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THE SEROLOGY OF AUTOLOGOUS IMMUNE COMPLEX NEPHRITIS IN THE RAT

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SUMMARY

The serological response has been studied in rats developing autoimmune complex nephritis, following injection of chemically modified kidney antigen. The results suggest that a change takes place from IgM to both IgM and IgG anti-kidney antibodies. This response can be distinguished from the naturally occurring IgM antitissue antibodies.

INTRODUCTION

Since the description by Heyman *et al.* in 1959 of renal disease in rats following sensitization with species-homologous kidney antigen in Freund's complete adjuvant, there have been considerable efforts at establishing the mechanisms underlying the pathogenesis of the lesion. The disease is a chronic progressive membranous glomerulonephritis characterized by proteinuria, hyperlipaemia and hypoalbuminuria. Granular deposits of γ-globulin and complement have been consistently found along the glomerular basement membranes (see review by Dixon, Edgington & Lambert, 1967). The nephritogenic antigen has been isolated as a membrane-bound phospholipoprotein of high molecular weight present in the apical portion of the epithelium of the proximal convoluted tubules—$RTE_{\alpha 5}$, and is found most abundantly in the mitochondrial fraction of the cell (Edgington, Glassock & Dixon, 1968).

The kidney antigen most frequently employed in producing experimental glomerulonephritis is the mitochondrial and/or microsomal fraction (Heyman *et al.*, 1959; Hess, Ashworth & Ziff, 1962). Chemically modified mitochondrial antigen has been found to be particularly effective (Watson & Dixon, 1966) and is used in the present study.

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The role of circulating anti-kidney antibody in the pathogenesis has been difficult to establish because of the presence in normal rat serum of naturally occurring non-tissue specific antitissue antibodies (Weir, 1963; Digby & Loewi, 1965; Weir *et al.*, 1966; Boss, Silber & Nelken, 1967). These antibodies, which are partly heat labile, have been shown to be of the IgM type and are also directed at an antigen which is most abundant in the mitochondrial fraction of the cell (Weir *et al.*, 1966; Pinckard & Weir, 1966). The antitissue antibodies are not considered to play a pathogenic role and may be implicated in the removal of tissue breakdown products (Elson & Weir, 1967). In contrast, the antibodies in the sera of humans and experimental animals with autoimmune disease have frequently been shown to contain IgG antibodies cytotoxic for cells in culture (Irvine, 1962; Appel & Bornstein, 1964), and IgG anti-kidney antibodies have been eluted from kidney tubules in experimental nephritis (Grupe & Kaplan, 1967).

These observations prompted us to attempt the differentiation of the naturally occurring IgM anti-mitochondrial antibodies from specific IgG anti-kidney antibodies in rats injected with kidney antigen and Freund's complete adjuvant. This report gives the results of fractionation studies of sera from rats taken at intervals during the development of the renal lesions and indicates a change from IgM to both IgM and IgG antibodies during this period.

MATERIALS AND METHODS

Animals

Wistar (Birmingham A A strain) rats of both sexes were used to provide kidney mitochondrial homogenate and sera.

Black and white hooded (inbred strain) adult female rats were used in the experiment to produce autologous immune complex nephritis. They were bled weekly by tail vein cut under light ether anaesthesia.

Preparation of antigen

Wistar rat kidney mitochondria was prepared by differential centrifugation as described by Sedgwick & Hübscher (1965). The purity of the preparation was checked by electron microscope (Barabas & Lannigan, 1968). Sulphanilic acid azo Wistar rat kidney mitochondria was prepared by the method of Vogel (1951). Ten per cent sulphanilic acid by weight to protein was used for making the conjugate. The chemically modified mitochondrial preparation was adjusted to 24 mg/ml by pressure dialysis and stored at $-20°C$ in small aliquots.

Injection schedule

Rats were injected intraperitoneally with 2 mg chemically modified rat kidney mitochondria in a volume of 0·25 ml in Freund's complete adjuvant four times at weekly intervals (Barabas & Lannigan, 1968).

Urinary protein estimation

Urine was collected from test animals twice before the start of experiment and thereafter weekly and analysed for protein using the Weichselbaum (1946) biuret protein estimation.

Complement fixation

This was as previously described (Weir, 1967), performed with tissue homogenates in MRC pattern Perspex trays using a four drop system and 1·25 MHD guinea-pig complement previously titrated against antigen.

Serum fractionation

Sucrose density gradient fractionation was performed by a modification of the method of Kunkel (1960) as previously described (Weir *et al.*, 1966; Coghlan & Weir, 1967). Layers of 40, 30, 20 and 10% sucrose in phosphate buffered saline, pH 7·0, are allowed to equilibriate for 24 hr at 4°C and serum diluted 1:2 is layered on top. Nine 0·5-ml fractions were taken after centrifugation at 35,000 rev/min at 4°C in a SR 40 rotor of an MSE superspeed 40 TC centrifuge and stored at −20°C. The peak of IgG activity is in fractions 3–4 and IgM activity in fractions 6–7.

TABLE 1

	No. of rats	Reciprocal CF titres			
		Unheated		Heated	
		Mean	Range	Mean	Range
Hooded kidney antigen	27	35	4–128	6·5	0–16
Wistar kidney antigen	9	20	16–32	8	0–32

RESULTS

Complement fixation titres of untreated rats against kidney antigen

The sera of twenty-seven untreated hooded rats was taken and tested unheated and heated (56°C for 30 min) against hooded rat kidney homogenate in the complement fixation test. Nine of these sera were tested against Wistar kidney antigen and the titres obtained are shown in Table 1. The unheated sera from eleven untreated rats was fractionated on sucrose density gradients and the nine fractions from each serum tested against Wistar and/or hooded kidney antigen. In every instance, the complement fixing activity was limited to the fractions containing the IgM globulins. These results are similar to the findings with normal rat sera tested with a variety of tissue antigens (Weir *et al.*, 1966). The IgM antibody is non-tissue-specific and affected by heating at 56°C for 30 mins.

Response to injection of rat kidney mitochondrial antigen

The results of anti-kidney antibody complement fixation tests with unheated rat serum taken from animals injected with the chemically modified antigen are shown in Fig. 1 during the period up to 21 weeks after the first immunization. There is an early rise of antibody and peaks of activity at 1 and 7 weeks with a gradual increase to 19 weeks. From about 8 weeks onwards marked proteinuria occurs and this roughly follows the increase

in antibody titre. The response was not simply a rise in the naturally occurring antitissue antibody as was shown by testing against Wistar liver antigen twenty-one sera from rats taken at periods up to 9 weeks after immunization with kidney. No increase in titre was noted.

Fractionation of rat sera during anti-kidney antibody response

Forty-four sera taken from six rats at intervals up to 9 weeks after the first injection of kidney antigen were fractionated on sucrose density gradients and the nine fractions from each serum tested against Wistar kidney antigen in the complement fixation test. Fig. 2 shows that in most instances there was an early shift from IgM antibody only to both IgM

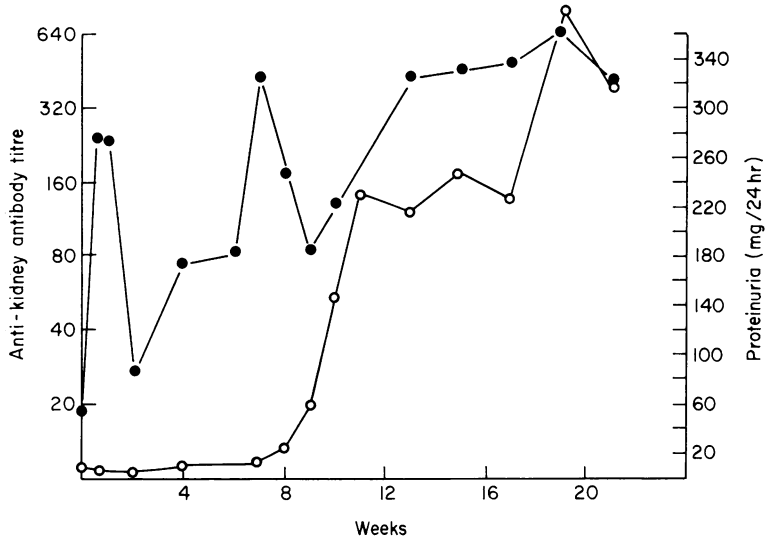

FIG. 1. Complement fixation titres with unheated rat serum and Wistar kidney homogenate at intervals after injection of chemically modified mitochondrial antigen at weekly intervals for 4 weeks. Mean titres of six animals. ●, Anti-kidney antibody titre; ○, proteinuria.

and IgG antibody and that after about 1 month all the animals were producing both classes of anti-kidney antibody. Fractions from ten of these sera taken from rats at various stages up to 9 weeks after injection were tested against hooded kidney antigen and gave an identical pattern of response developing IgG antibodies in addition to the IgM naturally occurring IgM antibody. The failure to obtain any activity with liver homogenate in the IgG containing fractions with sera taken from twelve rats 1–8 weeks after immunization with kidney shows that the IgG response was not an enhanced response to non-tissue-specific antigen. Anti-liver activity was found only in fractions containing the IgM globulins.

It was also noted that there was no increase in the IgM anti-liver antibodies during this period, the titres ranging between 1:4 and 1:16 with a mean of 1:8. In contrast, the IgM titres to hooded kidney antigen were in the range 1:16 to 1:128 with a mean of 1:66.

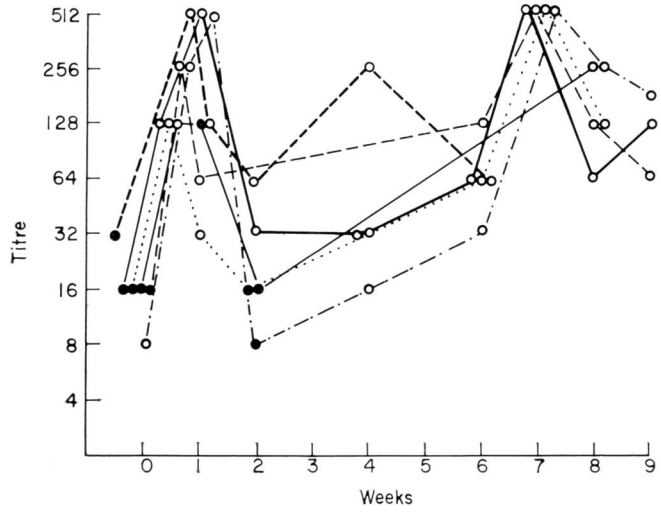

FIG. 2. Complement fixation titres of unheated rat serum of the six rats from Fig. 1. ●, Antibody activity was present only in the IgM sucrose density gradient fractions; ○, antibody was present in both IgM- and IgG-containing fractions.

DISCUSSION

The results obtained following immunization of rats by the method of Watson & Dixon (1966) using chemically modified kidney antigen show that there is an early development of anti-kidney antibodies in the IgG fraction of sera coincident with a raised level of activity in the IgM containing fractions. By means of the sucrose density gradient fractionation procedure, we have been able to separate the tissue specific response in the IgG containing fractions from the naturally occurring IgM response and to show that there is a gradual increase in anti-kidney antibody level at the same time as an increase in kidney damage, as indicated by the development of proteinuria. This is associated with histological and electron microscopic evidence of kidney damage reported elsewhere (Barabas & Lannigan, 1968).

It is not possible to conclude from the present data that there is a causal relationship between the antibody and the developing kidney lesions characteristic of this form of experimental nephritis. It is significant, however, that IgG anti-kidney antibodies have been found localized in the lesions of the disease and can be eluted from such lesions (Grupe & Kaplan, 1967).

The antigen responsible for the development of the anti-kidney response appears to differ from that which induces the formation of the naturally occurring non-tissue specific IgM antibody found in normal rats or after tissue damage (Weir, 1963; Digby & Loewi, 1965; Weir *et al.*, 1966; Weidermann, Reinhardt & Denk, 1966). Although in both cases it seems likely that the antigen is associated with the mitrochondrial fraction of the cell (Edgington *et al.*, 1968; Pinkcard & Weir, 1966; Weir *et al.*, 1966). The specificity of the antibody in the kidney immunized animals for kidney rather than an antigen widely distributed in tissues is supported by fluorescent antibody studies with such antisera which stained kidney tubules but failed to stain liver, stomach and pancreas (Barabas & Lannigan, 1968). The

explanation for the failure of the immunized rats to develop increased levels of the naturally occurring antibody is likely to be due to the considerable instability of the mitochondrial antigen reactive with the naturally occurring antibody (Pinckard & Weir, 1966) and this antigen would almost certainly be destroyed by the chemical modification procedure used in the present work.

Using the purified tubular antigen $RTE_{\alpha 5}$ Glassock *et al.* (1968) were able to induce immunization in rats with microgram quantities of the material in Freund's complete adjuvant. These workers conclude that autologous antigen is likely to be partly involved as the stimulus for antibody production. The present results suggest that autologous material may be released at 6–9 weeks, as evidenced by the peaks of anti-kidney antibody occurring at this time. It seem unlikely that the rise in antibody levels at this period can be due to the injected antigen as the last of the four injections was given at 4 weeks, i.e. 2 weeks before the start of the rapid rise in titre between 6 and 7 weeks and 5 weeks before the rise commencing at 9 weeks (Fig. 1).

The ability of chemically altered antigen to circumvent tolerance has been clearly established by the work of Weigle (1967) and Cinader, Rose & Yoshimura (1967). Weigle, for example, showed that rabbits tolerant to bovine serum albumin or thyroglobulin could be induced to produce anti-BSA or anti-thyroglobulin antibody by injection of sulphanilarsanil modified antigen. The injection of unmodified mitochondrial antigen appears to be a less reliable way of inducing kidney damage and proteinuria and fractionation of sera from three rats 8–12 weeks after injection showed the presence of antibodies in the IgG fractions in only one animal (Barabas, unpublished results). It is not possible to determine from the work reported here if rats are initially tolerant to the tubular antigen involved although Glassock *et al.* (1968) consider this likely. Presumably the chemical and physical procedures used in extracting the $RTE_{\alpha 5}$ antigen are sufficient to have a similar effect as that induced by the procedure used with the mitochondrial fraction in the present work and result in evasion of normal tolerance. It has, however, been established with the naturally occurring antitissue antibody that rats are not tolerant to the particulate mitochondrial antigen with which this antibody reacts (Elson & Weir, 1969) nor can rats be made tolerant neonatally to this antigen (Weir & Pinckard, 1967). Thus rats are not tolerant to such antigen in the classical sense that the immune system cannot respond to 'self' antigen. The antibody-forming tissues of these animals are capable of giving a limited but specific response to particulate subcellular antigen by producing antibodies of the IgM class. It seems likely that the additional production of IgG antibodies is brought about by breaking off this partially unresponsive state by means of chemically modified antigen. Whether or not this can occur in other disease states where autoantibodies are produced remains to be established.

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