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Immune Complexes in Glomeruli of Patients with Leprosy*

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Renal biopsy specimens from 7 patients with leprosy were studied by fluorescence microscopy after staining with fluorescein *iso*thiocyanate-labelled anti-human immunoglobulins IgG, IgM, and third component of human complement βiC. Bound immunoglobulins and complement were observed in the form of granular deposits along the glomerular capillary walls in 2 patients during reversal reaction and in one other active patient. It seems reasonable to conclude that renal impairment in some patients with leprosy may be regarded as an immune-complex disease.

Introduction

Autopsy studies on leprosy patients have shown that lesions in the kidney are a major cause of death. In this respect they are second in importance only to tuberculosis. Thus from the 5458 cases reported in the literature it has been possible to ascertain that at least 17.6% of these patients had pathological changes in the kidneys (Table 1), but the mechanism of the production of these changes was unknown.

From retrospective studies of the clinical histories of 498 patients from Argentina it is clear that renal complications shorten the lives of patients with lepromatous leprosy, especially those with lepra reactions (Brusco & Masanti, 1963). To date, however, specific granulomatous lesions of the kidney due to *Myobacterium leprae* have not been recorded.

The question was asked, whether deposition of immune complexes is responsible for the lesions, and in a clinical and pathological study made in an attempt to answer this problem, 7 patients were submitted to renal biopsy and investigations to detect the presence of immune complexes in the kidney.

Materials and Methods

The 7 patients were chosen from among the patients undergoing treatment at the Hospital for Tropical Diseases, London. All the patients were classified according to the method of Ridley and Jopling (1966) and their skin smears were assessed according to Ridley's bacterial and granularity indices (Ridley, 1964). The bacterial index, that is the logarithmic index, ranges from 0 to 64, while the

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TABLE 1

Summary of the autopsy findings in patients with leprosy

Name of worker and year reported	Country from which reported	No. of cases studied	Common pathological condition found at autopsy associated with leprosy			General remarks
			Tuberculosis of the lungs	Amyloidosis in any internal organ	Renal changes including amyloidosis of the kidney	
Hansen & Looft (1895)	Norway	89	40	52	67	Nodular leprosy cases
		36	14	10	13	Maculo-anaesthetic cases
Pineda (1924)	Culion, Philippines	300	72	nil	49	Nephritis in nodular type is 25.5%, neural type almost nothing
de Souza Arauja (1929)	Culion, Philippines	–	–	–	–	Same as Pineda (1924)
Ryrie (1934)	Malaya	33	not mentioned	not mentioned	32	
Muller *et al.* (1936)	East Java	225	61	not mentioned	18	
Mitsuda & Ogawa (1937)	Japan	150	82	not mentioned	20	
	Culion, Philippines	3155	1533	not mentioned	411	
Fujita (1938)	Japan	1200	456	not mentioned	240	
Kean & Children (1942)	Panama	103	24	not mentioned	22	16 chronic and 4 acute cases of glomerulonephritis
Powell & Swan (1955)	Carville, U.S.A.	50	7	23	19	
Shuttleworth & Ross (1956)	Carville, U.S.A.	20	0	9	9	
Franca (1961)	Brazil	30	not mentioned	1	30	
Wu Li Tien *et al.* (1962)	China	2	0	0	0	Borderline cases only
Hosaka Sakuri *et al.* (1964)	Japan	115	6	not mentioned	see remarks	On basis of histopathology the main causes of death were contracted kidney and pneumonia
Krishnamurthy & Job (1966)	India	25	not mentioned	2	2	
Desikan & Job (1968)	India	30	15	3	24	Lepromatous cases
		7	0	0	2	Non-lepromatous cases
Sachdev *et al.* (1969)	India	3	0	3	3	Report of 3 cases of amyloidosis

granularity index varies from 1 to 10. A granularity index of 1 is given if all the bacilli are in solid form, and 10 if all the bacilli are in granular form. Skin biopsy specimens were also taken from one or two skin sites.

From each of the above patients 2 kidney biopsy specimens were taken at the same time. One was snap frozen in liquid nitrogen, stored at $-70°$ C overnight, and on the next day sections 5μ in thickness were cut with a cryostat at $-18°$ C, using a guide plate to ensure flatness. The sections were transferred to separate slides at room temperature and dried for 2h in a current of air; no fixation was carried out. The sections were moistened with a drop of Coon's buffer and then stained with sheep fluorescein labelled anti-human immunoglobulin IgG, IgM (Wellcome Reagents) adsorbed with pig's liver powder to reduce non-specific staining, or with anti-βiC globulin respectively (the anti-βiC was kindly supplied by the Department of Immunology, Middlesex Hospital, London). Fluorescent-labelled anti-rabbit immunoglobulin (Wellcome Reagents) and anti-rat fluorescein-conjugated animal antiglobulin GAR/FITC (Fraburg Ltd.) were used as control staining solutions.

After staining for 30 min the slides were washed in Coon's buffer for 45 min. They were then dipped in 1% photographic gelatin and finally mounted in 75% glycerol buffer. The other kidney biopsy was fixed in S.U.S.A. and paraffin sections were prepared 5μ in thickness and stained by haematoxylin, eosin, periodic-acid Schiff, congo red for amyloid, and modified Fite-Faraco (Lowy & Ridley, 1954) for acid-fast bacilli.

The clinical details of the patients studied were as follows.

Case 1. An Indian male aged 30 years, suffering from near lepromatous leprosy for 2 years, had a severe reaction of 21 days' duration. The skin biopsy was

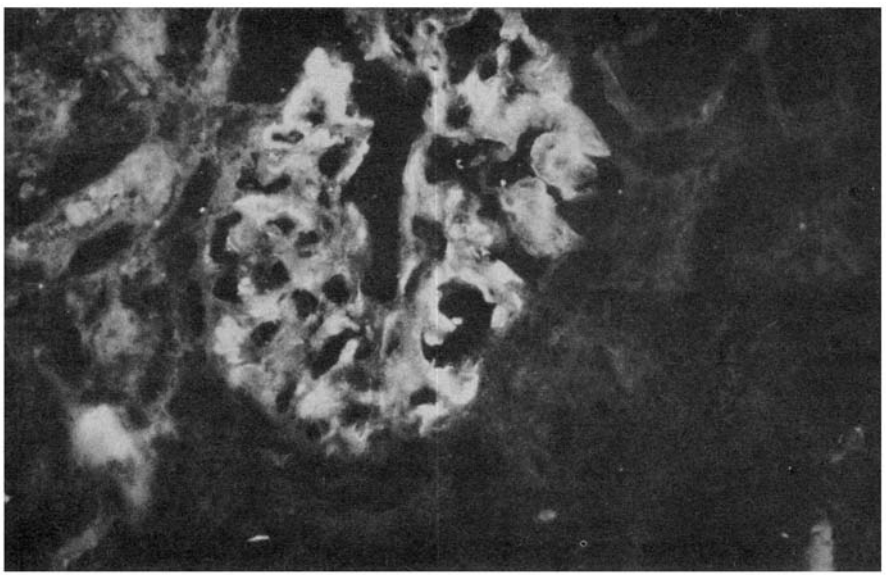

Fig. 1. Frozen section of kidney from patient no. 2, stained for IgG, showing irregular granular deposits of IgG globulin in the glomeruli.

reported as showing a reversal reaction, with a change from the original classification of BL to BB or BT. Skin smears revealed a bacterial index of 2.7 and a granularity index of 7.5. There was a trace of albumin in the urine, approximately 2 g in 24 h. The creatinine clearance rate was 76 ml per min. Renal biopsy examination showed normal glomeruli, but chronic inflammatory cells were present between the tubules.

Case 2. An Anglo-Indian female aged 39 years had been suffering from lepromatous leprosy (LI) for 4 years, and had had lepra reactions for 2 years. For the previous 3 months an acute reversal reaction had been present. Skin biopsy revealed suppuration and micro-abscess formation. A satisfactory clssification was not possible at this stage, but the condition was considered likely to move to BT. Skin smears taken 3 months prior to the renal biopsy showed a bacterial index of 2.7 and a granularity index of 8.2. It was also possible to demonstrate a few acid-fast bacilli engulfed within the monocytes in the blood during this reaction. The results of renal function tests were normal except for the presence of a trace of albumin in the urine; renal biopsy showed proliferation of cells in the glomeruli.

Case 3. A Cypriot male aged 57 had been suffering from borderline leprosy for 10 years. He came to hospital with oedema of the face and extremities. The skin biopsy showed an almost healed lesion with only one acid-fast bacillus in muscle; skin smears were persistently free from acid-fast bacilli. There was a trace of albumin in the urine, and the creatinine clearance rate was 68 ml per min. The patient had had no lepra reaction for the past year, and the cause of the oedema of the face and extremities was not clear. The kidney

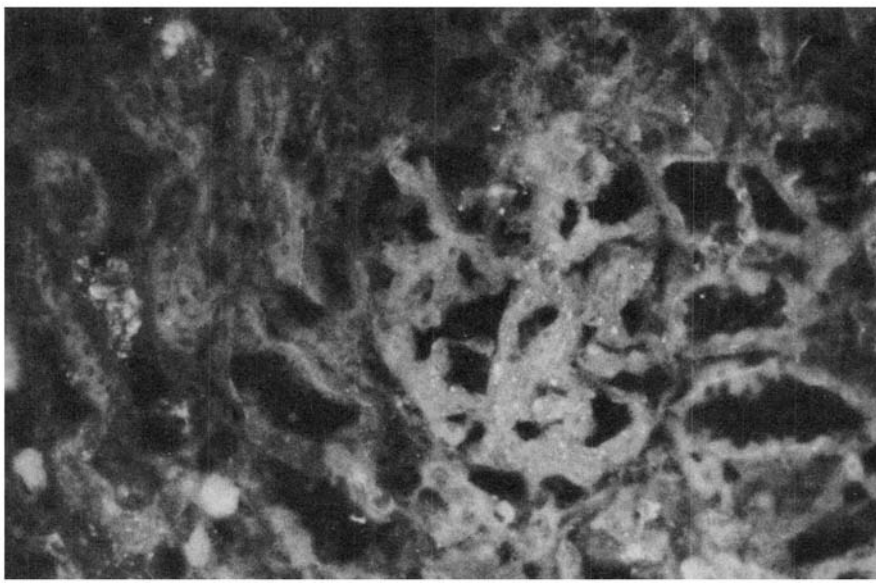

Fig. 2. Frozen section of kidney of patient no. 6, stained for anti-human IgG globulin. No deposits are seen.

biopsy showed chronic inflammatory cells between the tubules, with increased interstitial fibrous tissue.

Case 4. An Anglo-Burmese male aged 47 had a history of lepromatous leprosy of 32 years' duration. He had had leprosy reactions in the past, and at the time of renal biopsy showed signs of a mild reaction consisting of a few erythema nodosum leprosum lesions in the skin, redness of one eye, and swelling of the metacarpo-phalangeal joint of the thumb of one hand. His main problem was trophic ulcers of the legs and fingers. Skin smears showed a bacterial index of 1.0, and he had intermittent albumin in the urine. The creatinine clearance rate was 68 ml per min. Renal biopsy revealed that one third of the glomeruli were replaced by hyaline material.

Case 5. An Indian male, aged 26, had been suffering from lepromatous leprosy (LL) for 1 year. He was admitted to hospital with general wasting and with palpable spleen, liver, and some of the lymph glands. His skin smears showed a bacterial index of 3.8 and a granularity index of 8.6. His calves and thighs were tender and there were a few nodules in the muscles of the leg. Biopsy examination of one of these nodules showed extensive leprous interstitial myositis, with large foam-cell foci between the muscle bundles. Non-solid acid-fast bacilli were numerous in the lepra cells of one specimen. The clinical appearance of the patient suggested leprosy of the "diffuse lepromatous type", He had intermittent proteinuria, but renal function tests, as well as the kidney biopsy, gave normal results.

Case 6. A European male, aged 63, with a history of lepromatous leprosy of 34 years' duration. In response to treatment with DDS for 15 years he had become bacteriologically negative at one stage, but at the time of examination he was suffering a relapse. The bacterial index was 3.5 and the granularity index 5.2. He had also had hypertension for 20 years (blood pressure 180-200/110-120 mmHg). There was oedema of the legs and body and also a trace of protein in the urine. The glomerular filtration rate was 57 ml per min. He had no clinical or histological evidence of lepra reactions. Renal biopsy showed hypertensive nephropathy.

Case 7. An Indian male aged 51 years, who had suffered from lepromatous leprosy for the previous 28 years. He had been receiving regular antileprosy treatment for the past 6 years, but his progress was slow and interrupted due to reactions of the erythema nodosum leprosum (ENL) type. He also had had hypertension (blood pressure 180-150/110-190 mmHg) for the previous 4 years. He remained free from lepra reaction for 1 year, but 2 months before admission he collapsed at work and developed a right hemiplegia due to cerebral thrombosis. Later he developed the nephrotic syndrome. His blood urea level was 77 mg per 100 ml, creatinine clearance rate 77 ml per min, plasma cholesterol level 238 mg per 100 ml, urinary protein excretion 10 to 17 g per day, serum total protein value 5.3 g—albumin 1.8 g and globulin 3.5 g. Skin smears showed a bacterial index of 1.5, all in granular form. As only one kidney biopsy was obtained paraffin sections could not be prepared, but when the patient died 4 months after the biopsy the kidney showed amyloid deposits in all the glomeruli and on the blood vessel walls.

Results

In the kidney specimens of patients nos. 1, 2, and 5 immunoglobulins and complement were demonstrable by immunofluorescence on the glomeruli. The fluorescence was in granular form and was localized along the walls of the glomerular blood vessels. Fluorescence was observed on all the glomeruli, but apart from the glomeruli no deposits were seen on other areas of the kidney section. Both fine and coarse deposits were observed and there was not much variation in the size and distribution of the glomerular deposits. In general the fluorescence was stronger on sections stained with IgG than on those stained with IgM and βiC. The sections from patient no. 5 showed comparatively weak fluorescence deposits. Sections from the remaining patients showed no specific fluorescence after staining with either conjugate. None of the control sections stained with either anti-rabbit or anti-rat fluorescein-conjugated globulin showed any deposits.

Discussion

In a disease in which antigen persists over a long period of time and antibody response to the infecting agent develops, conditions are favourable for the formation of circulating soluble immune complexes. That antigen-antibody complexes in the blood stream can lead to serious tissue injury is unquestioned (Weigle, 1961). So in leprosy it is possible that such antigen-antibody complexes may be found in patients with lepromatous leprosy, in whom numerous leprosy bacilli are spread all over the body, and this especially during treatment, when much disintegration of bacilli takes place.

Epidemiological studies recently undertaken at the Hospital for Tropical Diseases, London, have shown that proteinuria in such patients is common, especially in those suffering from severe ENL as well as in those with reversal reactions. There are also records of patients with proteinuria without clinical reactions.

ENL is thought to be due to an Arthus reaction. It is often associated with acute vasculitis in the skin, iridocylitis, arthritis, epididymo-orchitis, and even glomerulonephritis (Turk, 1970). Histologically, the presence of polymorphonuclear leucocytes is the essential and predominant feature, especially in the early stage, and there is much cellular disintegration (Job *et al.*, 1964; Mabalay *et al.*, 1965). In this type of reaction Wemambu *et al.* (1969), using the indirect fluorescent antibody technique, had demonstrated the presence of granular deposits of immunoglobulin and complement in the acute lesions of the skin, but not in older forms of the skin lesions. During this type of reaction there is every likelihood of immunoglobulins and complement being deposited in the glomeruli of the kidneys. In this study patient no. 4, though suffering from a mild form of ENL, had no deposits of immunoglobulins and complement, probably because the lesion was old. There were no other patients with ENL in the series.

The second type of reaction is the reversal reaction in which the main infiltrating cell is the lymphocyte. This reaction, which resembles a tuberculin reaction, is the result of a recovery of the patient's cell-mediated immune response towards *Mycobacterium leprae* (Ridley, 1969; Turk, 1970).

Patients with acute reversal reaction can be very ill, but the reaction occurs primarily in the clinically apparent lesions of the skin—unlike ENL, in which the

reaction often occurs in crops of new lesions. It has not so far been postulated, therefore, that reversal reactions are due to a humoral mechanism. Nevertheless, reactions of this type are characterized by the elimination of bacilli from the skin lesions, and it is probable that much antigenic material is discharged into the blood circulation and filtered off by the renal glomeruli. This is probably the explanation for the finding of fluorescent deposits of immunoglobulin and complement in the glomeruli of patients nos. 1 and 2, both of whom had a severe reversal reaction associated with a sharp diminution in the number of bacilli in the skin and elsewhere, with an erythrocyte sedimentation rate (ESR) of over 100 mm in 1 h.

Patient no. 5 was in the early months of treatment, and though not in reaction his skin smears showed a granularity index of 8.6, indicating that most of the bacilli in his body were in fragmented or granular form, a condition which is seen in ENL. So the finding of weak immunoglobulins and complement in the renal glomeruli of this patient was not altogether unexpected.

Immunoglobulins and complement were not found in any of the other patients. In patient no. 3 the leprosy was almost cured, and there were no acid-fast bacilli (AFB) in his skin lesions. Patient no. 6 was suffering from relapse, probably due to drug resistance. His skin smears showed a bacterial index of 3.5 and a granularity index of 5.2, so antigenic breakdown was therefore limited; his proteinuria was probably secondary to hypertension. Patient no. 7 had long-standing chronic disease, with amyloidosis of the kidneys, but few signs of activity of his leprosy.

The sera of all the above patients gave a negative response to the test for Australia antigen and antibody, the anti-streptolysin-0 titre was less than 200 Todd units, and the malaria antibody titre less than 20. So it is not likely that the present finding of immune complexes in the kidneys of Patients 2, 3 and 5 was due to any other antigen than leprosy.

All the above evidence suggests therefore that the renal impairment in some leprosy patients is due to deposition of antigen-antibody complexes in the glomeruli.

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