IMMUNOCHEMICAL DETERMINATION OF OROSOMUCOID IN THE BLOOD SERUM OF LEPROSY PATIENTS

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Immunization of animals with specific serum proteins from heterologous species is usually done for the purpose of preparing antisera that will react specifically with the particular serum protein used as the antigen. By means of the quantitative precipitin technique such antisera may then be used to estimate the quantity of the given protein component present in an unknown serum, as shown by Goodman and associates ([1, 2, 3]). By such immunochemical methods a number of human serum proteins can be determined more accurately than by physical or chemical methods.

A great advantage of the immunochemical method for the estimation of specific proteins resides in its extreme sensitivity ([2]). Routine clinical use of the method, however, has been largely prevented by the difficulties encountered in producing specific antiserum of high titer. Rabbits, horses, and chickens have been employed for antibody production. The authors cited observed ([1]) that, in isotonic saline, chicken antiserum reacted poorly as compared with mammalian antiserum, and that a high concentration of NaCl (13%) was needed to effect complete precipitation. Recent work by them ([3]), based on this observation, has revealed the pronounced precipitin response of chickens to antigenic stimuli. By their techniques reliable antisera can be produced with relative ease, even to a relatively poor antigen such as orosomucoid.

My interest was stimulated by results reported by Goodman *et al.* in 1957 on the determination of serum protein components (albumin, transferrin, orosomucoid and gamma globulins) in cancer patients. Orosomucoid was affected by a gross change in the clinical state of the patient, increasing with clinical deterioration and decreasing with clinical improvement. Orosomucoid is an acid glycoprotein, or M-1 mucoprotein, homogenous both chemically and immunochemically and representing a single biochemical entity ([3]). It is a major component of the seromucoid, or mucoprotein, fractions of serum, which increase significantly in a number of diseases ([5]). Other mucoproteins are heterogeneous and provide a less meaningful index for evaluation of a clinical state.

MATERIALS AND METHODS

Sera were obtained from 100 leprosy patients, of which 91 were of the lepromatous type and 9 were tuberculoid. The controls consisted of sera from 17 nonpatient employees.

Of the 91 lepromatous cases, 52 were clinically active and bacteriologically positive, with *M. leprae* present in the skin scrapings in moderate numbers or numerous; 15 were

also classed as "clinically active" and bacteriologically positive, but with few or rare bacilli in skin scrapings; and 24 were clinically and bacteriologically "inactive." The 9 tuberculoid cases were inactive clinically and bacteriologically.

A generous amount of antichick orosomucoid antiserum was supplied by Goodman, and also antichick albumin, transferrin and gamma globulin. A quantity of standardized pooled serum from cancer patients was also supplied.

The serologic reactions were routinely carried out in 13 per cent NaCl. The antigen solutions were also made up to this same concentration of salt. A 1/15 dilution of the patient's serum was made, and 0.3 cc. of antichick orosomucoid antigen was then added. The reagent blank consisted of 0.3 cc. of 13 per cent NaCl and 3.0 cc. of the orosomucoid antigen. The tubes were incubated in a water bath at 38°C for one hour. After incubation the precipitate was centrifuged at 3,000 rpm for 20 minutes. The supernatant was decanted and the tube drained. Then to each tube was added 1.1 cc. of 13 per cent NaCl. The precipitate was resuspended and the turbidity determined in the Coleman Junior Spectrophotometer at 450 mμ against the reagent blank.

C-reactive protein (CRP) determinations were included in the study. Previous studies have shown that a positive test is a nonspecific but sensitive indicator of inflammation of infectious or noninfectious origin.

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 patient's serum of the same length. 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 (0 to 4+). The test was reported as either positive or negative.

RESULTS

Serum orosomucoid was found to be in highest amount in the bacteriologically positive lepromatous group, with numerous bacilli in skin scrapings, as shown in Table 1. In the entire group of 100 patients, 40 did not differ significantly from the control group, while 60 showed elevated values, the highest being 310 mgm. per cent. Of the 60 cases in which the serum orosomucoid level was higher than in the control

TABLE 1.—*Distribution of serum orosomucoid levels in 100 leprosy cases and 17 normal controls.*

Amount mgm./per cent (normal range 40-103)	Lepromatous			Tuberculoid	Controls (17 cases)
	Bacilli n-m[a] (52 cases)	Bacilli f-r[a] (15 cases)	Bact. negative (24 cases)	Bact. negative (9 cases)	
0- 49	4	1	2	1	2
50- 99	10	7	10	5	13
100-149	13	5	11	3	2
150-199	19	1	0	0	0
200-249	3	0	0	0	0
250-299	2	1	1	0	0
>300	1	0	0	0	0
>99 Total	38 (73%)	7 (47%)	12 (50%)	3 (33%)	2 (12%)

[a]n-m, numerous-moderate; f-r, few or rare.

group, 45 were bacteriologically positive and 12 negative. Normal values as compared to the control group were obtained in 34 lepromatous cases, of which 22 were bacillus-positive and 12 negative. Values within the normal range were found in 6 of the 9 tuberculoid cases, all of which were bacteriologically negative.

TABLE 2.—*Serum orosomucoid and C-reactive protein (CRP) in the sera of 100 leprosy patients and 17 controls.*

	Serum Orosomucoid		CRP	
Bacteriology	Elevated	Normal	Positive	Negative
Positive	45	22	27	40
Negative	15	18	11	22
Controls	2	15	0	17

The data of Table 2, they being the findings with both orosomucoid and C-protein, illustrate that, although the serum orosomucoid was elevated in 60 instances, there existed no definite relationship between the concentrations of the two substances. Of the lepromatous cases, 33 showed the presence of CRP with an elevated serum orosomucoid, while CRP was absent in 27 of the lepromatous cases in which the serum orosomucoid was elevated.

That serum orosomucoid levels need not be closely correlated is suggested on comparison to the presence or absence of CRP in the serum. The control group as well as the tuberculoid cases were negative for CRP. No definite correlation existed between the bacteriologic status of the group when compared with the presence or absence of CRP in the serum of patients with elevated serum orosomucoid or those patients showing normal values.

COMMENTS

Alterations in the level of serum mucoproteins (seromucoid) have been reported in a wide variety of unrelated diseases ([5]). It is known that tissue changes in leprosy result in the existence in the blood of abnormally large amounts of globulins. It can be reasoned that, depending upon the activity and the extent of the disease, there will be absorbed into the blood stream proportionate amounts of tissue substances responsible for increases in serum orosomucoid.

SUMMARY

Serum orosomucoid determinations were performed in 100 cases of leprosy, of which 91 were of the lepromatous and 9 of the tuberculoid type. The C-reactive protein content of the serum was determined in parallel with the serum orosomucoid. As controls, the serum of 17 nonpatients were similarly examined.

Elevations of serum orosomucoid above the normal range were found in 57 lepromatous cases, of which 45 were bacteriologically positive and 12 were negative.

Normal values were obtained in 34 lepromatous cases of which 22 were clinically and bacteriologically active and 12 cases which were inactive.

Values within the normal range were obtained in 6 of the 9 tuberculoid cases, all of which were inactive clinically and bacteriologically.

No definite relationship existed between the serum orosomucoid concentration and the presence or absence of C-reactive protein.

RESUMEN

Se ejecutaron determinaciones del sero-orosomucoide en 100 casos de lepra, 91 de los cuales eran de forma lepromatosa y 9 de forma tuberculoidea. Se determinó el contenido de proteína C-reactiva en el suero paralelamente al del orosomucoide. Como testigos, se examinaron en forma semejante los sueros de 17 sujetos que no eran enfermos.

Se descubrieron elevaciones del sero-orosomucoide sobre los límites normales en 57 casos lepromatosos, 45 de los cuales eran positivos bacteriológicamente y 12 negativos.

Obtuviéronse valores normales en 34 casos lepromatosos, 22 de los cuales eran clínica y bacteriológicamente activos y 12 inactivos.

Se obtuvieron cifras que quedaban dentro de los límites normales en 6 de los 9 casos tuberculoideos, todos los cuales eran clínica y bacteriológicamente inactivos.

No existió ninguna relación bien definida entre la concentración de sero-orosomucoide y la presencia o falta de proteína C-reactiva.

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