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Lymph node cells from rats with Heymann's nephritis produce *in vitro* autoantibodies directed against purified renal tubular antigen

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Summary. A method is described for the purification and identification of the nephritogenic glycoprotein in renal tubular epithelium (RTE-Gp) from Wistar and Lewis rats. This antigen is responsible for the induction of Heymann's nephritis (HN) in Lewis rats. RTE-Gp was detected in chromatographic fractions by an enzyme-linked immunosorbent assay using rat antibodies eluted from glomeruli of Lewis rats with active HN. In each step of the purification procedure, an absolute correlation between detection of RTE-Gp *in vitro* and nephritogenicity *in vivo* was demonstrated. Lymph node cells obtained from rats with HN produced *in vitro* autoantibodies in a linear dose–response fashion against both allogeneic and autologous RTE-Gp, as detected by an enzyme-linked immune protein sorbent assay. During a 2 hr incubation period, 10^6 lymph node cells produced 10–40 ng of IgG. Antibody production above background levels could be detected with an input of 10^5 lymph node cells. This study shows that the specific autoimmune response in rats with NH can be measured at the cellular level.

Abbreviations: HN, Heymann's nephritis; FCA, Freund's complete adjuvant; RTE-Gp, renal tubular epithelial glycoprotein; ELISA, enzyme-linked immunosorbent assay; ELIPSA, enzyme-linked immune protein sorbent assay; DOC, sodium deoxycholate, EDTA, ethylenediamine tetraacetate; SDS, sodium dodecyl sulphate; PAGE, polyacrylamide gel electrophoresis; GBM, glomerular basement membrane; IF, immunofluorescence; HRP, horseradish peroxidase.

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INTRODUCTION

Heymann's nephritis (HN) (Heymann *et al.*, 1959) has been used as an experimental model to study the disappearance of natural tolerance to renal autoantigens, which leads to the nephrotic syndrome due to membranous glomerulopathy. This autoimmune nephropathy can be induced in genetically susceptible rat strains (Stenglein, Thoenes & Günter, 1975; Zanetti *et al.*, 1980) by allogeneic immunization with a crude fraction of renal tubular epithelium (Fx1A) in Freund's complete adjuvant (FCA), resulting in the deposition of IgG and complement components in the glomeruli (Alousi, Post & Heymann, 1969; Couser, Stilmant & Darby, 1976; Mendrick *et al.*, 1980). The nephritogenic component in Fx1A has been isolated and identified by several authors by means of detergent-solubilized Fx1A (Edgington, Glassock & Dixon, 1968; Miettinen *et al.*, 1980; Kerjaschki & Farquhar, 1982) or enzyme-digested Fx1A (Naruse, Fukasawa & Niyakawa, 1975; Miettinen *et al.*, 1980; Masugi & Masamichi, 1982). The isolated proteins had different molecular weights depending on the method of preparation. Most investigators, however, agree on several points concerning the nature of this antigen. First, it is located on brush border membranes at the luminal side of the proximal tubules

(Edgington *et al.*, 1968; Meittinen *et al.*, 1980; Kerjaschki & Farquhar, 1982). Secondly, it is a glycoprotein with mannose and/or glucose residues (Makker, 1980; Miettinen *et al.*, 1980; Kerjaschki & Farquhar, 1982). Thirdly, some antigenic determinants may be shared by structures in the glomerulus (van Damme *et al.*, 1978; Makker, Kirson & Moorthy, 1982; Neale & Wilson, 1982). Investigations into the cellular mechanisms have as yet been limited to assays with the crude kidney fraction (Litwin *et al.*, 1971; Harmon, Grupe & Parkman, 1980). Since antibodies (Sugisaki *et al.*, 1973) and complement (Salant *et al.*, 1980) seem to be the mediators involved in the induction of proteinuria, the activation of autoreactive B cells is the pivotal event in the pathogenesis of this disease. In order to assess autoantibody production *in vitro* by B cells activated *in vivo*, the renal tubular epithelial glycoprotein (RTE-Gp) responsible for the induction of HN was purified from Fx1A derived from both Lewis and Wistar rat kidneys. Rat antibodies eluted from glomeruli of rats with HN were used in an ELISA to detect the antigen during the purification procedure. The *in vitro* production of autoantibodies by autoreactive B cells against purified RTE-Gp was assessed by a modified version of the enzyme-linked immune protein sorbent assay (ELIPSA) of Boerrigter, Vos & Scheper (1983). This study shows that the generation of autoimmune disease can be studied in this model at the cellular level.

MATERIALS AND METHODS

Animals

Inbred Lewis rats ($RT1^l$) were obtained from the Laboratory of Pathology, Leiden, and Wistar rats ($RT1^u$) were bred in the Laboratory of Pathological Chemistry, Leiden. Comparisons were made only between groups of rats of the same sex.

During *in vivo* experiments, the rats received distilled water containing 0·2 g/litre tetracylin-HCl (Sigma Chemical Co., St. Louis, U.S.A.) and purina chow pellets *ad libitum*.

Antisera and reagents

Rabbit anti-rat IgG (directed against heavy and light chain determinants) was raised in rabbits by repeated subcutaneous immunizations with purified rat IgG. Rabbit anti-rat C3 was prepared as described previously (Daha *et al.*, 1979). One and 2 weeks after each boost the animals were bled. Anti-whole rat serum was obtained from rabbits immunized with a 50% ammonium sulphate precipitate of normal rat plasma. A 33% ammonium sulphate precipitate of 80 ml of this antiserum was coupled to 200 ml of Sepharose 4B. Sepharose 4B, DEAE Sephadex A.50, Lentil lectin-Sepharose 4B and Sephacryl S-400 were obtained from Pharmacia Fine Chemicals Inc. (The Hague, The Netherlands).

Fx1A was prepared according to the method of Edgington *et al.* (1968) with minor modifications. For each preparative run, 250 kidney cortices in 200 ml of PBS, pH 7·4, containing 0·1% NaN_3 were pressed through a 150 mesh stainless steel sieve (Twente Inc., Hengelo, The Netherlands). Intact glomeruli and large particles were pelleted for 10 min at 400 ***g*** in a Sorvall RC-3 centrifuge at 4°. The supernatant was then centrifuged in a Beckmann ultracentrifuge with a PMT 30 rotor for 45 min at 79,000 ***g***. The supernatant obtained after ultracentrifugation (fraction 1B) was carefully removed by vacuum suction taking care that none of the whitish material on top of the pellet was discarded (Chant & Silverman, 1977). The pellet (fraction IA or Fx1A) was resuspended in cold distilled water containing 0·1% NaN_3 and recentrifuged at 79,000 ***g***. This procedure was repeated three times. The last pellet was dialysed overnight against distilled water at 4°, lyophilized and stored in airtight glass bottles at −30°. Each run of 250 kidneys resulted in 12–13 g of lyophilized Fx1A.

Induction of HN

Lewis rats (male and female) were immunized when 8 weeks old with 200 μl of a Freund's complete adjuvant (FCA) emulsion containing 1 mg M.tuberculosis HRa 37 (Difco, Detroit, USA) and 5 mg Wistar Fx1A. Equal amounts were injected into the front footpads.

Preparation of nephritic eluates

Lewis rats with prominent HN, as indicated by proteinuria and immunofluorescence on kidney biopsies, were selected. The rats were perfused with 50 ml of PBS by aortic puncture under either anaesthesia. The kidneys were subsequently snap-frozen in isopentane and stored at −70° until further use. The glomeruli were isolated from pooled Heymann kidney cortices, as described above, and then washed three times in PBS. The final pellet was resuspended in 2 M KSCN in 0·01 M phosphate buffer, pH 7·6. Five millilitres of elution buffer were added per 1 ml pellet. After being stirred at room temperature for 60 min, the glomeruli were spun down at 8000 ***g*** for 15 min at 4° in

a Sorvall RC-5 high-speed centrifuge. The supernatant was dialysed overnight in PBS with multiple changes of dialysis buffer. The precipitate that was formed during dialysis was spun down for 15 min at 17,000 ***g***. The supernatant was concentrated in an Amicon chamber with an XM50 filter to one-fifth of the original volume. It contained 160 μg IgG/ml, as determined by rocket electrophoresis at pH 8·0 and a purified rat IgG standard.

Detection in vitro *of RTE-Gp during purification*
During purification RTE-Gp was detected by mixing 10 μl samples of the column fractions with 90 μl of a 0·1 M carbonate/hydrogen-carbonate buffer, pH 9·6, in wells of flexible PVC microtitre plates (Dynatech Inc., Alexandria, U.S.A.). After overnight incubation at room temperature, the wells were rinsed with PBS containing 0·1% Tween 20 and incubated with 100 μl of 2% BSA in PBS-Tween, pH 7·4, for 60 min at 37°. The wells were rinsed again and incubated with 100 μl of nephritic eluate diluted 1/100 in PBS-Tween for 45 min at 37°. Rinsing was followed by incubation with immunospecific rabbit anti-rat IgG conjugated to horse radish peroxidase diluted in PBS-Tween. A final incubation with orthophenylene diamin (Sigma, St. Louis, U.S.A.) in a 0·1 M phosphate/citrate buffer (0·4 mg/ml), pH 5·0, was performed for 1 hr at room temperature in the dark. The optical density at 450 nm was measured in a Titertek ELISA scanner (Flow Laboratories, Zwanenburg, The Netherlands).

Nephritogenicty in vivo *during purification*
After each purification step, samples were taken from all protein pools, emulsified in FCA and injected into Lewis rats as described. Kidney biopsies were taken 6 weeks later and assessed for depositions of IgG and C3 by direct immunofluorescence microscopy of cryosections.

Urine samples were collected by keeping the rats in metabolic cages for 24 hr without food but with water *ad libitum*. Proteinuria was assessed by measuring 24 hr urine samples containing 0·1% Merthiolate by the biuret method using normal horse serum as a protein standard.

Purification of RTE-Gp

Solubilization of Fx1A with sodium desoxycholate (DOC). For each preparation, 5 g of lyophilized Fx1A were suspended in 250 ml 0·01 M Tris-HCl buffer, pH 8·0, containing 0·1 M EDTA, 0·1 M NaCl and 1% Na-DOC. After 1 hr of stirring at 4°, the suspension was cleared by ultracentrifugation for 60 min at 89,000 ***g***. Saturated ammonium sulphate was added drop-wise under constant stirring at 4° until 25% saturation was achieved. The precipitate formed was spun down by ultracentrifugation for 45 min at 79,000 ***g***. The supernatant was dialysed against 0·0175 M phosphate buffer, pH 6.5, containing 0·02% NaN_3; conductivity was 2 mS. It was subsequently concentrated to 20 ml by high pressure ultrafiltration in an Amicon chamber using an XM 50 filter and stored at −30° until further use.

Anion exchange chromatography. Fx1A DOC was applied to a 5 × 50 cm column of DEAE Sephadex A.50 equilibrated with the phosphate buffer described above. After rinsing with 2·5 litres of buffer at a flow rate of 100 ml/hr, a linear salt gradient of 2·5 litres was applied up to a conductivity of 80 mS. Protein and RTE-Gp were assayed by Folin analysis and ELISA, respectively. Fractions containing RTE-Gp were pooled and concentrated as described above, and dialysed against phosphate-buffered saline containing 0·02% sodium azide (PBS-NaN_3). All subsequent purifications were performed in the same buffer.

Affinity chromatography on anti-whole rat serum-Sepharose 4B. The pooled A.50 eluate was applied in portions to a 2·5 × 30 cm immunoabsorbent column of rabbit anti-whole rat serum coupled to Sepharose 4B. A counter-immunoelectrophoresis of fraction samples against rabbit anti-whole rat serum was used to test for the presence of serum components in the effluent. Fractions containing RTE-Gp, as detected by ELISA, without detectable serum proteins were pooled and concentrated to 10 ml.

Affinity chromatography on Lentil lectin-Sepharose 4B. The material obtained after the anti-whole rat serum column was applied to a Lentil lectin-Sepharose 4B column (1·5 × 30 cm). After the column was rinsed with 100 ml of PBS-NaN_3, at a flow rate of 20 ml/hr, the bound glycoproteins were eluted with a linear mannose gradient employing 50 ml of starting buffer gradually mixed with 50 ml of 0·6 M D-mannose (Sigma, St. Louis, U.S.A.). Pilot studies had shown that mannose was the most effective eluent. Pools of eluted glycoproteins were made on the basis of the ELISA and the protein profile, dialysed against PBS-NaN_3 in 3% glycerin and concentrated to 1·5 ml.

Gel filtration. A sephacryl S-400 column of 1·5 × 90

cm was equilibrated with PBS-NaN_3 in 3% glycerin and calibrated with normal human serum by measuring the elution volumes of IgM, IgG and albumin. The void volume was determined with Dextran blue. Fractions of 2·5 ml were collected. After a protein sample was applied, the column was rinsed with equilibration buffer at a flow rate of 9 ml/hr. Fractions containing RTE-Gp were pooled, concentrated and stored at −30°.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis. Aliquots of 40 μg of protein were incubated for 60 min at 37° in 10 M urea with 1·5% SDS and applied to 6% polyacrylamide gels containing 1% SDS. Electrophoresis was carried out at 8 mA/gel and 30° until the buffer front reached the end of the gel (Weber & Osborn, 1969). Bovine thyroglobulin, human C3, IgG and albumin served in parallel gels as molecular weight markers. Stained gels were scanned at 578 nm with an Eppendorf photometer (Eppendorf, Hamburg, W. Germany).

In vitro *assessment of autoantibody production*

Antigen-specific autoantibody production by lymph node cells was assessed *in vitro* in an enzyme-linked immune protein sorbent assay (ELIPSA).

The draining lymph nodes of the front legs, i.e. the brachial and axillary lymph nodes (Tilney, 1971), were

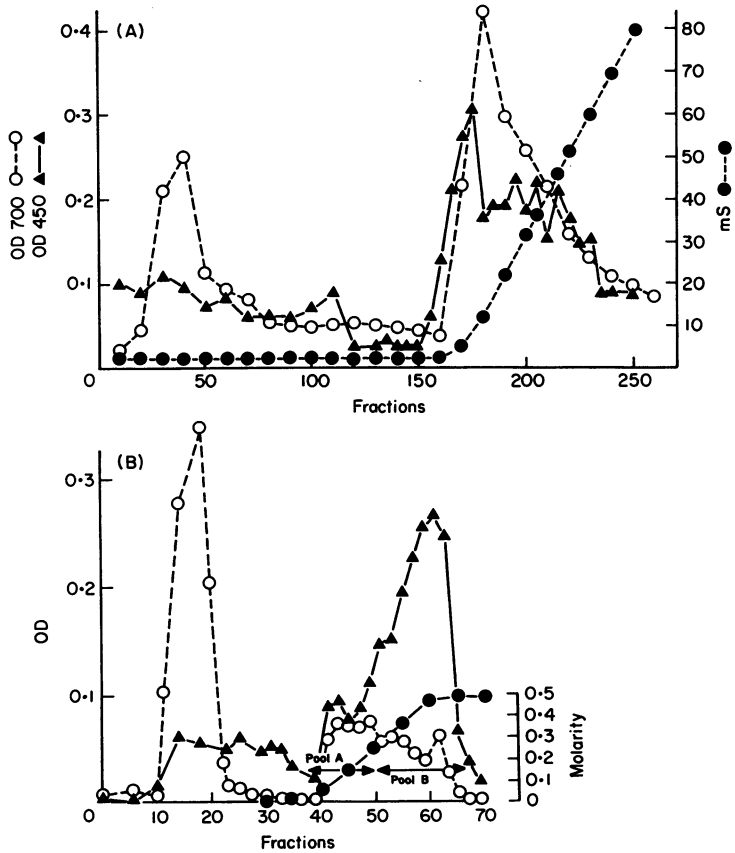

Figure 1. (A) DEAE-A50 chromatography of Fx1ADOC. Protein content, as measured by Folin analysis, (○) and ionicity (●) of the fractions are indicated. Presence of RTE-Gp in the fractions was assessed by ELISA (▲) using rat antibodies eluted from glomeruli of nephritic kidneys.

(B) Chromatography of pooled RTE devoid of rat serum proteins on Lentil lectin-Sepharose 4B. Protein content (○), presence of RTE-Gp (▲) ad molarity of D-mannose (●) are indicated. The eluate was divided into two protein pools (A and B), as indicated by the *arrows*.

removed under ether anaesthesia and pressed through a 100 mesh stainless steel sieve drenched in RPMI-1640 containing 4% foetal calf serum (Flow), 5×10^{-5} M 2-mercaptoethanol, 20 mM HEPES (Sigma), 2 mM glutamin and $NaHCO_3$ 1 g/litre (pH 7·4). The cells were washed three times by centrifugation at 300 *g* for 10 min at 4° and resuspended in medium. A final cell suspension of 10^8/ml was made and pipetted in two-fold dilutions into ELISA microtitre wells coated with 1 μg RTE-Gp per well. Tween 20 was omitted from the wash buffer until the cells were removed from the wells. After 2 hr of incubation at 37°, the cells were removed by vigorous rinsing with PBS. The wells were washed three times with PBS-Tween; HRP-conjugated rabbit anti-rat IgG was added to the wells and incubated for 45 min at 37°. The assay was completed as described for the ELISA. The amount of bound antibodies produced by lymph node cells within 2 hr was calculated from standard curves using protein A-purified IgG from nephritic eluate. For this purpose, a microtitre plate was coated with 1 μg purified RTE-Gp per well and incubated with a dilutiom series of known amounts of purified antibody and HRP-conjugated rabbit anti-rat IgG.

RESULTS

Antigen purification

To perform the ELIPSA it was necessary to purify the nephritogenic antigen from rat kidneys. For this purpose Fx1A was prepared from 500 kidneys and solubilized in DOC, yielding 2·6 g of protein. This protein pool was applied on a DEAE Sephadex A.50 column followed by linear salt elution up to 80 mS. The protein and ELISA profiles, as depicted in Fig. 1(A), show that relatively little RTE-Gp was present in the effluent fractions. Salt elution, however, resulted in a broad antigen peak ranging from 5 to 50 mS. Fractions 160–235 which contained 850 mg of protein were pooled, concentrated, dialysed and applied in 200 mg portions to an immmunoabsorbent column of rabbit anti-whole rat serum coupled to Sepharose 4B. The early fractions contained RTE-G- antigen, as detected with the ELISA, but no detectable rat serum proteins. The antigen pools of five consecutive separate absorption runs were pooled, yielding 60 mg of protein, and applied on a Lentil lectin-Sepharose 4B column.

Figure 1(B) shows that a minimal amount of RTE-Gp, as detected by ELISA, was present in the effluent fractions. Elution with a linear mannose gradient up to 0·5 M resulted in a broad protein peak. Fractions 50–65 contained the highest concentration of RTE-Gp. Fractions 38–50 and 51–65 were pooled and called pool A (2 mg) and B (20 mg), respectively. After concentration and dialysis both pools were

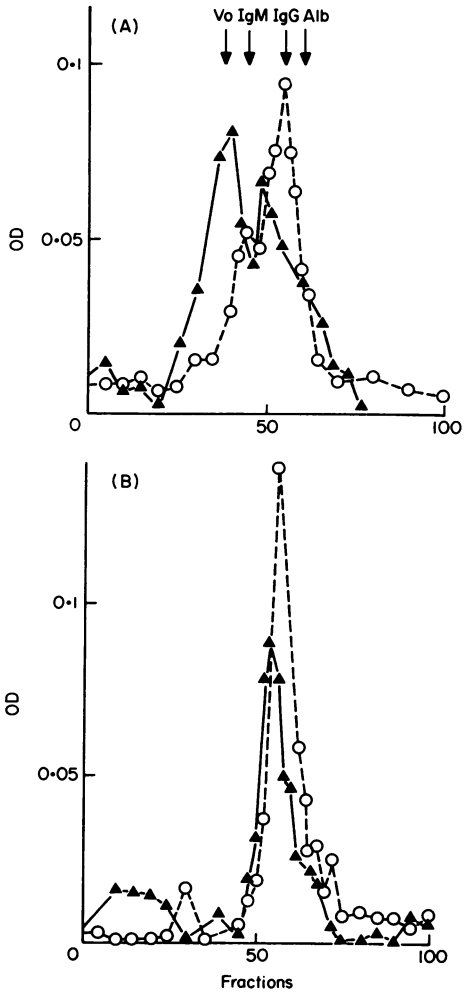

Figure 2. (A) Gel filtration on Sephacryl S-400 of concentrated pool A, eluting at low mannose molarity from the Lentil lectin column. Protein content (○) and presence of RTE-Gp (▲) are indicated. The filtration positions of Dextran Blue (Vo), IgM, IgG and albumin, as indicated by *arrows*, were determined previously.

(B) Gel filtration of the concentrated pool B, eluting at high mannose molarity from the Lentil lectin column. Protein (○), RET-Gp (▲) and molecular weight markers (*arrows*) are indicated.

applied separately to a Sephacryl S-400 column, as shown in Fig. 2(A, B).

Gel filtration of pool A resulted in a filtration profile in which RTE-Gp was present in two peaks of different molecular weight. The molecular weight of the material in the void volume fractions exceeded 1500K. The average size of the material in the second peak was slightly more than 150K (Fig. 2A). Fractions 25–45 were pooled, concentrated and stored at −70°.

Gel filtration of pool B (Fig. 2B) resulted in the elution of an antigen peak with a molecular weight slightly higher than 150K. Fractions 50–55 were pooled, concentrated and stored at −70°. Cross-absorption inhibition studies using the ELISA showed that the antibody activity of nephritic eluate against low molecular weight RTE-Gp from pool B could be absorbed completely by high molecular weight RTE-Gp from pool A, and vice versa. This suggested that pool A contained a high molecular form of pool B and that the material of pool A was a mixture of RTE-Gp of two molecular weights. SDS-PAGE analysis showed a pattern of protein bands of 130, 150 and 390K, regardless of whether high or low molecular weight samples were used. Part of the high molecular weight material remained on top of the gel (Fig. 3). Parallel unstained SDS-gels were sliced into 4 mm sections and assessed for RTE-Gp antigen by ELISA. The results, shown in Fig. 3, indicated that all stained bands were also able to react with nephritic eluate.

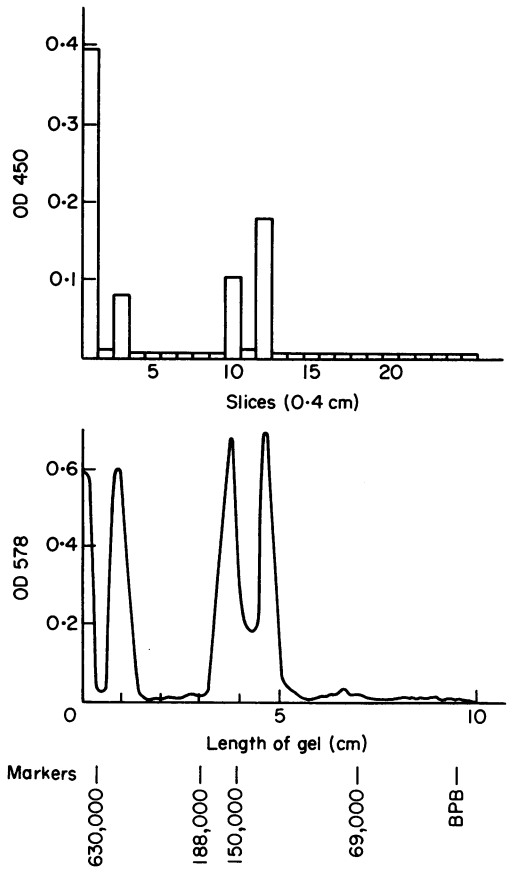

Figure 3. SDS-PAGE analysis of 40 μg of high molecular weight RTE-Gp. Molecular weight markers were run in parallel gels. Reactivity of protein bands from high molecular weight RTE-Gp to nephritic eluate was determined in a parallel gel by ELISA using eluted 4 mm gel slices.

Nephritogenicity *in vivo* of (semi-) purified RTE-Gp

After each purification step, a sample was taken from all protein pools obtained, emulsified in CFA and injected into groups of four Lewis rats. A kidney biopsy was taken after 6 weeks and proteinuria was measured between the 8th and 16th weeks. The time intervals were chosen on the basis of preliminary experiments with Lewis rats immunized with Fx1A in FCA. Protein excretion by normal rats never exceeded 5 mg/24 hr. Protein excretion in excess of 20 mg was regarded as significantly abnormal proteinuria.

As shown in Table 1, injection of 5 Mg DEAE A.50 eluate caused diffuse granular deposits of IgG and C3 along the GBM within 6 weeks without the development of proteinuria. Injection of an equal amount of the effluent pool had no nephritogenic effect. Injection of 800 μg of the effluent pool of the anti-whole rat serum immunoabsorbent caused an increased diffusely granular deposition of IgG without proteinuria. Injection of proteins eluted from the immunoabsorbent had no nephritogenic effect. Administration of 300 μg of the eluate of the Lentil lectin column caused heavy deposition of IgG and C3 along the GBM and the brush border of the proximal tubuli in all immunized rats. The protein excretion after 8 weeks was 0·6, 8·3, 24·6 and 40·8 mg/24 hr, indicating that two of the four immunized rats developed abnormal proteinuria. However, rats immunized with 500 μg of the effluent pool of the Lentil lectin column that was negative in the ELISA, exhibited no deposits and their protein excretion was $0{\cdot}5 \pm 0{\cdot}2$ mg/24 hr. Injections of 40 μg of the pools obtained after gel filtration caused granular deposits of IgG and C3 without abnormal proteinuria. High molecular weight antigen and low

Table 1. Steps of purification of RTE-Gp verified *in vivo* by injection of various fractions into groups of Lewis rats

Purification step	Protein pool	Amount injected	Immunofluorescence of kidney for IgG and C3	Number of rats with proteinuria (number of rats tested = 4)
Sieving and ultracentrifugation	Fx1A	5 mg	++++	4
Solubilization in 1% DOC	Fx1A-DOC	5 ng	++++	4
Anion exchange chromatography (DEAE A.50)	Effluent	5 mg	−	0
	Eluate 5–50 mS	5 mg	+	0
Removal of serum proteins (Seph. 4B-anti-whole rat serum)	Effluent	0·8 mg	++	0
	Eluate (acid)	2·0 mg	−	0
Affinity chromatography on Lentil lectin-Seph. 4B	Effluent	0·5 mg	−	0
	Eluate	0·3 mg	+++	2
Gel filtration Sephacryl S-400	Pool A	0·04 mg	+	0
	Pool B	0·04 mg	+	0

molecular weight antigen did not differ in their nephritogenic effect.

Assessment of autoantibody production *in vitro*

Four female Lewis rats were immunized with 5 mg Wistar Fx1A in FCA as described. An additional injection of *B. pertussis* (Difco, Detroit, U.S.A.) vaccine was given in the dorsum of each front footpad. Each rat received 5×10^7 bacteria. Four control rats only received the adjuvants. Four weeks after immunization, the rats were killed. Their kidneys were perfused with saline and a specimen of tissue was snap-frozen for immunofluorescent studies. Single cell suspensions of the draining lymph nodes were made and assayed in the ELIPSA against purified RTE-Gp. Immunofluorescence studies of kidney cryosections from rats immunized with Fx1A in FCA revealed the heavy granular deposits of IgG and C3 along the GBM and brush border of the proximal tubules characteristic of active HN (Fig. 4A). No granular deposits of IgG or C3 were visualized by IF on kidney cryosections from control rats (Fig. 4B).

As shown in Fig. 5, lymph node cells from rats with HN produced, in a linear dose–response fashion, autoantibodies against purified RTE-Gp. The antigen specificity of the assay was confirmed by addition of soluble purified antigen to the cells which inhibited up to 43% of the antibody binding. Non-nephritogenic proteins did not inhibit the assay. Furthermore no significant difference could be measured between *in vitro* production of antibodies against purified alloantigen (Wistar) and autoantigen (Lewis). Cells obtained from control rats, injected with adjuvants alone, did not produce measurable amounts of antibodies with this specificity. Incubation of lymph node cells from rats with HN in microtitre wells coated with non-nephritogenic components of Fx1A did not yield significant *in vitro* antibody production.

DISCUSSION

To detect RTE-Gp *in vitro* in column fractions during chromatography we used an ELISA in which we coated microtitre wells with samples of the column fractions. Eluted antibodies obtained from the glomerular deposits of rats with HN were used as antibody source. This method had several advantages. First, it selectively detected proteins carrying antigenic determinants which caused autoantibodies that were involved in the formation of deposits in the glomeruli *in vivo*. Secondly, it did not require a monospecific antiserum or purified antigen. Furthermore, we were able to detect as little as 10 ng of RTE-Gp with this assay. However, this required an antigen that binds

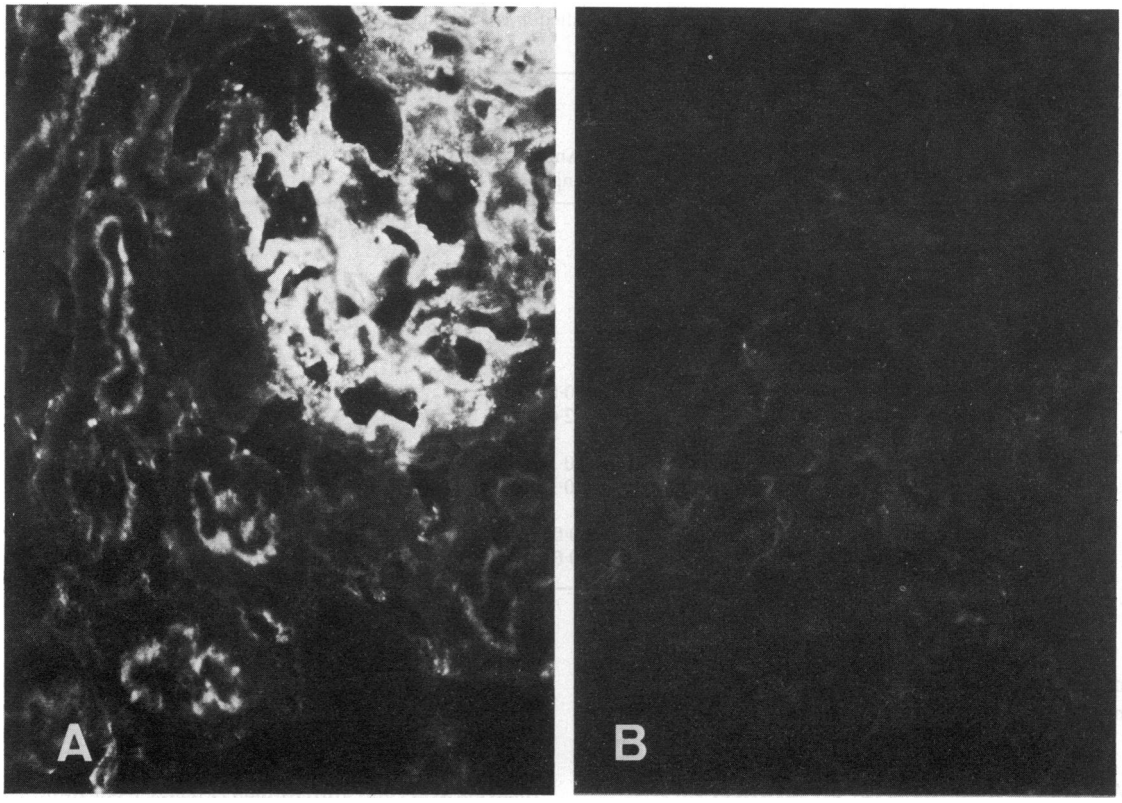

Figure 4. Diffuse granular deposits of rat IgG outlining the GBM of glomeruli of a Lewis rat with HN (A) combined with staining of the brush border of the proximal tubules. No deposits were seen on kidney cryosections of rats injected with adjuvants only (B). Stainig was accomplished by fluorescein isothiocyanate-conjugated anti-rat IgG; (magnification ×400).

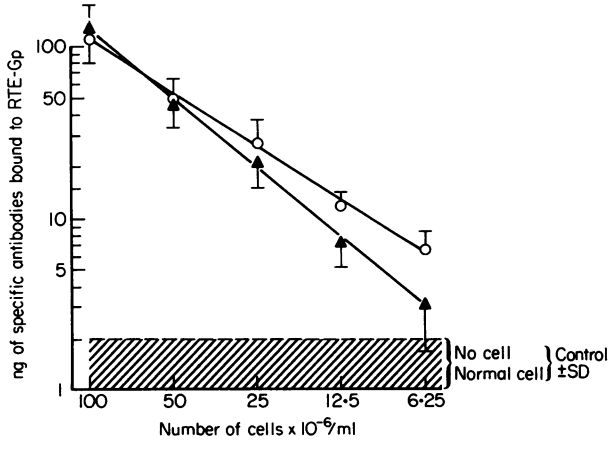

Figure 5. *In vitro* production of autoantibodies by a dilution series of lymph node cells from Lewis rats with HN in microtitre wells coated with purified RTE-Gp. After 2 hr of incubation at 37°, the cells were removed and the microtitre wells were incubated with HRP-conjugated rabbit anti-rat IgG and treated further according to an ELISA. Control values are indicated by the *hatched area* and the amount of antibodies bound to Lewis (▲) or Wistar (O) RTE-Gp, as calculated from standard curves, are shown.

readily to microtitre wells, which implies that non-adherent nephritogenic protein(s) could be missed in this assay. To exclude this possibility plus the possibility of loss of nephritogenicity during the purification procedure, protein samples from each purification step were emulsified in FCA and injected into Lewis rats. Table 1 shows that there was an absolute correlation between detection of RTE-Gp *in vitro* by the ELISA and nephritogenicity *in vivo*.

The purification of high molecular weight RTE-Gp by chromatography closely resembles the antigen purification described by Edgington *et al.* (1968). However, presence of lipid components in our antigen preparations could not be demonstrated. We calculated that 1 mg of lyophilized Wistar Fx1A contained approximately 35 μg of RTE-Gp, which is in the same order of magnitude as found by others (Glassock *et al.*, 1968). Solubilization in DOC and further chromatography lead to a reduction in nephritogenicity *in vivo*, since proteinuria was not caused by injections with (semi-) purified protein samples unless large amounts of highly purified antigen were administered. Similar results have been reported by others (Edgington *et al.*, 1968; Miettinen *et al.*, 1980; Kerjaschki & Farquhar, 1982). The insoluble form of RTE-Gp in Fx1A may persist longer at the site of administration, leading to a stronger autoimmune response. Furthermore, other components within Fx1A may serve as additional adjuvants for the induction of HN. On the other hand the elegant experiments of Kerjaschki & Farquhar (1982) demonstrate that only one immunogen in Fx1A seems to be responsible for the induction of HN. The fact that RTE-Gp appears in peaks after gel filtration and yields several bands on SDS-PAGE analysis suggests that this glycoprotein is fragmented by detergent treatment into fragments of different molecular size, each of which reacts with nephritic eluate. We have observed that overnight treatment of high molecular weight RTE-Gp (> 1500K) in 1% DOC and subsequent gel filtration resulted in the isolation of RTE-Gp of 150K and a small amount of lower molecular weight breakdown products (data not shown). The reported instability of RTE-Gp (Kerjashki & Farquhar, 1982) combined with its tendency to aggregate in highly concentrated forms (Edgington *et al.*, 1968) complicates the determination of the molecular weight of the intact molecule. Others have reported SDS-PAGE gels of lectin-purified RTE with a similar distribution of protein bands (Miettinen *et al.*, 1980; Singh & Makker, 1982). Our results indicate that RTE-Gp consists of a series of repetitive units or distinct polypeptides which are fragmented to various degrees by detergent treatment.

The ELIPSA seems to be a valid alternative for the Jerne plaque assay for the detection of antibody production by isolated cells. Only minimal amounts of purified antigen are required. As few as 5×10^5 cells/well can be used to measure antigen-specific antibody production above background levels. The observation that antibody formation by lymph node cells from rats with HN in wells coated with RTE-Gp could not be inhibited by non-nephritogenic kidney antigens obtained from the effluent fractions of the Lentil lectin column indicates that no immunochemical contaminants were present in our RTE-Gp preparations. No significant difference could be measured in the amount of antibodies produced against Wistar RTE-Gp and Lewis RTE-Gp. This indicates that only antigenic determinants common to the two rat strains have nephritogenic significance.

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