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EDITORIALS

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Serodiagnosis of Leprosy: The Past, the Present, and Some Prospects for the Future

Leprosy is a disease caused by *Mycobacterium leprae*. This bacterium has a generation time of about 12 to 14 days[1, 2], and the incubation period of the disease varies from one to 20 years[3].

Humans exposed to leprosy go through phases. The greater part of a population having contact with patients with multibacillary forms of leprosy may be infected. This will lead to a limited multiplication of *M. leprae*[4]. The (immune) defense in most cases will limit and arrest the multiplication of leprosy bacilli before signs of the disease appear. In some individuals, the disease will progress to clinical forms of leprosy. The earliest recognized form of the disease is indeterminate leprosy. This is an early, unstable form of leprosy in which the majority of the patients will control the infection leading to spontaneous healing without treatment[5]. Some patients with indeterminate leprosy will progress to more persisting disease with a spectrum from paucibacillary tuberculoid (BT-TT) leprosy to multibacillary lepromatous (LL-BL) leprosy. The last form is the most infectious and has the longest incubation period, an average of four years from exposure to when the clinical signs of the disease appear.

Sulfone therapy in leprosy was started in 1941, and it was believed that leprosy could be controlled with the help of this drug. The appearance of dapsone (DDS) resistant leprosy, and the need to continue DDS treatment for life in lepromatous leprosy patients, has led to a failure in the control of leprosy by DDS alone in most areas with leprosy. Today on a world scale leprosy might be on the increase[6].

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[1] Levy, L. Studies of the mouse footpad technique for cultivation of *Mycobacterium leprae*. 3. Doubling time during logarithmic multiplication. Lepr. Rev. **47** (1976) 103–106.

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

[3] Newell, K. W. An epidemiologist's view of leprosy. Bull. WHO **34** (1966) 827–857.

[4] Taylor, C. E., Elliston, E. P. and Gideon, H. Asymptomatic infections in leprosy. Int. J. Lepr. **33** (1965) 716–722.

[5] Lara, C. B. and Nolasco, J. O. Self-healing or abortive and residual forms of childhood leprosy and their probable significance. Int. J. Lepr. **24** (1956) 245–263.

[6] Chatterjee, B. R. A view from the window. In: Gandhi Memorial Leprosy Foundation Silver Jubilee Commemorative Volume. Chatterjee, B. R., ed. New Delhi, 1978, pp. 386–395.

The screening programs for leprosy in endemic areas started a long time ago. These programs have been, and still are, hampered by difficulties in the diagnosis of early leprosy and leprosy in the incubation period. At the same time, it is increasingly important that an early diagnosis of leprosy be made since early antileprosy treatment can limit the spread of leprosy, and leprosy is easier to treat in the early phases of the disease.

Epidemiological studies of leprosy have been difficult to carry out due to:

a) the lack of methods to identify humans infected with leprosy bacilli without developing the disease;

b) the long incubation period with no possibility to diagnose subclinical disease;

c) the difficulties of diagnosing early leprosy such as indeterminate disease.

Similar work with tuberculosis has been helped, especially in the developed countries, by means of skin reactions such as Koch's delayed hypersensitivity test with old tuberculin. These tests made it possible to determine the number of humans infected with *M. tuberculosis* and helped epidemiologists to calculate the percentage of infected people who later develop tuberculosis. The value of these "Koch" derived, delayed-type hypersensitivity skin tests is somewhat less in the developing countries due to the occurrence of other mycobacteria which causes positive reactions to old tuberculin or PPD made from *M. tuberculosis*.

The lepromin skin test was introduced by Mitsuda in 1919, as a test for leprosy similar to the "Koch" delayed-type hypersensitivity test for tuberculosis. The lepromin reagent was made from human lepromas and later from *M. leprae* isolated from livers of *M. leprae*-infected armadillos. The lepromin test gives two reactions:

a) the early, or Fernandez, reaction which occurs 24 to 48 hours after the injection, and

b) the late, or Mitsuda, reaction which reaches a maximum around 21 to 28 days after the injection.

The lepromin test, especially the late reaction, is negative in lepromatous leprosy patients and positive in tuberculoid leprosy patients with a histological picture similar to that seen in tuberculoid leprosy skin lesions[7]. This pattern of response in leprosy patients is almost unique and cannot be reproduced by "lepromin-like" reagents made in the same way with other mycobacteria. When the lepromin test is used as an indicator of cell-mediated immunity (CMI) to *M. leprae*, lepromatous leprosy patients seem to lack CMI to *M. leprae*, while tuberculoid leprosy patients have CMI to *M. leprae*. *In vitro* cultivation and stimulation of cell preparations containing lymphocytes and macrophages from leprosy patients with *M. leprae* have indicated a similar defect in CMI to *M. leprae* in lepromatous leprosy patients.

High percentages of people living in areas without leprosy can develop a positive late lepromin test reaction[8]. Several studies have shown that 10%–15% of people living in close contact with lepromatous leprosy patients have a negative lepromin reaction, and only 10% of these eventually develop leprosy, mainly lepromatous leprosy. Considerably fewer lepromin-positive people with contact with lepromatous leprosy patients develop leprosy, and then only tuberculoid leprosy. The children of these leprosy patients often develop transient, indeterminate leprosy. The majority of these children were lepromin positive when the lesions were discovered, and after spontaneous healing the lepromin reaction was even stronger. Therefore the lepromin test cannot be used to:

a) indicate protection from leprosy, since both lepromin-positive and lepromin-negative individuals can develop leprosy;

b) evaluate people infected with leprosy, since humans in non-endemic leprosy areas can develop lepromin-positive reactions;

c) detect subclinical leprosy, since humans developing lepromatous leprosy are likely to have a negative lepromin reaction and only 10% of lepromin-negative contacts to lepromatous leprosy patients eventually develop leprosy.

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[7] Sato, S. Human leprosy. In: *Mykobacterium und mykobakterielle Krankenheit*. Band 4. Meissner, G. and Schmiedel, A., eds. Jena: Veb. Gustav Fisher Verlag, 1967.

[8] Guinto, R. S. Skin tests in leprosy. Ann. N.Y. Acad. Sci. **154** (1968) 149–156.

The main value of the test seems to be:

a) for differentiation between tuberculoid and lepromatous leprosy.

b) If a human exposed to *M. leprae* will develop leprosy, the possibilities are that it will be lepromatous or tuberculoid leprosy. Therefore, it appears that most lepromatous leprosy patients go through phases of leprosy—from subclinical leprosy, through indeterminate leprosy and possible borderline leprosy to lepromatous leprosy with a consistent negative lepromin reaction.

The importance of other tests for the detection of subclinical leprosy and lepromatous leprosy in the incubation period would therefore be great, and the search for a serodiagnostic test in leprosy has been carried out since the beginning of this century. Such a test should optimally help to:

1. Diagnose:
   a) people infected with leprosy;
   b) subclinical leprosy, especially in those individuals whose disease will progress to lepromatous leprosy and the median incubation period is seven years;
   c) indeterminate leprosy.
2. Help:
   a) in the epidemiological studies of leprosy;
   b) to select humans for vaccination trials, and to evaluate the effect of a possible vaccination within a reasonable time scale.

To try to evaluate such a test, I will discuss the serological tests carried out in leprosy in the past, what is being done at present, and what can be envisaged in the near future. This discussion is divided as follows:

The past. Prior to the successful inoculation and multiplication of *M. leprae* bacilli in animal models

The present. The multiplication of *M. leprae* in the armadillo and the larger quantity of *M. leprae* available for research purposes

The future. The development of the hybridoma technique and the possible production of monoclonal antibodies against *M. leprae* antigenic components in almost unlimited quantities.

THE PAST

The complement fixation test

Around 1900, complement fixation tests were introduced for the diagnosis of several infectious diseases such as syphilis, plague, and typhoid fever. In 1906 Eitner[9] showed that antigen preparations from lepromas could fix complement with sera from leprosy patients. Later antigen preparations from *M. tuberculosis* cultures *in vitro* were used[10] for the detection of antibodies in sera from leprosy patients, and standardization of the antigenic preparation from *M. tuberculosis* was tried[11, 12]. Sera from lepromatous leprosy patients showed high reactivity in the complement fixation test, while sera from tuberculoid leprosy patients showed poor or no reactivity[13]. Based upon these and similar findings using different test systems for antibodies in sera from leprosy patients, paucibacillary tuberculoid leprosy patients have been classified as "poor antibody responders" and patients with multibacillary lepromatous leprosy as "good antibody responders" with an antibody response "almost outside control"[14]. This has also been reflected by an increased immunoglobulin concentration, especially of the IgG class, but also of IgA and IgM[15], and by an increased frequency of autoantibodies like rheumatoid factors, anti-thyroid anti-

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[9] Eitner, E. Ueber den Nachweis von Antikörpern im Serum eines Leprakranden mittels Komplementablenkung. Wien. Klin. Wochenschr. **19** (1906) 1555–1557.

[10] Frugoni, C. and Pisani, S. Vielfache Bindungseigenschaften des Komplementes einiger Sera (Leprakranker) und ihre Bedeutung. Berl. Klin. Wochenschr. **46** (1909) 1530–1532.

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[13] Brants, J. Komplementbindungsreaktion mit dem Tuberkulose-antigen von Witebsky, Klingenstein und Kuhn bei Lepra. Dermatol. Wochenschr. **47** (1932) 1688–1691.

[14] Bullock, W. E. Anergy and infection. Adv. Intern. Med. **21** (1976) 149–173.

[15] Gupta, R. M., Gupta, S. C., Singh, C. and Khanna, S. Immunoglobulins in leprosy. Int. J. Lepr. **46** (1978) 342–345.

bodies, and anti-nuclear antibodies[16] in sera from lepromatous leprosy patients.

Antigenic components from several mycobacterial and diphtheroid species were found to fix complement with sera from leprosy patients[17], and since this was later confirmed as mostly occurring in sera from lepromatous leprosy patients, it was looked upon almost as diagnostic for lepromatous leprosy[18]. Since the complement fixation test was usually negative in paucibacillary tuberculoid leprosy and in early, indeterminate leprosy[19, 20], the test could not help in the diagnosis of leprosy. The complement fixation test still gave very useful information and brought the field of antibody formation in leprosy patients several steps forward. It took a long time before other test systems could give substantially more information than this test. The complement fixation test clearly demonstrated:

a) good antibody production in leprosy patients;

b) a distinction between lepromatous leprosy patients with high titers in their sera and tuberculoid leprosy patients with low titers in their sera;

c) antibodies in sera from leprosy patients mainly against crossreactive antigens of most of the mycobacteria isolated at that time.

Hemagglutination tests

The next major step in the development of serological methods in leprosy was the development of hemagglutination tests, described in 1948 by Middlebrook and Dubos[21], for mycobacterial antigen-antibody reactions. Sheep red blood cells (SRBC) were found to be the best carrier cells[22], and better results in leprosy were obtained with SRBC sensitized with *M. tuberculosis* than with *M. leprae* from human lepromas[23]. Low positive titers were found in sera from controls, somewhat higher titers in sera from tuberculoid leprosy patients, and the highest titers in sera from lepromatous leprosy patients[24]. Recently, a modified hemagglutination test, using tanned SRBC coated with leprosy bacilli purified from the liver of infected armadillos and *M. tuberculosis* and *M. vaccae* absorbed leprosy patients' sera, showed positive results (titer above 1:32) in all sera from lepromatous leprosy patients and in some sera from tuberculoid leprosy patients, while sera from tuberculosis patients and household contacts to leprosy patients were all negative[25]. It is unlikely that this test is *M. leprae*-specific since similar absorbed lepromatous leprosy sera were shown to contain antibodies reacting with *M. lepraemurium, M. bovis, M. flavescens, M. gastri, M. gordonae,* and *M. nonchromogenicum* by the immunodiffusion method[26].

The hemagglutination titers could be related to bacteriological findings since sera from patients with higher bacterial counts in skin smears had higher titers than sera from leprosy patients with negative or low bacterial counts in skin smears[24]. In some lepromatous leprosy patients a decrease in titers could be demonstrated in sera taken some months after sulfone treatment com-

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pared to the sera taken prior to treatment[27]. An increase in titers could also be demonstrated in sera taken during an ENL reaction compared to sera taken before this reaction[28].

The titers of sera from patients with active pulmonary tuberculosis were lower and a lower percentage of the sera showed a positive titer in the hemagglutination test than was found in sera from leprosy patients[29]. The hemagglutination test in leprosy has also indicated that there is a large crossreaction in antigen-antibody reactions using antigens from several mycobacteria (also *M. lepraemurium*) and sera from leprosy patients[30].

The hemagglutination test primarily confirmed the previous findings from the complement fixation test, e.g., the ability of leprosy patients to produce antibodies, the differentiation of lepromatous leprosy patients from tuberculoid leprosy patients, and the demonstration of mainly crossreacting antibodies to other mycobacteria in sera from leprosy patients. Higher titers were found in sera from patients with a high bacterial index in skin smears, and DDS treatment led to a decrease in the hemagglutination titer. But this test did not come any nearer to a serodiagnosis of leprosy than the complement fixation test. The hemagglutination test was negative in a high proportion of sera from tuberculoid leprosy patients, gave variable results with sera from contacts of leprosy patients, and showed crossreaction with most of the other mycobacteria.

The Rubino test

The Rubino test was described by Rubino[31] in 1926 as a sedimentation reaction between formalin-treated SRBC and sera from leprosy patients. Sera from patients with diseases other than leprosy could not sediment these treated SRBC[32], and the Rubino test was mostly positive with sera from patients with lepromatous leprosy and negative with sera from patients with tuberculoid and indeterminate leprosy. This test was employed in Latin America until quite recently. It has lately been shown that Rubino reaction can be inhibited by antigens from several mycobacteria[33] and can be positive with sera from patients with chronic renal failure and kala-azar[34]. The test is therefore not specific for leprosy, and the value of this test seems to be very limited for serological studies in leprosy.

Antigen-antibody reactions in gels by double diffusion methods

The characterization of antigen-antibody reactions in gels by double diffusion was introduced in 1948 by Ouchterlony[35]. Burrell and Rheins[36] started to apply this technique with sonified *M. tuberculosis* for studies of antibody formation to tuberculosis. This demonstrated anti-mycobacterial antibodies in sera from 43% to 84% of patients with different types of tuberculosis and in 40% of non-tuberculous controls[37]. A comparison between antigen-antibody reactions in gel by double diffusion and the hemagglutination reaction for the demonstration of antibodies in sera from tuberculous patients and controls gave roughly the same results for these two tests[38].

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Lepromin and sera from lepromatous and tuberculoid leprosy patients and children of leprosy patients were used in double diffusion tests[39]. They demonstrated three precipitation lines, one of which was different from the lines between old tuberculin and rabbit anti-BCG antibodies. The formation of one of the lines between lepromin and sera from lepromatous leprosy patients, tuberculoid leprosy patients, and children of leprosy patients could not be blocked by the addition of old tuberculin. The use of cell fractions from *M. leprae* and rabbit antiserum against *M. leprae* produced a double diffusion pattern of five lines[40]. This was later extended to demonstrate 12 antigen-antibody reactions using serum from a lepromatous leprosy patient and a *M. leprae* preparation made from the liver of *M. leprae*-infected armadillos[41].

The difficulties of obtaining large quantities of *M. leprae* preparations led several researchers to use other mycobacteria grown *in vitro*. Myrvang, *et al.*[42] used the cross-reacting antibodies to *M. duvalli* in sera from leprosy patients to evaluate the anti-mycobacterial antibodies through the spectrum of leprosy. They found no line in 90% and one line in 10% of the sera from tuberculoid leprosy patients, and no line in 25%, one line in 25%, and two or more lines in 50% of the sera from lepromatous leprosy patients. From these results they constructed a model of good antibody response in lepromatous leprosy and poor antibody response in tuberculoid leprosy patients against mycobacterial antigens as a reflection of the mycobacterial antigenic load in leprosy patients.

Diffusion in gel methods using culture filtrate of *M. tuberculosis* and patient sera taken before and after DDS treatment demonstrated a decrease in anti-mycobacterial antibody activity from tuberculoid and lepromatous leprosy patients after a period of antileprosy treatment[43].

The immunodiffusion technique showed extensive crossreactions between different mycobacteria and *M. leprae*[44] but it has a detection limitation of 10 to 15 antigen-antibody reactions against this bacterium[44]. Increased sensitivity and a better comparison can be obtained by observing the changes induced by a serum on one or several reference systems instead of a direct reaction between the serum and different mycobacterial antigen preparations[45]. Even when using changes in a reference system, one must not judge too conclusively on the basis of the absence of precipitations but must analyze the total picture of the precipitation spectrum and its interaction with suitable reference systems[46].

Immunodiffusion has lately been used to investigate the specificity of an absorbed lepromatous serum pool used as a basis for the fluorescent leprosy antibody absorption test (FLA-ABS), and the absorbed serum pool was found to react with antigens from *M. lepraemurium, M. bovis* (BCG), *M. flavescens, M. gastri, M. gordonae* and *M. nonchromogenicum*[26]. Caldwell, *et al.* claimed to have isolated *M. leprae*-specific antigen(s) and tested these by double diffusion tests using the previously mentioned absorbed lepromatous serum pool[47]. Har-

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[46] Lind, A. and Norlin, M. A comparative serological study of *M. avium, M. ulcerans, M. balnei* and *M. marinum* by means of double diffusion in gel methods, a preliminary investigation. Scand. J. Clin. Lab. Invest. **15** Suppl. (1969) 152–163.

boe and Closs[48] tried to isolate the above-mentioned specific *M. leprae* antigen(s) by an identical method but found that the antigen(s) isolated by Caldwell, *et al.* cross-reacted with *M. avium* and *M. bovis* (BCG). The double diffusion in gel technique has a restricted capacity to detect different antigen-antibody reactions, and the technique has a lower sensitivity than other techniques such as crossed immunoelectrophoresis (CIE). The suggestions made by Norlin and Lind for the comparison of sera in double diffusion tests were not followed by Caldwell, *et al.* These factors can therefore explain the difference between the results of Caldwell, *et al.* and those of Harboe and Closs.

The immunodiffusion technique visualized the crossreaction between *M. leprae* and other mycobacteria[41] and thereby proved the findings of the complement fixation tests and the hemagglutination test, and showed that *M. leprae* had several antigens which crossreact with other mycobacteria. But the technique was not any better for the serodiagnosis of leprosy than the previous complement fixation test and the hemagglutination test due to the crossreactions between mycobacteria, the lower sensitivity compared to other tests such as CIE, and the detection of a limited number of antigen-antibody reactions (e.g., 10 to 15 of the at least 70 antigen-antibody reactions between *M. tuberculosis* and rabbit anti-*M. tuberculosis* antibodies detected by CIE). The immunodiffusion technique is still being used today since the method is cheap, easy to perform, needs little equipment, and gives nice precipitation lines which can be classified as being identical, partially identical, or non-identical, when compared with either a different antigen or a different antibody preparation[49]. But the technique has not been developed any further since the use of *M. leprae* purified from the liver of infected armadillos. The possible future isolation of *M. leprae*-specific antigenic components or the production of antibodies against *M. leprae*-specific components might make this technique useful for field work in leprosy.

Indirect immunofluorescence

Morris, *et al.* used the indirect fluorescent antibody technique for the demonstration of antibodies against mycobacteria in 1961[50], employing smears made from *M. leprae* and *M. tuberculosis* and sera from leprosy patients. They found positive reactions with smears from both *M. leprae* and *M. tuberculosis*. After the sera were absorbed with *M. tuberculosis*, they only reacted with smears made from *M. leprae*. This technique was further developed by Abe, *et al.*[51] ten years later and made more specific by absorbing the sera with BCG, *M. vaccae*, cardiolipin, and lecithin[51, 52]. They developed a test in which the absorbed sera primarily reacted with antigens from *M. leprae*, but one of 56 sera also reacted with several other mycobacteria. This serum reacted with smears from *M. leprae* after further absorption with *M. smegmatis*. The results from these tests without further absorption with *M. smegmatis* are shown in Table 1[53].

From this table we can see that a higher percentage of sera from household contacts to leprosy patients was positive in this test than of sera from tuberculoid leprosy patients. Bharadwaj, *et al.* used a similar fluorescent leprosy absorption test (FLA-ABS) on a group of Indian leprosy patients and

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TABLE 1. *Results of fluorescent leprosy antibody absorption test (FLA-ABS) from Abe,* et al.[53]

	Total no.	Percent positive[a]	Titer
BL-LL	144	99	1:856
BB	12	100	1:1146
BT-TT	25	80	1:452
Household contacts	62	92	
Pulmonary tuberculosis	18	0	
Schoolchildren, endemic area	173	63	
Patients in hospital, not in endemic area	138	1.5	

[a] Positive sera diluted 1:40.

TABLE 2. *Results of fluorescent leprosy antibody absorption test (FLA-ABS) from Bharadwaj,* et al.[54]

	No. of patients	Percent positive
Lepromatous leprosy	38	94.7
Borderline leprosy	34	88.2
Tuberculoid leprosy	9	66.6
Early tuberculoid leprosy	36	86.1
Indeterminate leprosy	19	68.4
Healthy contacts	58	67.2
Healthy non-contacts	19	0

family contacts of leprosy patients[54], and their results are shown in Table 2.

They mention neither controls concerning crossreactions with other mycobacteria nor the serum dilutions used to differentiate between positive and negative reactions. The demonstration of a higher percentage of positive results in the contact group than in the tuberculoid leprosy group in both these two investigations is difficult to understand. The FLA-ABS test for antibodies has been in use for many years, but its successful application seems to be limited to the two places mentioned above. Recently it has been shown that the absorbed sera could still react with antigens from *M. lepraemurium, M. bovis* (BCG), *M. nonchromogenicum, M. flavescens, M. gordonae* and *M. gastri*[26]. Therefore exposure to environmental mycobacteria and BCG vaccination may have an influence on the results from these studies.

The FLA-ABS continues to be an interesting test. The basis for the test is simple; a fluorescent microscope is necessary for the test, but it does not involve the use of radioactive materials or a gamma counter. The demonstration of an increase in the percentage of positive results in close contacts to leprosy patients indicates a possible way to estimate how many people in a given area are infected with leprosy. The controls so far seem to be insufficient, especially in relation to other mycobacteria, and the application of the test in other parts of the world, especially in non-leprosy endemic areas, ought to be carried out. The FLA-ABS test is not proven to be *M. leprae*-specific, and the restricted use of the test in other laboratories indicates that it is a difficult test to use and that the reading of the results are subjectively evaluated. In a way, this test does combine the past with the present situation in serological testing for leprosy.

THE PRESENT

Crossed immunoelectrophoresis

The present status of serological investigations in leprosy is the result of two major developments. The first of these was the success with the armadillo; it was possible to transmit leprosy to an animal model, and the *M. leprae* bacilli could multiply almost unlimitedly as in lepromatous leprosy patients[55]. Extensive studies showed that the *M. leprae* bacilli obtained from infected armadillos were identical to the *M. leprae* bacilli from human sources. This made available a much larger quantity of *M. leprae* bacilli for immunological investigations.

The second major development was crossed immunoelectrophoresis (CIE) with a considerably better resolving power in separating the different antigen-antibody re-

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actions than previously known. This technique was introduced by Laurell in 1965[56] in studies of human serum proteins. When applied to the isolation of mycobacterial antigens, sonified *M. tuberculosis* was first separated in CIE in a 1% agarose gel in barbital buffer, pH 8.6, by electrophoresis. Electrophoresis was subsequently run a second time, perpendicular to the first time, into an anti-*M. tuberculosis* antibody containing gel. In this method the antigen-antibody reactions were separated into about 60 different immunoprecipitates[57]. CIE separated sonified *M. leprae* bacilli into seven or eight distinct antigenic components by the use of sera from lepromatous leprosy patients[58] or sera from *M. leprae*-immunized rabbits[59]. By the use of an intermediate gel technique, all of these seven or eight components were found to crossreact extensively with *M. avium*, *M. bovis* (BCG), *M. lepraemurium*, *M. smegmatis* and *Nocardia asteroides*. Later, Closs, *et al.* demonstrated about 20 distinct antigenic components using a concentrated sonified preparation of *M. leprae* prepared from the liver of *M. leprae*-infected armadillos and immunized rabbit anti-*M. leprae* antibody globulins in CIE. Most of these 20 antigenic components crossreacted with other mycobacteria, again detected by the intermediate gel technique[60]. This gave a better resolution of the antigenic components than previously, but did not detect any *M. leprae*-specific antigenic components.

CIE has so far been used to:

a) detect antigenic components in sonified *M. leprae* preparations.

b) demonstrate crossreactions between *M. leprae* and other bacteria. CIE with intermediate gel is a sensitive and well-suited system for showing crossreactions between bacteria[61, 62].

c) demonstrate antibody activity in sera from leprosy patients. CIE could not detect any change in the precipitation pattern of sera from lepromatous leprosy patients taken before and after 12 months of DDS treatment[63].

d) demonstrate *M. leprae*-specific determinants (by the use of CIE with intermediate gel and tandem-crossed immunoelectrophoresis) on one of the antigenic components of *M. leprae*[64, 65].

The need for both concentrated sonified *M. leprae* and rabbit anti-*M. leprae* antibody made CIE unsuitable for antibody studies on sera from leprosy patients on a large scale.

CIE has also been used as a basis for the production of monospecific antibodies against individual antigenic components of mycobacteria by cutting out one precipitation arc in the agarose gel after CIE, and then immunizing rabbits with the preparation made from this line. In this way monospecific antibodies for several of the mycobacterial antigenic components were made and used for identification of specific antigen-antibody reactions in more complex CIE systems, such as the BCG anti-BCG reference system [66, 67, 68]. A further use

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of the monospecific antibodies would be to isolate mycobacterial antigenic components by immunosorbent techniques.

Radioimmunoassay (RIA)

Electrolytic iodination with ^{125}I of a crude BCG fraction which gave rise to a much simpler CIE pattern, gave almost a selective labeling of BCG antigen 60[67]. BCG 60 was shown to be a large, cell-wall glycoprotein component, and the reaction between the labeled BCG 60 and rabbit and human antibodies against BCG 60 was partially inhibited by polysaccharides from *M. tuberculosis*. BCG antigen 60 was found to crossreact extensively with antigens from many other mycobacterial species, including *M. leprae* and *M. tuberculosis*[67]. Antibodies against BCG 60 were measured using protein A containing staphylococci to separate antibody-bound labeled antigen 60 from free labeled antigen 60 in a radioimmunoassay (RIA). This RIA was sensitive and could detect antibodies against BCG 60 in sera diluted 1:10,000. The highest activity of antibodies against BCG 60 was found in patients with active pulmonary tuberculosis, somewhat lower activity in sera from lepromatous leprosy patients, and still lower activity in patients with tuberculoid leprosy. The lowest and almost equal activity was found in a group of patients with leishmaniasis and two control groups, one of which consisted of close contacts of lepromatous leprosy patients[69]. Two additional important observations were made in this work:

a) Sera from patients with active tuberculosis contained higher activity of antibodies against BCG 60 than sera from lepromatous leprosy patients, even though the group of patients with tuberculosis was small. This is in clear contrast to earlier findings using test systems such as the hemagglutination test and complement fixation tests, where a higher percentage of positive reactions and higher titers were found in sera from lepromatous leprosy patients than in sera from patients with active pulmonary tuberculosis, even when *M. tuberculosis* antigens were used. Therefore, RIA with labeled antigen 60 and/or other surface antigens should be further explored in the search for a serodiagnostic test in patients with active tuberculosis.

b) There was a large variation in the activity of antibodies against BCG 60 in sera from patients with a similar classification, e.g., tuberculoid leprosy patients or lepromatous leprosy patients, and a considerable overlap of the individual results between these two groups of patients. These findings severely challenged the accepted dogma that "lepromatous leprosy patients had a poor cellular immune response and a hyperactive humoral immune response, while tuberculoid leprosy patients had a good cellular immune response with almost non-demonstrable humoral immune response."

One antigenic component, *M. leprae* antigen 7, which crossreacts extensively with BCG 60, was thereafter preferentially labeled with ^{125}I by electrolytic iodination[70]. Testing showed that more than 95% of the radioactivity was localized to the antigen 7 component of *M. leprae*. This labeled component was thereafter used in RIA for antibodies against antigen 7 in sera from humans, rabbits, and armadillos.

The antigenic structure and crossreactions of *M. leprae* antigen 7 were studied by Harboe, *et al.*[71]. The antibody activity against *M. leprae* antigen 7 could be inhibited by the polysaccharides, arabinogalactan and arabinomannan, from *M. tuberculosis*, particularly in sera from tuberculoid leprosy patients but also, to some extent, in

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resis: A reference system. Scand. J. Immunol. **12** (1980) 249–263.

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sera from lepromatous leprosy patients. The *M. leprae* antigen 7 component therefore contains several antigenic determinants. The antibody reaction to some of these could be inhibited by arabinogalactan and/or arabinomannan from *M. tuberculosis*. Some of the sera from lepromatous leprosy patients contained antibodies which were not inhibited by these polysaccharides. So far, these different determinants of antigen 7 have not been classified, but one or several of them might be *M. leprae*-specific, as shown by Kronvall, *et al.*[64, 65] on another antigenic component of *M. leprae*.

Both rabbits injected with sonified *M. leprae*[59] and armadillos inoculated with live leprosy bacilli produced antibodies against *M. leprae* antigen 7[72]. There was a correlation between antibodies against antigen 7 and clinical signs of leprosy in the armadillos; an increase of antibodies against antigen 7 first appeared at the time that clinical signs of leprosy developed in the armadillo eight to 24 months after being inoculated with the leprosy bacilli[73].

The highest activity of antibodies against *M. leprae* antigen 7 was found in sera from untreated lepromatous leprosy patients, lower activity in sera from untreated tuberculoid leprosy patients, and the lowest in sera from contacts to leprosy patients[70], thereby confirming the results from earlier studies of antibodies against BCG 60 by Harboe, *et al.*[69]. Yoder, *et al.*[74] again confirmed the same decrease in median activity against antigen 7 through the spectrum of untreated leprosy patients.

DDS treatment of both tuberculoid (BT-TT) leprosy and lepromatous (BL-LL) leprosy patients[70] led to a reduction of antibody activity against antigen 7. The median reduction was later shown to be 30% of the pretreatment antibody activity in sera taken on an average of three years after the start of DDS treatment in a group of BL-LL leprosy patients[75].

There was a marked increase in the activity in the serum from one patient during the three-year period of DDS treatment. This patient developed pulmonary tuberculosis in addition to lepromatous (BL) leprosy after starting DDS. This indicates that the humoral immune response in BL leprosy can be stimulated to an even higher production of antibodies against *M. leprae* antigen 7 by a second mycobacterial disease.

From these findings it seems that the antibody activity against antigen 7 is partially dependent on the antigenic load, both in the human and in the armadillo[73].

Both Melsom, *et al.*[70, 75] and Yoder, *et al.*[74] showed a wide variation, with both low and high antibody activity against antigen 7, in sera from patients with a similar classification according to the Ridley-Jopling criteria, confirming the results of Harboe, *et al.*[69] with anti-BCG 60. This variation was so large that a considerable overlap in the activity of antibodies against antigen 7 could be demonstrated between individual sera from BL-LL patients and from BT-TT patients.

Ulrich, *et al.*[76] demonstrated antibodies against mycobacterial antigens both in the 7s globulin fractions and the 19s macroglobulin fractions of sera from leprosy patients. This indicated that both IgG and IgM anti-mycobacterial antibodies occur in sera from leprosy patients. This was confirmed by Abe, *et al.*[77] with the indirect fluorescent antibody test for IgM antibodies and by Melsom and Duncan[78] using RIA with la-

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beled antigen 7 on fractions of sera from leprosy patients obtained by gradient ultracentrifugation. This shows that the sensitive RIA can demonstrate both IgG and IgM antibodies against *M. leprae* antigen 7.

Congenital tuberculosis induced by the spread of *M. tuberculosis* across the placenta is well known[79, 80, 81]. Leprosy bacilli can also cross the placenta, and this has been shown by the presence of acid-fast bacilli in cords[82] and cord blood[83] from babies of lepromatous leprosy mothers and acid-fast bacilli in placentae of lepromatous leprosy women[84]. Melsom, *et al.*[85] demonstrated increased IgA concentration in cord sera from babies of lepromatous leprosy mothers, indicating a stimulation of the immune apparatus in the fetus by *M. leprae*. Ten of 20 sera taken two to four months after birth from babies of lepromatous leprosy mothers showed higher antibody activity against *M. leprae* antigen 7 than expected from the activity in the cord sera[86]. This increased activity indicated the production of IgG and/or IgM antibodies against antigen 7 in the fetus and newborn baby.

The isolation and labeling of one antigenic component from *M. leprae* has given new and important information to serology in leprosy. This assay has mostly been based upon the labeling of one, large antigenic component, antigen 7 of *M. leprae*, with many different antigenic determinants, several of which crossreact with similar determinants on antigenic components of other mycobacteria and of *Nocardia asteroides*.

A very sensitive RIA for the demonstration and quantification of antibodies against one antigenic component of mycobacteria has thus been developed, and a spectrum of antibody activity, with the lowest median activity in sera from contacts with leprosy patients, higher median activity in sera from untreated tuberculoid leprosy patients, and the highest median activity in sera from untreated lepromatous leprosy patients, has been shown. A decrease in activity after a period of DDS treatment has been demonstrated both in tuberculoid and in lepromatous leprosy patients. Sera from leprosy patients contained both IgG and IgM antibodies against antigen 7, and the assay was sensitive enough to indicate the production of antibodies against antigen 7 in the fetus and the newborn baby of lepromatous leprosy mothers.

The activity of antibodies against *M. leprae* antigen 7 has so far been determined in a limited number of sera from both leprosy patients and controls. The test should therefore be applied to larger number of sera from leprosy patients, patients with other mycobacterial diseases, and contacts exposed to leprosy patients and controls, especially from areas without leprosy, to evaluate the use for this assay in the serodiagnosis of leprosy.

In 1978 Harboe, *et al.*[87] isolated and labeled components other than *M. leprae* antigen 7. This labeled preparation was used in RIA with human sera absorbed with BCG sonicate, and thereafter only absorbed sera from leprosy patients reacted with this newly labeled preparation from *M. leprae*. The highest median activity was found in sera from lepromatous leprosy patients and lower median activity was found in sera from tuberculoid leprosy patients. Sera from all 8 Norwegian patients with tuberculosis, all 30 BCG vaccinated Norwegian medical stu-

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dents, and 17 of 19 staff members at a leprosy hospital in Addis Ababa, Ethiopia, were found to be negative. The two sera from staff members at the leprosy hospital were strongly positive without present signs of leprosy or a past history of leprosy in either of the two persons. Sera from rabbits immunized with *M. nonchromogenicum* and *M. avium* were, however, strongly positive even after absorption with BCG sonicate. This test with the described absorption with BCG sonicate is, therefore, not *M. leprae*-specific. Additional absorption with *M. nonchromogenicum* and *M. avium* removed virtually all antibody activity from the rabbit antisera; whereas lepromatous leprosy sera still contained high antibody activity, indicating that the test demonstrated antibodies reacting with determinants present in *M. leprae* and not detectable in *M. tuberculosis*, BCG, *M. nonchromogenicum*, *M. avium*, *M. duvalii*, *M. smegmatis*, *M. phlei*, and *Nocardia asteroides* by the present assay. The preparation of labeled *M. leprae* antigen(s) suitable for the demonstration of antibodies to *M. leprae*-specific determinants has been difficult, different batches of bacilli yielding different results. The requirements for absorption with other mycobacteria (and possibly other bacteria) may vary in different populations depending on the mycobacterial flora. The requirements for establishing a specific test for anti-*M. leprae* antibodies has thus not been met with either this RIA or Abe's FLA-ABS test.

Solid phase radioimmunoassay

Melsom, *et al.*[75, 88, 89, 90] developed a solid phase radioimmunoassay (sRIA) for the demonstration of IgA, IgM, and IgG anti-*M. leprae* antibodies. The sRIA should have a sensitivity similar to the RIA and should be immunoglobulin class-specific[91, 92]. The sRIA has been shown to be less affected by rheumatoid factors than other assays[93]. The use of bovine serum albumin (BSA) and Tween 20 in the dilution fluid reduced the background activity considerably[89]. The test is based upon the attachment of antigenic components of *M. leprae*[60] to the polystyrol test tube walls. Preliminary studies (Harboe, unpublished data)[75] indicate that antigen 7 in addition to several other antigenic components of *M. leprae* became attached. The sRIA thus will demonstrate both cross-reacting antibodies to other mycobacteria and antibodies against *M. leprae*-specific determinants such as the one demonstrated by Kronvall, *et al.*[64, 65]. No correlation could be demonstrated when the individual results of IgA, IgM, and IgG anti-*M. leprae* antibody activities were compared with the results of antibody activities against *M. leprae* antigen 7 in RIA[75].

A lepromatous serum pool (LSP) was collected from 40 lepromatous (BL-LL) leprosy patients either prior to or within six months of the start of antileprosy treatment[89] and used as a standard. IgA anti-*M. leprae* antibodies could be detected in LSP diluted 1:1000, and IgG and IgM anti-*M. leprae* antibodies in LSP diluted 1:10,000.

IgA, IgM, and IgG anti-*M. leprae* antibody activity increased through the leprosy spectrum with the lowest median activity in contacts, higher median activity in untreated tuberculoid (BT-TT) leprosy, and the

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highest median activity in untreated lepromatous (BL-LL) leprosy patients[75, 89, 90], confirming the results from the RIA with BCG 60 and *M. leprae* antigen 7. Previously, no increased antibody activity could be demonstrated in sera from patients with "strictly indeterminate" leprosy[94]. It was therefore particularly interesting to demonstrate increased IgA and especially IgM (virtually without overlap of the individual results) anti-*M. leprae* antibody activity in sera from "strictly indeterminate" leprosy patients compared to sera from close contacts of lepromatous patients[75].

A significant decrease in the IgG and IgA anti-*M. leprae* antibody activity, but not the IgM activity, could be shown in sera taken after (compared to sera taken before) DDS treatment of lepromatous leprosy patients[90] as previously shown with antibody activity against antigen 7[70]. There was a wide variation in IgA, IgM, and IgG anti-*M. leprae* antibody activities in individual sera from patients with a similar classification according to the Ridley-Jopling criteria. Again, this variation was so large that there was a considerable overlap of the individual results from sera of tuberculoid compared with sera of lepromatous leprosy patients[90].

The immunoglobulin class-specific sRIA was used on cord sera and sera taken after birth from babies of lepromatous leprosy mothers (Group 1) and babies of tuberculoid leprosy and non-leprosy mothers (Group 2). IgA and IgM anti-*M. leprae* antibody activity was present in 30% and only IgM activity in a further 20% of the cord sera from Group 1. No such IgA and IgM activity was detected in cord sera from Group 2[88, 89]. This was taken as an additional indication of transplacental passage of *M. leprae* in lepromatous leprosy women, causing fetal production of anti-*M. leprae* antibodies.

There was a gradual increase of IgA and IgM anti-*M. leprae* antibody activities in sera taken from the babies after birth, depending on the age of the babies. This could be demonstrated in sera taken a few weeks after birth and shows that the IgA and IgM activities in the cord sera could not have been transferred from the mothers[88]. Sera taken three to six months after birth from Group 1 showed a significantly higher IgA and IgM anti-*M. leprae* antibody activity than sera from Group 2[88]. The median IgA and IgM activities in sera taken 6 to 9, 9 to 15, and around 24 months after birth from both groups were the same. But the median IgM anti-*M. leprae* antibody activity in sera taken three to four years after birth from Group 1 (23% of LSP) was significantly higher ($p < 0.001$) than in sera from Group 2 (4.7% of LSP) (Melsom, unpublished observations). These findings are best explained by transplacental passage of live *M. leprae*. These bacilli probably have a limited multiplication in the fetuses and newborn babies until the immune apparatus stops the growth and starts to eliminate the bacilli, and this is indicated by the increased IgA and IgM activity in sera taken three to six months after birth and the increased IgM activity in sera taken three to four years after birth from Group 1 compared to Group 2. This is in agreement with the observation that 15% to 45% of babies with close family members (mother or father or both) with lepromatous leprosy developed transient, indeterminate leprosy[95, 96, 97]. About 75% of these children experience self-healing of their leprosy without any treatment[5, 98].

Both Group 1 and Group 2 babies will be exposed after birth to a large number of environmental mycobacteria and other bacteria with antigens crossreacting to *M. leprae*. This exposure, we believe, induced increased IgA and IgM anti-*M. leprae* antibody activity in sera taken 6 to 9, 9 to 15, and around 24 months after birth in Groups 1

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and 2 babies. These findings further show that fetal exposure to *M. leprae* does not induce tolerance in the humoral immune response to mycobacteria after birth.

IgG anti-*M. leprae* antibody activity decreased rapidly in sera taken until six to nine months after birth compared to the activity in cord sera from Groups 1 and 2[88]. This is due to catabolism of maternal IgG in the newborn babies[99]. There was no significant increase of IgG activity in sera taken two years after birth from Groups 1 and 2, while such an increase could be demonstrated in sera taken three to four years after birth from both groups. There was no increased IgG anti-*M. leprae* antibody activity in sera taken from patients with "strictly indeterminate" leprosy[90]. Both these findings show that IgG antibodies against *M. leprae* appear considerably later in the development of leprosy than IgM and IgA antibodies in humans. In the armadillo, antibodies against *M. leprae* antigen 7 (by RIA) started to increase six to 24 months after inoculation with leprosy bacilli. Clinical signs started to appear at the same time[73]. Since the RIA used demonstrated mostly IgG antibodies against *M. leprae* antigen 7, it seems that IgG antibodies also appear late in leprosy-infected armadillos.

Solid RIA for class-specific, anti-*M. leprae* antibodies is mainly based upon demonstrating antibodies to crossreacting antigenic components of *M. leprae*. The use of the assay has confirmed previous findings of increased median activity from the lowest in exposed contacts, to higher in untreated tuberculoid leprosy patients, and to the highest in untreated lepromatous leprosy patients. The assay showed a decrease in activity after DDS treatment of lepromatous leprosy patients and a large variation in individual sera from patients with a similar classification leading to a considerable overlap of individual sera from patients with untreated tuberculoid and lepromatous leprosy.

The assay has given new information to the serology of leprosy. Leprosy patients can produce IgA anti-*M. leprae* antibodies. Such IgA antibodies have been detected both in sera and in milk samples from women with leprosy (Melsom, unpublished observations). The significance of IgA anti-*M. leprae* antibodies is unknown, but they might help to stop the invasion of *M. leprae* across mucous membranes as an early defense against leprosy.

Patients with indeterminate leprosy have been shown to react immunologically to *M. leprae* by having increased IgM anti-*M. leprae* in their sera. Since IgM anti-BCG antibodies play an important role in diminishing granuloma formation after subcutaneous injection of BCG in rats[100, 101], the IgM anti-*M. leprae* antibodies may also be of importance in the control and elimination of *M. leprae* in indeterminate leprosy.

IgA and IgM anti-*M. leprae* antibodies could be detected in cord sera from babies of lepromatous leprosy mothers, indicating transplacental passage and multiplication of *M. leprae* in the fetus. This route of infection could have been the cause of leprosy in some of the infants and children less than three years old[102]. Congenital tuberculosis, appearing one to four weeks after birth, is well documented[79, 103]. The difference in generation time (1 day for *M. tuberculosis* and 12 to 14 days for *M. leprae*), makes it unlikely that clinical signs of leprosy due to intrauterine leprosy infection would appear before the child was one year old.

Other assays

The use of enzyme-labeled, anti-human IgA, IgM, and IgG, e.g., with alkaline phosphatase instead of with ^{125}I, makes it possible to carry out assays for IgA, IgM and

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IgG anti-*M. leprae* antibodies without the use of radioactive materials and a gamma counter. Therefore the enzyme-linked immunosorbent assay (ELISA) would be a very useful test for leprosy in leprosy endemic areas. ELISA has been used to demonstrate IgA, IgM, and IgG anti-*M. tuberculosis* antibodies in the sera from patients with tuberculosis, sarcoidosis, and Crohn's disease[104, 105]. ELISA has been used at this institute for the assay of IgG and IgM antibodies against *M. leprae* in human sera. The assay has a sensitivity similar to the sRIA and the RIA but is probably more unstable, making it difficult to obtain reproducible results.

The assay for antibodies against *M. leprae* can be carried out on whole blood samples obtained from venous or capillary blood[73] and on blood samples dried on filter paper (Harboe, unpublished results). With these developments, large-scale screening for anti-*M. leprae* antibodies can be carried out in countries with leprosy.

Crossed radioimmunoelectrophoresis (CRIE) has been developed to detect IgE antibodies against specific allergens in sera from allergic humans[106, 107]. Recently, Melsom, *et al.*[108] developed CRIE to detect antibodies of the IgG class against *M. leprae* antigenic components. This system is only in the experimental stage, but further development can give valuable results directly visualizing the immunoglobulin class-specific antibodies in human sera against different antigenic components of *M. leprae*. If CIE can be developed to detect *M. leprae*-specific components, CRIE may be an important tool for demonstrating *M. leprae*-specific antibodies in sera from leprosy patients. This can help in choosing particular antigenic components from *M. leprae* for further isolation and use in the serology of leprosy.

Discussion

Mycobacterium leprae contain antigenic components crossreacting with several other mycobacteria. These crossreacting components, both from *M. leprae* and from other mycobacteria, have been and are still used to detect and quantitate the activity of anti-mycobacterial antibodies in sera from leprosy patients. Assays based upon such cross-reacting antigenic components have shown:

a) increasing antibody activity through the leprosy spectrum with the lowest activity in sera from close contacts of lepromatous leprosy patients, higher median activity in sera from indeterminate leprosy patients (only of IgM and IgA antibodies), even higher median activity in sera from untreated tuberculoid leprosy patients, and the highest median activity in untreated lepromatous leprosy patients.

b) decreased activity after DDS treatment of both tuberculoid and lepromatous leprosy patients.

c) a large variation in the antibody activity of sera from leprosy patients with a similar clinical classification (according to the Ridley-Jopling scale).

d) a considerable overlap of results in individual sera from both tuberculoid and lepromatous leprosy patients.

Kronvall[109] discussed the value of various assays for anti-mycobacterial antibodies. If these assays are to be used for the serodiagnosis of leprosy, high specificity is particularly important. To be useful for leprosy, a serological test must have a very low frequency of false-positive results since the prevalence rate of leprosy is usually low. An assay for *M. leprae*-specific antibodies may fulfill this criterium.

Research for the development of assays for *M. leprae*-specific antibodies have so far

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led to the demonstration of *M. leprae*-specific antigenic determinants on one antigenic component[64, 65] and two assays, *M. leprae*-specific antibodies detected by RIA[87] and the fluorescent leprosy antibody (FLA-ABS) test[52, 53]. These two assays have not been documented as *M. leprae*-specific. Some of the antibodies crossreacting with other mycobacteria have been removed by absorption, but some crossreactive antibodies remained in sera from leprosy patients after absorption with other mycobacteria. This has been clearly demonstrated with the absorption used in FLA-ABS test[26].

The term "the original mycobacterial sin"[110] implies that the immune response in humans is strongly influenced by the first exposure to one species of mycobacterium. Later exposure to a different mycobacterium will lead to a strong immune reaction to antigenic components crossreacting with the first mycobacterium; whereas the immune response to the antigenic components of the second mycobacterium not crossreacting with the first will be much weaker. Evidence for this theory has been found in the reduction or lack of protection by BCG vaccination in humans previously exposed to certain environmental mycobacteria[111, 112].

The effect of previous exposure to environmental mycobacteria on the antibody response to *M. leprae* in humans infected with leprosy is virtually unknown. Leprosy may lead to increased production of antibodies against crossreacting antigens but a weak antibody response to *M. leprae*-specific determinants. Therefore, assays based on the antibody response to crossreacting mycobacterial antigenic components can be more sensitive indicators for subclinical and early leprosy than assays for *M. leprae*-specific antibodies.

PROSPECTS FOR THE FUTURE

Further developments within the serology of leprosy might depend on the hybridoma technique with the production of monoclonal antibodies against various antigenic determinants of *M. leprae*. Such monoclonal antibodies might help to:

a) purify several antigenic components from *M. leprae*, which might be of value in the continuous study of the immune response in leprosy patients and the interplay between the cellular and humoral responses. These purified antigenic components can be used as a basis for antibody assays similar to the already described *M. leprae* antigen 7 assay.

b) produce *M. leprae*-specific antibodies. Inhibition assays can be based on monoclonal *M. leprae*-specific antibodies to detect corresponding antibodies in sera from contacts of leprosy patients and leprosy patients. As mentioned previously, sera from contacts of leprosy patients and patients with early leprosy might not contain detectable *M. leprae*-specific antibodies.

c) distinguish *M. leprae* from other mycobacteria, particularly as it relates to the continued research on the growth of *M. leprae* on culture media and in animal models where *M. leprae* can multiply freely.

d) define the taxonomic position of *M. leprae*.

Monoclonal antibodies are an important new tool in immunology, but they often behave differently from conventional polyclonal antibodies. So far, we are aware that:

a) Monoclonal antibodies have a very fine specificity and are directed towards individual antigenic determinants which may be composed of only a few amino acids or polysaccharide units. An antigenic component usually contains many antigenic determinants. Some are crossreacting and others are species-specific; some are strong and some are weak as immunogens. Therefore, it is important to use a proper test system to select the most valuable monoclonal antibodies for further work. The fine specificity of the monoclonal antibodies can be compared to detect the "building blocks" of the antigenic components. These "building blocks" might be common for very different antigenic components, and we might be faced with crossreactions not only with

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other mycobacteria but also with completely unrelated species of bacteria.

Secondly, we do not know how immunogenic the *M. leprae*-specific determinants of the leprosy bacilli are in humans. Various observations indicate that the *M. leprae*-specific antigen determinants are weakly immunogenic in humans. It may therefore be difficult to detect antibodies against these determinants in human sera.

b) Monoclonal antibodies react differently with antigenic components than do polyclonal antibodies. Most of them do not precipitate in double diffusion tests. It may be necessary to change our concepts of interactions and establish new assay systems in work with these antibodies.

Monoclonal antibodies against *M. tuberculosis* have been produced which differentiate between *M. tuberculosis* and *M. bovis*, and even between different strains of these two bacteria[113]. When these monoclonal antibodies were used in an inhibition assay in attempts to develop a serodiagnostic test for tuberculosis, the results were as yet not too promising[114]. Monoclonal antibodies against *M. leprae* have also been produced[115], and some of them were found to be *M. leprae*-specific in the limited test systems used. Gillis and Buchanan[115] have not, so far, used these monoclonal antibodies in inhibition assays, but claim that monoclonal antibodies against *M. leprae* can be used to isolate antigenic components from *M. leprae* and to construct an antigenic and structural map of the surface of the leprosy bacilli.

The isolation and demonstration of *M. leprae* antigenic components from sera and other body fluids of leprosy patients is an area of great potential in the serology of leprosy. Such a demonstration would be diagnostic of leprosy. Subclinical and early untreated lepromatous leprosy patients have a very high load of leprosy bacilli. These patients are therefore the major infectious reservoir of leprosy. It should be possible to detect mycobacterial antigens in their sera. Screening programs based on *M. leprae* antigen(s) would be diagnostic for these patients, and the quantification of such antigens would be a good indicator of the effect of antileprosy treatment and the appearance of drug-resistant leprosy.

Other recent developments besides monoclonal antibodies can be important for the future of the serology of leprosy. The purification of highly purified MPB 70[116] from the culture fluid of *M. bovis* BCG, strain Tokyo, and the unique specific antigenic property of this antigen both in CMI (skin testing) and in antibody studies are promising. This antigenic component seems to be almost entirely specific (in both CMI and antibody response) for *M. bovis* BCG Tokyo and almost absent in related strains like *M. bovis* Copenhagen and in *M. tuberculosis*. This shows that it is possible by conventional methods to isolate antigenic components which are highly specific.

Other recent developments such as radioimmunoassay with labeled *M. leprae* antigen 7, solid phase radioimmunoassay for immunoglobulin class-specific antibodies, the ability to use fingerprick blood and blood dried on filter paper for serological testing, CRIE to visualize the specificity of the individual antibodies against *M. leprae* antigens in the different immunoglobulin classes, and production of polyclonal antibodies against individual antigenic components, are very promising. The possibility of finding a useful serodiagnostic test seems high. The use of both monoclonal and polyclonal antibodies needs to be explored to reach this goal.

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