In Vitro Synthesis of Humoral Factors (Immunoglobulins and Complement) in Lesional Skin of Leprosy Patients

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An in vitro culture technique was used to demonstrate the synthesis of immunoglobulins and complement in lesional skin of patients representing the entire clinico-histopathological spectrum of leprosy. The results indicate that immunoglobulin G is produced in different amounts in the various forms of leprosy. Classification of the patients according to the three main groups shows that a small amount of immunoglobulin G synthesis occurred in tuberculoid leprosy, a distinct amount occurred in borderline leprosy, and a large amount occurred in lepromatous leprosy. Contrary to expectation, synthesis of C3 was found only in some of the cultures of these three forms of leprosy. The function of the locally synthesized immunoglobulin G and the findings concerning C3 synthesis are discussed.

In humans different patterns of immunoglobulin production are found in the mucous membranes of the gastrointestinal and respiratory tracts (8, 10, 22, 23) and in the conjunctival, nasal, vaginal, and oral mucosa (9). Normal skin does not synthesize immunoglobulins, but pathological skin of patients suffering from certain skin diseases produces these proteins variably (9).

Synthesis of several complement components has been demonstrated in the mucosa of the gastrointestinal and respiratory tracts (3, 4, 8, 10), in normal and pathological skin (9), in the liver (2, 3, 21), and in the lymphoid tissues (11, 21, 22). The cellular localization of complement synthesis has been proven to be in mononuclear phagocytes (monocytes and macrophages) (5, 11, 20).

Leprosy, a chronic infectious disease caused by *Mycobacterium leprae*, shows a wide range of clinical forms, the spectrum ranging from polar tuberculoid at one end to polar lepromatous at the other. The clinical manifestations reflect the degree of host resistance to *M. leprae* (17). The tuberculoid type represents the highly resistant, self-limiting form, and the lepromatous type represents the weakly resistant, progressive form.

Histologically, the tuberculoid lesion is characterized by a granuloma consisting of epitheloid cells, lymphocytes (most probably T-cells), some

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giant cells, and few or no bacilli (15–17, 19). The lesions of lepromatous leprosy are characterized by the presence of large numbers of macrophages loaded with *M. leprae* and a small number of lymphocytes, most probably B-lymphocyes (15–17, 19).

The cellular immune response to *M. leprae*, as measured by the lymphocyte transformation and leukocyte migration inhibition tests, showed a continuous decrease from the tuberculoid to the lepromatous end of the spectrum (14). On the other hand, the presence and titer of antimycobacterial antibodies in sera increased toward the lepromatous end of the spectrum (13). The results of Abe et al. (1) and Harboe et al. (6), obtained with an indirect immunofluorescent antibody method and a radioimmunoassay technique, respectively, were in agreement with those of Myrvang et al. (13). These authors found an increase in the proportion and titer of *M. leprae*-specific antibodies in sera toward the lepromatous pole. However, the sera of a considerable proportion of the subpolar tuberculoid and borderline tuberculoid patients showed high titers of *M. leprae*-specific antibodies (6).

To obtain more information about the functional activities of the cells in the lesions of various forms of leprosy, two characteristics were investigated, namely, the synthesis of immunoglobulins specific for B-lymphocytes and the synthesis of complement component (C3), a characteristic of mononuclear phagocytes (monocytes and macrophages).

MATERIALS AND METHODS

Tissues. Biopsy samples were taken from untreated patients who were between 9 and 76 years old. The clinical and histopathological diagnoses were based on the criteria of Ridley and Jopling (17), Ridley and Waters (18), and Myrvang et al. (14). The patients were classified into the following eight groups: indeterminate, polar tuberculoid, subpolar tuberculoid, borderline tuberculoid, borderline, borderline lepromatous, indefinite lepromatous-subpolar lepromatous (15), and polar lepromatous.

The skin specimens were taken with a biopsy punch under local anesthesia with 2% lidocaine, without previous disinfection of the skin. For each patient three samples with a diameter of 3 to 6 mm were taken from one lesion or, when this was not possible, from two similar lesions. One sample with a diameter of 6 mm was used for culture study, and the two other samples were used for histological and immunofluorescence investigations.

Histological examination. The biopsy samples were studied histologically to confirm the clinical diagnosis. After fixation in 10% formaldehyde, dehydration, and embedding in paraffin, the specimens were cut into 6-μm-thick sections and stained with hematoxylin and eosin and, for the demonstration of acid-fast bacilli, with the Fite-Faraco staining procedure (25), with some minor modifications.

Demonstration of protein synthesis in vitro. The method used for the study of immunoglobulin and complement synthesis in vitro and the specificity of the technique have been described in a previous paper (9). Briefly, the method, which was originally described by Hochwald et al. (7) and later in detail by van Furth et al. (24), was as follows. After removal of the epidermis with a surgical blade, the skin specimens (weighing 120 to 160 mg) were minced in Hanks solution. These tissue fragments were incubated in roller tubes for 48 h at 37°C in 1 ml of modified Eagle medium containing 1 μCi of L-[¹⁴C]lysine (specific activity, 312 mCi/mmol; Schwarz Bio Research, Inc., Orangeburg, N.Y.) per ml and 1 μCi of L-[¹⁴C]isoleucine (specific activity, 312 mCi/mmol; Schwarz Bio Research) per ml. To this medium gentamicin (25 μg/ml) was added. After incubation, the cultures were frozen (−20°C) and, after being thawed, dialyzed against 0.015 M phosphate buffer (pH 7.6) to remove excess radioactive amino acids. Next, the culture fluid was concentrated by lyophilization, dissolved in 0.1 ml of twice-distilled water, and analyzed by micro-immunoelectrophoresis. Because concentrated culture fluids often contain too little protein to provide well-defined precipitation lines, an appropriate carrier serum was used.

The synthesis of immunoglobulin E (IgE) was investigated with the micro-Ouchterlony technique, because immunoelectrophoresis did not give reproducible precipitation lines. The newly synthesized proteins were detected and identified by autoradiography of the immunoelectrophoretic pattern of the culture fluid and of the Ouchterlony plate. The plates were exposed to Kodak Royal-X Pan film (ASA 1200) for 21 days and developed with 10% Rodinal solution (Agfa).

Antisera. The antisera used were horse anti-human serum, sheep anti-IgA, sheep anti-IgM, rabbit anti-IgE, and rabbit anti-complement, which contained antibodies against C3c (=β1A), C3d (=α2D), and C4 (=β1E) (all obtained from the Central Laboratory of the Red Cross Blood Transfusion Serivce, Amsterdam, The Netherlands); also used was swine anti-IgD from Nordic Immunological Laboratories, Tilburg, The Netherlands.

Evaluation of the synthesized proteins. The intensity of the autoradiographic lines, which indicates the amount of protein synthesized, was classified, by comparison with a standard pattern, according to the following scale: −, negative; (+), just visible; +, clearly visible, to a maximum of ++++. All readings were made independently by two observers. The results are presented on the following basis: in each category of cultures the intensity of the autoradiographic line was scored [− = 0; (+) = 1; + = 2; ++ = 3; +++ = 4; ++++ = 5], and the mean of the scores was calculated and expressed according to the classification described above.

RESULTS

Immunoglobulin synthesis. The analysis of immunoglobulin synthesis in the skin lesions of various forms of leprosy gave the following results (Table 1). In indeterminate leprosy, no synthesis of immunoglobulins was demonstrable. Half of the cultures of polar tuberculoid leprosy synthesized small amounts of IgG. All but one of the cultures of subpolar tuberculoid leprosy produced IgG in small amounts. In borderline tuberculoid, borderline, and borderline lepromatous leprosy, IgG synthesis was consistently found. The amounts were small in the borderline tuberculoid and borderline forms and distinct in the borderline lepromatous form. In about half of the cultures of subpolar lepromatous leprosy IgG was produced in distinct amounts, whereas all cultures of the polar lepromatous form synthesized IgG in considerable amounts (Fig. 1).

IgA was found in only one culture of polar lepromatous leprosy, and the amount was small; synthesis of IgM could not be demonstrated in any of the cultures. Synthesis of IgD and IgE was assessed in a limited number of cultures of all groups of patients; none of these cultures showed synthesis of these immunoglobulins.

Synthesis of complement (C3 and C4). None of the cultures of indeterminate leprosy showed synthesis of C3 or C4. Some of the cultures of polar tuberculoid leprosy (5 of 14) synthesized C3 in small amounts. In the cultures of subpolar tuberculoid, borderline tuberculoid, borderline, and subpolar lepromatous leprosy, synthesis of C3 could not be detected. In some of the cultures of borderline lepromatous leprosy (2 of 6 cultures) and polar lepromatous leprosy (3 of 11), the production of small amounts of C3

TABLE 1. *Synthesis of immunoglobulins and complement in lesions of leprosy patients*

Form of leprosy	Total no. of cultures	IgG		C3	
		No. of positive cultures	Mean intensity of positive cultures[a]	No. of positive cultures	Mean intensity of positive cultures[a]
Indeterminate	4	0	−	0	−
Polar tuberculoid	14	7	(+)	5	(+)
Subpolar tuberculoid	6	5	(+)	0	−
Borderline tuberculoid	4	4	(+)	0	−
Borderline	3	3	(+)	0	−
Borderline lepromatous	6	6	+	2	(+)
Subpolar lepromatous	8	5	+	0	−
Polar lepromatous	11	11	++	3	(+)

[a] Intensity of autoradiographic lines: −, negative; (+), just visible; +, clearly visible, to a maximum of ++++.
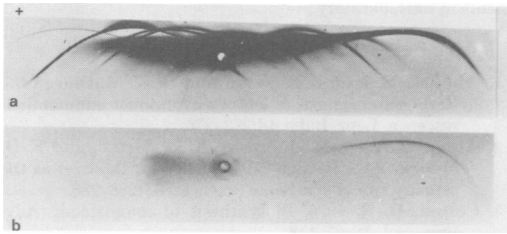

FIG. 1. *Polar lepromatous leprosy. (a) Immunoelectrophoretic pattern of carrier serum and culture fluid developed with horse anti-human serum. (b) Autoradiograph with clearly labeled IgG line.*

was demonstrated (Table 1).

Synthesis of a small amount of C4 was found in only one culture of polar lepromatous leprosy; the cultures of the other forms of the disease did not show C4 production.

Control experiments. Experiments demonstrating the specificity of the method used to assess immunoglobulin and complement synthesis in vitro have been described in detail elsewhere (9, 24). In the present study one of these control experiments was performed, i.e. culture of dead tissue. Two biopsy samples were taken from several patients with borderline lepromatous and polar lepromatous leprosy. One of these specimens was cultured as routinely done; the other was cultured after killing of the cells by freezing and thawing. The autoradiographs of the culture fluids of dead tissues showed no labeled lines, whereas the cultures of living tissues showed labeled IgG lines.

DISCUSSION

The results of this study show that IgG is synthesized in different amounts in the various forms of leprosy and that complement (C3) is produced in about one-fourth of the cultures of polar tuberculoid leprosy and sporadically in the other forms.

What is the function of these locally synthesized immunoglobulins? In all probability they include antibodies directed against *M. leprae* antigens. This view is supported by the following finding: when the leprosy patients are classified according to the three main forms, namely tuberculoid (polar tuberculoid plus subpolar tuberculoid), borderline (borderline tuberculoid plus borderline plus borderline lepromatous), and lepromatous (subpolar lepromatous plus polar lepromatous) leprosy, a spectrum of immunoglobulin synthesis is found that ranges from a small amount in tuberculoid leprosy, to a distinct amount in borderline leprosy, to a considerable amount in the lepromatous form (Table 2). This pattern is reflected in the results obtained by Abe et al. (1), Myrvang et al. (13), and Harboe et al. (6), all of whom found an increase in the amount and titer of antimycobacterial serum antibodies, including *M. leprae*-specific antibodies, from the tuberculoid toward the lepromatous end of the spectrum. Therefore, it is conceivable that the locally produced immunoglobulins are antibodies directed against *M. leprae* antigens. In preliminary experiments using crossed immunoelectrophoresis with intermediate gel and autoradiography, we found antibodies directed against several *M. leprae* antigens in the culture fluids of skin biopsies from patients with borderline and lepromatous leprosy; culture fluids from dead tissue were negative (manuscript in preparation).

Synthesis of small amounts of C3 was found in one-fourth of the cultures of tuberculoid leprosy and in about one-sixth of the cultures of the other forms (Table 2). This finding is contrary to expectation, since mononuclear phagocytes occur in the lesions of all forms of leprosy and these cells are known to produce certain complement components (3, 5, 11, 20, 22). Failure to demonstrate C3 synthesis could be due to production below the level of detection, but this is

TABLE 2. *Synthesis of immunoglobulins and complement in lesions of leprosy patients*

Form of leprosy	Total no. of cultures	IgG		C3	
		No. of positive cultures	Mean intensity of positive cultures[a]	No. of positive cultures	Mean intensity of positive cultures[a]
Indeterminate	4	0	−	0	−
Tuberculoid[b]	20	12	(+)	5	(+)
Borderline[c]	13	13	+	2	(+)
Lepromatous[d]	19	16	++	3	(+)

[a] Intensity of autoradiographic lines: −, negative; (+), just visible; +, clearly visible, to a maximum of ++++.

[b] Polar tuberculoid plus subpolar tuberculoid.

[c] Borderline tuberculoid plus borderline plus borderline lepromatous.

[d] Subpolar lepromatous plus polar lepromatous.

unlikely, because C3 synthesis has been demonstrated in almost all of the investigated skin diseases in which mononuclear phagocytes are involved (9). However, these diseases are not infectious in nature; in another infectious disease (cutaneous leishmaniasis) the cultures of skin biopsies from lesions also did not synthesize complement (unpublished data). The second possibility is that C3 is produced but is not excreted. This possibility too is very unlikely, because in the present study C3 would have been released from the mononuclear phagocytes during freezing and thawing. The third possibility is that the macrophages do not produce complement because they are loaded with *M. leprae*, which might hamper the synthesis of complement. However, this explanation is unlikely, because the small number of cultures showing C3 synthesis are equally distributed over the three forms of leprosy; furthermore, macrophages containing many *M. leprae* are found in lesions of the lepromatous and borderline forms, whereas the lesions occurring in the tuberculoid form seldom contain these bacilli. The fourth possibility is that C3 is not produced due to a functional defect of the macrophages. In this respect it is interesting to mention that Matsuo and Skinsnes (12) demonstrated a β-glucuronidase deficiency in macrophages derived from lepromatous leprosy patients, whereas the macrophages from tuberculoid leprosy patients did not show this deficiency. Further studies are needed to determine whether there is a functional defect in the macrophages from leprosy patients and, if so, whether the defect is more pronounced in the lepromatous than in the tuberculoid form.

ACKNOWLEDGMENTS

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