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EDITORIAL

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Immunological Aspects of Leprosy: Ten Years' Activity at the Armauer Hansen Research Institute and Prospects for Further Work

The Armauer Hansen Research Institute (AHRI) was inaugurated in March 1970. The 10th anniversary of the Institute was celebrated in Addis Ababa on 12 March 1980, and a consideration of the work performed at the Institute during these years shows that this was a very significant event in the field of leprosy.

What were the concepts, and who were behind the foundation of this institution?

The Swedish and the Norwegian Save the Children Federations took the initiative in 1966, but the concept that these organizations should make a major contribution to development of leprosy research in a leprosy-endemic country was not new at that time. The knowledge of leprosy was far behind that of other important communicable diseases, and the experience gained since the introduction of sulfones in 1941 showed that the existing methods were inadequate to establish efficient leprosy control programs on a large scale and to reduce the incidence of the disease to a truly significant extent. Swedish and Norwegian physicians engaged in leprosy fieldwork in Africa saw the need for increased knowledge of the disease and thus for research. The matter was discussed at a collaborative meeting between the two organizations in 1962. Various projects were considered, but the plans were not made concrete until 1966 when it became clear that a leprosy research institute might be associated with the All Africa Leprosy and Rehabilitation Training Centre (ALERT) and the Medical Faculty at the University of Addis Ababa. ALERT was founded in 1965 and developed to become a major institution for training of health personnel for leprosy work. At ALERT a leprosy hospital and an outpatient clinic with large numbers of patients would be available, leprosy control work would be undertaken in a large area around Addis Ababa, and a leprosy teaching institution would be in need of and benefit from close cooperation with a leprosy research institute. These two institutions have their separate budgets and their separate boards but are closely collaborating and in essence mutually dependent institutions. It was felt natural that the institute should be named after Dr. Gerhard Armauer Hansen who discovered the leprosy bacillus in Ber-

gen[1] and be affiliated with the University of Bergen.

According to the statutes, the function of the Armauer Hansen Research Institute is to contribute to our knowledge of leprosy through basic research. During the discussion which led to selection of the main area for work at the institute, immunological aspects of leprosy were brought into consideration early. At the time it was realized that the highly bacilliferous form of the disease, lepromatous leprosy, was characterized by a marked deficiency of cell mediated immune reactions against *Mycobacterium leprae*. Evidence for this view had been obtained both through clinical and experimental studies. The classification of leprosy in a five group system by Ridley and Jopling[2] had immunological considerations as a main background. The pathogenesis of "reactions" in leprosy had been considered, and their classification was basically founded on immunological considerations. In many other infectious diseases, immunological techniques and considerations had been of major importance in development of efficient prophylactic measures. That the same might be done in leprosy was, and still is, a main hope. Last, but not least, immunology had experienced a period of marked expansion and application in various medical disciplines. It had also expanded in leprosy, but it appeared that a lot remained to be done in this area. Immunology was then selected as a main subject for the research work at AHRI, and the institute was staffed and equipped accordingly.

At the 10th anniversary it seems appropriate to consider the work and achievements at AHRI. As the first Director of AHRI and present member of the AHRI Board, my view will be biased. But all scientific work and accounts of it are biased, and the essence is to keep personal views and bias controlled to an extent that observations and views still may be valid and trusted. In considering the work performed at AHRI, the information obtained is certainly of more general interest since the work at AHRI to a great extent reflects immunological studies of leprosy as they have evolved during the 1970s. Towards the end of this account, I shall also present my views on the prospects for immunological studies of leprosy during the next decade.

Leprosy may to a great extent be described and considered as an immunological disease. The initial events occurring shortly after infection with *M. leprae* are to a great extent unknown. When the defense acts properly, the individual will not show any clinical symptoms after infection, and this is what usually takes place. If resistance is inadequate, the bacilli multiply with development of clinical disease. The leprosy bacillus is remarkably non-toxic and may be present in vast numbers in tissues with very few clinical symptoms. Most symptoms of the disease are in fact caused by immune reactions against various components of the bacillus, leading in turn to tissue damage and clinical symptoms. *M. leprae* is an obligate intracellular parasite. In such infections, cell mediated immune reactions are of main importance for protective immunity[3] and studies of cell mediated immunity have therefore been a major part of the work at AHRI.

The lymphocyte transformation test (LTT) and its significance. Studies of the response of lymphocytes to stimulation *in vitro* by mitogens and specific antigens had provided extensive new information relating to cell mediated immune reactions in the late 1960s. This test was developed for use in leprosy through independent studies of the response of lymphocytes *in vitro* to stimulation with *M. leprae* antigens by Bullock and Fasal[4], Godal, *et al.*[5] and Han, *et al.*[6].

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At AHRI, Godal, *et al.* developed a lymphocyte transformation test (LTT) for use in leprosy. They studied the degree of morphological blastoid transformation *in vitro* after addition of washed *M. leprae* to lymphocyte cultures prepared from patients with different clinical forms of leprosy and various healthy individuals from leprosy endemic and leprosy non-endemic countries. They made three major observations:

Godal and Kesete Negassi showed that lymphocytes from healthy individuals who had been in contact with leprosy patients on the whole responded more strongly to stimulation with *M. leprae* than lymphocytes obtained from healthy individuals who had not been exposed previously to leprosy patients[7,8]. This is the first demonstration that tests of blood lymphocytes can indicate whether an individual has been exposed to *M. leprae*. Cross-reaction between *M. leprae* and other mycobacteria in the LTT[9,10] complicates the evaluation of the results of the LTT test, but this early work at AHRI meant that immunological evidence of exposure to *M. leprae* can be obtained in healthy individuals. Application of the test among the Gurage people in a rural area of Ethiopia by Menzel[10] revealed that lymphocytes obtained from contacts of patients with active lepromatous leprosy respond more strongly to stimulation with *M. leprae in vitro* than lymphocytes obtained from individuals with a lower degree of exposure. It has thus been shown that the test can be applied in a field project, albeit with difficulty. Diagnosis of subclinical infection in leprosy represents a main challenge. It is not yet clear whether one should rely on tests of cell mediated immunity or on tests for humoral antibodies or both, but these early findings in the LTT test show that we are on the way to being able to assay the "infectious load" of leprosy in different populations. This type of information is essential to understand how leprosy is spread in the population and thus to obtain a better basis for prevention and leprosy control work in different areas.

Godal and Myrvang[11] found that patients with reversal reactions presenting with episodes of acute inflammation in skin lesions and nerves had particularly strong responses in the LTT test to stimulation with *M. leprae*. This was a direct demonstration that reversal reactions in man are associated with increased cell mediated immunological reactivity against *M. leprae*.

Myrvang and Godal made important observations on the LTT test in patients throughout the clinical spectrum of leprosy. Patients with tuberculoid leprosy usually reacted strongly to *M. leprae* in culture. They recorded the mean response of groups of patients clinically and histologically classified according to the extended Ridley and Jopling scale[2,12,13] and found that the mean response showed a continuous decrease towards the lepromatous end of the spectrum where the lymphocytes did not respond to stimulation with *M. leprae in vitro*. The paper[12] describing and commenting on the strong correlation between the mean LTT response in groups of patients and their classification according to the Ridley and Jopling scale has later been widely cited.

This was the "first stage" of the LTT work at AHRI. The second stage involved further work with technical development of the test which was transferred to a micro scale and to quantitation of DNA synthesis by determination of incorporation of labelled thymidine into newly synthesized DNA. These developments permitted expression of the results on a more quantitative basis, and more tests could be done

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on a single blood sample from each patient so that dose response curves could be established for simultaneous stimulation with a variety of antigens.

Bjune at AHRI collaborated closely with Barnetson at ALERT in a prospective study of the LTT test in patients with borderline leprosy where LTT was correlated with continuous and careful clinical observations of the patients. Patients with stable disease usually showed an LTT response to *M. leprae* which was almost constant. In patients with reversal reactions there was a marked increase in the LTT response to antigens of *M. leprae* during reactions, and these subsided in the post-reaction period[14]. The lymphocytes were tested against various antigenic preparations made from *M. leprae*. It was demonstrated that individual patients showed a markedly different response to stimulation with washed *M. leprae* and to a sonicated preparation of *M. leprae*; the former induced the strongest response in patients with predominant symptoms of the skin and the latter in patients with nerve reactions[15]. These observations indicated that the cell mediated immune response observed in different groups of patients may be directed against different components of the leprosy bacillus and that reactivity to certain antigens may be associated with a certain clinical picture.

Bjune and Barnetson also found[14] that the variation in LTT response in patients with tuberculoid leprosy earlier observed by Myrvang and Godal[12] was related to clinical symptoms. Patients with BT leprosy with silent skin lesions usually had fairly low LTT responses whereas BT leprosy patients with lesions showing signs of inflammation usually had strong LTT responses. Similar observations were made in the borderline lepromatous group in which patients with lesions showing signs of inflammation had fairly strong LTT responses, and these might even be stronger than the responses in borderline tuberculoid patients with lesions showing no signs of inflammatory reactivity. These observations indicate strongly that the LTT response is more related to hypersensitivity than to resistance to infection in the individual patient. This work on reversal reactions illustrates the importance of the interaction between basic work at AHRI and clinical work at ALERT. Concepts and findings were interchanged and rapidly led to improved treatment of neuritis and better prevention of deformity. From an immunologist's point of view it is the logical procedure that patients with reversal reactions should obtain their anti-leprosy treatment continuously to reduce the bacterial load, and during the acute episodes immunological damage should be reduced by additional drug treatment aiming at a reduction of inflammatory symptoms, e.g., by prolonged administration of steroids.

It is widely recognized that patients with lepromatous leprosy are unable to mount an efficient cell mediated immune response to *M. leprae*. Evidence for this view is obtained *in vivo*, e.g., through the consistent finding of a negative lepromin test[16], and *in vitro* through the lack of response to stimulation by *M. leprae* in the LTT test[5, 12, 17]. The uninhibited growth of *M. leprae* is generally considered to be due to this defect. The nature of the defect has remained a puzzle. Epidemiological studies indicate that susceptibility to lepromatous leprosy is, at least partly, genetically determined[18]. Godal, *et al.* demonstrated that patients with lepromatous leprosy lacked circulating lymphocytes with ability to respond to *M. leprae* in the LTT test[5, 17]. Recently, Stoner has approached this problem in a new way by studying the LTT response in patients with lepromatous leprosy and their siblings[19]. His main observation is that HLA-

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D identical siblings of a patient with lepromatous leprosy show equally strong LTT responses to *M. leprae* as HLA-D non-identical siblings of the same patients[19]. The HLA region is expected to contain genes essential for development of immune reactions as it corresponds to the H-2 region in the mouse which contains a series of important immune response genes[20]. If the immunodeficiency in lepromatous leprosy is genetically determined, this may have serious implications concerning the possibility for prevention of lepromatous leprosy by immunological techniques. Stoner's findings imply that the deficient cell mediated immunity in lepromatous leprosy is not associated with genetic factors closely linked with the major histocompatibility locus. Its possible association with other genetic factors and the importance of these concerning the possibility of prevention of a deficient cell mediated immunity or restoration of immunodeficiency remain to be defined. Cells from lepromatous leprosy patients and their HLA-D identical siblings are particularly useful in LTT tests on cell mixtures prepared from patients and healthy persons to study if the defect in CMI in lepromatous leprosy is located in the macrophages or in the T-lymphocytes. Experiments based on mixture cells from different individuals had earlier been initiated at AHRI by Hirschberg[21]. They are difficult to interpret when the individuals are HLA-D non-identical, both due to increased background values in the cultures caused by stimulation with allogeneic cells and to decreased efficiency of cellular cooperation in HLA-D non-identical combinations[22]. Stoner's approach is also valuable in further attempts to establish if circulating suppressor cells are essential for development of the cellular immunodeficiency in lepromatous leprosy.

Analysis of cell mediated immune reactions *in vivo* or *in vitro* is often difficult since identical results may be obtained even if different components of the mycobacterium act as the stimulating agent. Work on isolated and defined antigenic components of *M. leprae* is therefore required. Through collaboration between Closs and Reitan[23], a highly purified preparation of *M. leprae* antigen 7 has been shown to be a powerful stimulator in the LTT test using lymphocytes from patients with borderline tuberculoid leprosy and in skin tests. This is the first published study on the activity of a purified antigenic component of *M. leprae* in cell mediated immune reactions in leprosy.

Erythema nodosum leprosum (ENL). ENL is another form of "reaction" in leprosy of great current interest. Occurring with high frequency in patients towards the lepromatous end of the spectrum[24], it leads to development of small tender subcutaneous nodules which persist for a few days, and new lesions may develop constantly. It is often difficult to handle clinically. This reaction is considered as a classical form of an immune complex disease[25]. The nature of the antigen involved was only incompletely known when AHRI started its work. The first experimental work at AHRI was on an experimental Arthus reaction using the same antigen, human serum albumin (HSA), in soluble and corpuscular form[26]. HSA induced acute inflammatory reactions both in soluble and in corpuscular form

terium leprae of HLA-D-identical siblings of lepromatous leprosy patients. Lancet **2** (1978) 543–547.

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when injected into rabbits with precipitating anti-HSA antibodies in serum. In the first instance, the lesions showed pronounced vasculitis with local accumulation of neutrophilic granulocytes in and around vessel walls. When HSA was used in corpuscular form, the inflammatory response was similar with regard to cell type but occurred more diffusely in the tissues with no apparent tendency to localized vasculitis. When compared to the histological features of ENL in man[27], this work strongly indicated that a soluble antigen of *M. leprae* is essential for development of ENL. Later investigations by Bjorvatn, *et al.*[28] have revealed characteristic changes in the complement system during ENL. They found an increased concentration of the complement split product C3d in serum in patients with active ENL. As there was no apparent correlation between amounts of circulating immune complexes and occurrence of ENL, this indicated that the immune complex formation leading to ENL mainly occurs extravascularly. At present, Ayele Belehu, *et al.* are working on quantitation of immune complexes in ENL and on isolation of these complexes. This is a logical approach to identify those antigens of *M. leprae* that are of importance in a defined complication of the disease. Undoubtedly, key features of ENL are still unresolved, and there is a great need for further work in this area. AHRI and other institutions with simultaneous availability of laboratory facilities and careful continuous clinical studies of patients are those that are needed for this type of work.

***In vitro* culture of *M. leprae*.** Samuel, *et al.*[29] attempted to culture *M. leprae* in human macrophages *in vitro*. In 27 out of 55 experiments a 2- to 9-fold increase in the number of acid-fast bacilli was observed over a period of 1½ to 3 months of cultivation. No such increase was observed with heat-killed bacilli, in the absence of macrophages, or on artificial media. A close correlation was found between the increased number of acid-fast bacilli and changes in viability as determined by the morphological index. The increases in acid-fast bacilli were inhibited by addition of DDS or rifampin to the medium. The multiplication of *M. leprae in vitro* did not appear to depend on the origin of the macrophages and was obtained in macrophages from healthy individuals and patients with tuberculoid and lepromatous leprosy. The applicability of the method has been limited by the restricted survival of human macrophages *in vitro*. The method has been adopted in other laboratories[30], and the principle should be intensively explored for development of *in vitro* techniques for demonstration of drug resistance.

Antigenic structure of *M. leprae* and diagnostic procedures. Until recently very little information was available on the antigenic structure of *M. leprae*. The availability of larger amounts of *M. leprae* due to the fact that it can grow and establish a systemic mycobacterial infection in the armadillo[31] has made studies of the antigenic constitution of *M. leprae* possible. A series of different antigenic components of *M. leprae* have been identified and numbered[32]. By the use of concentrated antigenic preparations and concentrated rabbit anti-*M. leprae* immunoglobulins, more than 20 distinct antigenic components have been demonstrated in *M. leprae* by crossed immunoelectrophoresis[33]. Kronvall, *et al.*

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initiated the work on the antigenic structure of *M. leprae* at AHRI. They studied *M. leprae* and related mycobacteria by crossed immunoelectrophoresis to characterize the antigenic makeup of *M. leprae* and the specificity of anti-mycobacterial antibodies in individual patients[34,35]. Their studies in this field provided new, essential information on the antigenic composition of *M. leprae*, on the specificity of the antibody response in leprosy patients, and on the usefulness of antibodies in patient sera as reagents for immunological studies of mycobacteria. Studies of antigen 21 of *M. smegmatis*, which cross-reacts with antigen 4 of *M. leprae*, led to the demonstration of antigenic determinants that are specific for the leprosy bacillus on the latter component[35,36]. This work shows that antibodies occurring in selected sera from patients with lepromatous leprosy are unique reagents to characterize the immunological relationship between *M. leprae* and other mycobacteria. The occurrence of *M. leprae* specific determinants on cross-reacting components offer possibilities for development of antibody assays specific for *M. leprae* by various absorption procedures[37,38]. Strictly species specific components occur only rarely in mycobacteria[33,39] and have so far not been conclusively demonstrated in *M. leprae*.

Diagnostic procedures in leprosy are important from various points of view. At AHRI, Heidar Abu Ahmed[40] worked on skin smears taken from various sites of the same patients. He obtained information pointing to the importance of selection of site of sampling and showed that skin smears obtained from the fingers are particularly rich in *M. leprae* and viable bacilli. These findings have been confirmed and extended by other authors[41]. Diagnosis of subclinical infection with leprosy is important from an epidemiological point of view and may provide information essential for the development of better leprosy control procedures. Immunological evidence of exposure to *M. leprae* in individuals without clinical symptoms of leprosy was first obtained at AHRI through work with the LTT[7,8]. Demonstration of antibodies reacting with high specificity with *M. leprae* may also be useful for diagnosis of subclinical infection. The fluorescent leprosy antibody absorption test of Abe[37] represents a major approach in the antibody work. By the use of ^{125}I labeled *M. leprae* antigen and concomitant absorption with a sonicate of another related unlabelled mycobacterium (BCG), a sensitive radio immunoassay has been developed which permits demonstration of *M. leprae* specific antibodies in high titers in most leprosy patients[38]. Work along the latter line and studies of antibodies against *M. leprae* antigen 7 have been a major undertaking in my laboratory in Oslo, being carried out in close collaboration with AHRI and ALERT. There is a current revival of interest in antibody studies in leprosy. Increased amounts of antibodies against *M. leprae* antigen 7 occur frequently in lepromatous leprosy. The median concentration decreases through the spectrum towards the tuberculoid end, but there is a marked variation in antibody concentration in individual patients with simi-

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lar clinical and histological classification[42]. Studies on anti-*M. leprae* antibodies in patients with lepromatous leprosy have revealed that the specificity of the antibodies is a characteristic feature of each individual patient and remarkably constant throughout the first 1½ years of DDS treatment[43] whereas the concentration of anti-*M. leprae* 7 antibodies decreases slowly[42,44]. In BT leprosy, studies of groups of patients treated for various periods with DDS revealed that the anti-*M. leprae* 7 concentration decreased quite markedly during the first 3 years of DDS treatment. Suspected and proven clinical relapse of BT leprosy was associated with renewed synthesis and increased concentration of anti-*M. leprae* 7 antibodies[42].

Immunological studies in "related" diseases and during pregnancy. Leprosy is in many ways a unique disease due to the extremely slow multiplication rate of *M. leprae* and the low toxicity of the bacillus. But in the wide spectrum of immunological features and reactions, it resembles other diseases that are also described as "spectral" diseases. Cutaneous leishmaniasis is a notable example. Important studies on cutaneous leishmaniasis have been made in Ethiopia; it occurs in different forms and one form, "diffuse cutaneous leishmaniasis," may easily be mistaken for lepromatous leprosy[45,46]. Studies on leishmaniasis are of interest in the context of this disease and in comparison with leprosy since information obtained from the study of one disease may provide us with a better understanding of the other. At AHRI, Ayele Belehu has obtained data through his studies of leishmaniasis that are of immediate interest to immunological studies of leprosy, e.g., in work on development of diagnostic procedures through studies of serum antibodies, through studies of the LTT test, and in studies of immune complex formation. Another disease studied at AHRI in the past is syphilis. Immuno-suppressive plasma factors were demonstrated in Ethiopians with early syphilis[47]. Their disappearance in late cardiovascular syphilis[47] is interesting in relation to disappearance of similar factors in leprosy patients during development of reversal reactions[48]. Combined leprosy and tuberculosis programs are currently developed in several African countries. Immunological studies in parallel of leprosy and tuberculosis are also envisaged to be valuable for our views concerning both diseases.

Barnetson and Bjune found a significant LTT reactivity to *M. leprae* in babies of mothers exposed to *M. leprae*[49]. This is one of the first reports indicating that a transfer of cell mediated immune reactivity from mother to baby may take place. Melsom and Duncan studied immunoglobulins and anti-*M. leprae* antibodies in babies of mothers with various forms of leprosy. Babies of mothers with active lepromatous leprosy had a statistically increased concentration of IgA in cord serum[50]. This indicated that the immune system of these babies was stimulated to immunoglobulin production *in utero*, possibly by transfer of *M. leprae*

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[42] Yoder, L., Naafs, B., Harboe, M. and Bjune, G. Antibody activity against *Mycobacterium leprae* antigen 7 in leprosy: Studies on variation in antibody content throughout the spectrum and on the effect of DDS treatment and relapse in BT leprosy. Lepr. Rev. **50** (1979) 113–121.

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antigens or live *M. leprae* bacilli across the placenta. A solid radio immunoassay was developed for demonstration of anti-*M. leprae* antibodies of the IgA and IgM classes[51], and anti-*M. leprae* 7 antibodies were assayed by radio immunoassay[52] in serial blood samples obtained through the first months of life. Many babies of mothers with active lepromatous leprosy had IgM and IgA anti-*M. leprae* antibodies in cord blood[51] and showed signs of active anti-*M. leprae* 7 antibody formation during the first months of life[52]. This indicates an early exposure to *M. leprae* antigens sufficient to stimulate the immune system of the offspring. The clinical consequences of this early exposure should be carefully followed up.

PROSPECTS FOR FURTHER WORK ON THE IMMUNOLOGY OF LEPROSY DURING THE NEXT DECADE

Development in basic immunology. The demonstration of B- and T-lymphocytes as separate cell populations with different functions, but still closely interdependent, in the late 1960s and the 1970s implied a profound change in immunology. As is apparent from this paper, it also changed completely the approach to immunological studies of leprosy. Recently, the "hybridoma" technique has been developed[53]. By fusion of a normal antibody producing mouse B-cell with a suitable mouse plasmacytoma cell, hybrid cells may be obtained capable of continuous growth and secretion of homogeneous, monoclonal antibodies in large amounts. Hybridomas have been made which produce antibodies against a wide variety of antigens. We are only at the beginning, and this technique will probably in a few years' time revolutionize antibody production and the methods for preparation of immunological reagents.

The development of immunology in the 1980s cannot be foreseen and may have an equally profound influence on immunological studies of clinical disease. Our challenge is to insure that advances in basic immunology are rapidly and carefully applied to the study of clinically important problems in leprosy.

The stage for leprosy research. Leprosy research will continue to be carried out in different types of institutions and in different administrative contexts. ARHI is a laboratory with modern equipment and competence in leprosy research being situated in a leprosy-endemic country and closely collaborating with ALERT which is a prominent teaching institution of leprosy. This combination will continue to offer unique opportunities for work in the immunology of leprosy and for further significant contribution in this area.

Leprosy has been included as one of the 6 diseases in WHO's Special Programme for Research and Training in Tropical Diseases. The immunology of leprosy (IMMLEP) component of this program has been a major stimulating factor in the work on leprosy. Among the priorities set by IMMLEP for its work are production of *M. leprae* in large amounts through experimental inoculation of armadillos, development of new diagnostic reagents particularly for diagnosis of subclinical infection, and development of efficient methods for preventive work with the main emphasis being put on development of a leprosy vaccine. There is wide agreement with regard to these goals. Opinions vary more with regard to the procedures and techniques to be followed to reach these goals. In particular, it is uncertain if sufficient basic knowledge is yet available for selection of the best approach for development and testing of a leprosy vaccine.

Until now, leprosy research has attracted relatively few laboratories and research workers. The number of people engaged in leprosy research needs to be expanded. This is particularly important both to increase the capacity for work and to bring new concepts and approaches to the field. In this area of research promotion,

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IMMLEP has a particularly important role to play by providing purified *M. leprae* bacilli to the investigators. Supply of *M. leprae* has been a major restrictive factor in the past for the development of leprosy research.

Various types of institutions in leprosy non-endemic countries have also contributed very significantly in the past to the study of leprosy.

Our resources are limited and must be carefully used. We have to use all existing knowledge and stimulate collaboration between various institutions so that the special opportunities of each institution are considered and explored. It is particularly important that careful clinical studies be carried out directly in connection with laboratory work in leprosy-endemic countries. Long term studies with careful serial observations on patients have been rather few in the past. They are difficult to carry out but are expected to be especially important in leprosy with a protracted chronic course interrupted by "reactions" with increased signs of inflammation in the lesions, particularly since the latter are responsible for a great part of the deformities often associated with leprosy.

Mechanisms of protective immunity. In certain infections, antibodies are mainly responsible for induction of protective immunity. The mechanisms of protection are in many cases understood in great detail. Antibodies against certain antigens lead to protection whereas antibodies against other constituents of the same microorganism do not have this effect. Antibodies of a particular specificity may be quantitated and related to protective effect both in a given individual and in population studies.

In cases where cell mediated immune reactions are of main importance for resistance, much less is known about the mechanisms of protective immunity. We do not know if certain antigens in the microorganisms are particularly important for development of protective immunity, and in several systems it is still not clear how these antigens shall be demonstrated and defined. Cell mediated immunity depends on T-cell functions. In several species the T-cell compartment has been divided into different subpopulations, each with characteristic functions. It is not known which subpopulation is of main importance for the development of protective immunity in leprosy. The relation between resistance and hypersensitivity has been the subject of intense debate and controversy for many decades, and the question is not yet settled. This applies to several mycobacterial infections, including leprosy. Laboratory methods are available (e.g., the LTT test) in which the result in individual patients is closely correlated with hypersensitivity whereas correlation to protective immunity is much less pronounced. It appears that at present we have no reliable method for assay *in vitro* in man of a person's ability to resist a mycobacterial infection, including leprosy. Further work on experimental models is thus greatly needed to obtain more basic information on these truly essential points.

The selection of appropriate experimental models is still difficult. Shepard's demonstration[54] of limited multiplication of *M. leprae* in the foot pad of the normal mouse was a major contribution in experimental leprosy and is currently the method of choice for studies of the effect of drugs and development of resistance[55]. The T-cell deprived mouse develops a generalized infection similar to lepromatous leprosy after experimental inoculation and provided basic new information on the mechanisms of reversal reactions[55,56]. It is uncertain to what extent the normal mouse can provide information on acquired immunological resistance to leprosy infection since *M. leprae* is almost non-pathogenic in this species. A main advantage of the mouse is, however, our extensive knowledge of its immune system and the ample opportunities for manipulation of the immune system, e.g., by cell transfer.

The armadillo has one major advantage. It is naturally susceptible to infection with *M. leprae*[31], and individual animals show a varying degree of susceptibility since intravenous inoculation of armadillos with 1–10 $\times$ 10^7 *M. leprae* leads to development

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of a systemic mycobacterial disease in about 40–60% of the animals. A serious drawback is our fragmentary knowledge of its immune system.

The use of another slow-growing mycobacterium in the mouse appears to be particularly valuable, and *M. lepraemurium* (MLM) has been widely used. The taxonomic position of MLM is not defined; our data indicate that it is antigenically related to *M. leprae* but more closely so to *M. avium*. A major advantage of this model is that different inbred strains are available with completely different levels of natural resistance to MLM infection[57]. This offers a unique opportunity to study the genetic factors involved in resistance to a leprosy-like infection. MLM infection also offers a relevant model to study induction of and the mechanisms involved in protective immunity[57,58,59].

Antigenic structure of *M. leprae*. Crossed immunoelectrophoresis with rabbit antibodies against *M. leprae* has permitted a precise definition of a series of antigenic components in *M. leprae*[32,33]. Antibodies against components no. 2, 4, 5, 6 and 7 are most readily produced in rabbits, and these antigens are present in most *M. leprae* sonicates. An advantage of this approach is that the technique defines directly different components of *M. leprae* that are involved in induction of an immune response. The antibodies most readily formed against *M. leprae* components in rabbits correspond to the most frequently occurring antibody specificities after *M. leprae* infection in man[32,33] and the armadillo[60] and in MLM infection in the mouse[61]. Use of concentrated *M. leprae* antigens and concentrated antibody reagents in crossed immunoelectrophoresis has permitted identification of more than 20 distinct antigenic components in *M. leprae*[33]. This system is more reagent consuming and difficult to apply on a larger scale but should be intensively exploited for studying the taxonomic relationship of *M. leprae* to other mycobacteria and for further definition of the specificity of the humoral immune response in leprosy.

The antigenic determinants on individual components should also be studied in detail. On one component, some determinants may be cross-reactive while others may be highly species specific for *M. leprae*[35,36,38]. Antibodies against the latter type of determinants would be particularly valuable for immunological identification of *M. leprae*, and the hybridoma technique should be explored to produce monoclonal antibodies with this type of specificity.

Additional knowledge of the antigenic structure of *M. leprae* is required for further work on development of diagnostic reagents and to establish reliable methods for control of *M. leprae* antigenic preparations. Simultaneous studies on the reactivity of highly purified components in the humoral and the cell mediated immune response in various clinical forms of leprosy are also greatly needed.

Immunodeficiency in lepromatous leprosy. The lack of cell mediated immune reactivity against *M. leprae* is responsible for the uninhibited growth of *M. leprae* in these individuals, leading to highly bacilliferous forms of the disease, and these patients are of major importance for dissemination of the infection. The nature of this immunodeficiency has been explored by various investigators. Alternative explanations are still favored[5,17,21,62,63,64], and the

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basic nature of this defect is not known. I think that an increased insight in this defect is of fundamental importance to understand leprosy. The available evidence indicates that prolonged drug treatment does not reverse the defect[17], and this is apparently the main reason for the high risk of relapse in lepromatous leprosy, a risk even more important in the light of the recent demonstration of frequent occurrence of dapsone resistant *M. leprae* in Ethiopia[65]. Detailed understanding of this basic defect may provide new means for immunotherapy directed against correction of this defect, which would be of utmost importance for prevention of relapse in patients with lepromatous leprosy.

Antigens of *M. leprae* that reside within macrophages must be expressed on the cell surface or be liberated from the cells to stimulate the immune system of the host. We know almost nothing about this essential process. The immunological consequences of occurrence of *M. leprae* in different cell types (e.g., in muscle cells, Schwann cells, or in the endoneurium versus macrophages) should be carefully studied since it may be an important escape mechanism permitting *M. leprae* to avoid destruction during an early phase of the infection and thus favor development of chronic disease[64].

Reactions. Immune reactions are responsible for important clinical complications, mainly nerve damage, which in turn is the cause of the deformities so often associated with leprosy today. Immunological studies of reversal reactions have led to better understanding of their nature and improved methods of treatment. Further work on reversal reactions and ENL is strongly indicated, particularly long term studies with simultaneous recording of careful clinical observations and a variety of laboratory investigations. Immunofluorescence studies of ENL defined the condition as an immune complex disease[25]. The lack of additional published immunofluorescence studies of this disorder is striking.

Diagnosis of subclinical infection. This is important for epidemiological studies to assay the infectious load and occurrence of *M. leprae* infection in different communities as a basis for leprosy control programs and better methods of prevention. Further work is required both with assays of cell mediated immunity and serum antibodies. Skin tests and *in vitro* techniques for assay of cell mediated immune reactions should be further explored, particularly with regard to their specificity. New techniques should be sought for improving their specificity. Further work is also required on tests for demonstration of *M. leprae* specific antibodies such as the fluorescent leprosy antibody absorption test of Abe[37] and radio immunoassays[38]. Additional information is required on the development of cell mediated reactivity and formation of antibodies after initial infection to establish which test is best for diagnosis of significant subclinical infection with *M. leprae*. A combination of the two procedures may be required since early detection of the individuals whose disease progresses towards the lepromatous pole is particularly important in view of their high contagiousness and since these patients are expected to have negative tests for cell mediated reactivity.

Vaccination. Priority should continue to be given to work aiming at development of a vaccine against leprosy. To me, several data indicate that the time is not yet ripe for decision on the contents of a vaccine. Selection of one or a few principles for production of a vaccine would be considerably facilitated if tests for ability to develop increased resistance were available since this would make it possible to test several different procedures in pilot experiments on smaller groups of individuals. Extensive preliminary investigations have to be carried out to establish the optimal conditions for field trials of a leprosy vaccine, the cost of such procedures, and the reliability of the evaluation of the protective effect of the vaccine.

Leprosy as a model. The study of immune reactions in leprosy has been one of the most active fields of leprosy research during the last decade. Previously, leprosy was far behind other infectious diseases with regard to basic information and un-

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derstanding. This has rapidly changed during the last decade. It has become increasingly apparent that leprosy offers unique opportunities for studies of the relationship between the host and the parasite during a chronic infection where most symptoms are due to the immune response to antigens of an otherwise virtually non-toxic microorganism. Leprosy is thus developing as a "model disease" which provides essential information on the importance of immune reactions in several chronic infectious diseases.

—Morten Harboe