous antigenic determinants common to the *Mycobacterium* species have been distinguished,[52] but only one determinant specific to *M. leprae* has been identified.[53]

In summary, three categories of people can be considered in context of the lepromin test.

Patients with leprosy. Negative cutaneous reactions are usually observed for lepromatous subjects, weakly positive reactions for borderline subjects, and positive reactions for tuberculoid patients. The presence of human-tissue antigen can sometimes be responsible for a weak positive reaction in the lepromatous case. There is also an absence of positive cutaneous reactions to lepromin in lepromatous patients even after many years of drug treatment.[18]

Healthy subjects living in an endemic zone. The percentages of Fernandez and Mitsuda reactions are similar for healthy subjects and for patients with tuberculoid leprosy. This demonstrates that the tests have prognostic but not diagnostic value.

There is an increase in the percentage of Mitsuda reactions with age, indicating the probable participation of acquired sensitization in granuloma formation. It could also mean that children have an immature capacity to form granulomas in response to killed *M. leprae.*

Healthy subjects living outside an endemic area. The percentage of positive Mitsuda reactions among this group is of the same order of magnitude as that among contacts and patients with tuberculoid leprosy. Therefore, it seems that the great majority of the population, whether or not they have had contact with *M. leprae,* are capable of producing an immunological granuloma in its presence.

From this one can see the importance of using standardized lepromin containing the minimum amount of human tissue antigens so that clear epidemiologic evidence can be collected. At this time, therefore, the lepromin test is not a good method for determining whether a person has been infected with *M. leprae,* i.e., it is not a good diagnostic test. It has, however, been claimed that a positive lepromin test is of prognostic value in contacts of leprosy cases, indicating diminished risk of developing the disease.[54] In this respect, the Mitsuda reaction is a sign of a nonspecific ability to form a granulomatous reaction in the presence of foreign antigen. The ability of a patient to respond with the Mitsuda reaction appears to parallel the ability to eliminate *M. leprae* from the body. Thus, lepromin reactions are negative toward the lepromatous side of the leprosy spectrum and strongly positive in tuberculoid leprosy. The lepromin test also aids in predicting the direction in which a patient with indeterminate leprosy will move. A patient with indeterminate leprosy who is lepromin positive may either recover spontaneously or develop leprosy on the tuberculoid side of the spectrum. A patient who is lepromin negative will not have the capacity to eliminate the organism, and is more likely to develop the lepromatous type of leprosy.

In addition to the lepromin test, CMI is assessed by the two *in vitro* tests mentioned previously, the LTT and the LMIT. The results of these tests indicate only the final effect of extremely complex reactions to

[52] Harboe, M., Closs, O., Bjorvatn, B., Kronvall, G. and Axelsen, N. H. Antibody response in rabbits immunized with *M. leprae.* Infect. Immun. **18** (1977) 792–805.

[53] Kronvall, G., Bjune, G., Stanford, J., Menzel, S. and Samuel, D. Studies of mycobacterial antigens with special reference to *M. leprae.* Infect. Immun. **13** (1976) 1132–1138.

[54] Dharmendra and Chatterjee, K. R. Prognostic value of lepromin test in contacts of leprosy cases. Lepr. India **27** (1955) 149–152.

which many variables contribute. In spite of this, impaired host resistance to *M. leprae* at the lepromatous pole is associated with a failure of the CMI response in the LTT and LMIT to specific *M. leprae* antigens as well as with a negative Mitsuda reaction. The results of these *in vitro* tests correlate with those of *in vivo* tests which both measure the ability to recognize the sensitizing antigen. *In vivo* tests include the lepromin test, delayed hypersensitivity to various skin-test antigens, skin allograft survival, graft-versus-host reaction, and examination of lymph nodes of patients, and particularly the absence of a delayed-hypersensitivity reaction of the Fernandez type.

Two studies[55, 56] have established correlations between *in vitro* tests and CMI, and Dierks and Shepard[57] have shown that when lepromin was added to the cultures of lymphocytes from lepromatous leprosy patients a very low percentage of blast transformation was seen. Lymphocytes undergo blast transformation and proliferation in the presence of antigens to which they have been previously sensitized, and this is measured either by counting lymphoblasts or by measuring the incorporation of ^{3}H-thymidine into DNA (the rate of synthesis of DNA increases during blast transformation). In addition, lepromin did not cause blast transformation in cells from lepromatous patients who had been treated, even though they responded strongly to BCG and purified protein derivative; this has been confirmed by Godal, *et al.*[58] These results have shown that untreated lepromatous subjects have a profound specific deficiency in cellular responses to *M. leprae,* that they never recover this deficiency, even after prolonged treatment, and this deficiency is unique to *M. leprae* because the responses to other mycobacterial antigens are normal.

The immune profile of the lepromatous patient in regard to *M. leprae* is that of an individual with depressed *in vitro* and *in vivo* responses to different antigenic preparations of *M. leprae.* There is a profound deficiency of CMI that cannot be recovered after treatment, and which includes lack of delayed hypersensitivity and granuloma formation and the inability to develop acquired immunity to *M. leprae.*

The immune profile of the patient with tuberculoid leprosy in regard to *M. leprae* is that of an individual with normal positive cutaneous responses, i.e., the patient is able to respond by delayed hypersensitivity and granuloma formation. However, some studies suggest that there is some nonspecific suppression of immune responses in patients with tuberculoid leprosy, although less than in lepromatous individuals.[59]

Immune responses in leprosy

When bacteria invade a host they initiate the various elements of the specific immune system. The type of immune defense system deployed depends upon many factors, including the type of bacteria, its mode of penetration and dissemination into the host, its virulence and metabolism, and its intra- or extracellular mode of multiplication. Bacteria that multiply extracellularly lead to humoral responses with the production of specific antibodies; bacteria that multiply intracellularly cause cellular immune responses dominated by T lymphocyte-macrophage interactions.

A feature common to many chronic diseases caused by facultative intracellular organisms is that they show a failure or an absence of host resistance. This lack of reactivity or "anergy," described by von Pirquet in 1911[43] as the antipode of allergy, can be regarded as a failure of the immune system. In diseases caused by intracellular organisms, the failure is chiefly in CMI since the organisms appear to be protected from the

[55] Bloom, B. R. and Bennett, B. Mechanism of a reaction *in vitro* associated with delayed-type hypersensitivity. Science **153** (1963) 80–82.

[56] David, J. R., Al-Askari, S., Lawrence, H. S. and Thomas, L. Delayed-type hypersensitivity *in vitro.* I. The specificity of inhibition of cell migration by antigens. J. Immunol. **93** (1964) 264–273.

[57] Dierks, R. E. and Shepard, C. C. Effect of phytohemagglutinin and various mycobacterial antigens on lymphocyte cultures from leprosy patients. Proc. Soc. Exp. Med. Biol. **127** (1968) 391–395.

[58] Godal, T., Myrvang, B., Froland, S. S., Shao, J. and Melaku, G. Evidence that the mechanism of immunological tolerance (central failure) is operative in the lack of resistance in lepromatous leprosy. Scand. J. Immunol. **1** (1972) 311–321.

[59] Bullock, W. E. Studies on immune mechanisms in leprosy. I. Depression of delayed allergic response to skin test antigens. N. Engl. J. Med. **278** (1968) 298–304.

action of circulating humoral antibody. Anergy is now thought to be not simply the absence of reactivity but a disturbance in the dynamic equilibrium between helper- and suppressor-cell activity. This lack of reactivity can be seen in both CMI and DTH, which are normally viewed as being functionally interrelated.

It has been observed in the case of tuberculosis that there is no correlation between resistance to infection and the level of antibody. However, various levels of specific and nonspecific antibody are found to be present in these infections. It can therefore be concluded that the existence of the antibody production response does not necessarily mean that it participates in the immune defense mechanism.

Different levels of antibody to *M. leprae* are, however, associated with the different forms of leprosy. Subjects with the polar or borderline form of tuberculoid leprosy may have low levels of antibody or none at all; whereas subjects with borderline or borderline lepromatous leprosy invariably have low or moderate levels of antibody. Subjects with the subpolar or polar form of lepromatous leprosy have high titers of antibody,[60] whether they have been treated or not. It would appear from this evidence that antibodies are not necessarily protective in leprosy, nor are they diagnostic of disease.

With the recent successful growth of *M. leprae* in the armadillo, and hence the large scale production of *M. leprae* antigens, the search for specific antibodies can now progress more rapidly. It has been found that specific IgM and IgG, but not IgA, are produced in response to the antigens, and to date, as many as 20 different antigens of *M. leprae* have been detected.[61] It is likely that more *M. leprae* antigens will be detected. The techniques used to measure the antibodies are described by Sansonetti and Lagrange.[17]

In contrast to this, it appears that lepromatous subjects produce as many or more antibodies directed against antigens other than those of *M. leprae* than do tuberculoid or normal subjects.[62] The main increase is in the IgG immunoglobulin level and, to a lesser extent, in IgA, although a small but significant increase in IgM has also been found. Tuberculoid patients have a moderate increase in IgG but normal levels of IgA, IgM, IgD, and IgE. In addition, there is a large elevation in serum complement in both of the polar forms, but especially in lepromatous subjects, and also an increased level of circulating immune complex in lepromatous individuals. Furthermore, subjects with borderline lepromatous and lepromatous forms of leprosy show a large number of circulating autoantibodies.

Acquired cellular immunity

Infections caused by intracellular facultative parasites, such as tubercle and leprosy bacilli, viruses like smallpox, and parasites such as toxoplasma, give rise to an immune reaction in the host that is controlled by T lymphocytes and is apparently independent of B cells. The T cells have surface receptors which are both antigen sensitive and antigen specific. The nature of these receptors is still under investigation but, it is known that the receptor is able to recognize antigen in association with Class II molecules of the Major Histocompatibility Complex (MHC) on the surface of the antigen-presenting macrophage. The contact between the antigen-presenting macrophage and the T cell leads to the release of the soluble mediator interleukin-1 (IL-1) from the macrophage which synergizes with the signal received through the T-cell receptor to activate the lymphocytes. A calcium-mediated increase in the intracellular cGMP concentration occurs in the early stages of T-cell activation. This increase in cGMP together with the increase in calcium ions derepresses the appropriate genes in the resting lymphocyte nucleus and new synthesis of RNA and protein occurs, producing blast-like cells with nuclear and cytoplasmic enlargement and surface receptors for a soluble T-cell growth factor known as interleukin-2 (IL-2). The production of

[60] Myrvang, B., Feek, C. M. and Godal, T. Antimycobacterial antibodies in sera from patients throughout the clinico-pathological disease spectrum of leprosy. Acta Pathol. Microbiol. Scand. [B] **82** (1974) 701–706.

[61] Axelsen, N. H., Harboe, M., Closs, O. and Godal, T. BCG antibody in tuberculoid and lepromatous leprosy. Infect. Immun. **9** (1974) 952–958.

[62] Waters, M. F. R., Turk, J. L. and Wemambu, S. N. C. Mechanisms of reactions in leprosy. Int. J. Lepr. **39** (1971) 417–428.

IL-2 itself is initiated by similar events in a separate T subset which, in turn, acts to expand the blasts bearing the IL-2 receptors. It is known that there are several different T-cell subpopulations, all of which are concerned with various immune functions. Some of these, upon activation, release a number of soluble factors; others develop cytotoxic powers; while a proportion become memory cells which can produce an enhanced secondary response.

The cell-mediated immune response acts via two major effector mechanisms: the generation of cytotoxic T cells and the release of lymphokines. This occurs through the stimulation of two distinct T-cell subpopulations, the cytotoxic cell precursor (Tcp) and the T-helper/inducer/delayed-type-hypersensitivity cells (Th/dth) which themselves can probably be divided into functional subsets. The lymphokines are biologically active, soluble factors (e.g., IL-1 and IL-2) which modulate the behavior of other cells, particularly the mononuclear phagocytes (e.g., monocytes and macrophages) via substances such as macrophage migration inhibition factor, macrophage chemotactic factor, and macrophage activating factor. The different lymphokines may be produced by different lymphocyte subsets.

As has been mentioned, several of the lymphokines directly influence the movement and activity of macrophages. Macrophage chemotactic factor causes an accumulation of mononuclear phagocytes at the site of antigen-mediated lymphokine release. Once attracted, the cells are prevented from leaving by the macrophage migration inhibition factor (MIF), and are stimulated by the macrophage activating factor (MAF), also known as gamma interferon. Stimulation by MAF produces morphological changes in the appearance of the cell, an increase in its lysosomal enzyme content, and a heightened ability to kill off ingested intracellular organisms. The movement of monocytes from blood vessels into the extravascular spaces is facilitated by another lymphokine, the skin-reactive factor, which also increases capillary permeability.

The T-cell subpopulations can be divided into functionally distinct groups according to the molecules they exhibit on their surface membranes. The two most notable antigens expressed on the lymphocyte membrane are OKT4+ and OKT8+. The helper and inducer T-cell subpopulation exhibits surface OKT4+, and these cells comprise around 65% of all the T lymphocytes. The suppressor and cytotoxic cells show OKT8+ on their surfaces, and this subpopulation accounts for the remaining 35%. T cells expressing OKT4+ see antigen in relation to Class II glycopeptides of the MHC, whereas OKT8+ cells see antigen in relation to Class I glycopeptides of the MHC. This is referred to as MHC restriction. The MHC is a complex of linked genes coding for cell surface transmembrane proteins, and is located in man on chromosome 6.

Class I antigens are expressed on all cells of the body except mature red blood cells. The three Class I Human Leucocyte Antigen (HLA) loci are identified as HLA-A, -B and -C according to the position of their coding gene on the chromosome. Class II antigens, also known as HLA-D and -DR, are expressed in B lymphocytes, monocytes, endothelial cells, and certain epidermal cells. It has been found that, in mice, the ability to produce an immune response in the form of specific antibodies appears to be under genetic control linked to the MHC.[63] In humans, the MHC probably contains a comparable region.[64] The spectrum of leprosy may well be determined by the HLA system and, consequently, have a genetic component.

There is good evidence that certain DR phenotypes correlate with the type of disease developed in an individual infected with *M. leprae*. It has been found that HLA-DR3 occurs at a relatively high frequency in polar tuberculoid leprosy, and is very rare in lepromatous leprosy in a mixed African-Caucasian population in Surinam, and that HLA-DR2 is associated with tuberculoid leprosy in multiple-case families in India.[65]

[63] McDevitt, H. O. and Sela, M. Genetic control of the antibody response. I. Demonstration of determinant specific differences in response to synthetic polypeptide antigens in two strains of inbred mice. J. Exp. Med. **122** (1965) 517–531.

[64] Back, F. H. and van Rood, J. J. The major histocompatability complex—genetics and biology. N. Engl. J. Med. **295** (1976) 806–813 and 927–936.

[65] van Eden, W., de Vries, R. R., D'Amaro, J.,

It may be instructive that healthy adult Caucasians who give negative responses to at least four different mycobacterial skin-test reagents are rarely, if ever, DR3 positive, although this allele has a frequency of 0.26 in Caucasians. It has been argued that because the DR antigens are involved in antigen presentation for T cells and macrophages, the observed correlations can be explained by an effect of these DR phenotypes on the ability to respond to critical antigenic determinants on the leprosy bacillus.[20]

Because suppressor T cells, by means of negative influences, are thought to regulate the immune response, many experiments have been performed to study their function in patients with leprosy. Consequently, much evidence has accumulated to indicate that suppressor T cells play an important role in the modulation of humoral and cellular immune responses.[66] There are numerous types of suppressor cells; some suppress responses to one antigen only, while others, once activated, are nonspecific in their effects. Some suppress induction of responses; others suppress the effector phase. The situation is more complicated because, although the cells mediating macrophage activation and delayed hypersensitivity usually express the OKT4 marker, and suppressor cells usually express OKT8, all three activities can be found in both subpopulations.[20] Therefore, terms such as delayed hypersensitivity, suppression, cell-mediated immunity, and macrophage activation each cover more than one phenomenon, mediated by more than one cell type. Human leprosy is characterized by an inverse relationship between humoral and cellular immunity, and this may indicate that the immune regulation is defective. Immune regulation can be thought of as the result of a dynamic equilibrium between suppressor and helper cells. Experiments have shown contradictory results. Some have shown that tuberculoid patients with high resistance and good immune regulation show a better ability to generate suppressor activity, and that this suppressor activity is lost in the lepromatous individual;[67] others have shown the converse to be the case. However, in leprosy it seems that T-cell suppression correlates, in some as yet undefined way, with optimal immune responses.

In the immune response to facultative intracellular microorganisms described so far, in which T lymphocytes and mononuclear phagocytes act together, only the latter (macrophages) have the ability to destroy the bacterial inoculum. Acquired resistance in these cases is expressed by an increased bactericidal capacity of the macrophages, which is itself T-cell mediated. A breakdown in the immune response could be due to defects in the activity of the macrophage, in the T cells, or in the macrophage-lymphocyte cooperation.

Because macrophages are involved in many phases of the immune response, an alteration in one macrophage function could account for the absence of a global response. In lepromatous lesions, there appears to be uncontrolled proliferation of *M. leprae* within macrophages, and this observation has led to the proposition that there is an intrinsic defect in these cells in lepromatous subjects.

One possible explanation would be that the macrophages lack bactericidal activity, and that this is related to a deficiency in some specific enzyme. Many studies have produced contradictory evidence for this, some showing that there is no difference in the ability of macrophages from tuberculoid and lepromatous subjects to digest washed and heat-killed *M. leprae,* and finding no differences in the enzyme content of these macrophages, while others show the opposite to be true.[17] Moreover, macrophages from lepromatous individuals seem to be perfectly normal in their ability to phagocytose and kill *Candida albicans* and a variety of gram-negative and -positive bacteria.[20] Similarly, lepromatous macrophages seem to respond normally to lymphokines in the LMIT *in vitro.*

Another possibility is that there is a defect in presentation of specific antigen, either

Schreuder, I., Leiker, D. L. and van Rood, J. J. HLA-DR-associated genetic control of the type of leprosy in a population from Surinam. Hum. Immunol. **4** (1982) 343–350.

[66] Gershon, R. K. T cell control of antibody production. Contemp. Top. Immunobiol. **3** (1974) 1–40.

[67] Nath, I. Immunological aspects of human leprosy. Lepr. India **55** (1983) 752–762.

during induction of the immune response or when it is expressed. However, if this is the case, since there appears to be an increased antibody production in lepromatous subjects, it could imply that the process of specific antigen production and presentation by macrophages to T lymphocytes must be different for the cell-mediated immune response and antibody production.[68] This means that an anomaly in macrophage function could provide for antigen presentation that is inadequate for induction of cellular immunity but is adequate for production of antibody.

In conclusion, cellular-mediated immunity can be considered under three fundamental concepts: acquired cellular resistance; delayed hypersensitivity, and granuloma formation. This distinction may seem crude because it is known that these three are different manifestations of the same immune response and also that interactions occur between the humoral and the cellular mechanisms, but it is a useful functional distinction to make.

If, as we suspect, there is a deficiency of cellular immune function in lepromatous leprosy, several questions arise: Is the immune deficiency specific to the response to *M. leprae* or nonspecific, thus leading to general immunodepression? Is this deficiency primary, thus leading to the lepromatous form, or secondary to the infection and the bacterial load? At the same time, it is important to know whether the suppression of the response to *M. leprae* originates at the lymphocyte or the macrophage level, or at both levels since these two populations are involved in the development of acquired cellular resistance.

Many researchers have attempted to answer these questions and have found that lepromatous subjects, and to a lesser degree tuberculoid subjects, have a nonspecific deficiency of CMI. The evidence for this comes from a mixture of *in vivo* and *in vitro* studies, and contains many inconsistencies in the experimental results. The evidence includes studies of rejection of allogenic skin grafts,[69] and lymphoblast transformation tests in the presence of mitogens such as phytohemagglutinin.[70] Lepromatous subjects respond to the tests in the same manner as do immunodepressed individuals, and a qualitative and quantitative deficiency of T lymphocytes seems to be responsible for this pattern of response. Nath, *et al.* have found that in lepromatous leprosy there is a decrease in the number of circulating T lymphocytes compared with that in healthy persons or those with other forms of leprosy.[71] A further relationship has been found to exist in the lepromatous individual: that the number of T cells varies inversely with the bacterial or antigenic load,[72, 73] a relationship found to exist in syphilis, miliary tuberculosis, and other granulomatous diseases. This deficiency disappears in successfully treated, *M. leprae*-free, patients and therefore appears to be secondary, being the result and not the cause of the disease. A profound nonspecific cellular immune deficiency, however, does not exist because there is no evidence that lepromatous individuals are more susceptible to opportunistic bacterial, mycotic, or viral infections, or have a higher incidence of cancer.[74] On the other hand, it has been found that subjects with secondary cell-mediated immune deficiency have an 80-fold increase in risk of cancer and a 350-fold higher risk of lymphoma.

In contrast to the reversibility of the nonspecific immunodepression in lepromatous subjects after effective treatment, the defi-

[68] Howard, J. C., Courtenay, B. M. and Desaymard, C. Equivalent responsiveness to branched polysaccharides and their dinitrophenyl conjugates in the Biozzi high and low responder lines of mice. Eur. J. Immunol. **4** (1974) 453–457.

[69] Han, S. H., Weiser, R. S. and Kau, S. T. Prolonged survival of skin allografts in leprosy patients. Int. J. Lepr. **39** (1971) 1–16.

[70] Nelson, D. S., Nelson, M., Thurston, J. M., Waters, M. F. R. and Pearson, J. M. H. Phytohemagglutinin-induced lymphocyte transformation in leprosy. Clin. Exp. Immunol. **9** (1971) 33–43.

[71] Nath, I., Curtis, J., Sharma, A. K. and Talwar, G. P. Circulation T cell numbers and their mitogenic potential in leprosy—correlation with mycobacterial load. Clin. Exp. Immunol. **29** (1977) 393–400.

[72] Nelson, D. S., Penrose, J. M., Waters, M. F. R., Pearson, J. M. H. and Nelson, M. Depressive effect of serum from patients with leprosy on mixed lymphocyte reactions. Influence of antileprosy treatment. Clin. Exp. Immunol. **22** (1975) 385–392.

[73] Mendez, N. F., Kopersztych, S. and Mota, N. G. S. T and B lymphocytes in patients with lepromatous leprosy. Clin. Exp. Immunol. **16** (1974) 23–30.

[74] Purtilo, D. T. and Pangi, C. Incidence of cancer in patients with leprosy. Cancer **35** (1975) 1259–1261.

ciency in the specific response to *M. leprae* seems to be profound and irreversible, and appears to be a primary deficiency that may well give rise to the lepromatous form of the disease.

There is as yet no immunological explanation for tuberculoid leprosy, in which cell-mediated responses to *M. leprae* seem to be intact. There are two possibilities for the appearance of this form of disease. Firstly, there may be a delay before the onset of cell-mediated immunity so that when it appears the bacilli are established in vulnerable tissues which are then damaged by the inflammation. Secondly, cell-mediated immunity could be directed against antigenic components which are released only by organisms that have been killed, so that the immunological attack occurs in the wrong place around dead or leaking bacilli.

It seems clear that the development of any one form on the leprosy spectrum is a multifactorial problem in which genetically controlled factors, fortuitous situations, or a combination of both, play a large part. The compilation of specific data that would allow the deconvolution of these predisposing conditions is made difficult by the long incubation period of leprosy and the large number of possible events between the moment of initial infection and the appearance of the first clinical signs. It is this long and critical period that predicts, to a large extent, the future position of the patient in the spectrum. Added to this is the problem that the tests available at this time are merely prognostic and not diagnostic of the disease.

Granulomas in leprosy

Immunohistological analysis of leprosy lesions has been performed using monoclonal antibodies to define the T-lymphocyte subpopulations, and considerable agreement has been reached about the extreme tuberculoid and lepromatous forms, although the picture is not so clear for the borderline cases.[75, 76] In the granulomas of tuberculoid leprosy, approximately half of the cells are lymphocytes, and most of these express the OKT4+ marker. There are a few of the suppressor/cytotoxic (OKT8+) phenotype which tend to be situated around the periphery. In lepromatous granulomas, on the other hand, there are altogether fewer lymphocytes and these include a greater proportion of OKT8+ cells. Moreover, the OKT8+ cells are not confined to the periphery but are located throughout the granuloma.

Wallach, *et al.*[77] have reproduced these results and report that in untreated lepromatous leprosy T-suppressor cells are increased in the peripheral blood, and in the granulomas T-suppressor cells are, on average, twice as numerous as T-helper cells. In treated lepromatous patients, the circulating T-suppressor cells were found to be at normal levels, and the total T-cell content of the granulomas was not different from the untreated subjects. However, T-helper cells were more numerous in the granulomas, and the helper/suppressor ratio in the dermis was significantly higher than the ratio in untreated subjects. The helper/suppressor ratio in treated lepromatous patients was found to be similar to that found in tuberculoid patients.

Because of this evidence, it has been suggested that the OKT8+ cells are exerting a suppressive influence in the lepromatous lesions. There are, however, two caveats one must mention in regard to this hypothesis. Firstly, OKT8+ cells are not always suppressor cells, nor are all suppressor cells OKT8+. Cells that show suppressor activity in delayed hypersensitivity and cells that show suppression in lymphoproliferation *in vitro* express both OKT8+ and OKT4+ antigens. Secondly, it has been argued that although most of the lymphocytes in lepromatous lesions are OKT8+, the actual number is so small that even if they were suppressor cells it seems unlikely that they would exert a sufficiently strong suppressor effect. Furthermore, it is not clear just how

[75] Modlin, R. L., Gebhard, J. F., Taylor, C. R. and Rea, T. H. *In situ* characterization of T-lymphocyte subsets in the reactional states of leprosy. Clin. Exp. Immunol. **53** (1983) 17–24.

[76] Modlin, R. L., Hofman, F. M., Taylor, C. R. and Rea, T. H. T-lymphocyte subsets in the skin lesions of patients with leprosy. J. Am. Acad. Dermatol. **8** (1983) 182–189.

[77] Wallach, D., Flageul, B., Bach, M.-A. and Cottenot, F. The cellular content of dermal leprous granulomas: an immunohistological approach. Int. J. Lepr. **52** (1984) 318–326.

many lymphocytes are revealed by the monoclonal antibodies in lepromatous lesions.[75]

Peripheral blood suppressor cells which can be activated by specific antigen to exert nonspecific suppressor effects are commonly found in the late disseminated phases of infections with protozoa, worms, and bacteria, and are usually considered to be consequences of increasing antigen load rather than the initial cause of failure to control infection. Moreover, in mice the equivalent suppressor cells are readily evoked by large mycobacterial loads, but appear to have no effect on susceptibility to virulent infection.[20] With this knowledge, one could argue that the OKT8+ suppressor cells are the consequence of the high antigen load rather than the cause of it. However, the presence of these suppressor cells together with the imbalance of OKT8+ and OKT4+ cells found in the lesions of lepromatous cases must give rise to some suspicion that this is a worthwhile line of research.

Mode of infection

Recently, there has been some speculation about the route of infection and antigen presentation in the development of leprosy. As has already been mentioned, macrophages which express Class II glycopeptides can present antigen to T cells and so initiate T-cell-mediated responses. There are, however, other cell types that can do this, e.g., the Langerhans' cells of the epidermis and the dendritic cells which are found in other tissues and in very small numbers in the blood.[78] There is some evidence that different routes of antigen presentation (intradermally, via the respiratory tract, or orally) can evoke a qualitatively different type of response, involving the generation of effector cells which are in some way able to avoid suppression. In contrast, the response evoked by a route that results in more macrophage-mediated antigen presentation produces a more powerful suppressor cell component.[79] Because of this difference, the route of infection is now thought to be of some importance, although the actual infecting mechanisms are still only speculative.

In the past, most leprologists believed that infection is contracted through the skin, but some believed that it could be contracted via the upper respiratory tract. The mode of spread of tuberculosis is almost always due to inhalation of droplet nuclei, infectious particles of respiratory secretions aerosolized by coughing, sneezing or talking, which are sufficiently small to dry while airborne and to remain suspended for long periods. Recent studies have challenged the importance of the skin in transmission because there are very few acid-fast bacilli shed from the intact skin of lepromatous patients. These studies, however, have shown the importance of the mouth and nose as sources of transmission where large numbers of bacilli are found in the nasal discharge and the sputum. These bacilli remain viable in dried nasal secretions for several days, thus increasing the likelihood of acquiring the infection through the respiratory tract. Furthermore, Hubscher, *et al.* found more leprosy bacilli in the mouth than in the nose.[80] In the past, contracting tuberculosis due to ingestion of milk contaminated with *M. bovis* was common, but bovine tuberculosis is now rare.

Leprosy bacilli entering the skin would have their antigens presented by Langerhans' cells, and this would be expected to be particularly immunogenic. The intradermal route has been shown to be the best way of immunizing mice with *M. leprae,* and the immunogenicity of this route is diminished by ultraviolet irradiation of the skin, which damages the Langerhans' cells.[81] Rook has suggested that perhaps infection via the skin leads to tuberculoid leprosy or subclinical disease.[20]

Infection via the respiratory tract is now

[78] Steinman, R. M. and Nussenzweig, M. C. Dendritic cells: features and functions. Immunol. Rev. **53** (1980) 127–147.

[79] Britz, J. S., Askenase, P. W., Ptak, W., Steinman, R. M. and Gershon, R. K. Specialized antigen presenting cells. Splenic dendritic cells and peritoneal exudate cells induced by mycobacteria activate effector T-cells that are resistant to suppression. J. Exp. Med. **155** (1982) 1344–1356.

[80] Hubscher, S., Girdhar, B. K. and Desikan, K. V. Discharge of *M. leprae* from the mouth in lepromatous leprosy patients. Lepr. Rev. **50** (1979) 45–50.

[81] Shepard, C. C., Walker, L. L., Van Landingham, R. M. and Ye, S. Sensitization or tolerance to *Mycobacterium leprae* antigen by route of injection. Infect. Immun. **38** (1982) 673–680.

commonly believed to be the dominant route. Experimentally, immunologically suppressed mice have become infected after being exposed to an aerosol containing *M. leprae*.[82] Exposure to the bacilli by this route might result in more macrophage-mediated antigen presentation and, consequently, to a greater suppressor cell component, although this is not reconcilable with our knowledge of aerosol infection in tuberculosis. Oral intake of organisms might well prime suppressor-cell mechanisms, and this has been demonstrated by Rook.[20] It might be significant that in lepromatous leprosy the nasal epithelium sheds large numbers of bacilli, most of which are swallowed. The presence of large numbers of mycobacterial species in water supplies in many endemic areas as well as in sphagnum bogs may contribute to this primed suppressor-cell mechanism. The inhabitants of the Bergen area of Norway obtained their water supplies from such bogs, and large numbers of bacilli must have been consumed. This could explain why leprosy was common around Bergen until late in the 19th century.

It has also been suggested that it might be significant that *M. leprae* has a predilection for peripheral nerves, and that nerves lack lymphatic drainage.[83] Because of this, antigens released in the nerves tend to leak into the bloodstream and travel to the spleen rather than to the lymph nodes. Experimentally, this has been shown to lead to an antibody response with relative suppression of CMI.[84] In experiments of this kind it was found that specific immunity could be induced in mice by subcutaneous injections of small quantities of *M. lepraemurium*; however, large doses introduced intraperitoneally abolished the immunity. The response observed in the latter case was a perturbation of circulating lymphocytes that are not necessarily engaged in the specific response, and the animals developed granulomatous disease characterized by a histiomonocytic infiltration with giant cells and foamy histiocytes of the T-cell-dependent areas of the spleen and peripheral lymph nodes.

To further investigate this point, radiolabeled lymphocytes obtained from normal rats were injected into normal rats and those infected with *M. lepraemurium*. Fewer labeled cells were found in the thoracic duct of the infected recipients, indicating a perturbation in the recirculating lymphocytes. It was postulated that the transfusion of the radiolabeled normal lymphocytes into infected rats causes an increase in the trapping in the spleen. When the same experiment was performed with splenectomized rats, a significant increase in the number of labeled cells was found in the thoracic duct of infected rats as compared with normal splenectomized rats.

This alteration in the numbers of recirculating noncommitted lymphoid cells could explain the peripheral depression in CMI seen in subjects with lepromatous leprosy, although the alteration appears to diminish during the course of treatment which decreases the bacterial load.

Vaccination in leprosy

According to the latest report by the World Health Organization,[85] of the six diseases selected in their program, leprosy is the one for which research on a vaccine has made the most progress. In spite of this optimism, the possibility of developing a powerful vaccine against *M. leprae* that can be used once to generate full protection seems unlikely at this time. Unlike many other diseases that have either been eradicated or controlled by vaccination, leprosy appears to take advantage of a specific deficiency in the host's immune system. Research, to date, has shown no simple method of reversing this deficiency.

The vaccines tried so far include the use of cobalt-irradiated or autoclaved *M. leprae* with or without the addition of BCG vaccine, the ICRC bacillus from Bombay, and "*Mycobacterium w*" from Delhi, all described elsewhere.[33] In Venezuela, author-

[82] Rees, R. J. W. and McDougall, A. C. Airborne infection with *Mycobacterium leprae* in mice. Int. J. Lepr. **44** (1976) 99–103.

[83] Kaplan, H. J. and Streilen, J. W. Do immunologically privileged sites require a functioning spleen? Nature **251** (1974) 553–554.

[84] Bullock, W. E. Leprosy: a model of immunological perturbation in chronic infections. J. Infect. Dis. **137** (1978) 341–357.

[85] *Special Programme for Research and Training in Tropical Diseases. Tropical Disease Research, Seventh programme report.* Geneva: World Health Organization, 1985.

ities have begun testing a vaccine consisting of killed *M. leprae* and BCG against 60,000 people exposed to leprosy in their homes. It will be many years before the results of this trial become apparent.

The existence of common mycobacterial antigens shared by *M. leprae,* BCG, and other species has been discussed, and it would seem likely that BCG would give protection against leprosy as well as against tuberculosis. Several vaccination trials with BCG have had variable success in leprosy. Good protection was seen in Uganda,[86] but little in Burma where it was confined largely to the younger age groups.[87] These common antigens could themselves be the targets of specific effector T cells or, as Stanford, *et al.*[88] have found, they could enhance the recognition of the species-specific ones. In addition, it has been found that BCG and killed *M. leprae* can protect mice in the footpad model of *M. leprae* infection, and that in trials of immunotherapy BCG is said to act as an adjuvant and to enhance the response to *M. leprae* when they are injected simultaneously.[20]

Research has also focussed on a search for nonpathogenic mycobacteria that cross-reacts with *M. leprae* and that would allow attempts at vaccination. A close relationship has been found between *M. leprae* and *M. duvalii,*[89] the latter being able to distinguish clearly between lepromatous and tuberculoid leprosy by delayed hypersensitivity. Similarly, people immunized with the vole bacillus (*M. microti*) gave a stronger response to *M. leprae* after vaccination than did the group immunized with BCG.[89]

A successful leprosy vaccine would have to prime the cell-mediated immune system to respond to antigenic components of *M. leprae* when they subsequently appear in the host and to protect both immunocompetent and immunodeficient individuals. The vaccine would have to be more effective in stimulating the host's immunological defenses than the antigen itself if it is to overcome the cell-mediated defect that occurs in some leprosy-infected individuals. However, because of the long time course of the disease a full trial takes many years to perform. Therefore, vaccines have also been used for immunotherapy in lepromatous patients, particularly in those who have developed resistance to dapsone. However, there have been no controlled trials which assessed whether the addition of *M. leprae* to immunotherapeutic regimes really makes any difference to the course of the disease.

Experiences with chemotherapy and vaccination in tuberculosis may provide valuable insight into the treatment of leprosy. Soon after isoniazid became available for the treatment of tuberculosis its efficacy and freedom from side effects, as then perceived, led to its widespread use as a preventative agent. It was even suggested that all tuberculin reactors should be treated in an attempt to eradicate the disease. This optimism has waned for two reasons. Firstly, it is now realized that isoniazid has significant hepatotoxicity. Secondly, it is now clear that neither isoniazid nor any other chemotherapy has much effect on an infection in which microbial multiplication is minimal or absent. Accordingly, the term prophylaxis is somewhat misleading, for what is actually taking place when chemotherapy is effective is treatment of a subclinical but active infection.

Combined drug treatments have been developed that reduce the length of the course of therapy and, therefore, improve patient compliance, and also combat the emergence of resistant strains that occur when only one therapeutic agent is used.

Although the data are conflicting, most evidence indicates that BCG vaccination when given to tuberculin-negative people

[86] Brown, J. A. K., Stone, M. D. and Sutherland, I. BCG vaccination of children against leprosy in Uganda: results at end of second follow-up. Br. Med. J. **1** (1968) 24–27.

[87] Bechelli, L. M., Lwin, K., Gallego Garbajose, P., Mg Mg Gyi, Uemura, K., Sundaresan, T., Tamondong, C., Matejka, M., Sansarricq, H. and Walter, J. BCG vaccination of children against leprosy: nine-year findings of the controlled WHO trial in Burma. Bull. WHO **51** (1974) 93–99.

[88] Stanford, J. L., Rook, G. A. W., Samuel, N., Madlener, F., Khameini, A. A., Nemati, T., Modabber, F. and Rees, R. J. W. Preliminary immunological studies in search for correlates of protective immunity carried out on some Iranian leprosy patients and their families. Lep. Rev. **51** (1980) 303–314.

[89] Godal, T., Myrvang, B., Stanford, J. L. and Samuel, D. R. Recent advances in the immunology of leprosy with special references to new approaches in immunoprophylaxis. Bull. Inst. Pasteur (Paris) **72** (1974) 273–310.

will result in a 60%–80% decrease in the incidence of tuberculosis in a given population.[90] However, some trials have shown no benefit from vaccination. The vaccinated individuals become tuberculin positive, but there is no agreement as to whether they also acquire resistance to a virulent infection. The 60%–80% decrease in the incidence of tuberculosis, if real, is reasonable as a public health measure in situations of quite high prevalence, greater than exists in the Western World today. Most experts believe that BCG vaccination is inadvisable in areas of a low incidence of tuberculosis, where most young adults are tuberculin negative, because the opportunity is lost to detect the onset of infection and potential disease by monitoring the tuberculin reaction.[91] Opinions are divided on whether BCG should be used in highly endemic areas. However, the preventative efficacy of vaccination is almost certainly at least equal to that of chemoprophylaxis. Vaccination does not prevent infection but rapidly limits its proliferation, usually to such a degree that clinical disease does not develop,[92] and the effectiveness of vaccination in the prevention of serious disseminated disease in young children is especially striking.[93] In the opinion of some authorities, vaccination still makes good sense in the case of certain high-risk groups, such as people who require residence in areas with a serious tuberculosis problem.

In the absence of an effective antileprosy vaccine, Lagrange and Stach[94] suggest various approaches in the control of the disease. Initially, BCG vaccination could be performed at birth to characterize the immune system and as a means of prophylaxis, followed a year later by analyses on vaccine efficiency and immunology using tuberculin and the Mitsuda reaction of lepromin. This would distinguish responders from nonresponders. The nonresponder group would constitute a part of the primary nonreactive population and would be further investigated with various *in vitro* tests to determine the seriousness of their nonresponse status. With true nonresponders, active and potential carriers would have to be detected and treated with polychemotherapy.

It seems clear that anyone in an endemic area with a negative Mitsuda skin test, and hence a nonresponder, should have a serological study to determine whether they have been infected with *M. leprae.* Those with a negative serology should be vaccinated with BCG and serologically tested at regular intervals. Those with a positive serology should have their bacterial load determined, their immune response checked with tests for BCG growth in their macrophages, and their *in vitro* lymphocyte responses measured, and subsequently treated with chemotherapy to reduce their bacterial load and prevent them from being carriers. Perhaps in this way the spread of leprosy can be minimized, and the people who either have a specific immune deficiency to *M. leprae* or who are already infected can be identified and treated. It is worth remembering that when chemotherapy is given to patients who show the subpolar lepromatous, borderline lepromatous, or borderline forms of leprosy reversal reactions can occur. The reversal reaction marks a sudden increase in the patient's CMI to *M. leprae* and can lead to serious peripheral nerve damage.

The issue of vaccination and herd immunity to infectious diseases has recently been discussed and analyzed mathematically by Anderson and May.[95] In their study, they claim that the persistence of infectious disease within a population requires the density of susceptible individuals to exceed a critical value, such that, on average, each primary case of infection generates at least one secondary case. It is, therefore, not necessary to vaccinate everyone within a community to eliminate infection; the level of herd immunity must simply be sufficient to

[90] Luelmo, F. BCG vaccination. Am. Rev. Respir. Dis. **125** (no. 3 pt. 2) (1982) 70–72.

[91] Centers for Disease Control. Recommendations of the Public Health Service Advisory Committee on Immunization Practices: BCG vaccine. MMWR **28** (1979) 241–246.

[92] Sutherland, I. and Lindgren, I. The protective effect of BCG vaccination as indicated by autopsy studies. Tubercle **60** (1979) 225–231.

[93] Smith, D. T. Isoniazid prophylaxis and BCG vaccination in the control of tuberculosis; high-risk groups. Arch. Environ. Health **23** (1971) 235–242.

[94] Lagrange, P. H. and Stach, J. L. Strategy for leprosy control. Int. J. Lepr. **53** (1985) 278–288.

[95] Anderson, R. M. and May, R. M. Vaccination and herd immunity to infectious diseases. Nature **318** (1985) 323–329.

reduce the susceptible fraction to below the critical point. This leads to the central epidemiological questions: What proportion of the population should be vaccinated to achieve elimination locally? eradication globally? or a defined level of control?

In order to answer these fundamental questions, we need to understand the interaction between the transmission dynamics of the disease agent and the level of naturally acquired immunity to infection. This relationship is complex and depends on such factors as the precise course of infection within an individual, the demography of the host population, the duration of acquired immunity and maternally derived protection, age-related changes in the degree and intimacy of contacts among people, and the prevailing levels of genetic, spatial and behavioral heterogeneity in susceptibility/resistance to infection.[95] The information with regard to these factors in leprosy is sadly inadequate. However, we can take comfort in the knowledge that eradication is possible because of evidence that leprosy has disappeared from previously endemic areas without reappearing again, as has been seen in Norway.

According to the model of Anderson and May, to eradicate an infection by mass immunization it is necessary to reduce the number of secondary cases produced by one primary case to below unity. This can be achieved by vaccinating a proportion of the community. It is known that generally vaccination during the period of maternally derived protection usually fails to protect the child adequately from subsequent infection.[96] It is believed that, on the average, the first infection in leprosy is probably at a very young age. The disease shows a maximal occurrence in the second and third decade of life, and is known to have an incubation period of about this length. Because of this, the creation of a level of herd immunity high enough to eliminate leprosy appears impractical in endemic regions. Instead, accurate serological or skin-testing methods that are diagnostic of the disease, rather than just prognostic, are necessary in order that disease-free and infected individuals may be identified early in life. Infected individuals thus identified could then be treated with immunotherapy so that they do not become carriers. Highly specific immunoassay methods have been developed for the early detection of the humoral immune response of subclinical leprosy infection.[97]

The maintenance of transmission is probably due to the carriers alone and, therefore, chemotherapy in lepromatous individuals is necessary in order to reduce this number. In this context, the number of resistant strains, both primary and secondary, that are evolving is worrying. Obviously, combination therapies must be devised, as in the treatment of tuberculosis, to circumvent this problem. In this way, in the absence of an insurmountable immunological barrier as was achieved with smallpox eradication, a solid chemotherapeutic wall can be built around the high-risk subjects.

Conclusion

Obligate intracellular organisms often cause chronic diseases, and their physiopathology is due more to the immune response of the host than to the virulence of the organism itself. In leprosy, the presence of a disease spectrum provides a parameter which is, in some complicated way, a reflection of the quality of that immune response. It may be tempting to speculate on this immune response in the various forms of the disease, but any conclusions must be tentative because our knowledge about the mechanism of the immune system, and of T cells in particular, is not complete.

It has been recognized that T cells exist in several subpopulations and that these subpopulations perform different but complementary functions in the immune response to foreign antigen. In leprosy research, attention has been focussed on the role of T-suppressor and T-helper cells, in particular. However, we are now beginning to recognize many more cell types that may be important in the immune response, and especially in the cell-mediated response. In

[96] Heyman, D. L., Mayben, G. K., Murphy, K. R., Guyer, B. and Foster, S. O. Measles control in Yaounde: justification of a one-dose nine-month minimum-age vaccination policy in tropical Africa. Lancet **2** (1983) 1470–1471.

[97] Young, D. B. and Buchanan, T. M. Serological test for leprosy with a glycolipid specific for *M. leprae*. Science **221** (1983) 1057–1059.

order to understand the response properly it is important to discover the various roles these cells play, individually and in unison. Also, many of the T cells which were given specific names because they were thought to have well-defined functions are now thought to be merely showing an activity that may be manifested by a large number of cell types. For example, natural killer (NK) cells have been named and their activity is believed independent of antibody and complement. We now suspect that NK activity can be manifested by T cells, non-T lymphocytes, myeloid cells, etc. The discovery that about 30% of all of the target-binding cells in the cell-mediated immune response are NK cells emphasizes that NK cells may well be more important than was originally thought. NK cells have also been implicated in immunoregulation. When non-T-type NK cells are activated with interferon and mixed *in vitro* with fresh T cells, the T cells become active suppressor T cells. It has been found that patients with systemic lupus erythematosus have low levels of *in vitro* NK cell activity, making one suspect that this explains the finding that they also have little or no T-suppressor cells. It is interesting to speculate whether individuals showing the lepromatous form of leprosy have a lowered NK activity. It is also believed that NK cells contribute to the regulation of progenitor cells in the bone marrow. Similarly, another subpopulation of named cells, the antibody-dependent cytotoxic cells (ADCC) show a specific activity which is independent of complement but needs antibody, but the ADCCs are not a homogeneous group of cells. Macrophages, T cells, polymorphs, platelets, etc., can all show ADCC activity, and the cells that regulate NK and ADCC activity overlap to some extent.

These examples illustrate that we may not be aware of the full repertoire of the immune response, and more research is needed to characterize the workings of the immune system before we can draw clear conclusions about its suspected malfunctions. In fact, our knowledge of it is so poor that even the significance of the different T4 and T8 ratios in tuberculoid and lepromatous granulomas is not understood. Further work in this direction might increase our understanding of the connection between the allergic response to *M. leprae* antigens and the host's immune response. This would allow better diagnostic methods to be developed for identifying not only those at risk but also those already infected. An animal model would be invaluable at this stage to enable the study of why some forms of the disease produce granulomas and how these granulomas damage victims' nerves.

Several lines of research into vaccines are possible. One would be to investigate various ways to engineer *Escherichia coli* and other organisms to produce the antigens of *M. leprae.* Alternately, "anti-idiotype antibodies" could be used rather than attempting to build replicas of the antigen itself. For each foreign antigen there is an antibody whose variable region is complementary to an area on the antigen, called an epitope. The configuration on the antibody is an internal image of the antigen and, hence, is potentially antigenic in its own right. An antibody to the specific idiotype (the variable region complementary to the antigen) would mimic the original antigen. These anti-idiotypes would reproduce exactly the antigenic determinants that the lymphocytes recognize, avoiding the problems with configuration inherent in the vaccines that use synthetic peptides. In addition, the epitopes of sugars (the carbohydrate component of glycoproteins) could be recognized. Anti-idiotypes are still, however, a research tool.

Research into vaccines has entered a new era. The use of new adjuvants, such as iscoms (immunostimulating complexes), to present antigens in a more immunogenic manner; new synthetic proteins; sequenced genes inserted into vectors; and anti-idiotype antibodies mark the beginning of new forms of preventative therapy.

—Michael Maier, D.Phil.