C-REACTIVE PROTEIN IN SERUM OF PATIENTS WITH LEPROSY

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An abnormal serum protein, called the C-reactive protein (CRP), has been observed in a wide variety of inflammatory processes. This abnormal constituent of serum was first described in 1930 by Tillett and Francis (13), who found that the somatic C polysaccharide of the pneumococcus formed a precipitate when added to the sera of patients with acute penumococcal pneumonia. The reaction, however, was found not to be specific for pneumococcal infections, being also observed with sera from patients with acute rheumatic fever, staphylococcal osteomyelitis, and subacute bacterial endocarditis.

Subsequently, Ash (3) demonstrated a similar reaction with sera from patients with infections caused by gram-negative bacilli of the colon-typhoid group. Hedlund (6), utilizing the observation that C-reactive protein caused capsular swelling with certain strains of pneumococci (8), was able to study CRP in a very wide variety of pathological conditions.

A common denominator in the various disease conditions with CRP in the serum seemed to be the presence of an inflammatory process. More recent investigators (2, 4, 12, 15) have described the use of CRP determinations to follow the course of rheumatic activity in acute rheumatic fever. One report (7) has suggested the possible value of CRP determinations to indicate the presence of myocardial necrosis with its associated inflammatory response in patients with coronary atherosclerosis.

A large body of information has been accumulated regarding the physicochemical properties of the C-reactive protein. Abernathy and Avery (1) demonstrated that CRP was associated with the albumin fraction of the serum proteins after ammonium sulphate precipitation; and Wood and associates (14) have recently shown by electrophoresis that the CRP is a member of the beta globulins. MacLeod and Avery devised a method for the isolation and purification of CRP, and were able to produce a specific antiserum to it in rabbits (9, 10). This has made it possible to detect very small amounts of CRP in sera by a precipitin reaction using the specific C-reactive protein antiserum, and it is with this method that the present investigation has been carried out.

Although determinations of CRP have been performed in a large number of different diseases, we have been unable to find any reports

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of its study in patients with leprosy. In view of the unusual host-parasite relationship in the infection with *Mycobacterium leprae*, and the frequent difficulty encountered in evaluation of the degree of inflammatory response to the organism by clinical or available laboratory methods, a study of levels of CRP in 100 patients with various types of leprosy in both active and arrested states was undertaken.

MATERIALS AND METHODS

Sera were collected from 100 patients with leprosy at the U. S. Public Health Service Hospital at Carville, Louisiana.[2] On the basis of clinical information, examinations of skin scrapings and skin biopsies, these patients were first divided into lepromatous (88 cases) and tuberculoid (12 cases) types, according to the classification of the VI International Congress ([5]). The former group was subdivided into active lepromatous and apparently arrested lepromatous.[3] On the basis of clinical data, chemical examinations of urine and blood, and congo-red tests, 24 of the 100 patients were thought to have secondary amyloidosis, and this group is given special consideration in the tabulations of the data.

C-reactive protein in the sera was determined by a capillary precipitin test using specific antiserum produced by Schieffelin & Co., the method used for the determination being that recommended by the manufacturer. Capillary tubes approximately 9 cm. in length and having an external diameter of 1 mm. were used. A column of CRP antiserum 1.5 cm. long was drawn into the capillary and followed by a column of the patient's serum of the same length, care being taken to insure that the columns of antiserum and serum were in contact. The entire column was then allowed to run to the center of the capillary tube, which was then placed upright in a plasticine block. This was incubated at 37°C. for 2 hours and then placed in the 4°C. refrigerator overnight. On the following morning the degree of reaction was read in terms of millimeters of precipitate in the column, and this was then graded one-plus (1+) for each millimeter of precipitate.

RESULTS

The results of the determinations of CRP in 100 patients with leprosy are summarized in Table 1 and Text-fig. 1.

Of the 47 patients with active lepromatous leprosy, 37 (79%) had CRP in the sera. As shown in Table 2, the reactions varied from 1+ to 4+, with most of them 1+ or 2+. There were 10 patients in this group without detectable CRP as determined by examination of a single serum (Table 1). Two of these patients were under treatment with cortisone at the time the serum samples were collected, because of episodes of erythema nodosum leprosum. The remaining 8 had cutaneous lesions and positive skin smears which were essentially similar to those of the 37 patients with CRP in their sera.

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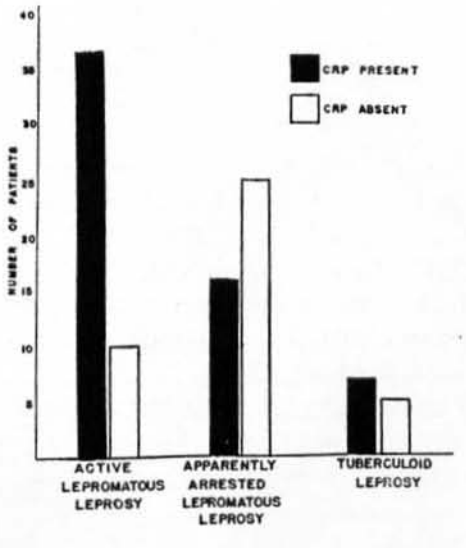
[2] I wish to express my gratitude to Sister Hilary Ross who collected the sera and performed the examinations of the skin scrapings, and to Drs. E. Gordon, Jr., R. Wolcott, and J. Shuttleworth who gave permission for the study and furnished clinical information.

[3] This is not to say that the term "active" signifies progressive disease; it means only "disease not apparently arrested." Many of the "active" cases were retrogressing and more or less improved under the sulfone treatment general at Carville.

TABLE 1.—*Determinations of C-reactive protein in patients with leprosy.*

Case Group	No. of sera examined	No. positive for CRP	Per cent positive
All cases (100)			
Active lepromatous	47	37	79
Arrested lepromatous	41	16	39
Tuberculoid	12	7	58
Total	100	60	60
Cases with amyloidosis (24)			
Active lepromatous	15	13	87
Arrested lepromatous	9	6	67
Total amyloid	24	19	79
Cases without amyloidosis (76)			
Active lepromatous	32	24	75
Arrested lepromatous	32	10	31
Tuberculoid	12	7	58
Total	76	41	54



TEXT-FIG. 1.—C-reactive protein in the sera of Carville leprosy patients, comparing the relative frequencies of positive and negative sera in the case groups discussed.

Only 16 (39%) of the 41 patients with the disease apparently arrested, clinically and bacteriologically, were found to have CRP, and 6 of them had active amyloidosis. One of the patients with inactive leprosy but demonstrable CRP had had a recent myocardial infarct, another had had a recent pulmonary infarct, and a third had had recent plastic surgery. Two of these patients had chronic ulcers of the lower extremities. In the remaining 5, no adequate explanation for the presence of CRP was found; however, it seems quite possible that some undetected inflammatory process may have been present.

TABLE 2.—*Semiquantitative estimates of CRP in patients with leprosy.*

Case group	Degree of precipitation				
	4+	3+	2+	1+	0
Active lepromatous	0	1	7	16	8
Arrested lepromatous	0	0	3	7	22
Tuberculoid	0	0	3	4	5
Amyloidosis	1	2	9	7	5
Total	1	3	22	34	40

The small group of tuberculoid cases has not been subdivided according to activity, although most of them were described as old quiescent cases with little or no clinical evidence of activity. Of the 7 with CRP (58%), one had malignant exopthalmos with ocular inflammation, another had widespread sarcoidosis, and a third was receiving radiation therapy at the time the serum sample was collected.

The 24 patients with amyloidosis are of special interest and are considered in the second section of Table 1. All of these patients had lepromatous leprosy, and, as noted in the table, 15 were in the active group while 9 were apparently arrested. No less than 13 of the 15 active cases with amyloid disease had CRP in their sera, while 6 of the 9 apparently arrested cases with amyloidosis also showed CRP. When all of the patients with amyloidosis are considered together, it can be seen that 19 of the 24, or 70 per cent, were positive for CRP. As shown in Table 2, in the group with amyloidosis there seems to be a larger proportion of 2+ reactions than in the others. Two of the 3 cases with 3+ reactions, and the only one with a 4+ reaction, are in the amyloidosis group.

The third section of Table 1 shows the results of the CRP determinations in all the patients without amyloid disease. There, the percentage of patients with CRP in the active lepromatous group is more than twice that in the group with inactive disease.

DISCUSSION

The presence of C-reactive protein in the sera of a large proportion of patients with active lepromatous leprosy is not surprising. Lepromatous leprosy is characterized by a histiocytic inflammatory response, and CRP is found in sera of persons with a wide variety of inflammatory diseases.

The finding of a small group of patients with active lepromatous leprosy, as that designation is used here, but without CRP merits some comment. This failure to develop CRP may represent some significant difference in host response. Whether this represents a factor of favorable or unfavorable prognostic significance can be determined only by long-term study of these cases.

Since almost all of these patients were receiving sulfone therapy at the time the sera were collected, the absence of CRP may be an early manifestation of a favorable therapeutic response. This would be in accord with observations of the disappearance of CRP in patients with acute rheumatic fever during cortisone therapy (12). In this connection, it is also of interest to note that 2 of the active lepromatous cases without CRP were under treatment with cortisone for erythema nodosum at the time the sera were collected.

Only 16 of the 41 apparently arrested lepromatous cases had CRP, and as said a number of them had known inflammatory diseases unrelated to their leprosy. In only 5 could no explanation for the presence of CRP be found, although it seems quite possible that undetected inflammatory foci existed. The possibility that some of these patients did have an active inflammatory response to *M. leprae* in spite of clinical and bacteriological evidence of inactivity cannot be eliminated, and, again, subsequent observation of the course of these patients is planned and may clarify the matter.

The results in the 12 cases of tuberculoid leprosy are difficult to interpret. All of the 7 cases with CRP were thought to be clinically arrested, and in only 2 of these could an adequate cause for the CRP be found. As with the apparently arrested lepromatous cases, the possibility of undetected inflammatory foci unrelated to the *M. leprae* infection, as well as that of active tuberculoid inflammation in spite of clinical signs of inactivity, must be considered.

The large proportion of patients with secondary amyloidosis who had CRP may be of significance. As shown in Table 1, second section, 79 per cent of the 24 patients with amyloidosis had CRP, including 6 of the 9 inactive lepromatous ones. There is at present no information available on the relationship of CRP to the development of amyloidosis in either patients or experimental animals. It is of interest, however, that Richter (11) has described the appearance of a beta globulin in the serum of animals developing amyloidosis in response to injections of sodium ribo-

nucleate; and that Wood and his associates (14) have recently shown that CRP is a beta globulin.

At the time the present study was initiated, it was hoped that determination of CRP might be of value in detecting the presence and degree of inflammatory response to *M. leprae* infection. It was further hoped that this information might be of some prognostic importance, or of aid in evaluating response to therapy. From the results presented above it seems possible that CRP determinations may have such values. Nevertheless, any real importance the test may have can only be evaluated by long-term studies, with correlation of serial measurements of CRP and the clinical course in the patients. Such studies are planned in this laboratory, and it is hoped that other investigators who have access to patients with leprosy will also utilize this simple test in order to determine its place, if any, in the study of leprosy.

SUMMARY

The levels of C-reactive protein (CRP) in the sera of 100 patients with leprosy have been determined. This abnormal protein was found in 79 per cent of 47 cases of the lepromatous leprosy classed as "active" (i. e., not arrested), 30 per cent of 41 cases of apparently arrested lepromatous leprosy; and 58 per cent of 12 tuberculoid cases. Possible explanations for the absence of CRP in some of the active cases, and its presence in some of the apparently arrested cases, have been offered. The importance of long-term studies, with correlation of clinical course and serial determinations of CRP, is stressed.

The presence of CRP in 19 of 24 cases of leprosy with secondary amyloidosis has been described. The relationship of CRP to amyloidosis is as yet unknown, but the present findings would suggest that the problem merits further study.

ACKNOWLEDGMENT

Credit should be given to Mr. Miller Turner for his assistance in carrying out the study and in the preparation of the graph and tables.

RESÚMEN

Al determinar las concentraciones de proteína C-reactiva (PCR) en los sueros sanguíneos de 100 leprosos, descubrióse esta proteína anormal en 79 por ciento de 47 casos de la lepra lepromatosa clasificada como "activa" (es decir, no estacionada), 30 por ciento de 41 casos de lepra lepromatosa aparentemente estacionada y 58 por ciento de 12 casos tuberculoideos. Ofrécense posibles explicaciones de la ausencia de PCR en algunos de los casos activos y de su presencia en algunos de los casos aparentemente estacionados. Recálcase la importancia de estudios a largo plazo, con correlación de la evolución clínica y las determinaciones seriadas de PCR.

Se describe la presencia de PCR en 19 de 24 casos (79 por ciento) de lepra en que había amiloidosis secundaria. No se conoce todavía la relación de la PCR con la amiloidosis, pero los hallazgos presentados sugieren que el problema es acreedor a estudios ulteriores.

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