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The relationship between hyperglycaemia and renal immune complex deposition in mice with inherited diabetes

C. J. MEADE, D. R. BRANDON, W. SMITH, R. G. SIMMONDS, SHEILA HARRIS* & C. SOWTER* *Lilly Research Centre, Windlesham, Surrey, and *Department of Histopathology, Clinical Research Centre, Harrow, UK*

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SUMMARY

Kidney lesions were studied by light microscopy and immunofluorescence in diabetic (db/db) and obese (ob/ob) mutant mice. The db/db mutation was studied both on the C57Bl/KsJ genetic background (where it produces severe hyperglycaemia) and on the C57Bl/6J background (where hyperglycaemia is only mild). In all cases, more IgG, IgM and C3 were deposited in the renal glomeruli of mutant mice than in the glomeruli of normal (+/?) mice of equivalent age. First signs of immunoglobulin deposition occurred at a slightly younger age than first signs of C3 deposition or histological change (mainly mesangial thickening). Insulin deposits were occasionally seen in the glomeruli of older mutant mice and immunoglobulin eluted from diabetic mouse kidneys had anti-insulin activity. Increased anti-DNA activity was present in the serum of older mutants. In those mutants with severe hyperglycaemia, the macula densa and distal convoluted tubules also contained immunoglobulin deposits, probably derived from the glomerular mesangium. Urine from diabetic mice contained high molecular weight material reacting with antisera to Fab or κ but not the Fc portion of immunoglobulin. We conclude that diabetic mice have immune complexes in the kidney containing antibodies against insulin and possibly other antigens. We find no evidence that hyperglycaemia itself is the direct cause of glomerular immune complex deposition, although there may be a link between hyperglycaemia and tubular dysfunction.

INTRODUCTION

Some of the renal complications of human diabetes have been suggested to be immunological in origin (Blumenthal *et al.*, 1962; Burkholder, 1965). However, though many workers have reported IgG or IgM deposits in the kidneys of at least some diabetic patients, it has frequently been impossible to demonstrate that these deposits either contain complement or have the ability to fix heterologous complement, arguing against the immunoglobulins being present in immune complexes (Westberg & Michael, 1972).

Mice homozygous for the recessive mutations 'obese' (ob) or 'diabetic' (db) spontaneously develop obesity, diabetes and insulin resistance. They are thus closest, as animal models, to maturity-onset diabetes. We have used these mutants to examine renal changes not only in established diabetes but also during development of the disease. The expression of the mutations depends on genetic background (Coleman, 1978). On a C57Bl/6J background the mutations cause modest hyperglycaemia associated with pronounced hyperinsulinaemia. On a C57Bl/KsJ background, the pattern of disease is initially similar to that on the C57Bl/6J background, but after

Correspondence: C. J. Meade, Ph.D., Lilly Research Centre Ltd, Erl Wood Manor, Windlesham, Surrey GU20 6 PH, UK.

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about 8 weeks of age the pancreatic islets necrose, insulin production falls and there is severe hyperglycaemia. By comparing older C57Bl/6J and /KsJ mutant mice we can compare two syndromes, similar in many respects but differing markedly in the degree of hyperglycaemia present.

MATERIALS AND METHODS

Mice. C57Bl/KsJ db/db mice and their lean littermates were bred by Mr J. Mullen and Miss Sue Robinson from a nucleus kindly supplied by Dr P. Trayhurn, Cambridge. Littermates of genotype +/+ could be distinguished from those of genotype +/db by the presence of the linked gene 'misty' on the normal (+) chromosome. C57Bl/6J db/db mice were bred by Dr C. Hetherington from a nucleus kindly supplied by Dr D. L. Coleman, Bar Harbor. C57Bl/6J ob/ob mice came from the Laboratory Animals Centre, Carshalton. Mice were fed Spratt's laboratory diet number 1 with water *ad libitum*.

Immunohistochemistry. One kidney from each mouse was fixed in 10% formol saline for histology, the other snap-frozen in 2-methyl butane. Four-micron frozen sections were cut and subsequently stained by indirect immunofluorescence and immunoperoxidase techniques. All antisera used were from Miles Laboratories, except for anti-C3 which was from Dr I. McConnell, and the same antisera batch numbers were used throughout in the quantitative studies. In the immunofluorescence technique, the sections were fixed in acetone for 20 min at 4°C. The first-layer antisera, rabbit anti-μ, -γ or -C3 and guinea-pig anti-porcine insulin, were used at the greatest dilution consistent with maximum fluorescence of a known positive. The second layer was fluorescein isothiocyanate-conjugated sheep anti-rabbit IgG (fluorescein/protein = 3·8) or rabbit anti-guinea-pig IgG (fluorescein/protein = 4). A chequerboard titration was used to select a titre of fluorescent antiserum sufficiently high to give optimum fluorescence at all titres of first-layer antiserum. Both layers were left on for 30 min and all dilutions and washes between each stage were in FA buffer (DIFCO). The sections were mounted in 50% glycerol in FA buffer.

Controls included omission of the first-layer antiserum and addition to the section of 400 μg/ml pure IgG or μ determinant-bearing myeloma protein (from Dr G. Klaus) prior to addition of anti-immunoglobulin antisera. One hundred micrograms per ml mouse insulin (Novo) was used to block anti-insulin. Criteria on which the fluorescence score was decided were frequency of positively-staining glomeruli and proportion of each positive glomerulus covered by deposits. Animals scoring 2, 3 or 4 had deposits in virtually all glomeruli so in these animals differences in scores only represent differences in the extent of deposition within glomeruli. Scores have no quantitative significance except to indicate a rank order of disease intensity. Statistical comparison was therefore an appropriate non-parametric test (Wilcoxon rank order test where two groups were compared; Kruskal–Wallis test where more than two groups).

The immunoperoxidase technique was based on Burns (1975), with the following modification. The sections were fixed in 3% glutaraldehyde for 20 min, washed and then digested using 1% pepsin in 0·01 M HCl for 15 min at 37°C (Reading, 1977). The action of the enzyme was stopped by placing the sections in ice-cold Tris buffer, pH 7·6. The second layer was a horseradish peroxidase-labelled anti-IgG antiserum.

Double-staining techniques to identify the type of tubule in which immunoglobulin deposits were located. Three techniques, all needing acetone-fixed frozen sections, were used. In all, IgM was identified with fluorescein-conjugated goat anti-mouse μ chain (Nordic). Antiserum was added after anti-brush-border antiserum, but first in the enzymic assays.

An anti-brush-border antiserum from a rat with Heymann's syndrome was kindly supplied by Dr C. Wilson. Sections were treated with this antiserum (diluted 1:10), then a tetrarhodamine isothiocyanate-conjugated anti-rat immunoglobulin from which anti-mouse immunoglobulin activity had been absorbed by passage down a column of purified insolubilized mouse immunoglobulin. Finally, fluoresceinated anti-μ was added. Brush-border fluoresced red and IgM green.

Alkaline phosphatase was demonstrated by a modification of the technique described by Gomori (1939). The substrate, 19 mM sodium-β-glycerophosphate, was incubated with sections for 5 min at 37°C and pH 9·0, in the presence of 72 mM Ca^{++} and 4 mM Mg^{++}. Calcium phosphate

formed was reacted with 2% cobalt nitrate, which then yielded a black precipitate with ammonium sulphide.

Succinic dehydrogenase was demonstrated by reduction of nitroblue tetrazolium (Nachlas *et al.*, 1957).

Elution of antibodies from the kidney. Each experiment used renal homogenates from between 10 and 20 C57Bl/KsJ db/db mice and their normal littermates, aged 8 to 24 weeks. Immunoglobulin was eluted with 3 M potassium bromide, pH 9 (Bartolotti, 1977). Eluate was brought to pH 2·2 with 1 N hydrochloric acid and dialysed first against 0·1 M glycine-HCl buffer, pH 2·4, then against phosphate-buffered saline, pH 7·2. Precipitated material was spun off after each dialysis.

Anti-insulin antibodies were assayed by addition of 1 ng ^{125}I-insulin (Radiochemical Centre, Amersham, item IM38), incubation for 48 hr at 4°C and precipitation of immunoglobulin with 50% ammonium sulphate. Presence of anti-kidney tissue antibodies was investigated by a sandwich technique using eluate as first layer and fluorescein-labelled goat anti-mouse immunoglobulin as second layer. The test frozen sections were cut from the kidney of a week-old normal (+/+) C57Bl/KsJ mouse. Anti-DNA antibodies were assayed by incubating eluate with 25 μg/ml DNase (DN-100, Sigma) for 30 min at 37°C and separating antibodies from enzyme and undigested DNA fragments by absorption onto Sepharose-insolubilized rabbit anti-mouse immunoglobulin. Antibody was recovered by washing the immunoabsorbent with 0·2 M glycine–HCl buffer, pH 2·4, then dialysed into borate-buffered saline, pH 8·0, and tested for ^{125}I-DNA binding as for serum (see below).

Urinalysis. Urine was collected directly with a Pasteur pipette or, on a 24-hourly basis, from mice in modified diuresis cages (Tecniplast, type 1760). It was assayed for protein (Lowry *et al.*, 1951), albumin (Mancini, Carbonara & Heremans, 1965) and total immunoglobulin. The last named was assayed by passing urine (dialysed into phosphate-buffered saline) down a column of insolubilized low-affinity anti-mouse immunoglobulin and estimating the protein content of the solution eluted by 0·2 M glycine–HCl, pH 2·4.

Urine proteins were characterized by immunoelectrophoresis or fused rocket electrophoresis at pH 8·6 and by isoelectric focusing in LKB ampholine polyacrylamide gel plates with a pH range 3·5–9·5 (Winter, Ek & Andersson, 1977).

Analysis of plasma or serum. Blood was collected by cardiac puncture into fluoride/oxalate tubes. Plasma glucose was assayed by oxidation of 4 amino-phenazone (Trinder, 1969), plasma insulin by radioimmunoassay (Hales & Randle, 1963), and anti-DNA (on heat-decomplemented serum, not plasma) by binding to double-stranded ^{125}I-DNA with subsequent precipitation of immunoglobulins with 50% ammonium sulphate (Pincus, 1971).

RESULTS

Glomerular changes

More IgG, IgM and C3 were deposited in the glomeruli of ob/ob or db/db mutants than in the glomeruli of normal mice of equivalent age (Fig. 1). The extent of immunoglobulin deposition in 24-week-old mice differed little whether the db/db genotype was present on a C57Bl/6J or C57Bl/KsJ background, although plasma glucose levels were very much higher in the latter case (Table 1). In C57Bl/KsJ db/db mice, C3 deposits were significantly ($0·05 > P$) greater in 12-week- than 24-week-old animals, although plasma glucose levels were higher in 24-week-old than 12-week-old mice. Immunoglobulin deposition also showed the same trend.

The pattern of glomerular staining with antisera against immunoglobulins or C3 was irregular and granular. Deposits were predominantly localized within the mesangium, but occasionally involved the juxtamesangial portions of the capillary loops. In severely affected glomeruli expansion of the mesangium occurred at the expense of the capillary lumen (Fig. 2a, b & c). In fixed sections stained with haematoxylin and eosin, mesangial thickening was observed. This resulted from accumulation of mesangial matrix associated with proliferation and clustering of mesangial cells. All 24-week-old mutant mice showed mesangial thickening, whether C57Bl/KsJ or C57Bl/6J. All 12-week-old C57Bl/KsJ db/db and most (seven out of 10) C57Bl/6J ob/ob mice showed

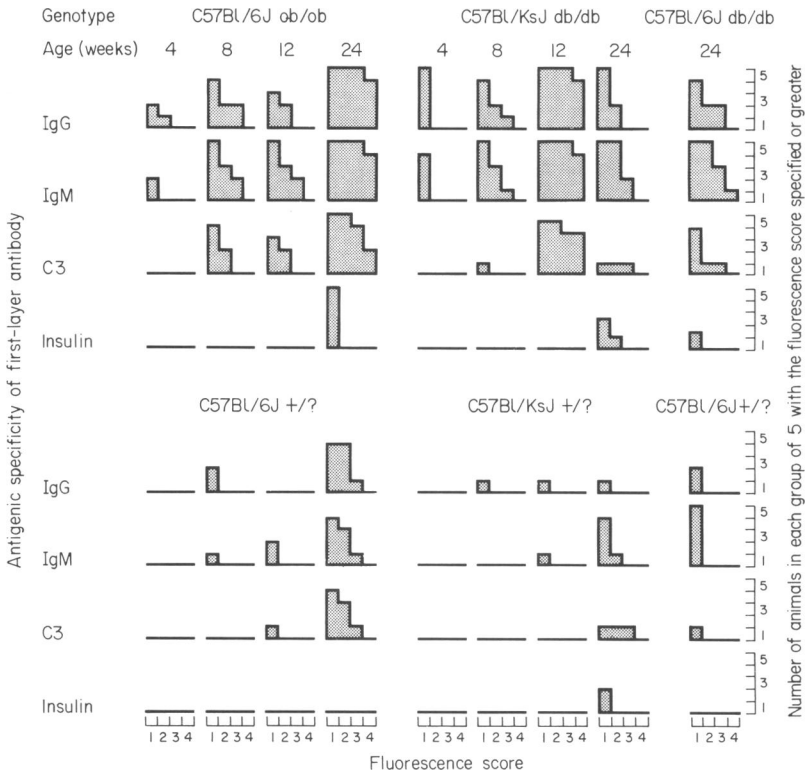

Fig. 1. Bar chart of fluorescence intensity of glomerular staining after treatment of kidneys from male mice of different ages and genotypes with antisera against IgG, IgM, C3 or insulin. Blocked controls were all negative. Mice were tested in groups of five of each age and genotype. Mutant mice and their control groups were examined together. The two 24-week-old C57Bl/6J control groups are shown separately because they were examined at different times and bred from different colonies.

mesangial thickening, but we could not detect histological glomerular changes in mice younger than this.

Limited insulin deposits were present in eight out of 15 24-week-old mutant mice examined, chiefly in the hilum of the glomerulus (Figs 1 and 2d). Only two of 15 normal controls showed any staining with anti-insulin, and in both cases these were borderline positives. Immunoglobulin eluted from db/db mouse kidneys had about eight times the insulin-binding capacity of immunoglobulin from control kidneys. Immunoglobulin from both db/db and control kidneys bound ^{125}I-DNA. Glomeruli did not stain with anti-albumin.

Tubular changes

Granular deposits were present in tubular cells of 4- or 8-week-old mutant mice of all strains, and to a lesser extent, in tubular cells of C57Bl/KsJ db/db mice of greater ages. Deposits stained heavily with antisera against γ_1, γ_{2a}, γ_{2b}, γ_3, μ, Fab, and κ chain, but only weakly with antisera against λ or C3 and not at all with antisera against albumin or fibrinogen. Stained tubules were identified by the following criteria.

(i) In peroxidase-stained sections, the tubule lumen appeared larger, cells flatter and nuclei more crowded than in the majority of other tubules (Fig. 3).

(ii) Tubules did not stain with antiserum against brush-border (Fig. 4).

(iii) They lacked alkaline phosphatase (Fig. 5).

(iv) They contained succinic dehydrogenase, though at lower concentrations than in most surrounding tubules.

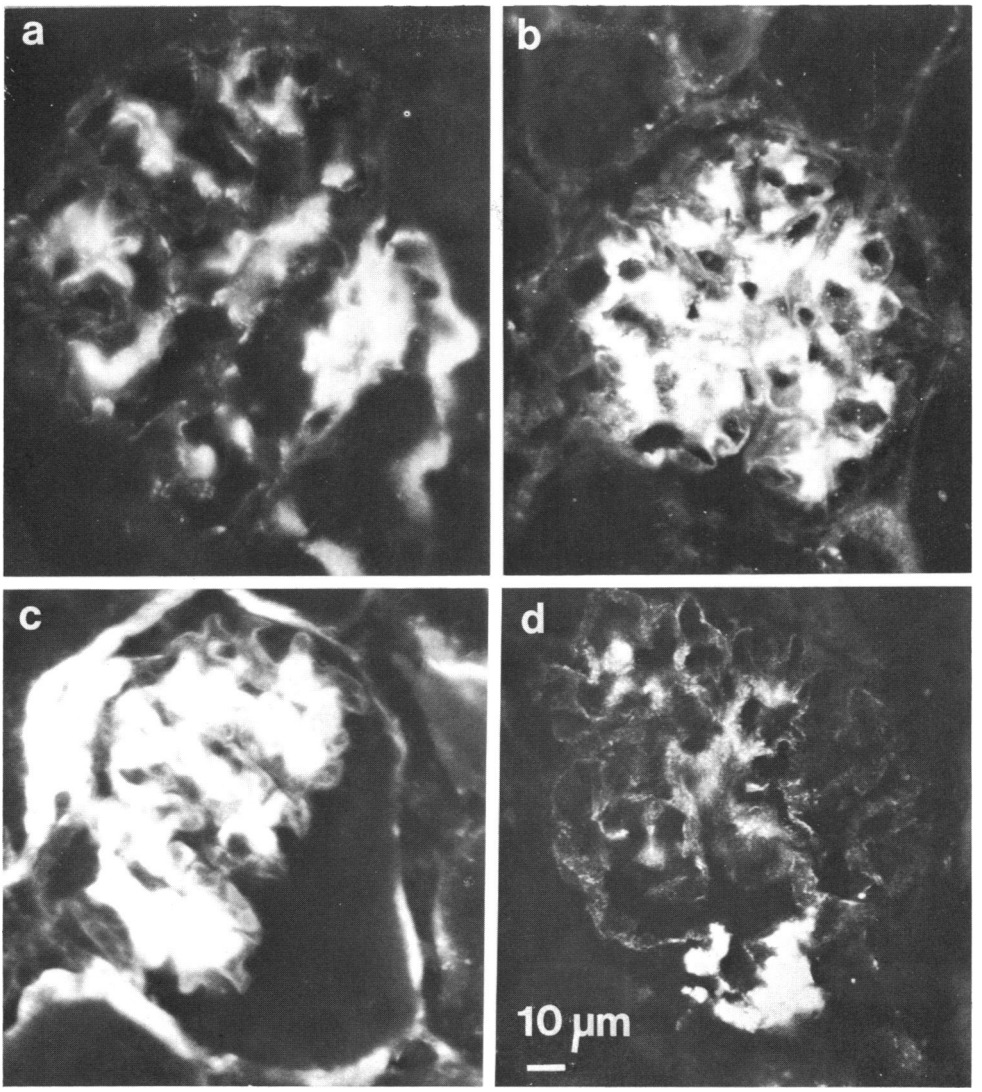

Fig. 2. High-power photomicrographs of kidneys from 24-week-old C57Bl/6J ob/ob mice stained with rabbit anti-mouse antisera and fluorescein-conjugated sheep anti-rabbit IgG. (a) First-layer anti-IgM. Mesangial staining. The peripheral capillary loops have been spared. Deposition is greater in the hilar region. (b) First-layer anti-IgG. Same animals as above, but more severely affected glomerulus. Although staining is still predominantly mesangial, some staining of the capillary loop walls is also evident. (c) First-layer anti-C3. (d) First-layer guinea-pig anti-porcine insulin, second-layer fluorescein-conjugated rabbit anti-guinea-pig IgG. Staining was entirely blocked by 100 μg/ml mouse insulin.

Not all tubules fulfilling the above criteria were immunoglobulin-positive; those that were tended to be located near glomeruli. Tubule staining was usually associated with staining of the glomerular mesangium, and in some cases there appeared to be a continuity of deposits from the hilum of the glomerulus into the macula densa and across to the abutting distal tubule. In peroxidase-stained sections, the epithelium of immunoglobulin-positive tubules sometimes showed signs of damage.

Renal eluates from C57Bl/KsJ db/db mice had no anti-tubule activity.

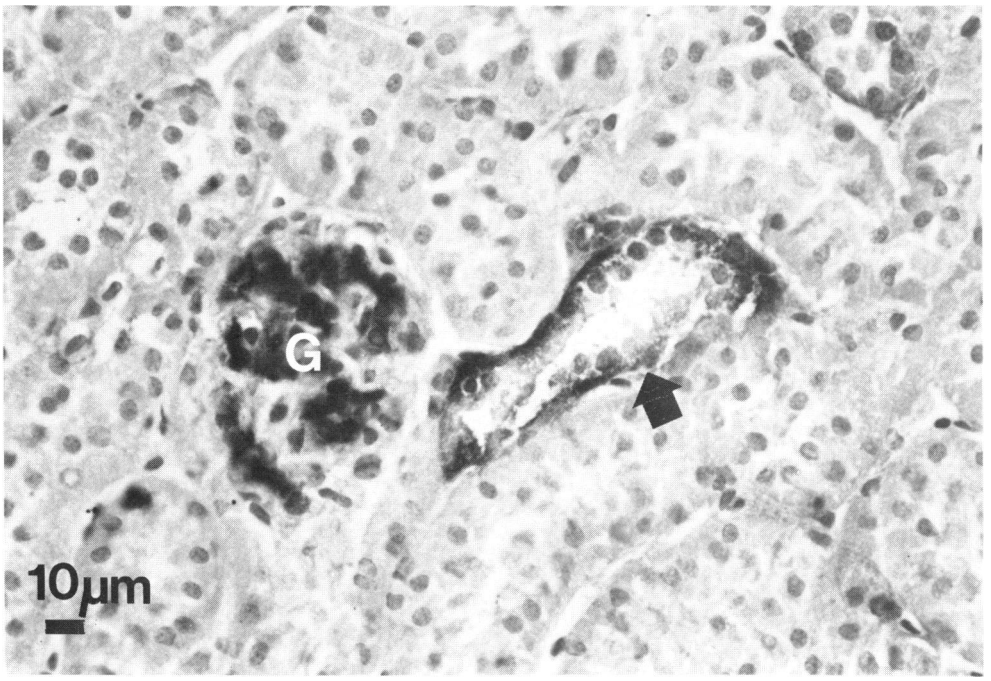

Fig. 3. High-power photomicrograph of kidney from 24-week-old C57Bl/KsJ mouse stained with rabbit anti-mouse IgM and peroxidase-conjugated goat anti-rabbit IgG, then counterstained with haematoxylin. Only one tubule (*arrow*) contains immunoglobulin deposits. It lies close to a glomerulus (G).

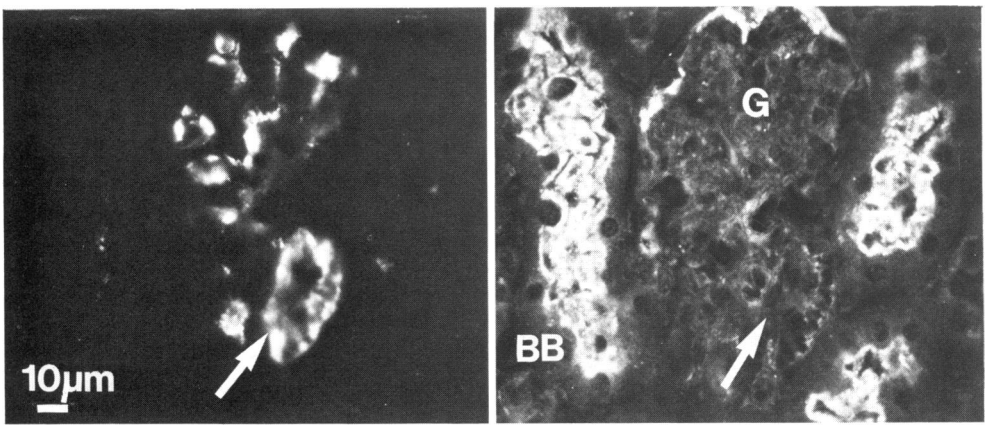

Fig. 4. Section from an 8-week-old C57Bl/KsJ db/db mouse kidney stained with fluorescein-conjugated anti-μ, then rat anti-brush-border and rhodamine-conjugated anti-rat immunoglobulin. The left-hand photograph shows green fluorescence. One tubule (*arrowed*) near the glomerulus (G) is fluorescent. The right-hand photograph of the same section shows red fluorescence. The arrowed tubule is not fluorescent and therefore lacks brush-border (BB).

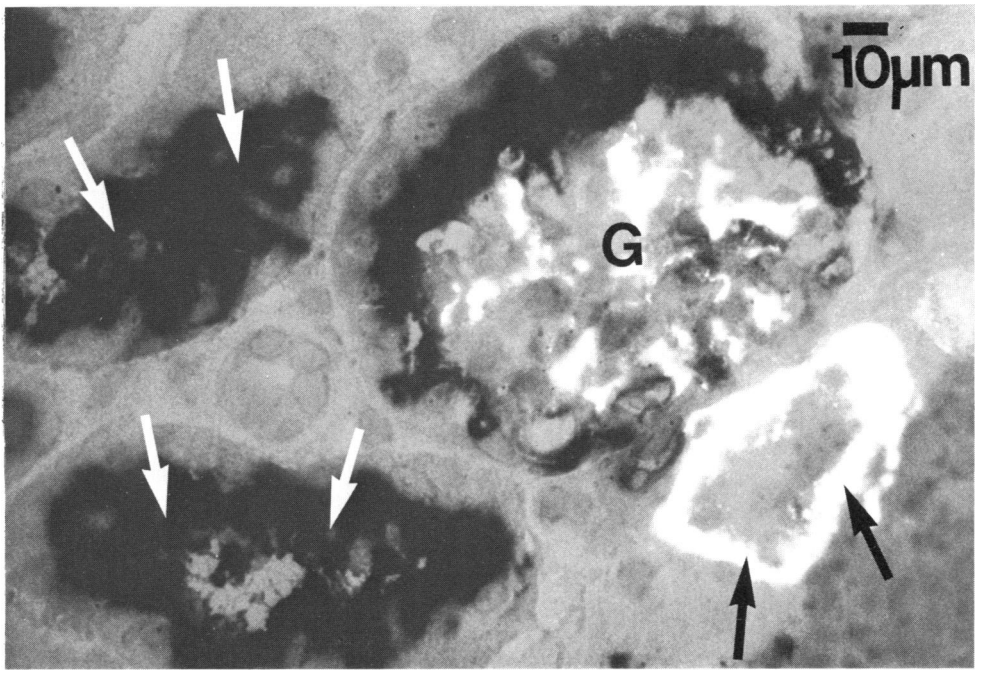

Fig. 5. Frozen section of a kidney from an 8-week-old C57Bl/KsJ db/db mouse. Cells containing alkaline phosphatase stain black. Tubules with cells positive for the enzyme are indicated with white arrows; alkaline phosphatase-positive cells also surround the glomerulus except at its hilum. Only one tubule (*black arrows*) is fluorescent following treatment with fluorescein-conjugated goat anti-μ. This tubule is located close to the hilum of the glomerulus and lacks alkaline phosphatase.

Urinalysis

The principal proteins identified by immunoelectrophoresis and isoelectric focusing in urine from male mice (and, to a lesser extent, female mice) resembled the sex hormone-controlled major urinary proteins (mups), described by Rumke & Thung (1964). Measurements of total urine protein output per 24 hr largely reflected changes in levels of 'mups'. Total urinary excretion of protein per 24 hr was not increased in male 24-week-old C57Bl/KsJ db/db mice in comparison with normal (+/?) littermates, and in females was only slightly increased. By contrast, C57Bl/KsJ db/db mice, especially older animals, had much more albumin in their urine than normal littermates. Twenty-four-hourly output of albumin per mouse was 14 ± 1 μg in normal 24-week-old male C57Bl/KsJ mice, but 930 ± 170 μg in db/db littermates. The figures for 6-week-old mice were 18 ± 3 and 170 ± 30 μg respectively (mean $\pm$ s.e.). Total immunoglobulin outputs were (mean values): male 24-week-old db/db mice 240 μg; normal littermates 70 μg; 6-week-old db/db mice 150 μg; normal littermates 70 μg.

However, there were clearly components in diabetic mouse urine which were present in much higher concentrations relative to normal mice than would be anticipated from the changes in total immunoglobulin or albumin levels. One of these components was a protein of molecular weight 20,000 to 40,000 (by Sephadex G-200) and reacting with antisera to whole serum, Fab and λ, but not to whole IgG. Another, the concentration of which was particularly elevated, reacted with antisera to whole serum, Fab and κ, but not to γ, μ or α or to C3. This component was excluded on Sephadex G-200. Rechromatography of the G-200-excluded peak on Sepharose CL-4B gave a small excluded peak and a series of fused peaks corresponding to a molecular weight of about 1,000,000. Fig. 6 illustrates some of these urine proteins.

Table 1. Body weight, plasma glucose and plasma immunoreactive insulin in non-fasting mice used for immunochemistry

		Genotype				
	Age (weeks)	C57Bl/6J +/?	C57Bl/6J ob/ob	C57Bl/KsJ +/?	C57Bl/KsJ db/db	C57Bl/6J db/db
Body weight (g)	4	9·8±1·5	12·0±1·4	13·6±1·8	21·6±0·9	
	8	24·8±1·6	32·6±1·3	22·6±1·5	40·2±2·4	
	12	26·6±1·5	42·4±3·9	20·2±1·8	48·2±2·9	
	24	29·2±5·7	62·0±4·5	22·0±2·6	40·6±4·2	46·6±6·1
Plasma glucose (μmol/ml)	4	9·3	13·8	10·0±1·3	16·7±0·6	
	8	13·3±3·1	28·4±4·3	12·4±0·7	29·3±3·0	
	12	11·8±1·4	13·4±2·8	11·7±2·2	35·5±3·0	
	24	16·5±2·3	14·8±3·6	14·0±2·9	45·3±3·5	14·8±2·4
Plasma immunoreactive insulin (μU/ml)	4	40	1,700	60 (30–90)	2,350 (1,950–2,740)	
	8	80 (40–120)	n.d.	80 (20–180)	630 (60–1,790)	
	12	60 (30–90)	3/10 in excess of 5,000; rest mean 1,540	60 (30–90)	460 (60–1,900)	
	24	180 (100–400)	All in excess of 5,000	30 (10–40)	310 (150–620)	All in excess of 5,000

Body weights and plasma glucose are given with their standard deviations, plasma insulins with their ranges. Plasma from 4-week-old mice was combined in one (C57Bl/6J mice) or two pools (C57Bl/KsJ mice) for assay. The insulin assay was not precise for insulin levels in excess of 5,000 μU/ml.

n.d. = Not done.

Analysis of plasma or serum

Changes in plasma insulin and glucose levels are shown in Table 1. Serum DNA binding was 40 to 80% elevated in C57Bl/KsJ db/db mice of 12 or 24 weeks age (statistically significant, $0{\cdot}05 > P$).

DISCUSSION

Nathorst-Windahl & Hellman (1964) and Bergstrand, Nathorst-Windahl & Hellman (1968) reported glomerular changes in ob/ob mice and Like *et al.* (1972) showed similar histological changes in C57Bl/KsJ db/db animals. Immunoglobulin and C3 deposits were reported in the kidneys of C57Bl/KsJ db/db mice by Mauer *et al.* (1976). We have confirmed renal deposits of immunoglobulin and complement in C57Bl/KsJ db/db mice and shown that these also occur in C57Bl/6J ob/ob or db/db animals. We have also identified a possible antigen (insulin) and shown antibody activity against this antigen in eluted immunoglobulin. Insulin was only detected in some older mice kidneys and deposits were limited in extent. Possibly the small size of the insulin molecule makes it hard to detect bound to immunoglobulin in complexes. Our results suggest injection of 'foreign' insulin is not necessary for anti-insulin antibodies to develop in diabetic animals. Insulin deposits have also been occasionally reported in human diabetes (Berns *et al.*, 1962) and kidneys of some human diabetic patients (even those not receiving insulin therapy) will bind fluorescein-conjugated insulin (Berns *et al.*, 1962; Farrant & Shedden, 1965). However, neither in man nor in our experimental model is it likely that insulin is the only antigen involved and the elevated anti-DNA titre of serum from C57Bl/KsJ db/db mice suggests at least one other possibility.

The deposition of significant quantities of immunoglobulin in tubules was an unusual feature of the renal disease in our mouse models. Deposition appeared to be related to hyperglycaemia in that

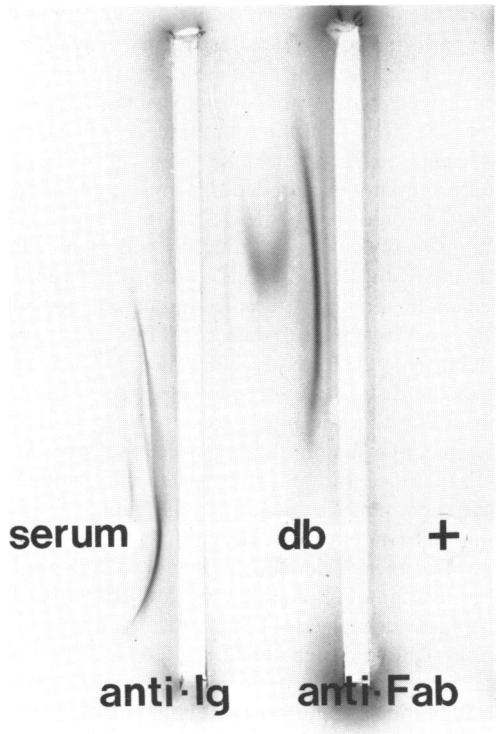

Fig. 6. Immunoelectrophoresis pattern. Conditions: pH 8·6, 10 V/cm, 1 hr. Urine was concentrated so that equal volumes of concentrate were obtained from urine collected over equivalent time periods. db and + identify urine concentrates from 6-week-old C57Bl/KsJ db/db mice and normal littermates. Anti-Ig is a mixture of antisera to IgM, IgG and IgA (heavy and light chains). Urine from diabetic (but not normal) mice gives strong lines with anti-Fab. With anti-Ig, urine and serum give quite different patterns. The urine gives a faint line in the same position as that given by anti-Fab. The serum gives the expected series of lines in the γ position corresponding to the different immunoglobulin classes. Hence, anti-Fab reactivity is not just a result of reaction with the Fab portion of whole immunoglobulin molecules.

only 8-week-old C57Bl/6J ob/ob mice showed prominent tubule staining, whilst tubule staining was present in kidneys from C57Bl/KsJ db/db mice of all ages examined. Twenty-four-week-old mice with the db/db genotype on a C57Bl/6J background (normoglycaemic) lacked tubule staining, but with the db/db genotype on a C57Bl/KsJ background (hyperglycaemic) showed prominent staining. The only exception to the relationship between tubule deposits and hyperglycaemia was that tubule staining was more intense in 8-week-old C57Bl/KsJ db/db mice than 24-week-old C57Bl/KsJ db/db mice, although hyperglycaemia was greater in the older animals. The tubule in which immunoglobulin was deposited appeared, in immunoperoxidase-stained sections, to resemble the distal convoluted tubule or early portion of the collecting ducts. Absence of brush-border or alkaline phosphatase excluded the proximal tubule, whilst the predominantly cortical location and presence of succinic dehydrogenase excluded the thin limb of Henle or the latter part of the collecting tubule (Wachstein, 1955). Even when the close relationship of each immunoglobulin-containing tubule to the hilum of an associated glomerulus was not (as in many cases) immediately apparent, our immunochemical and enzymatic assays suggested that the affected part of the tubule could not be far removed from that segment which runs close to the glomerulus. Localization of immunoglobulin deposits only in distal, and not in proximal, tubules was a remarkable feature of diabetic mice. In human immune complex diseases such as systemic lupus erythematosus, tubule deposits do also occur, but in both proximal and distal tubules (Brentjens *et al.*, 1975).

We consider that the immunoglobulin in the tubules of diabetic mice is probably derived from

the mesangia of associated glomeruli. Our chief reason for supposing this is the continuity frequently observed in the pattern of deposits across glomerular hilum, macula densa and distal tubule. Although in any particular section a glomerulus associated with a segment of tubule containing immunoglobulin deposits was not always visible (it would not be expected that all sections would be cut through, both glomerulus and tubule together), where an associated glomerulus was visible this always contained mesangial immunoglobulin deposits. Various other explanations for the origin of tubule immunoglobulin deposits are, we consider, unlikely. Tubule staining can occur as a result of production of antibodies to the tubule itself (Lehman, Wilson & Dixon, 1975). The pattern of staining we observed was 'granular' rather than 'linear' as is usually the case when the tubule itself is reacting with antibody. Further, anti-tubule antibodies are often associated with anti-glomerular basement membrane antibodies and basement membrane staining. The latter we did not observe. Finally, we were unable to detect any anti-kidney antibodies in either serum or eluted renal immunoglobulin from C57Bl/KsJ db/db mice. Neither do we consider it likely that immunoglobulin present in the distal tubules is derived by resorption from the tubule lumen, because although albumin was present in much greater concentration than immunoglobulin in C57Bl/KsJ db/db mouse urine, no albumin deposits were ever observed in the tubules. Further, 8-week-old C57Bl/KsJ db/db mice showed stronger tubule staining than 24-week-old mice although urine from the latter contained more immunoglobulin.

The means by which immune complexes are cleared from the glomerular mesangium is unclear. Intracellular degradation by mesangial cells has been suggested as a primary mechanism (Farquhar & Palade, 1962) but the localization of immune complexes between, rather than within, mesangial cells (Striker, Mannik & Tung, 1979) suggests other pathways may be more important. Studies with a number of particles such as colloidal carbon (Elema, Hoyer & Vernier, 1976) and iron-dextran (Leiper, Thomson & MacDonald, 1977) have delineated a pathway whereby such materials pass along channels in the mesangium to the glomerular hilum and thence via the macula densa to the associated distal tubule. We suggest that immune complexes may also be cleared by this pathway, and that either alteration in glomerulotubular balance associated with the marked diuresis of C57Bl/KsJ db/db mice, or damage to the distal tubule, may cause the rate of clearance of immunoglobulin from the distal tubule to fall below the rate of entry into the pathway from the glomerular hilum, and accumulation of immunoglobulin in the distal tubule and macula densa. The weak anti-C3 staining of tubular deposits in comparison with mesangial deposits suggests that during passage to the distal tubule, reorganization of the immune complex lattice or breakdown or release of C3 may occur.

Immunoglobulin can be cleared from the distal tubule because tubule deposits disappear in older C57Bl/6J ob/ob mice whose plasma glucose has normalized, but the route of clearance is uncertain. The presence of a high molecular weight complex reacting with anti-Fab and anti-κ, but not anti-Fc or anti-C3, in the urine of C57Bl/KsJ db/db mice is compatible with immune complexes being partially digested by proteases within the distal tubule before being released into the tubule lumen. Our complexes reacted with anti-whole serum as well as anti-Fab. Like *et al.* (1972) also reported a protein, reacting with anti-mouse serum, in C57Bl/KsJ db/db mouse urine even before the appearance of glomerular lesions.

By contrast to the distal tubule changes, both histological and immunological changes in the glomerulus were very similar in db/db mice whether the mutation was present on the C57Bl/KsJ or C57Bl/6J background, and also resembled the changes in C57Bl/6J ob/ob mouse glomeruli. Mesangial proliferation and deposition of immunoglobulins or C3 could occur in the absence of either hyperglycaemia or tubule staining. It is thus unlikely that glomerular staining can be explained solely by disturbance of a pathway of immune clearance involving the juxtaglomerular apparatus and distal tubule. Immunoglobulin and complement deposition in the glomerulus may be related to the hormonally-mediated disturbances in T cell function described in C57Bl/6J ob/ob mice (Sheena & Meade, 1978; Meade, Sheena & Mertin, 1979) and in C57Bl/KsJ db/db mice (Mahmoud *et al.*, 1976; Fernandes *et al.*, 1978). The only difference between C57Bl/6J and C57Bl/KsJ mice glomeruli was observed in 24-week-old animals. C57Bl/KsJ db/db of this age (whose plasma insulin levels were lower than those of either C57Bl/6J mice of the same age or C57Bl/KsJ db/db mice of 12 weeks) also had less C3 deposited in their glomeruli than either

C57Bl/6J mice (db/db or ob/ob) of the same age, or C57Bl/KsJ db/db mice of 12 weeks.

To conclude, although the tubular changes observed are an unusual feature of diabetic mice, the glomerular changes resemble those in many animal models of immune complex disease, for example the NZB mouse (Comerford, Cohen & Desai, 1968).

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