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Detection of circulating immune complexes in alcoholic liver disease

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SUMMARY

Sera of twenty-five patients with alcoholic liver disease and forty normal control sera were screened for circulating immune complexes by means of the anti-antibody neutralization test and by Raji-cell membrane immunofluorescence assay. IgG-containing immune complexes were detected in thirteen out of twenty-five patients with alcoholic liver diseases and in one out of forty normal individuals; in addition, IgA-containing complexes were demonstrated in seven out of thirteen sera positive for IgG complexes. The presence of immune complexes was restricted to alcoholic hepatitis and active cirrhosis, thus indicating a relationship with disease severity.

INTRODUCTION

Immunological abnormalities have been described in patients with alcoholic liver disease. Thus, it is well established that polyclonal immunoglobulin elevations, predominantly of IgG and IgA, frequently occur in this condition (Bailey *et al.*, 1976). Furthermore, the immune response to alcoholic hyalin, both humoral (Chen *et al.*, 1975) and cellular (Zetterman, Luisada-Opper & Leevy, 1976), has been demonstrated in alcoholic hepatitis. In addition, in liver and kidney of patients with this disease, immune complexes have been detected by immunohistological techniques (Callard *et al.*, 1975) and by elution experiments (Kanagasundaram *et al.*, 1977). The presence of circulating immune complexes in sera of patients with alcoholic liver disease has been suggested by Jori *et al.* (1977), based on cryoprecipitation experiments, and Thomas, Potter & Sherlock (1977), who showed Clq binding activity in sera of patients with this disease.

In the present study, sera of patients with documented alcoholic liver disease were screened for circulating immune complexes by means of the anti-antibody (AA) neutralization test and Raji-cell membrane immunofluorescence assay.

MATERIALS AND METHODS

Human sera. Sera of five patients with fatty liver, ten patients with severe alcoholic hepatitis and ten patients with active alcoholic cirrhosis were studied. Most sera were obtained from the 2nd Univ. Clinic of Gastroenterology and Hepatology, University of Vienna, Austria. In addition, selected samples and case histories were kindly provided by Dr L. A. Katz of the E. J. Meyer Memorial Hospital, Buffalo, N.Y. All patients had a history of chronic alcoholism and morphological evidence of alcoholic liver disease in liver biopsy (Leevy, Popper & Sherlock, 1976). None of the sera studied were positive for HBsAg or anti-HBs as determined by commercially available solid-phase radioimmunoassays (Ausria/Ausab, Abbott Laboratories, Diagnostics Division, North Chicago, Illinois). Forty sera of healthy staff members of this department served as controls.

Detection of immune complexes. The AA neutralization test was employed as described previously (Kano *et al.*, 1978). This procedure detects IgG antibodies which formed immune complexes in reaction with their corresponding antigens.

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Briefly, an AA-containing serum at a dilution corresponding to three agglutinating units was mixed with an equal volume of a 1 : 5 dilution of the serum tested for complexes, and the mixture was incubated at 20°C for 60 min. Thereafter, equal volumes of the above mixture and of a 1% suspension of Rh-positive erythrocytes, sensitized by incomplete Rh antibodies, were placed on a glass slide, stirred with a wooden applicator and further incubated in a moist chamber at 20°C for 20 min. The agglutination of sensitized erythrocytes was then viewed macroscopically.

For Raji-cell membrane immunofluorescence, a modification of a procedure originally developed by Theofilopoulos *et al.* (1974) was used as described by Albini, Ossi & Andres (1977). Briefly, 10^7 Raji cells were washed three times in RPMI 1640 medium and suspended in 1 ml of this medium containing 0·7 mM sodium azide. Washing and staining of the cells was directly in microtitre plates (Cooke Engineering Co., Alexandria, Virginia) with round-bottom wells as described by Schauenstein, Wick & Kink (1976). Serial dilution of the tested serum in 5 µl volume were placed in the wells and one drop of the Raji-cell suspension was added. After 30 min of incubation, the cells were washed and then incubated for 20 min with FITC-conjugated rabbit antisera to human immunoglobulins diluted 1 : 6 in RPMI 1640 medium. The following conjugates were used: anti-whole IgG, F/P 2·3, 8 U/mg/ml; anti-IgG (heavy chain specific), F/P 2·2, 8 U/mg/ml; anti-IgM (heavy chain specific), F/P 2·1, 4 U/mg/ml; anti-IgA (heavy chain specific), F/P 2·6, 8 U/mg/ml. The anti-whole IgG antiserum was obtained from Hyland Diagnostics Division, Travenol Laboratories, Costa Mesa, California: all heavy chain specific antisera were purchased from Cappel Laboratories, Downington, Pennsylvania. After three washings in PBS, containing 0·7 mM sodium azide, the cells were placed on a glass slide. The preparation was mounted with a cover-slip using buffered glycerol and examined with the fluorescence microscope. As a control for binding of aggregated immunoglobulin to Fc receptors, sera were used in which complement was destroyed by heat inactivation at 56°C for 30 min.

In order to identify the antigen in the complexes, excess of putative antigen was added to the tested serum. According to previous experiences, excess of the antigen would convert AA-neutralizing complexes into complexes that do not affect AA. The following antigens were used: Hanganutziu-Deicher (H-D) antigen, ubiquitous tissue antigen (UTA), and liver-specific lipoprotein. H-D antigen was kindly provided by Dr J. Merrick from this department. The extraction procedure has been described in detail elsewhere (Merrick, Zadarlik & Milgrom, 1978). UTA, described by Kasukawa *et al.* (1967) and Palosuo, Andres & Milgrom (1976), was prepared according to the procedure of D'Amelio & Perlmann (1960), with modifications described by Kasukawa *et al.* (1967). Liver-specific lipoprotein was obtained following the method of Meyer zum Büschenfelde & Miescher (1972).

RESULTS

Sera of twenty-five patients with alcoholic liver disease and forty normal control sera were assayed for the presence of circulating immune complexes. As seen in Table 1, by means of AA neutralization test, immune complexes were detected in twelve out of twenty-five (48%) patients with alcoholic liver disease and in one out of forty (2·5%) control individuals. The Raji-cell assay for IgG detected immune complexes in the same twelve sera and in one serum that gave negative result in the AA neutralization test. In addition, the Raji-cell assay detected IgA-containing immune complexes in seven alcoholic liver disease sera, all of which were positive for IgG complexes.

Immune complexes were detectable only in patients with the more severe form of alcoholic liver disease, alcoholic hepatitis and active cirrhosis, but not in fatty liver. The titres of positive sera were

TABLE 1. Immune complexes in alcoholic liver diseases

	Total number of sera tested	Numbers of positive sera				
		AA neutralization test*	Raji-cell assay†			
			IgG	IgA	IgM	Total
Fatty liver	5	0	0	0	0	0
Alcoholic hepatitis	10	7	8	4	0	8
Alcoholic cirrhosis	10	5	5	3	0	5
Normal individuals	40	1	1	0	0	1

* The dilution of the sera 1 : 5

† The dilution of the sera 1 : 4

comparable in both methods: AA neutralization test 5–40, Raji-cell assay 4–32. The highest titres were observed in alcoholic hepatitis.

In order to underline the apparent relationship between disease activity and presence of circulating immune complexes, sequential serum samples were collected from two patients with alcoholic hepatitis. In both patients, immune complexes, which were initially present in high titres, disappeared during resolution of the illness.

Our attempts to identify the antigen(s) in the complexes have been unsuccessful in studies involving H-D antigen, UTA and liver-specific lipoprotein.

DISCUSSION

Our findings provide strong evidence that circulating immune complexes containing IgG and/or IgA occur with a high frequency in patients with active alcoholic liver disease and extend previous studies which have shown deposition of complexes in kidney and liver in this condition.

The two methods used to detect circulating immune complexes, namely the AA neutralization test and the Raji-cell assay gave comparable results for complexes containing IgG. AA is an IgM serum factor, similar to rheumatoid factors, specifically combining with the $F(ab)_2$ fragment of IgG antibodies, which underwent molecular transformation in reaction with their corresponding antigen (Milgrom, Dubiski & Wozniczko 1956). As previously shown by Kano *et al.* (1978), aggregated IgG does not give rise to false positive results in the AA neutralization test. The Raji-cell assay, on the other hand, may give rise to false positive results with aggregated IgG (Theofilopoulos *et al.*, 1974), but it is also capable of detecting immune complexes containing antibodies of immunoglobulin classes other than IgG. IgA in immune complexes could activate the complement system via the alternative pathway, as has been described for aggregated IgA (Götze & Müller-Eberhart, 1971). While heat-inactivated sera of patients positive for IgA-containing complexes did not bind to the Raji-cells via the Fc receptor, the possibility of aggregated immunoglobulin being detected in this assay cannot be ruled out completely.

So far, we have not been able to identify the antigen(s) in the complexes detected. However, we assume that circulating immune complexes in alcoholic liver disease might represent the sequelae of severe tissue destruction, resulting in the release of immunogenic material into circulation and the subsequent formation of immune complexes. Only recently, Bailey *et al.* (1976) have reported an increased incidence of antibodies to smooth-muscle in patients with alcoholic liver disease. In similar studies conducted by us (Penner & Milgrom, in preparation), release of a microsomal UTA and subsequent antibody formation have been observed in alcoholic liver disease. Attempts to identify the antigen(s) participating in immune complex formation in patients with alcoholic liver disease will be continued using several antigens, first of all, alcoholic hyalin and antigens extracted from the intestinal tract.

Our findings of IgA-containing complexes merit further attention. These data could indicate a possible intestinal origin of the antigen involved in complex formation. On the other hand, the finding that alcoholic hyalin binds IgA (Zinnemann *et al.*, 1974) could provide a clue to the composition of circulating immune complexes in alcoholic liver disease.

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