C-Reactive Protein, Immunoglobulin and Serum Protein Analyses of Sera from Cases of Lepromatous Leprosy and Tuberculosis[1,2]

Elizabeth Tamblyn and Y. Hokama[3]

Analysis of C-reactive protein (CRP) in various conditions of leprosy, reported by several investigators ([1, 3, 4, 8, 11, 17]), has proven fruitful. Results of the recorded examination could be summarized as follows: (a) High frequencies of CRP-positive reactors are found in patients with active lepromatous leprosy with or without concurrent erythema nodosum leprosum (ENL), and thus its appearance could be employed for the determination of efficacy of therapy in those cases; and (b) patients with residual or relatively inactive leprosy show a low or no frequency of CRP occurrence in their sera.

Recent examinations of immunoglobulin (Ig) levels in leprosy and tuberculosis have indicated variations in the levels of these proteins. For example, Lim and Fusaro ([6, 7]) hare shown a high frequency of elevated immunoglobulin A (IgA) among patients with tuberculoid leprosy and tuberculosis. Tamblyn *et al.* ([16]) similarly showed a significant quantitative increase in IgA in the plasma of far advanced and moderately advanced tuberculous cases.

Since concurrent utilization of several laboratory tests might prove more useful in prognosis and possible differential diagnosis, we have examined the appearance of CRP, immunoglobulin analysis, and serum protein profiles in patients with lepromatous leprosy and tuberculosis. This paper presents the preliminary results of such a critical examination.

MATERIALS AND METHODS

Thirteen cases of lepromatous leprosy selected from the Hale Mohalu Leprosarium, Honolulu, Hawaii, were examined in this study. The group consisted of six females with a mean age of 39.8 years (range 30-55 years) and seven males with a mean age of 43.1 years (range 25-64 years). The duration of hospitalization varied from six months to 19 years.

A parallel study of 9 cases of far advanced and eight cases of chronic-minimal tuberculosis was carried out. The far advanced tuberculous group consisted of seven males and two females with a mean age of 71.0 years (range 38-87 years). The chronic-minimal group was comprised of five males and three females with a mean age of 61.1 years (range 53 to 82 years).

A control group of normal healthy nonpatients consisted of 16 individuals, nine females and seven males, with a mean age of 50.0 years (range 31 to 67 years). No significant illnesses were noted at the time the blood samples were taken.

The following ethnic backgrounds were represented in both the normal group and the groups of patients with leprosy and tuberculosis donating sera for this examination: Filipino, Samoan, Hawaiian, part-Hawaiian, Japanese and Chinese.

Blood was collected by venipuncture and permitted to clot at room temperature. The serum samples obtained were then kept at −20°C until use.

1 Received for publication 22 July 1968.

2 This work was supported in part by U. S. Public Health Service Grants R01-CA-10671-01 and R01-CA-10130-02 from the National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014.

3 E. Tamblyn, M.S., Assistant Professor of Microbiology and Public Health, California State College at Los Angeles; Y. Hokama, Ph.D., Professor of Pathology, Department of Pathology, University of Hawaii School of Medicine and Leahi Hospital, 3675 Kilauea Avenue, Honolulu, Hawaii 96816.

Quantitation of the Ig was carried out by radial immunodiffusion in agar ([15]). Immunoglobulin standards and their homologous antibodies in standardized prepoured agar plates from the Hyland Laboratories, Los Angeles, California, were used.

Standard Ig's were examined in parallel with each group of serum samples, and the standard values were plotted as diameters of precipitate-rings against concentration of the standard. The values of the serum samples were obtained by interpolation from this standard curve over the standard range examined. Serum samples with results falling outside the standard values were appropriately diluted so that, upon reexamination, the results would fall within the standard values. Immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) were examined by this procedure.

C-reactive protein was determined by the capillary precipitation method of Anderson and McCarty ([2]), using horse anti-CRP (HCRPA). This antiserum was obtained from the Waimanalo Research Labortory, Waimanalo, Hawaii. The results were recorded as millimeters of packed precipitate.

All serum samples were examined on cellulose polyacetate strips (Sepraphore III) by electrophoresis according to the procedure of Nerenberg ([10]). Sepraphore III was obtained from the Gelman Instrument Company, Ann Arbor, Michigan. Briefly, the sequential steps in electrophoresis were as follows: A serum sample of 1 μl. was applied to Sepraphore III and the electrophoresis was carried in barbital buffer, pH 8.6. Following electrophoresis the strip was stained with Ponceau S and the excess stain then removed with 5 per cent acetic acid. The strip was allowed to air-dry between two sheets of filter paper. Finally, the strip was cleared by immersing it in a solution of 15 per cent acetic acid in ethyl alcohol for one to two minutes, and then removed by placing a clean microslide beneath it and flattening it by rolling a clean test tube over it, thus draining off the excess solvent. The slide was placed at a slight angle and allowed to dry in air to a clear translucent membrane. The cleared strip containing the protein patterns was examined with a densitometer containing an integrator. The concentrations of gamma (γ), beta (β), alpha-$_2$ (α_2), and alpha-$_1$ (α_1) globulins, and albumin were calculated from the densitometric tracings and the total protein analysis. Total serum protein was determined by the biuret procedure ([5]) and reported as gm./100 ml.

RESULTS

Six of 13 leprosy patients showed CRP in their peripheral blood. This represented 46.1 per cent CRP-positive reactors among the total lepromatous cases examined, and was essentially similar to that shown by the chronic-minimal tuberculous group. However, in marked contrast, all of the far advanced tuberculous patients showed CRP in their peripheral serum. The leprosy patients showed a significant increase in total serum protein ($P<.001$). Similarly, an increase in total serum protein was noted for both of the tuberculous categories ($P<0.05$). Elevation of alpha-$_2$ and depression of alpha-$_1$ globulins was demonstrated for the lepromatous group, as shown in analysis on cellulose polyacetate strips. The far advanced tuberculous group showed a significant elevation in the gamma and a depression in the alpha-$_1$ globulins. In the chronic-minimal tuberculous group a significant decrease in the beta globulins was shown ($P<0.05$). The results are summarized in Table 1. The probability values were calculated from the 16 normal serum samples examined concurrently.

Table 2 summarizes the immunoglobulin and CRP data for lepromatous, far advanced and chronic-minimal tuberculous patients, and normal individuals. Of significant interest are the marked differences noted between lepromatous and far advanced tuberculous patients with respect to CRP appearances and the immunoglobulin profile. Associated with consistent CRP appearance is the elevated IgA in far advanced tuberculosis. In contrast, lepromatous patients showed essentially normal IgA values with moderate frequency of positive CRP response. In addition, IgG levels were significantly lower in leproma-

TABLE 1. *C-reactive protein appearance and serum protein levels in lepromatous and tuberculous patients and normal individuals.*[a]

Disease category	No. examined	CRP		Serum proteins in gm./100 ml.					
		mm. ppt.	No. positive	Total	Gamma	Beta	Alpha-2	Alpha-1	Albumin
Lepromatous leprosy	13	1.56 (0–4.0)	6	7.90[b] ±0.42	1.79 ±0.42	1.02 ±0.26	0.74[c] ±0.69	0.23[c] ±0.10	4.20 ±0.64
Far advanced tuberculosis	9	2.20 (1.0–4.5)	9	7.56[d] ±0.42	2.73[c] ±0.89	0.84 ±0.36	0.63 ±0.21	0.21[c] ±0.14	3.40 ±1.00
Chronic-minimal tuberculosis	8	2.60 (0–5.5)	4	7.50[d] ±0.51	1.89 ±0.83	0.70[d] ±0.27	0.65 ±0.29	0.27 ±0.17	3.97 ±0.62
Normal	16	negative		7.27 ±0.35	1.67 ±0.95	0.99 ±0.23	0.57 ±0.15	0.40 ±0.17	3.57 ±1.30

[a] Values are scored as means ± standard deviation for serum proteins and means with ranges in parentheses for CRP.
[b] $P < 0.001$.
[c] $P < 0.01$.
[d] $P < 0.05$.

TABLE 2. *Appearance of C-reactive protein and immunoglobulin levels in lepromatous, tuberculous, and normal individuals.*[a]

Disease category	Number examined	CRP		Immunoglobulins in gm./100 ml.		
		mm. ppt.	No. positive	IgG	IgA	IgM
Lepromatous leprosy	13	1.56 (0–4.0)	6	1.14[c] ±0.19	0.26 ±0.10	0.11[b] ±0.06
Far advanced tuberculosis	9	2.20 (1.0–4.5)	9	1.76 ±0.58	0.43[b] ±0.05	0.10[b] ±0.09
Chronic-minimal tuberculosis	8	2.60 (0.5.5)	4	1.78[d] ±0.39	0.32 ±0.09	0.05 ±0.03
Normal	16	negative		1.51 ±0.33	0.26 ±0.10	0.06 ±0.01

[a] Values are scored as means ± a standard deviation for the immunoglobulins and means with ranges in parentheses for CRP.
[b] $P < 0.001$.
[c] $P < 0.01$.
[d] $P < 0.05$.

tous than in tuberculous patients. IgM appeared to be significantly elevated in lepromatous and far advanced tuberculous patients in contrast to the normal individuals examined. However, these levels are within normal values reported for other ethnic groups, while the values for the normal group in this study appear to be lower ([13]).

DISCUSSION

The frequency of occurrence of CRP in the cases of lepromatous leprosy examined was comparable with some of the frequencies reported previously ([1,3,4,8,12,17]). Ross *et al.* ([14]) reported 49 per cent CRP-positive cases occurring sporadically in patients with lepromatous leprosy, negative for acid-fast bacilli. However, frequency of 65.7 per cent CRP-positive cases was found among similar patients, with demonstrable acid-fast organisms. Reich and Tolentino ([12]) found 58.0 per cent CRP-positive reactions in patients with lepromatous leprosy with ENL: In contrast, Abe and Hirako ([1]), in an examination of 84 lepromatous patients with ENL, found 86.0 per cent CRP-positive cases, while 1.5 per cent of patients with residual lepromatous activity were positive for CRP. As with many other disease processes, the general conclusion obtained can be summarized as follows: The frequency of CRP positivity is higher among the active lepromatous cases associated with an active inflammatory condition such as ENL. Hence, the use of CRP for analysis would probably be limited to following the efficacy of drug therapy in active cases where it occurs. Nevertheless, its use could be enhanced when concurrent examinations of immunoglobulins are carried out with the same serum samples, as indicated by the results presented here and elsewhere ([16]).

Previous studies by Lim and Fusaro ([6, 7]) have demonstrated that the frequency of increase in IgA levels in tuberculoid leprosy was 91.0 per cent, and in the lepromatous type, 51.0 per cent. Conversely, IgM increase was observed to be higher in lepromatous (99.0%) than in tuberculoid (62.0%) leprosy. In addition, patients with pulmonary tuberculosis showed a higher frequency of IgA than did patients with lepromatous leprosy. In this respect, tuberculoid leprosy appeared similar to pulmonary tuberculosis. Our previous observations

([16]) and this study are in general agreement with the findings of Lim and Fusaro ([6, 7]). However, our results failed to show high frequency of IgA increase in lepromatous leprosy (Table 2). This discrepancy. might be attributed to the difference in procedure utilized. The values obtained by Lim and Fusaro ([6, 7]) were obtained qualitatively and depended on the precipitation of the immunoglobulin after immunoelectrophoresis following dilution of the serum sample, whereas the values obtained in our study are based on quantitation of the specific Ig by radial immunodiffusion.

The concurrent analyses of CRP and Ig, as previously reported ([16]) for various categories of tuberculosis, strongly suggested that such combined determinations could be a useful adjunct to other laboratory tests. For example, it has been shown that the presence of CRP and an increase in IgA levels are good indicators of an active tuberculous process, whereas the presence of CRP with normal IgA levels but elevated IgM levels, might be indicative of active leprosy. A leprosy patient positive for CRP and an elevated IgA should be examined for possible concomitant tuberculosis.

Examination of tuberculoid leprosy was not attempted in this study. Therefore, it would be of interest to examine further the concurrent appearance of CRP and immunoglobulins in serum of these patients, since it has been demonstrated qualitatively that IgA levels appear to be elevated in tuberculoid leprosy ([7]).

Finally, it may be of value to reiterate that variations in the frequency of appearance of CRP in a given disease process, obtained by various investigators, may be attributable to variations in the source of CRP antisera. This has been suggested previously by Nakamura *et al.* ([9]).

SUMMARY

Sera of lepromatous and tuberculous patients were examined concurrently for C-reactive protein (CRP), immunoglobulins and serum proteins by electrophoresis and immunodiffusion. Sera of lepromatous patients showed a significant increase in total serum protein, reflected by α_2 globulin elevation. CRP was present in the sera of 46.1 per cent of the patients examined. Sera of far advanced tuberculous patients showed a high frequency of occurrence of CRP, with concomitant significant increase in immunoglobulin A levels. Chronic-minimal tuberculous patients had lower frequency occurrence of CRP and appeared similar to the lepromatous group. Total serum proteins were increased significantly in the tuberculous and lepromatous groups. It is suggested by these studies that concurrent examination of CRP and immunoglobulins would be a valuable means of determining the prognostic course and possible differential diagnosis in diseases caused by mycobacterial organisms.

RESUMEN

Sueros de enfermos lepromatosos y tuberculosos fueron examinados concurrentemente en búsquede de proteína reactiva-C (CRP), e inmunoglobulinas y sero proteínas por electroforesis e inmunodifusión.

El suero de enfermos lepomatosos mostró un aumento significante en el total de proteínas del suero, que se reflejó en un alza del nivel de globulina-α_2. CRP estaba presente en el suero del 46.1 por ciento de los pacientes examinados. El suero de enfermos tuberculosos avanzados mostró una alta frecuencia de CRP con un alza concomitante y significative en los niveles de inmunoglobulina A. Enfermos tuberculosos crónicos mínimos tuvieron frecuencias menores de CRP y parecían similares al grupo lepromatoso. El total de proteínas del suero aumentaron significativamente en los grupos de enfermos tuberculosos y lepromatosos. Estos estudios sugieren que el exámen concurrente de CRP e inmunoglobulinas serían elementos valiosos para determinar el pronóstico y posible diagnóstico diferencial en enfermedades producidas por organismos mycobacterianos.

RÉSUMÉ

Au moyen de l'électrophorèse et de l'immuno-diffusion, on a examiné des échantillons de sérum de malades lépromateux et de malades tuberculeux, afin d'étudier simultanément la protéine C-réactive (CRP), les immunoglobulines et les protéines du sérum. Le sérum obtenu chez des malades lépromateux

a montré une augmentation significative des protéines totales du sérum, dont témoignait l'élévation des globulines α_2. Le CRP était présente dans le sérum de 46.1% des malades examinés. Le sérum de malades tuberculeux fort avancés montrait une frequence élevée de CRP, avec une augmentation significative concomitante dans les niveaux d'immunoglobulines A. Les malades atteints de tuberculose chronique et minimale présentaient des niveaux plus faibles de CRP et paraissaient semblables au groupe lépromateux. Les protéines totales du sérum étaient significativement augmentées dans les groupes tuberculeux et lépromateux. A la suite de ces études, on suggère que l'examen simultané de la CRP et des immunoglobulines pourrait être une méthode valable pour déterminer l'évolution future et les diagnostics différentiels possibles dans les affections causées par des organismes mycobactériens.

Acknowledgments. We wish to thank Dr. Joseph C. Hathaway, Chief of Hale Mohalu Leprosarium in Honolulu, for donations of lepromatous leprosy blood samples.

REFERENCES

1. Abe, M. and Hirako, T. C-reactive proteins in leprosy. I. The parallelism between the C-reactive proteins and erythema nodosum leprosum. La Lepro **30** (1961) 186-189. (*Abstract in* Internat. J. Leprosy **30** (1962) 526)
2. Anderson, H. C. and McCarty, M. The occurrences in the rabbit of an acute phase protein analogous to human C-reactive protein. J. Exper. Med. **93** (1951) 25-36
3. Bush, O. B. C-reactive protein in leprosy. Internat. J. Leprosy **26** (1958) 123-126.
4. Kabakov, E. N. Detection of C-reactive protein in cases of leprosy. Zhur. Mikrobiol., Epidemiol. i Immunobiol. (Moscow) (Transl.) **32** (1961) 448-455.
5. Kingsley, G. R. The direct biuret method for the determination of serum proteins as applied to photoelectric and visual colorimetry. J. Lab. & Clin. Med. **27** (1942) 840-845.
6. Lim, S. D. and Fusaro, R. M. Leprosy II. IgA and IgM immunoproteins in leprosy sera. Internat. J. Leprosy **35** (1967) 355-360.
7. Lim, S. D. and Fusaro, R. M. Leprosy III. A comparison of IgA and IgM immunoproteins of patients with pulmonary tuberculosis and leprosy. Internat. J. Leprosy **35** (1967) 361-365.
8. Montestruc, E. La "C-reactive protein" dans l'infection lépreuse. Bull. Soc. Path. exot. **52** (1959) 561-564.
9. Nakamura, R. M., Magsaysay, R., Ford, J. and Kunitake, G. M. Significant variations in commercial C-reactive protein antiserums. American J. Clin. Path. **44** (1965) 290-295.
10. Nerenberg, S. T. Electrophoresis: A Practical Laboratory Manual. Philadelphia, F. A. Davis Co., 1965.
11. Rabson, A. S. C-reactive protein in serum of patients with leprosy. Internat. J. Leprosy **23** (1955) 155-161.
12. Reich, C. V. and Tolentino, J. G. The relationship of C-reactive protein levels to erythema nodosum leprosum in lepromatous leprosy. Internat. J. Leprosy **35** (1967) 470-476.
13. Ritzmann, S. E. and Levin, W. C. Polyclonal and monoclonal gammopathies. Lab. Synopsis (Hoeschst Pharmaceutical Co.) **2** (1967) 9-50.
14. Ross, H., Butler, C. F. and Laukaitis, R. B. C-reactive proteins in the sera of patients with leprosy. Internat. J. Leprosy **27** (1959) 129-133.
15. Stiehm, E. R. and Fudenberg, H. H. Clinical and immunological features of dysgammaglobulinenum Type I. Report of a case diagnosed in the first year of life. American J. Med. **40** (1966) 805-815.
16. Tamblyn, E., Ching, G. A. and Hokama, Y. Quantitative analyses of C-reactive protein, immunoglobulins and serum proteins by electrophoresis and immunodiffusion of sera of tuberculous patients during therapy. (*Submitted to* American Rev. Resp. Dis., 1968).
17. Terencio de las Aguas, J. La proteina C reactive en la lepra. Rev. Leprol. (Fontilles) **4** (1959) 445-453.