INDUCTION OF ANTINUCLEAR ANTIBODIES BY MERCURIC CHLORIDE IN MICE

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INTRODUCTION

Reports suggest that exposure to low doses of mercury or mercuric compounds in man could lead to proteinuria or nephrotic syndrome (Kazantzis, 1962; Belghiti et al, 1986). Immunologically mediated glomerulonephritis (GN) of IC type has been described in such patients (Gaultier et al, 1968; Tubbs et al, 1982). The closer mechanism of action in human mercury-induced glomerular disease remains unknown, but there is evidence that mercury can modify the immunoregulation (Charpentier et al, 1981).

Bariety et al (1971) described the induction of immune-mediated membranous GN by mercury in Wistar rats. Brown-Norway (BN) rats develop anti-glomerular basement membrane (anti-GBM) disease after exposure to mercurials by different routes (Sapin et al, 1977; Bernaudin et al, 1981). Circulating immune complexes develop, and granular IC deposits replace the linear anti-GBM antibodies (Druet et al, 1978; Bellon et al, 1982). A possible pathogenetic mechanism in BN rats could be T-cell dependent polyclonal B-cell activation (Hirsch et al, 1982) with antibodies to single-stranded DNA (SS-DNA) (Bellon et al, 1982). Such antibodies have also been found in other inbred rat strains susceptible to induction of GN by mercuric chloride (Druet et al, 1982), and antibodies to DNA-associated proteins occur in both serum and renal immune deposit (Weening et al, 1978; Weening et al, 1980).

Exposure to inorganic mercury causes mesangial IC-GN in certain mouse strains (Enestrom and Hultman, 1984), whereas other strains are resistant (Hultman and Enestrom, 1987). The susceptible strains develop systemic IC deposits and raised serum immunoglobulin concentrations with an isotype pattern dependent on the strain and any immunopotentiation used (Hultman and Enestrom, 1987). Antinuclear serum antibodies without IC deposits have been described in mice (Robinson et al, 1984). The present study was undertaken to characterize the autoantibodies, and to study the relation between these antibodies and the renal IC deposits.

MATERIAL AND METHODS

Experimental Procedure. Twenty two female SJL mice aged 8-10 weeks were given subcutaneous injections of 1.6 mg HgCl₂/kg body weight at 3-day intervals over a period of 2, 4, or 12 weeks. The same number of controls received 0.1 ml of a sterile 0.9% NaCl solution.
Serum Autoantibody Test. Serum obtained at the end of the experimental period was tested by indirect immunofluorescence using normal and mercury treated mouse kidney and HEp-2 cells as substrates (Enestrom and Hultman, 1984). Anti-native DNA anti-bodies were studied by the Crithidia luciliae assay (Aarden et al, 1973). Acid extraction was performed on HEp-2 slides and cryostat sections of acetone-fixed mouse kidney by incubation in acetate buffer (Weening et al, 1980) or 0.1-N HCl (Tan et al, 1976). For histone reconstitution calf thymus histones were freshly prepared in a 0.01-M PBS solution (pH 7.2) in a concentration of 25 ug/ml, and the acid eluted slides were immersed in this solution for 60 min. Sera were absorbed with calf thymus double- or single-stranded DNA, histones, calf liver ribonucleic acid (Sigma Chemical Co), 5S- and 16S/23S RNA from E coli (Boehringer Mannheim GmbH), whole chicken-erythrocyte nuclei, and purified chromatin (Sung et al, 1977). The absorbents were always used in excess, and were incubated with the sera for 1 h at 37°C and then at 4°C overnight. Sera were analysed by double immunodiffusion for the presence of antibodies to the non-histone nuclear antigens Sm, ribonucleoprotein (RNP), SS-A: and SS-B. The antigens were obtained by saline extraction of calf thymus using a modified version of the method described by Sharp et al (1972), and standard sera containing antibodies were included as reference agents.

Kidney studies. Direct immunofluorescence was performed on cryostat sections of kidney blocks using antibodies to IgG and C3c, and the deposits were titrated as described elsewhere (Hultman and Enestrom, 1987). Light- and electron microscopy studies were performed as described elsewhere (Enestrom and Hultman, 1984). Kidneys from mercury treated and control SJL mice and female MRL mice were acid eluted by Bartolotti’s method (1977).

RESULTS

Serum Autoantibodies. A strong nucleolar (Fig. 1a) and a weaker homogeneous (Fig. 1b) ANA pattern with strongly stained chromosome regions in metaphase cells (Fig. 1c) were found after 4 and 12 weeks’ mercury treatment. No autoantibodies to mouse kidney structures apart from ANA could be demonstrated. Acid extraction resulted in weakening of the homogeneous staining (Fig. 2b), which was not restored after reconstitution with histones (Fig. 2c). Absorption with whole chicken-erythrocyte nuclei (Fig. 2e) or purified chromatin (Fig. 2f) abolished the homogeneous staining, leaving only the nucleolar fluorescence. The other absorbents caused no reduction in the homogeneous or the nucleolar staining. No precipitating antibodies to the tested antigens were found.

Kidney studies. Mercury treatment for 4 weeks or longer resulted in significantly increased deposition of IgG and C3c in the glomerular mesangial regions (Fig. 3a) and in the walls of the interlobular arteries and arterioles. A slight increase in endocapillary cells and a widening of the mesangial zones took place after mercury treatment. Electron dense deposits were found in the mesangium (Fig. 3b) and vessel walls. Eluates of kidneys from mice treated with mercury for 4 weeks showed a strictly nucleolar ANA pattern (Fig. 3c); no homogeneous staining was detected. The eluate from control mice showed no staining, and the eluate from MRL mice gave strong homogeneous staining.

DISCUSSION

Mercury induced a polyclonal autoantibody response in mercury treated SJL mice, with two main ANA patterns, homogeneous and nucleolar. The