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The Relationship of C-Reactive Protein Levels in Lepromatous Leprosy to Erythema Nodosum Leprosum[1]

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C-reactive protein (CRP) is a component of serum that is present only under certain pathologic conditions. Tillett and Francis ([8]) first reported it in the sera of patients with acute pneumococcal pneumonia. It was demonstrable as a nonantibody protein having the capacity to form a precipitate with the C polysaccharide of *Diplococcus pneumoniae*. CRP has since been demonstrated in a number of disease states, and it is now generally accepted that its presence indicates an active inflammatory process ([3, 4, 5]).

In 1955, Rabson ([6]) reported that 37 out of 47 patients with active lepromatous leprosy had CRP in their sera. Of the 10 CRP-negative cases in this group, two were under cortisone treatment for erythema nodosum leprosum (ENL). Bush ([2]) reported the presence of CRP in the sera of 39 out of 73 patients with "nodular" leprosy. He was concerned to a great extent with the effect of "complications" on the CRP level in this disease and concluded that "the CRP test is a more accurate guide to progress of complications in leprosy than the sedimentation rate." Ross *et al.* ([7]) investigated the CRP reaction in a group of leprosy patients over a period of two years. Of 280 bacteriologically positive lepromatous cases, 121 were reported to be inconsistently positive and 63 were consistently positive.

With the exception of the indirect reference to two patients under cortisone therapy mentioned in the study by Rabson, these investigations were made without question of the effect of ENL on the CRP test results. In these two patients, Rabson was primarily concerned with the effect of cortisone on the CRP test. Abe and Hirako ([1]) reported that "CRP was absent in the sera of ENL patients whose symptoms were relatively light, but always present in the severe cases." Of 135 residual lepromatous patients, not in the reactional state at the time of the test, they reported only two (1.5%) as being positive. Of 84 lepromatous patients with ENL, 72 (86%) were CRP-positive. On the basis of these data, they suggested that the CRP test would be useful for the differentiation of ENL from other lepra reactions and as an indication for cortisone treatment of ENL.

This paper reports an additional investigation to test the concept that CRP and ENL are directly related in lepromatous patients.

MATERIALS AND METHODS

The C-reactive protein was determined with the use of Bacto C Protein antiserum furnished with kits, including the capillary tubes, and control sera, by Difco.[3]

The test is easily and rapidly accomplished in the manner of a standard capillary precipitin ring test. In the CRP test, however, the antiserum is applied to test for an antigen in the patient's serum. After

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incubation, a positive test results in a flocculation, which settles to the lower air-liquid interface in the capillary tube. Quantitation is based on a direct measure of the height of the column of flocculate in millimeters, i.e., 1 mm. = 1+, 2 mm. = 2+, etc. All tests were run within 24 hours after withdrawal of the blood; however, tests on refrigerated sera showed no significant changes in reaction character of the sera over a period of weeks.

Venous blood was withdrawn with a 25 gauge needle and a 2 ml. syringe. The small needle and syringe were found to be desirable in order to relieve the apprehension of patients from whom repeated specimens might be required. One to two ml. of blood was withdrawn and immediately expelled into 10 x 75 mm. test tubes for clotting and subsequent aspiration of the serum for testing.

The bleeding schedule was arranged to accomplish three phases of study. In the first phase, blood was drawn from 312 patients with leprosy, in the Eversley Childs Sanitarium, without regard to degree of activity or clinical classification of the disease.

The second phase of the study was conducted over a period of three months, during which time a selected group of "reaction-prone" patients was tested twice weekly for CRP. "Proneness" was defined as a characteristic of those patients whose clinical history indicated that they would most probably encounter a period of erythema nodosum leprosum during the study period. On termination of this phase, 17 patients had completed a sequential series of from seven to 19 semi-weekly clinical examinations and simultaneous CRP tests. Of these patients, 11 were ambulatory and six were confined to the infirmary. On the scheduled bleeding dates, and prior to bleeding, each patient was examined closely for the clinical signs and symptoms as indicated in Table 2, and the extent of each sympton was graded, according to severity, on a scale of 0 to 3, i.e., 0 for absence, 1 for mild, 2 for moderate, and 3 for severe. The values for fever were based on a scale of 1 for body temperatures between 37.1° to 37.9°C, 2 for 38.0° to 38.9°C and, 3 for temperatures over 38.9°C.

In the third phase of the study, periodic and random bed checks were made of the infirmaries. Any patient with an oral temperature in the range 37.5° to 40°C was bled, on a voluntary basis, for a test of the CRP levels. The bleeding was accomplished immediately after the temperature was taken, and accordingly the CRP-temperature relationships reported here are for simultaneous readings. The same thermometer was used in all readings to assure relative values. The selection of patients was with respect to temperature in an attempt to spread the tests evenly over the study range. Any patient with a temperature identical to that of another from whom a specimen was taken earlier, was not bled. There were a few exceptions to this rule. This limitation on the number of tests was necessary because of the understandable dissatisfaction with continued blood-letting on the part of febrile bed patients, who generally also had neuritis, a problem especially evident in the higher body temperature ranges.

RESULTS

Table 1 is a summary of the results of the first phase of this study. It illustrates the scope of the results of CRP tests conducted in a random survey of a group of 312 out of approximately 500 patients within the Eversley Childs Sanitarium. The sera were taken without regard to extent or clinical classification of the disease. It was seen that 181 patients, or 57 per cent, were found to be positive at the time of testing. Of these,

TABLE 1. *A survey of C-reactive protein levels in lepromatous patients.*

CRP level	No. of patients	Per cent
0	131	42
1	95	30
2	44	14
3	36	12
Over 3	6	2
Total	312	100

95 patients (30%) gave 1+ CRP reactions; 44 (14%) gave 2+; 36 (12%) gave 3+; and 6 patients (2%) had reactions higher than 3+. In this last group, one patient had a CRP of 9+. He died within three weeks of testing. The cause of death was not determined.

In the second part of the study, the sera were taken from 17 patients selected on the basis of a history of ENL. All of the patients in this phase showed acute manifestations of ENL during the period of observations except one, who went through a period of six months with only negligible signs of this condition. He was retained in the study because his record of having had periodic episodes of ENL satisfied the criterion for inclusions.

These patients were classified in Table 2 into two groups, one group of 11 ambulatory and another of six infirmary cases. The sum of the mean values of all the signs and symptoms of ENL for each patient for the entire period of observation ranged from 0.5 to 6.5 The corresponding mean of CRP levels ranged from 0 to 3.0. Table 2, however, shows that this relationship was not linear. In general, it could be stated that the more severe the ENL, the higher the level of CRP. An interesting observation was that when the ENL was so severe that it required confinement of the patients, the CRP values were markedly elevated, so that the difference between the CRP levels in ambulatory and infirmary patients was highly significant.

Table 3 shows this relationship between the severity of ENL and the levels of CRP even more vividly. The data in Table 3 are taken from Table 2 and the patient numbers refer to the same individuals, but they are now arranged in the order of the severity of their ENL, based on the sum of the means of the clinical signs and symptoms. Values between 0 and 2 were classed as mild, 2 to 5 as moderate and over 5 as severe.

It can be seen in Table 3 that the mild, moderate and severe cases had mean temperature values of 0.1, 0.5 and 1.0 respectively. The corresponding values for CRP were 0.3, 1.0 and 2.5. There was, therefore, a remarkable correlation in the rise in temperature, increase in CRP and severity of ENL in a group of patients selected on the basis of "proneness" to ENL.

The patients in the third phase of this study were selected on the basis of body temperature. The results, as seen in Table 4, indicate that while fever and CRP do coexist in ENL patients the degrees of each are not necessarily related. Patient number 19 entered the study at three grades of fever, 37.6°, 38.4° and 38.7°C. The respective CRP results were 3+, 5+ and 3+. Patient number 18 had body temperatures of 37.5° and 37.9°C with respective CRP values of 4+ and 4+.

As could be expected, fever was, very frequently, not related to ENL. Two tests each on patients numbered 20 and 24 with different grades of fever, one as high as 39.1°C, showed negative CRP tests. The clinical records made without regard to this study showed no ENL present on those occasions.

Two patients, numbers 26 and 29, died subsequently to these studies. The autopsy report for patient number 26 showed death as a result of miliary tuberculosis. At the time of this study this patient had a 2+ CRP with a fever of 38.8°C. The autopsy report for that patient number 29 read ". . . histologic changes in the liver consisting of acute inflammation, focal necrosis and micro-abscess formation." The corresponding fever and CRP for this patient were 39.9°C and 7+. In the former case, the low CRP was in agreement with the finding that it was not a reactional condition, while the presence of a very high CRP in the second case was consistent with the reaction-related findings on autopsy.

DISCUSSION

The overall survey of CRP reactions in leprosy patients indicated that a significant response was evident in a population of this kind. The question might be asked about the absence of a similar negative control group. However, this study was conducted on the premise that CRP is an accepted indicator of a pathologic condition. It is true that the reaction is considered nonspecific.

TABLE 2. *Clinical data of lepromatous patients with erythema nodosum leprosum (ENL).*

	Patients																
	Ambulatory											Infirmary					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
No. Exams[a]	19	12	19	12	12	12	19	7	12	19	7	19	8	8	8	8	8
ENL % of days[b]	100	0	90	67	25	50	100	71	100	100	100	100	100	100	100	100	100
Severity of ENL[c]																	
Papules	1.9	0.8	0.5	0.6		0.3	1.8	0.9	1.6	0.9	1.0	1.5	1.5	1.3	0.6	0.4	1.4
Nodules	2.6		0.6	0.2	0.3	0.4	1.3	0.4	0.8	1.4	1.4	1.9	2.1	2.0	1.6	1.6	1.5
Blebs	0.1						0.1						0.3			0.8	
Pustules						0.1										0.1	
Ulcers							0.2						0.3		1.8	1.8	
Patches							0.1					0.1	0.4			0.3	0.6
Infiltration				0.2													0.6
Neuritis							0.1					0.1		0.1	0.1		
Chills													0.1			0.5	1.0
Fever	0.1		0.1		0.2		0.2	0.4	0.3	0.2		1.1	1.5	1.1	1.5	1.0	1.0
Headache							0.1					0.1					
Joint pains	0.6																
Total[d]	5.3	0.8	1.2	1.0	0.5	0.8	3.9	1.7	2.7	2.5	2.4	4.8	6.2	4.5	5.6	6.5	6.1
CRP[e]	1.4	0	0.4	0.3	0	0	0.7	1.1	0.2	0	0	2.6	2.4	2.4	2.8	2.9	3.0

[a] Continuous sequence of semiweekly examinations.
[b] Per cent of examination days on which a diagnosis of erythema nodosum leprosum was made.
[c] The adjacent values are the means of the sums of the estimations of the severity of the clinical signs and symptoms of ENL on a daily examination scale of 0 to 3. Where no value is recorded the value is 0.
[d] The sum of the means in *c* above.
[e] The means of all CRP tests made on examination days.

TABLE 3. *Relationship of the severity of ENL, fever, and CRP in lepromatous leprosy.*

Patient No.	Total ENL[a]		Fever		CRP	
5		0.5	0.2		0	
2		0.8	0		0	
6	Mild	0.8	0	0.1[b]	0	0.3[b]
4		1.0	0		0	
3		1.2	0.1		0.4	
8		1.7	0.4		1.1	
11		2.4	0		0	
10		2.5	0.2		0	
9	Moderate	2.7	0.3	0.5[b]	0.2	1.0[b]
7		3.9	0.2		0.7	
14		4.5	1.1		2.4	
12		4.8	1.1		2.6	
1		5.3	0.1		1.4	
15		5.6	1.5		2.8	
17	Severe	6.1	1.0	1.0[b]	3.0	2.5[b]
13		6.2	1.5		2.4	
16		6.5	1.0		2.9	

[a] Total of the mean values for "severity of ENL" as recorded in Table 2.
[b] The mean of the adjacent values.

TABLE 4. *Relationship between fever and C-reactive protein in lepromatous patients.*

Temperature °C	Patient No.	Date	CRP	Comment
37.5	18	3/17	4	
37.6	19	3/23	3	
37.7	20	3/23	0	Not ENL
37.8	21	3/15	4	
37.8	22	4/6	7	
37.9	18[a]	3/21	4	
38.2	24	4/4	0	Not ENL
38.3	20[a]	5/31	0	Not ENL
38.4	19[a]	3/15	5	
38.6	25	4/6	6	
38.7	19[a]	4/4	3	
38.8	26	4/29	2	Died 8/15/66 Autopsy-tuberculosis
39.1	24[a]	3/15	0	Not ENL
39.2	27	3/28	7	
39.8	28	4/5	3	
39.9	29	4/11	7	Died 10/11/66 Autopsy-acute liver inflammation
40.0	30	4/29	4	

[a] Repeat patient, different temperatures.

The survey reported here indicates that the CRP test is not positive for the state of leprosy *per se*. No correlation could be found between the level of CRP and the extent of clinical infection. If CRP is appearing as a function of leprosy it must be as a result of one or more of the various complications associated with this disease, and not due to the disease itself. The most probable leprosy complication to implicate is ENL. The relationship of ENL to CRP would be consistent with the nature of the CRP response in other diseases where it assumes a reactional character. The results of this study gave strong evidence that a relationship of this kind does indeed exist.

It is difficult to define degrees of ENL among various individuals with any certainty. The various manifestations are subject to arbitrary values and interpretations on the part of the clinician, as well as differences in the febrile responses and pain thresholds on the part of the individual patient. For these reasons an objective comparison of ENL and CRP was not possible. Nor was any single manifestation of ENL directly related to CRP. On the other hand, the cumulative effect of the various manifestations did appear to be related to CRP.

As a single entity, fever showed the most consistent association with CRP. However, it is clear that this association existed as a qualitative relationship, without regard to the degree of either fever or CRP. While CRP must be accepted as a nonspecific response, it was obvious from this study that, with respect to ENL in a given population of leprosy patients, fever was even less specific. The two febrile patients without CRP were evaluated as being without ENL on a completely "blind" basis. The two patients who died four and six months, respectively, after the study are also evidence of the greater specificity of CRP. The first of these patients, subsequently confirmed as a case of miliary tuberculosis, had 2+ CRP. This low value was not a direct result of ENL, and was generally consistent with the opinion, formed during this study, that ENL *per se* rarely confined a patient to the infirmary until the CRP level was above 2. The CRP response in this instance could have been either a low level response to tuberculosis itself or to a nonspecific activation of the lepra reaction by a related infectious agent. On the other hand, the autopsy report on the other patient showed evidence that could be indicative of a severe reactional state, as was indicated by the earlier high CRP. Since the deaths occurred after a relatively long period of time following the tests, these explanations are speculative. Subsequent CRP tests were not conducted on the tuberculosis patient because of the blind aspect of the study. However, the results of the study were being analyzed on the occasion of the second death and a request was made to conduct a CRP test on the postmortem serum of patient number 29. This serum gave a 4+ reaction, a fact that is probably more interesting than enlightening.

The sedimentation rate and CRP levels have also been equated in many studies. In data from this study, but not reported here, this relationship was not evident. A reasonable number of comparisons were actually made where it was also possible to take a heparinized sample of blood for a sedimentation rate determination. A CRP-sedimentation rate association was apparent, but no direct relationship was observed either in direction or degree. It was our opinion that the CRP clearly represented a more direct measure of the extent of ENL and was less influenced by nonspecific factors than the sedimentation rate.

During this study one patient was observed to undergo marked changes in both CRP and temperature within 24 hour periods. This patient was one within the group selected on the basis of body temperature. When this response was questioned, it was disclosed by the clinician in charge, that this patient was under steroid therapy. A check of the records revealed that the observed reductions in CRP levels closely followed the times of steroid administration. Further examination of the records revealed an additional patient on steroid and two patients under treatment with the nonsteroid anti-inflammatory agents Pyrazone and Butazolidine. In this case also, the steroid administration was evidenced by a subsequent drop in CRP level, but the anti-inflammatory agents had no effect on

the CRP levels. The data on these patients were not included in the material presented here. It was also observed, on numerous occasions, that aspirin could influence the body temperature, but had no significant effect on the CRP level.

The foregoing observations support a conclusion that CRP affords a means to an effective, objective measure of ENL. This conclusion opens the corollary premise that the CRP test will be a valuable tool in the evaluation and treatment of ENL and in the investigation of new drugs for ENL therapy.

SUMMARY

C-reactive protein (CRP) was evident at the 1+ or higher levels in 57 per cent of 312 leprosy patients at the Eversley Childs Sanitarium, Cebu, Philippines. The normal level of CRP is 0. A definitive study indicated that the elevated CRP levels in leprosy patients were related to the cumulative effect of the various manifestations of ENL. No single manifestation afforded a better indicator of the extent of ENL than the CRP. It is suggested that CRP determinations could be used as a measure of the success of ENL therapy, both clinically and experimentally.

RESUMEN

Proteina C-reactiva (CRP) fué evidente a 1+ o mas altos niveles en 57 por ciento de 312 pacientes de lepra en Eversley Childs Sanitarium, Cebu, Philippinas. El nivel normal de CRP es 0. Un estudio definitivo indicó que niveles elevados CRP en pacientes de lepra estaban relacionados al efecto acumulativo de varias manifestaciones de ENL. Ninguna manifestación presentó un mejor indicador de la extención del ENL que el CRP. Se sugiere que las determinaciones del CRP puede ser usada como una medida del éxito del tratamiento con ENL, tanto clínica como experimentalmente.

RÉSUMÉ

Chez 57 pour cent parmi 312 malades de la lèpre de l'Eversley Childs Sanitarium, à Cebu aux Philippines, on a pu mettre en évidence la protéine C réactive (CRP) à des niveaux 1+ ou plus élevés. Le niveau normal de CRP est zéro.

Une étude définitive a révelé que l'élévation des niveaux de CRP chez les malades de la lèpre était associée avec l'effet cumulatif de diverses manifestations d'ENL (erythème noueux lépreux). Aucun autre signe isolé, sinon le CRP, n'a permis de mieux mesurer l'étendue de l'ENL. On suggère que les déterminations de la CRP pourraient être utilisées comme mesure de l'efficacité des thérapeutiques de l'ENL, à la fois cliniquement et expérimentalement.

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