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Activation of Complement (C3) in Patients with Leprosy

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Evidence of activation of complement (C3), detected by altered mobility on immuno electrophoresis, was found in the fresh plasma of patients with lepromatous leprosy and proteinuria, with or without lepra reactions, but not in patients with other forms of leprosy. This may result from circulating antigen-antibody complexes. Such immune complexes if deposited in the capillary walls of the skin might cause erythema nodosum leprosum, and if deposited in the kidneys might cause proteinuria.

Introduction

Proteinuria is frequent in leprosy patients with a high bacterial index in skin smears, an acute erythema nodosum leprosum (ENL) reaction or reversal type of reaction, and occurs in some patients with lepromatous leprosy without skin reactions. Using the indirect fluorescent antibody technique, Wemambu *et al.* (1969) demonstrated deposition of immunoglobulins and complement in the skin lesions of patients with acute ENL. Also, by the fluorescent antibody technique, Tin Shwe (1971) has demonstrated the depositions of immunoglobulins and complement in renal glomeruli of 2 patients suffering from proteinuria and reversal reaction, and in one other patient with proteinuria without skin reaction.

Since such immune complexes activate complement, a study of C3 levels and immuno-electrophoretic characteristics of this complement component (βic in immuno-electrophoresis nomenclature) was undertaken.

Patients and Methods

Twenty-one leprosy patients attending the Hospital for Tropical Diseases, London, were chosen for study. All the patients were classified according to the criteria of Ridley and Jopling (1966). A control group of 4 members of the staff of the Hospital for Tropical Diseases was included in the study. Specimens of

blood from patients and controls were collected at the same time in tubes containing EDTA (ethylenediamine tetra-acetic acid). After immediate centrifugation, the fresh plasma specimens were subjected to electrophoresis on the same agar slide (barbitone buffer I = 0.9, pH = 8.6) at +4° C for 1 hour (Soothill, 1967). Specific anti-βic-βia antisera prepared by Behringwerke were used. The slides were examined 24 h after electrophoresis. Two of the patients in whose serum alteration of the complement component was detected were followed at intervals for up to 4 months.

Quantitative estimation of C3 was made on the same plasma samples using Hyland immunoplates for radial diffusion. The results were expressed in mg per 100 ml, using the standards supplied with the plates. Proteinuria was detected by the sulphosalicylic acid test.

Results

Immuno-electrophoresis of plasma of all normal subjects used as controls revealed a single normal (uncoverted) β ic arc (Fig. 1). This normal result was also obtained in the 4 patients with non-lepromatous leprosy and in the 7 patients

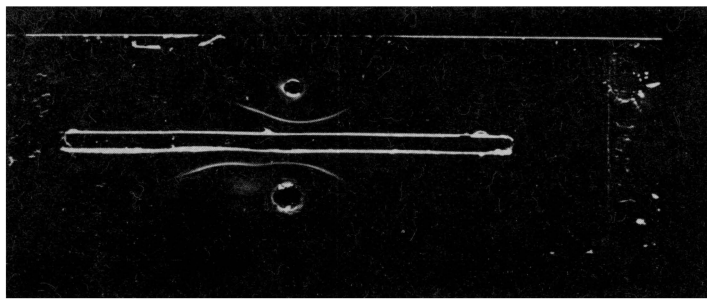

Fig. 1. Altered complement component in leprosy patient with proteinuria and reactions (above) compared with normal control (below).

with lepromatous leprosy but with no skin reactions and no proteinuria. Altered βic was detected in all 3 patients with recent ENL (duration less than 2 weeks) and proteinuria. The patient who had lepromatous leprosy and ENL for 18 months but no proteinuria did not show the reacted β-ic.

Of the other 6 patients with lepromatous leprosy and proteinuria, 3 showed this complement change. One had a reversal reaction, but the other 2 had no present skin reaction. Of the 3 who were negative, one had had a renal transplant for chronic renal failure and was receiving immunosuppressive treatment, one had renal failure due to amyloid, and the 3rd patient, who had had leprosy for 30 years, suffered a relapse.

Immunochemical estimation of C3 gave a wide range of results (Table 1). Only patient No. 25, who had had reacted βic, gave a low value, and he had had ENL for the shortest time, namely one week.

Two patients were followed sequentially. In patient no. 23 (LL with proteinuria and skin reactions), altered βic was detected 3 months prior to, and also during, the period of clinical skin reaction (ENL). The βic electrophoresis

TABLE 1

Clinical and immunological features of patient groups

Patient group	Serial No.	Age	Sex	Classification	Special clinical features	Serum C3 level (mg/100 ml)	Altered complement component βic/βia
Controls	1	36	M			135	–
	2	47	M			132	–
	3	31	F			110	–
	4	20	M			145	–
Non-lepromatous leprosy	5	36	M	BB		148	–
with no proteinuria	6	20	F	BB		130	–
	7	38	F	TT		142	–
	8	23	M	BT		105	–
Lepromatous leprosy, no reactions	9	34	M	LL	No ENL for 1 yr	80	–
and no proteinuria	10	47	M	LL	Trophic ulcer 6 months	170	–
	11	39	F	BL	Reversal reaction 1 yr. ago	285	–
	12	71	F	LL	Trophic ulcer 8 yrs	210	–
	13	62	M	LL	No reactions in the past	120	–
	14	71	M	LL	No ENL for 3 yrs	196	–
	15	38	M	LL	No reactions in the past	185	–
Lepromatous leprosy with chronic ENL–no proteinuria	16	46	M	LL	ENL 18 months	150	–
Lepromatous leprosy and proteinuria	17	21	F	BL	Reversal reaction	152	+
	18	25	M	LL	Hepatosplenomegaly, no skin reaction	210	+
	19	31	F	LL	No reaction in skin	200	+
	20	33	M	LL	Renal transplant; 6 yrs on immunosuppressives	85	–
	21	52	M	LL	Renal failure due to amyloidosis for 9 months	160	–
	22	64	M	LL	Leprosy 30 yrs, in relapse hypertension 20 yrs	210	–
Lepromatous leprosy with acute ENL and proteinuria	23	23	M	LL	Acute ENL 2 weeks	220	+
	24	47	M	LL	Acute ENL 2 weeks	165	+
	25	46	M	LL	Acute ENL 1 week	60	+

then became normal as the ENL subsided, but abnormal βic was again detected prior to and during the period of proteinuria which followed. Subsequently, further studies gave normal results (Fig. 2). Patient no. 24 (LL with proteinuria and skin reactions) had proteinuria and ENL at the time when electrophoretically altered βic was first detected. This disappeared after the ENL and proteinuria cleared up.

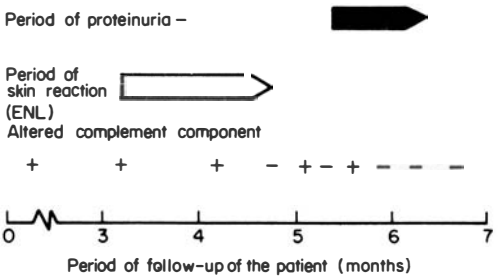

Fig. 2. Time relationships between the presence of altered complement in the plasma and the presence of skin reactions and proteinuria in patient no. 23.

Discussion

It is believed that the low serum complement level frequently found in patients with acute glomerulonephritis is partly due to utilization of complement components in an antigen-antibody reaction. This provides indirect evidence for an immunological basis for glomerulonephritis. Electrophoretic alteration of βic can be induced by *in vitro* exposure of normal plasma to an antigen-antibody reaction (Müller-Eberhard & Nilsson, 1960). Soothill (1965, 1967) has demonstrated that this change occurs *in vivo* in patients with acute glomerulonephritis (with or without low complement levels) and in some patients with the nephrotic syndrome due to proliferative glomerulonephritis, and membranous glomerulonephritis. Morse *et al.* (1962) and Lachmann (1963) had also demonstrated a similar phenomenon in patients with active systemic lupus erythematosus. One possible cause could be the presence of circulating soluble antigen-antibody complexes. In the present study we have demonstrated the presence of electrophoretically altered complement in all 3 of the 3 patients with a recent ENL reaction and proteinuria, and in 3 out of 6 lepromatous leprosy patients with proteinuria. The 3 patients with proteinuria, but no reacted complement, had other likely explanations for their proteinuria, namely renal transplant, amyloid, and hypertension respectively; 12 other patients without proteinuria showed no βic changes.

Towards the end of the first year of treatment most patients with lepromatous leprosy have ENL and some have reversal reactions. During this phase, bacterial morphology in skin smears changes from the solid to the granular form, indicative of cell death. This is presumed to be associated with antigen release and formation of complement activating antigen-antibody complexes. Clinically, this phase coincides with the development of ENL, reversal reactions, and proteinuria. The demonstration of altered βic at this stage in the disease is consistent with this hypothesis. Indeed the electrophoretic change may precede proteinuria or skin reaction. As recovery occurs the βic electrophoresis becomes normal.

In patients with chronic lepromatous leprosy, although the acid-fast bacilli may still be present as dead granular forms, there is no more active degranulation and possibly there is no further release of antigen into the circulation. In these patients and in those patients with no bacilli in skin smears such altered complement was not detected.

This study provides evidence of complement activation, perhaps by circulating antigen-antibody complexes. It is suggested that such immune complexes when deposited in the capillary walls of the skin cause ENL, and when deposited in the glomeruli of the kidneys cause proteinuria.

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