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EDITORIALS

Editorial opinions expressed are those of the writers.

Significance of Antibody Studies in Leprosy and Experimental Models of the Disease[1]

Infection with *Mycobacterium leprae* induces a complex immune response of the host involving both cellular immune reactions and antibody production.

Since *M. leprae* is an obligate intracellular parasite, cell-mediated immune reactions have been considered to be of main importance in the defense against the infection[2], and antibodies have generally not been considered to be of importance in this regard. This view has been widely held for many years. The immune response is, however, far more diversified than previously realized. The classic view now needs to be analyzed in more detail based on the new information on T cell subsets and the occurrence of various cell-mediated immune reactions which may have entirely different effects on the infection.

The reaction of mycobacterial antigens with sensitized T cells resulting in the liberation of various lymphokines and macrophage activation is considered to be the main mechanism for defense against mycobacterial infection[3]. T helper cells are mainly responsible for the release of macrophage activating lymphokines[4]. However, stimulation of other T cells with the induction of suppressor mechanisms may directly favor bacterial growth. An imbalance favoring T suppressor cell activity is a major current hypothesis to explain the lack of resistance in multibacillary forms of leprosy[5,6,7].

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[1] The Armauer Hansen Research Institute (AHRI) in Addis Ababa, Ethiopia, has organized an annual Armauer Hansen Memorial Lecture. The major objectives of this annual lecture are a) to bring new research ideas in the immunology of leprosy to AHRI, b) to motivate on-going research, and c) to attract new immunologists into leprosy research by bringing external investigators to AHRI. The JOURNAL is honored to publish this guest editorial by Professor Morten Harboe based on the first annual Armauer Hansen Memorial Lecture given by him on 12 February 1982.

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Why are antibodies of interest, and why should we study them?

We can use them as probes to learn and try to understand what goes on during the disease. We have to study antibodies to learn whether there is an interaction between the humoral and cellular immune responses in which aberrant antibody production may lead to decreased cellular immune response and decreased resistance. Another main question is: Do antibodies arise after infection and prior to the development of clinical symptoms? If this is the case, we can study the epidemiology of leprosy infection and not just the epidemiology of the disease and its complications, as has mainly been the case until today. Finally, antibodies are an essential tool for studying the immunogenic structure of *M. leprae*.

A thorough knowledge of the immunogenic structure of *M. leprae* is required for further development and understanding of immunological tests and immunological aspects of the disease process in leprosy.

After the introduction of the armadillo model[8], *M. leprae* became available in much greater amounts than in the pre-armadillo era. This made detailed studies of the immunogenic structure of *M. leprae* possible.

In the pre-armadillo era, a few *M. leprae* antigens were demonstrated. These were a polysaccharide antigen[9, 10], the beta and delta antigens of the Gothenburg group[11], and the "nodule extract protein," the NEPR antigen of Abe, *et al.*[12, 13]. Recent findings by Closs (unpublished observations) indicate strongly that the latter antigen is not a constituent of *M. leprae* itself but an antigen of host origin induced by the granulomatous process.

After armadillo-grown *M. leprae* became available, we studied the antigenic composition of *M. leprae* by crossed immunoelectrophoresis (CIE), using sonicates of purified *M. leprae* for immunization and testing and rabbit antisera as the antibody reagent in the top gel. CIE is a potent technique for precise identification of immunogenic components of *M. leprae* which induce formation of precipitating antibodies after immunization of rabbits. Initially, seven distinct antigenic components of *M. leprae* were identified and numbered[14]. All of them are involved in the induction of a humoral immune response in leprosy, although the specificity varies in individual patients. Antibodies against *M. leprae* antigen 2, 5 and 7 are most frequently formed[14].

Components No. 2, 5 and 7 are particularly strong immunogens during the development of systemic *M. leprae* infection in the armadillo[15] and after immunization of rabbits with ultrasonicated purified *M. leprae*. The pattern with only seven lines in CIE was markedly different from the pattern obtained with antisera against other mycobacteria produced both in our laboratory[14, 16, 17] and in other laborato-

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nosuppression during a *Mycobacterium leprae* infection determine the leprosy spectrum? Lepr. Rev. **52** (1981) 1–10.

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ries[18,19]. By the use of concentrated antigen for immunization and testing, more than 20 distinct antigenic components were later demonstrated in *M. leprae*[20]. This implies that we can study the immunogenic structure and the immune response to *M. leprae* at almost the same level as the response to other mycobacteria. CIE systems producing more than 20 precipitate lines with *M. leprae* are, however, very expensive with regard to reagents, and difficult to establish. Due to the limited availability of reagents, we have not been able to do the number of experiments needed with this system. The use of polyacrylamide gel electrophoresis with and without SDS combined with "immunoblotting" is currently being explored for its value in extending the information obtainable by conventional crossed immunoelectrophoresis.

Various terms have been used in this field during the last decade: *M. leprae* specific component, *M. leprae* specific antigen, and *M. leprae* specific antigenic determinant. There is an obvious need for precise definitions of terms. By the terms *M. leprae* specific component and *M. leprae* specific antigen, we mean a component which is present in *M. leprae* but not in other mycobacterial species[21]. As far as we know, no *M. leprae* specific component has yet been demonstrated. On a component which is present in *M. leprae* and crossreacts with other mycobacteria, there may be some antigenic determinants which are specific for *M. leprae*, as initially demonstrated by Kronvall, *et al.*[22]. Based on the principle of absorption, assays may be developed for the demonstration of antibodies against *M. leprae* specific determinants irrespective of their occurrence on crossreacting components[23,24,25].

For the demonstration and quantification of antibodies against a crossreacting component of *M. leprae*, a radioimmunoassay for antibodies against *M. leprae* antigen 7 was developed[26] based on prior experience with the radioimmunoassay of antibodies against BCG antigen 60[27,28]. *M. leprae* antigen 7 may be labeled with iodine quite selectively. It is localized in the cell wall of *M. leprae* and crossreacts extensively with similar antigens in other mycobacterial species, *e.g.*, with BCG antigen 60[14] and antigen 38 of *M. lepraemurium*[14]. Other properties related to our selection of this antigen for study is that humoral immune responses in leprosy are very frequently directed against this component[14], and it is closely related to the main precipitating component of tuberculin PPD[17]. Later experiments have shown that preparations in which essentially only antigen 7 can be demonstrated by CIE have a strong stimulating capacity in the lymphocyte stimulation test with cells from patients with tuberculoid leprosy; whereas it fails to stimulate lymphocytes from lepromatous leprosy patients[29,30]. Antibodies against *M. leprae*

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antigen 7 react with different determinants on this micromolecule, some being of polysaccharide nature and others of non-polysaccharide nature[31].

By definition, an increased concentration of anti-*M. leprae* 7 antibodies is not specific for leprosy, but is also expected to occur in other mycobacterial infections. Antibody assays involving crossreacting components may, however, be particularly sensitive for detecting mycobacterial infection[32]. In several instances tests of this sort may be particularly valuable in epidemiological and field studies. Other tests for antibodies against *M. leprae* antigens which do not claim specificity have also been developed, *e.g.*, by using labeled protein A as an indicator of antibody binding[33], and immunoglobulin class specific assays for antibodies against ultrasonicated *M. leprae* bacilli using radiolabeled anti-IgG, anti-IgA or anti-IgM antibodies as indicators[34,35], or in corresponding ELISA techniques.

The first generation assays for antibodies against *M. leprae*-specific antigenic determinants were based on radioimmunoassay[25] or immunofluorescence[23,24], employing absorption with other mycobacteria to remove antibodies against crossreactive determinants. The application of this type of absorption in model experiments indicates that a major part of the antibodies in lepromatous leprosy sera are directed against determinants which are highly specific for *M. leprae*[25]. Assays for antibodies of this kind would be very important to obtain serological evidence of infection, provided that the test shows a sufficient and entirely reproducible specificity. In applications of our original radioimmunoassay for *M. leprae*-specific antibodies in field studies, we have encountered some difficulties, and absorption with other mycobacteria in addition to BCG is necessary to obtain complete specificity for *M. leprae*. The mycobacteria to be used for absorption may vary in different populations, depending on the occurrence of other mycobacterial species in the area tested. Similar difficulties in obtaining sufficient specificity have been encountered in the immunofluorescent test of Abe, *et al.*[36].

The highly variable course after infection with *M. leprae* indicates that antibody studies may be valuable to characterize important immunological features of the disease and the subclinical stage. After exposure to *M. leprae*, some individuals control the infection to such an extent that there will be no visible lesions. This is denoted "subclinical infection." The factors responsible for the induction of this course are partly of an immunological nature but, to a great extent, they are unknown. If the proportion of individuals with this course after infection could be increased, it would be an important factor in reducing the extent of disease in the population. Improvement in socio-economic conditions, hygiene, and nutrition all contribute to enlarge the number of individuals in this group.

Some individuals develop a few lesions which heal spontaneously. Most of these are clinically diagnosed as "indeterminate leprosy." Improved methods for a more precise diagnosis of indeterminate leprosy are essential in order to provide more reliable information on the frequency of this form of the disease and its clinical consequences, spontaneous healing, or progression to persisting disease.

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Persisting disease is classified as a spectrum between two polar forms[37]. Tuberculoid (TT) leprosy is characterized by a few lesions containing few bacilli, development of cell-mediated immune reactions, and relatively high resistance. In polar lepromatous (LL) leprosy uninhibited bacterial growth eventually results in lesions containing vast amounts of bacilli which represent an enormous antigenic load. The immune system must be intensely stimulated, but on the effector side the response is virtually only by production of antibodies. There is no cellular immunity against *M. leprae*, and this specific immunodeficiency is probably directly responsible for this particular clinical course. It is also responsible for the high risk of relapse after stopping drug treatment. Various mechanisms have been proposed for this specific immunodeficiency[38,39,40,41], and a major current view is that increased suppressor cell activity is responsible for the lack of development of efficient cell-mediated immunity[5,6,7]. There is a great need for further work to characterize the specificity and the basic nature of the cellular immunodeficiency in lepromatous leprosy. It is not adequately established whether increased suppressor cell activity is a primary or a secondary phenomenon, and it is entirely possible that different mechanisms may be responsible for the development of this immunodeficiency.

Few data are available on the site of production of anti-*M. leprae* antibodies in lepromatous leprosy. By *in vitro* culture of biopsies of local skin lesions from patients with lepromatous leprosy in media containing radioactive amino acids, it has been shown that anti-mycobacterial antibodies are produced locally in skin lesions with different specificities in individual patients[42]. It has also been demonstrated that antibodies are produced locally, not only in skin lesions but also at other locations, *e.g.*, in the nasal mucosa, in the larynx, and in lymph nodes[43]. Antibodies are thus produced locally in various sites where leprosy bacilli probably provide a local stimulus for antibody synthesis. It is indeed striking that the specificity of the immune response may vary from one location to another, *e.g.*, from the skin to the mucous membrane of the nose, in individuals with such an enormous load of bacillary antigen affecting the immune system[43]. Several investigators have shown that the median antibody concentration is higher in lepromatous than in tuberculoid leprosy[32,44]. This is to be expected if it is the number of bacilli, that is the antigenic load, which mainly determines the antibody concentration. What we think may be even more striking is the wide variation in antibody concentration in patients with a similar clinical classification[44] which has been demonstrated in patients towards both the lepromatous and the tuberculoid end of the clinical spectrum of leprosy. The reason for this wide variation in antibody activity is not known, and is important to delineate. We have found that patients with relapsing borderline tuberculoid (BT) leprosy tend to have higher anti-*M. leprae* antibody activity than patients with early forms of BT leprosy[45]. This might be expected from an immunological point of view; repeated liberation of antigen during relapse is expected to be a particularly strong stimulus for the immune system.

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The wide variation in antibody activity in patients with a similar clinical classification makes it imperative to study experimental models to see whether antibodies can be a reliable indicator of infection in individual patients. Evidence on this point was sought through studies of armadillos inoculated with *M. leprae*. The anti-*M. leprae* 7 antibody activity was assayed in serial samples from individual armadillos, and the findings in the antibody assay were correlated with various criteria for the development of systemic mycobacterial infection. The animals were regularly examined for nodules at the inoculation site and elsewhere, and for acid-fast bacilli in their blood (buffy coat), ear clip preparations, and nasal washings. The data have not been fully analyzed but have been published in part[32].

Some animals showed completely flat curves with no signs of antibody formation; other animals showed an initial flat plateau followed by a marked increase in antibody concentration. There was a close correlation between positive findings in the antibody assay and the development of systemic mycobacterial infection after inoculation: among 35 inoculated armadillos, 25 developed signs of systemic infection; 24 of these were positive, and 1 was negative in the antibody assay. Nine out of the ten animals which did not develop signs of systemic infection were negative in the antibody assay. In one animal with a false-positive result, the antibody assay became positive 24 months after inoculation, which is later than in all but one of the animals that developed clinically detectable systemic infection.

A crucial question is: Does the antibody activity increase significantly before the animal shows definite clinical signs of disease?

A nodule at the inoculation site was usually the first sign of mycobacterial infection in these intravenously inoculated animals. This often occurred slightly before the antibody assay became positive. The development of a nodule at the inoculation site has certain distinctive features, and is quite different from other signs of systemic infection. A positive result in the antibody assay was thus obtained before the appearance of nodules at other sites. A positive antibody assay was also obtained earlier than the other criteria of systemic mycobacterial infection which became positive in the following order: acid-fast bacilli in peripheral blood, acid-fast bacilli in ear clips, and acid-fast bacilli in nasal washings. Increasing anti-*M. leprae* antibody activity is thus an early indicator of infection in the armadillo. It should be borne in mind, and it may be an essential point, that the histological features of the lesions in the armadillo correspond closely to lepromatous and not to tuberculoid leprosy in man. The relevance of these findings for diagnosing infection before the development of clinical signs may thus apply particularly for disease towards the lepromatous end of the leprosy spectrum.

Assays for anti-*M. leprae* antibodies in the various immunoglobulin classes have also provided interesting results which need careful consideration and further work. IgG anti-*M. leprae* antibody activity is low in healthy controls and in patients with indeterminate leprosy, with a higher median value in borderline tuberculoid leprosy, and a markedly higher median value in lepromatous leprosy. Also, in this system, there is marked variation in antibody activity in individual patients with similar clinical classification. In the assay for IgM anti-*M. leprae* antibodies, it was found that the antibody activity was higher in the indeterminate leprosy group than in the control group[46]. Contrary to earlier statements, this indicates that the immune system has indeed already been triggered in indeterminate leprosy and that the assays for IgM anti-*M. leprae* antibodies may provide additional information related to the diagnosis of this condition. Further studies are needed, however, to substantiate this finding.

In studies of intrauterine infection, it is well known that antibodies of various immunoglobulin classes are of different diagnostic value due to the transfer of maternal IgG across the placenta and the lack of transfer of IgA and IgM. The presence of IgA or IgM antibodies in cord sera is therefore an important indicator of intrauterine infection. Melsom, *et al.* found IgM and IgA

[46] Melsom, R., Harboe, M., Myrvang, B., Godal, T. and Belehu, A. Immunoglobulin class specific antibodies to *M. leprae* in leprosy patients, including the indeterminate group and healthy contacts as a step in the development of methods for sero-diagnosis of leprosy. Clin. Exp. Immunol. (in press).

anti-*M. leprae* antibodies in cord serum from 30% to 50% of the babies of mothers who had active lepromatous leprosy during pregnancy; whereas such antibodies were not found in babies of mothers with tuberculoid leprosy or nonleprosy controls[34]. The activity in the assays for IgA and IgM anti-*M. leprae* antibodies increased early after birth; the concentration of IgM anti-*M. leprae* antibodies between two months and four months of age being higher in babies of mothers with bacilliferous leprosy than in babies of mothers with paucibacillary BT leprosy[35]. These findings indicate a transfer of *M. leprae* antigen or live *M. leprae* bacilli with the stimulation of the immune system *in utero* and early after birth. The consequence of this early stimulation of the immune system should be established by careful clinical and immunological follow-up studies.

The relationship between mycobacterial antigen content in the body and stimulation of the immune system is also evident from studies of antibody activity during treatment. In lepromatous leprosy, dapsone (DDS) treatment is associated with a gradual decrease in antibody activity as detected in several types of assays[26, 27]. The median decrease in antibody activity during approximately three years of treatment corresponds to the difference in median antibody activity between untreated lepromatous and tuberculoid leprosy[47]. In tuberculoid leprosy, the course may be different. Studying groups of patients with BT leprosy treated for various periods of time, Yoder, *et al.* initially demonstrated that the median antibody activity decreased during the first three years of DDS treatment, and that suspected or clinically proved relapse was associated with renewed synthesis and increased anti-*M. leprae* 7 antibody activity[44]. Studies of serial samples from individual patients have later indicated that in some patients there is a gradual decline in antibody activity, usually associated with clinical improvement. In other patients, the antibody activity does not decrease, and this tends to be associated with lack of clinical improvement. In patients with newly diagnosed BT leprosy treated with DDS, there is often an initial rapid increase in anti-*M. leprae* antibody activity followed later by a decrease correlated with clinical improvement. This indicates that treatment may induce the liberation of mycobacterial antigens which strongly stimulate the immune system, resulting in increased antibody synthesis[48]. Similar findings have been made in tuberculosis[49].

Antibodies are considered responsible for erythema nodosum leprosum (ENL). In lepromatous patients we have an ample release of antigens from the bacillus and usually a high concentration of anti-mycobacterial antibodies in the blood and tissue fluid. The formation of antigen-antibody complexes is thus expected to occur, which may initiate complement activation and local inflammatory reactions. In the medical literature ENL is one of the classical examples of an immune complex disease. This view is not so firmly established as generally thought. After the first paper showing immune complexes at the site of ENL lesions[50], there was a striking lack of additional papers confirming and extending this observation. So, it is apparently very difficult to demonstrate mycobacterial antigens and immune complexes at the site of early lesions of a disease which nevertheless has been presumed to be a classical example of an immune complex disease. There is an obvious need to look at ENL with a free mind, exploring other mechanisms for inducing or contributing to the tissue damage and vasculitis so characteristic of this condition. Recent investigations have revealed an imbalance of immune regulatory mechanisms in patients with ENL with the ratio between T helper and T suppressor cells rising markedly during[51] and even preced-

[47] Melsom, R., Harboe, M. and Naafs, B. Class specific anti-*M. leprae* antibody assay in lepromatous (BL-LL) leprosy patients during the first 2–4 years of DDS treatment. Int. J. Lepr. **50** (1982) 271–281.

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ing[52] attacks of ENL. Recent immunohistological studies of skin biopsies from patients with lepromatous leprosy with and without ENL[53] showed that the presence of acute local vasculitis and inflammatory infiltrates did not correlate with the presence or absence of immunoglobulin or complement deposits in early ENL lesions. Immune complex deposits may, therefore, not be an essential feature of early ENL lesions, and such complexes may be secondary rather than primary in ENL.

Finally, I will present a personal view on further work with antibodies in leprosy and corresponding experimental models. How are we going to study antibodies in the future and what are the challenges and the possibilities in this area?

We are, of course, in the fascinating beginning of work with monoclonal anti-*M. leprae* antibodies produced by the hybridoma technique. Monoclonal antibodies produced by this technique are exquisitely specific and, by definition, each antibody that we are able to produce will be directed against one antigenic determinant present on the surface or inside *M. leprae*. One problem with the technique is that it is difficult to predict what type of determinant we will be able to produce antibodies against. At present, at least five major laboratories are involved in experiments on producing monoclonal antibodies against *M. leprae*. International collaboration with exchange of reagents is needed for optimal use of this technique, and the specificity of the antibodies produced in different laboratories needs to be compared and defined with regard to reactivity with defined components of the bacillus.

How useful will these antibodies be? If they are directed against determinants specific for *M. leprae*, they will become the reagents of choice for identification of acid-fast bacilli as *M. leprae*. If we are lucky and the determinants are sufficiently immunogenic during natural infection, we can demonstrate by inhibition assays antibodies of the same specificity in the sera of single individuals after infection resulting in significant bacterial multiplication, but hopefully before they develop clinical symptoms. This would be a great advance and, combined with studies of *M. leprae*-specific cell-mediated immune reactions, we would be in an entirely different situation in leprosy epidemiology. We would no longer study only the epidemiology of the disease but also the epidemiology of *M. leprae* infection, and be able to study the factors of importance for the transition from infection to disease.

There is a great need for further work on experimental models. Various models are valuable for various purposes, and monkeys are of great current interest. Some monkey species are naturally susceptible to leprosy and develop systemic infection after inoculation with *M. leprae*[54]. The immune system of monkeys is probably more similar to man than the immune system of the armadillo. The mouse is obviously an animal of great importance in some aspects of leprosy research, but in others it is difficult to evaluate its significance; the main point being that *M. leprae* is not a natural pathogen of the mouse. The study of protective immunity and methods to increase resistance needs work in a species in which *M. leprae* is a natural pathogen in order to define the conditions required for increasing in a specific way the ability to resist the infection. In this area *M. leprae*-susceptible monkeys would appear to be particularly useful, and antibody assays a suitable tool for following the development of *M. leprae* infection in the animals under different experimental conditions.

IgA anti-*M. leprae* antibodies and their significance need to be further explored. The gut associated immune system appears to be an immune system partly of its own in humans and in experimental animals. It illustrates more than probably anything else the importance of cell traffic in the immune system and its remarkable functional adaptation. Local antigen stimulation in the gastrointestinal tract leads to local produc-

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tion of IgA antibodies that are of main importance for local defense against infection in the gut. Cells involved in the development of local IgA antibody production in the gut mucosa not only do their job there, but some of these cells move from the gut to the mammary glands so that maternal antibodies of the same specificity may be transferred through the milk to the offspring to protect its gastrointestinal tract. This defense mechanism has been demonstrated to be active against several toxins of gastrointestinal bacteria, *e.g.*, cholera toxin[55]. IgA anti-*M. leprae* antibodies have been demonstrated in larger amounts in milk samples from mothers with lepromatous leprosy than in milk samples from nonleprosy patients from the same area[56]. We have to study those antibodies further to learn more about them and whether they are an important indicator of gastrointestinal exposure to *M. leprae*.

There is a great need for further combined studies of antibodies and cell-mediated immunity, not only at the level of *M. leprae* component 7, which is the only component that has been studied specifically in this regard through collaboration between the Armauer Hansen Research Institute and our laboratory in Oslo[29,30], but also for other defined components of the bacillus. It remains a possibility that antibodies and cell-mediated immunity may interact and influence each other in a way that is of fundamental importance in leprosy. Aberrant antibody production may be of importance for the induction of deficient cell-mediated immunity, and thus for the development of multibacillary disease. We do not know. We need to know.

Prevention of leprosy—this is certainly what we are aiming at and working to develop efficient methods for. A main approach to this problem is the development of a leprosy vaccine which is the major priority of the WHO Immunology of Leprosy Programme. Further work toward the development of an antileprosy vaccine is certainly very important. There are, however, other approaches that should be explored, and the current state of knowledge indicates that we should keep a multifaceted approach in our work to attain better methods for leprosy control. The individuals with deficient cell-mediated immunity are the most important ones in many aspects. They are the patients with the most bacilli, and they are mainly responsible for the bacillary load in the population and thus for the spread of the disease. Early diagnosis, before they have spread a lot of bacilli, cannot be made by tests for cell-mediated immune reactions because this is what they lack. Therefore, we are left with the study of antibodies to try to provide the tool for early detection of this form of the infection. We do not yet know if we can provide antibody assays for reliable identification of single individuals at this stage. If they can be detected early, and if they can be effectively treated by intensive combined chemotherapy courses, we should concentrate our efforts on these key individuals, and we may have a new and another efficient way of preventing the spread of the disease.

So, in the beginning of the 1980s, I think that antibody studies in leprosy are not on the way out. Although antibodies are not considered to be of direct importance for resistance against the infection, they still provide us with a challenge to develop another logical way to combat the leprosy problem.

—Morten Harboe, M.D., Ph.D.

Professor of Immunology
Institute for Experimental Medical Research
Ullevaal Hospital
Oslo 1, Norway

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