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Extensive Complement Activation in Hereditary Porcine Membranoproliferative Glomerulonephritis Type II (Porcine Dense Deposit Disease)

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Massive glomerular deposits of C3 and the terminal C5b-9 complement complex (TCC), but no immune complex deposits were detected by immunofluorescence in porcine membranoproliferative glomerulonephritis type II. TCC deposits were always observed with concomitant deposits of vitronectin (S-protein) in membranoproliferative glomerulonephritis, in contrast to a piglet with mesangial glomerulopathy where TCC was present without vitronectin co-deposition. Enzyme immunoassays revealed extensive systemic complement activation in 1-week-old affected piglets, observed by low plasma C3 (about 5% of normal) and high plasma TCC (about 10 × normal). Affected piglets revealed some plasma complement activation already at birth, 3 to 4 weeks before recognizable clinical disease. It is concluded that porcine membranoproliferative glomerulonephritis represents a nonimmune complex-mediated glomerulonephritis caused by unrestricted systemic complement activation with C3 consumption, TCC formation, and glomerular trapping of complement activation products. A pathogenetic mechanism of a defective or missing complement regulation protein is suggested. (Am J Pathol 193, 143:1356–1365)

Porcine membranoproliferative glomerulonephritis (MPGN) with intramembranous dense deposits (porcine dense deposit disease) is a recently discovered hereditary glomerulonephritis in Yorkshire piglets.[1] It affects both sexes, and in clinicopathological terms it represents a rapidly progressive glomerulonephritis.[2] Affected piglets reveal clinical signs of disease and elevated serum urea and creatinine values at approximately 3 to 4 weeks of age, and they eventually die from uremia at 4 to 10 weeks of age. The disease has morphological and immunohistochemical similarities with human MPGN type II.[1] Human MPGN type II is associated with hypocomplementaemia caused by alternative pathway activation of complement. Usually, a C3 nephritic factor (C3NeF) is found, an autoantibody directed against neoantigenic determinants on the alternative pathway C3 convertase. The C3NeF acts by stabilizing the alternative C3 convertase, thereby leading to sustained complement activation and pronounced consumption of C3. However, the pathogenic basis of human MPGN type II is completely unclear, and the nature and composition of the intramembranous dense deposits is unknown.[3,4]

Spontaneous hereditary animal models of hypocomplementaemic glomerulonephritis have previously been reported.[5,6] These have been morphologically described as analogues to human MPGN type I, which is believed to represent immune complex-mediated glomerulonephritis. In these animal models, the glomerulonephritis has been associated with a genetic deficiency of C3 and a pathogenetic mechanism of reduced immune complex clearance has been proposed in such disease.[3,7]

The present hereditary MPGN in piglets may be the first animal model analogous to human MPGN type II. The disease has occurred in a species that readily produces a large number of offspring, which

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offered a unique opportunity to produce affected offspring from parent animals associated with the disease. However, because the availability of antibodies to porcine complement antigens was limited and only polyclonal antibody to porcine complement component C3 was commercially available, we sought to determine the cross-reactivity of polyclonal and monoclonal antibodies to human complement components with the corresponding porcine complement antigens. Once determined, such cross-reactions could be exploited in immunofluorescence detection of glomerular complement deposits and in enzyme immunoassays (EIA) for determining plasma and urine levels of complement in healthy and diseased piglets.

Materials and Methods

Antibodies

The present study included use of the following antibodies: antibodies to porcine antigens: Fc-specific goat antiserum to swine immunoglobulin G (IgG), IgM, and IgA (Nordic Immunological Laboratories, Tilburg, The Netherlands) and rabbit polyclonal antibodies (PAb) to swine C3 (Organon Teknika BCA/Cappel, West Chester, PA); and antibodies to human complement antigens: goat antisera to human C4, C5, and factor B (Quidel, San Diego, CA); murine monoclonal antibodies (MAbs) to human C3c, C6, vitronectin (S-protein), and properdin (≠1) (Quidel); murine MAb aE11 (anti-TCC, anti-C9 neo),[8] and rabbit PAb to human vitronectin (S-protein).[9]

Screening Procedure for Determining Cross-reactivity of Antibodies

Pooled normal human serum and pooled normal pig serum were diluted 1/1,000 in phosphate-buffered saline (PBS) and coated into microtiterplates overnight at 4 C. The MAbs were diluted to 1 μg/ml, antisera were diluted 1/1,000. The antibodies were added to the plate that had previously been washed with PBS containing 0.1% Tween 20 (Sigma Chemical Co., St. Louis, MO). Subsequently, a peroxidase-linked secondary antibody was added; either sheep anti-mouse Ig or donkey anti-rabbit Ig (Amersham, Buckinghamshire, UK) or mouse anti-goat Ig (Jackson ImmunoResearch Laboratory Inc., West Grove, PA). All antibodies were diluted in PBS containing 0.2% Tween 20, and all incubations were done for 1 hour at 37 C. The plates were developed with 2,2′-azino-di-[3-ethylbenzthiazolinsulphonate] (Boehringer Mannheim, Mannheim, Germany) and H_2O_2. The OD of wells coated with pig serum were compared to wells coated with human serum and uncoated wells. EIA screening for cross-reactivity against pig of anti-human complement antibodies was graded arbitrarily as strong, medium, or weak.

Animals

Piglets affected by MPGN type II and healthy littermates were produced by test matings of parent animals associated with the disease as previously described.[1]

Plasma Samples

Blood samples were obtained by venopuncture of the jugular vein and collected into ethylenediaminetetraacetic acid-containing tubes. The samples were cooled, centrifuged, and the plasma samples were stored at −70 C as recommended.[10] Seven litters from parents known to be carriers were analyzed, 62 offspring altogether. A newborn sample was collected from 19 of the piglets within 3 hours after birth. New samples were collected once a week until the piglets died or were killed. Plasma from 3-week-old, healthy Yorkshire piglets (n = 15), that were not related to the diseased piglets were used as controls. A pool made from these plasma samples was designated normal pig plasma.

Urine Samples

Urine was collected from four healthy and 10 MPGN piglets after killing. The urine was obtained by suprapubic puncture of the urinary bladder and collected into glass tubes containing ethylenediaminetetraacetic acid, then centrifuged, and stored at −70 C.

Immunofluorescence

Renal specimens from a total of 19 piglets were obtained by autopsy. These included 15 piglets with a histological diagnosis of MPGN between 5 and 48 days of age,[1] three healthy littermates of glomerulonephritic piglets killed at 28, 39, and 41 days of age, and one healthy littermate of glomerulonephritic piglets with a histological diagnosis of mesangial glomerulopathy at 84 days of age.

The right kidneys were perfused with Ringer's acetate via the renal artery. Slices of renal tissue that included both cortex and medulla were frozen in

chlorodifluoromethane (Isceon, DOT - 39 NRC 260/325 M 1001, ISC Chemicals Ltd., Rhône-Poulenc, U.K.), cooled in liquid nitrogen. Cryostat sections, 2- to 4-μ thick, were stained by indirect immunofluorescence technique.

Primary antibodies used were goat antiserum to IgG, IgM, IgA; rabbit PAb to C3; murine MAb to properdin, C6, TCC, and vitronectin; goat antiserum to C4, C5, and factor B, and rabbit PAb to vitronectin. Control sections from each kidney were treated separately with normal goat, normal rabbit, or normal mouse sera (Sigma) instead of the primary antibody.

Primary antibodies were diluted in phosphate buffered saline (PBS) containing 3% bovine serum albumin. The following antibodies were diluted 1/1,000: PAb C4, PAb factor B, and PAb C5. The MAb properdin was diluted 1/100, whereas all other antibodies were diluted 1/2,000. Fluorochrome-labeled secondary antibodies used were Sigma's rabbit-anti-goat-IgG-fluorescein isothiocyanate (FITC), goat-anti-rabbit-IgG-FITC, goat-anti-mouse-FITC, and goat-anti-mouse-tetramethyl rhodamine isothiocyanate (TRITC). Dilution of fluorochrome-labeled antibodies was 1/100 for all stainings. The sections were examined in a Leitz Laborlux K (light) microscope equipped with a 50 W Hg lamp and a Leitz Ploemopac filter holder, using Leitz I 3 (FITC) and Leitz N 2.1 (TRITC) filter blocks. Dark-field fluorescence micrographs were taken with a Leica MPS52 photoautomat. The intensity of the fluorescence was graded arbitrarily as 0 to 3+.

EIA for C3

Rabbit PAb C3 diluted 1/10,000 was used as capture antibody. Normal pig plasma was used as standard, defining 100%. Plasma samples were diluted 1/5,000. Plasma samples containing less than 12% C3 were even diluted 1/500. Urine samples were diluted 1/5. The MAb to C3c was used as secondary antibody diluted 1/10,000. Peroxidase-linked anti-mouse Ig was used as developing antibody. All incubations were for 45 minutes at 37 C.

EIA for TCC

TCC was quantitated essentially as described previously.[11,12] The capture C9 neoepitope specific MAb aE11 was purified from ascites by protein A affinity chromatography and MonoQ HPLC (Pharmacia LKB Biotechnology, Uppsala, Sweden). A zymosan-activated human serum pool was used as standard, defining 1,000 arbitrary units per ml (AU/ml). Plasma samples were diluted 1/10. Samples containing more than 25 AU/ml were even diluted 1/100. Urine samples were assayed undiluted after the addition of 0.2% Tween 20. Subsequently, MAb to C6 and a subclass specific peroxidase-linked goat anti-mouse IgG1 (Southern Biotechnology Associates, Inc., Birmingham, AL) was added. A zymosan-activated pool of pig serum was found to contain 420 AU/ml by this assay.

Statistical Analysis

To test the significance of differences between different groups, the Mann–Whitney two sample test was used.

Results

EIA Screening for Crossreactivity of Antibodies

The MAb to C6 and vitronectin showed strong cross-reactivity, whereas the MAb to C3c, properdin, and TCC showed medium cross-reactivity. Goat antisera to C4 and factor B and rabbit PAb to vitronectin showed weak cross-reactivity. Goat antiserum to C5 showed no cross-reactivity by this method (this antiserum did, however, cross-react in immunofluorescence).

Immunofluorescence

The results of the immunofluorescence stainings are summarized in Table 1. Deposits of IgG, IgM, or IgA could not be detected in glomeruli from the examined MPGN piglets (aged 5 to 48 days). However, glomerular immunofluorescence for C3 (3+, Figure 1A), C5 (3+, Figure 1B), C6 (3+, Figure 1C), and TCC (3+, Figure 1D) was very strong. Likewise, immunofluorescence for vitronectin was very strong using both PAb (Figure 1E) and MAb to vitronectin (3+). The glomerular fluorescence patterns for TCC and vitronectin were identical (Figure 1, D and E) in all MPGN piglets examined. A granular mesangial fluorescence for all complement components was observed in all glomeruli of MPGN piglets. In renal sections double labeled for detection of C3 and TCC, this mesangial fluorescence was most abundant for C3, whereas the intensity of fluorescence was equally strong for C3 and TCC along the glomerular capillary walls (Figure 1F). Immunofluorescence for all complement components showed a linear fluorescence staining along the glomerular

Table 1. *Results of Immunofluorescence Staining of Renal Sections from Piglets with MPGN, Healthy Littermates, and a Control Piglet with Mesangial Glomerulopathy*

	MPGN $n = 15$	Healthy* $n = 3$	Mes GP* $n = 1$
Antibodies to porcine antigens			
PAb IgG Fc-spec.	0	2	2
PAb IgM Fc-spec.	0	2	3
PAb IgA Fc-spec.	0	0	3
PAb C3	3	1	3
Antibodies to human antigens			
PAb C4	0	1	2
PAb C5	3	0	2
MAb C6	3	0	1
MAb TCC (aE11)	3	0	3
PAb vitronectin	3	0	0
MAb vitronectin	3	0	0
PAb factor B	0	0	0
MAb properdin	0	2	1

Immunofluorescence was scored as 0 to 3+. Mes GP = mesangial glomerulopathy.
* Mesangial and paramesangial deposits.

capillary walls. This fluorescence was often split in two parallel lines with a nonstaining intermediate zone, creating a peculiar image of double lines along the glomerular capillary walls (Figures 1C and 2).

The 84-day-old piglet with a histological diagnosis of mesangial glomerulopathy was used as a control in immunofluorescence. It showed mesangial and paramesangial deposits of IgG (2+, Figure 3A), IgM (3+, Figure 3B), IgA (3+, Figure 3C), C3 (3+, Figure 3D), and TCC (3+, Figure 3E). Immunofluorescence for vitronectin with both PAb (Figure 3F) and MAb were negative. Small mesangial and paramesangial areas of fluorescence positive for C4 (2+), C5 (2+), and properdin (1+) were detected.

Glomeruli from healthy littermates killed parallel to glomerulonephritic piglets at 28, 39, and 41 days of age, showed scattered small, segmental, mesangial and paramesangial deposits of IgG (2+), IgM (2+), C3 (1+), C4 (1+), and properdin (2+). No deposits of IgA, C5, C6, TCC, or vitronectin were observed. Glomeruli from all piglets were negative for factor B.

Plasma C3 and TCC

The plasma concentrations of C3 and TCC in healthy and MPGN offspring are shown in Table 2 and Figure 4. MPGN piglets showed lower C3 and higher TCC concentrations than their healthy littermates at any age. At birth, all piglets showed low C3 (median 7.4%) and low TCC concentrations (median 1.0 AU/ml). Importantly, median C3 concentration of newborn MPGN piglets was only 22% of the values found in age-matched healthy littermates (1.9% vs. 8.5%, $P = 0.009$), whereas the median TCC concentration was four times higher (4.2 AU/ml *versus* 1.0 AU/ml, $P = 0.004$). During the first week of life, C3 concentrations increased substantially to about 50% in the healthy piglets, but remained low (about 5%) in the MPGN piglets. TCC remained low (about 2 AU/ml) in the healthy piglets but increased to 20 AU/ml in the MPGN piglets. Consequently, the minor overlap between healthy and MPGN piglets with respect to C3 and TCC values seen at birth, rapidly disappeared as the values changed to two strictly separate groups (Table 2).

The distribution of C3 concentrations in the offspring from mated carriers of the glomerulonephritic trait and age-matched controls from healthy families are compared in Figure 5. The C3 concentrations of the healthy littermates of MPGN piglets showed a broader distribution compared to the controls, and the existence of three populations may be suggested: the MPGN piglets (C3 < 20%), an intermediate population (C3 = 20 to 80%), and the healthy (C3 > 80%). Consequently, a few control piglets may represent carriers of the glomerulonephritic trait. Despite the fact that median C3 concentration was lower in healthy littermates of MPGN piglets than in control piglets (68.3% compared to 89.4%), the difference was not significant ($P = 0.18$). The C3 concentrations of the parents to glomerulonephritic piglets were 56.7, 124.0, 128.7, and 28.1% respectively, showing that these carriers do not necessarily have low C3.

Urine C3 and TCC

TCC was detected in the urine from seven of the 10 MPGN piglets (range 0 to 0.30 AU/ml), but not in the urine from any healthy piglet ($n = 4$) (detection limit 0.01 AU/ml). Urinary C3 was maximally 0.02% in healthy piglets. In contrast, urinary C3 was more than 0.02% in five of the 10 MPGN piglets (0.21, 0.20, 0.18, 0.10, and 0.07% respectively). Four of these urines also contained detectable amounts of TCC. Urinary C3 amounted up to 5% of the corresponding plasma C3 value, whereas maximal urinary TCC amounted 2% of the corresponding plasma TCC value.

Discussion

The results of the screening for cross-reactivity with porcine of antibodies produced against human complement components, showed that complement

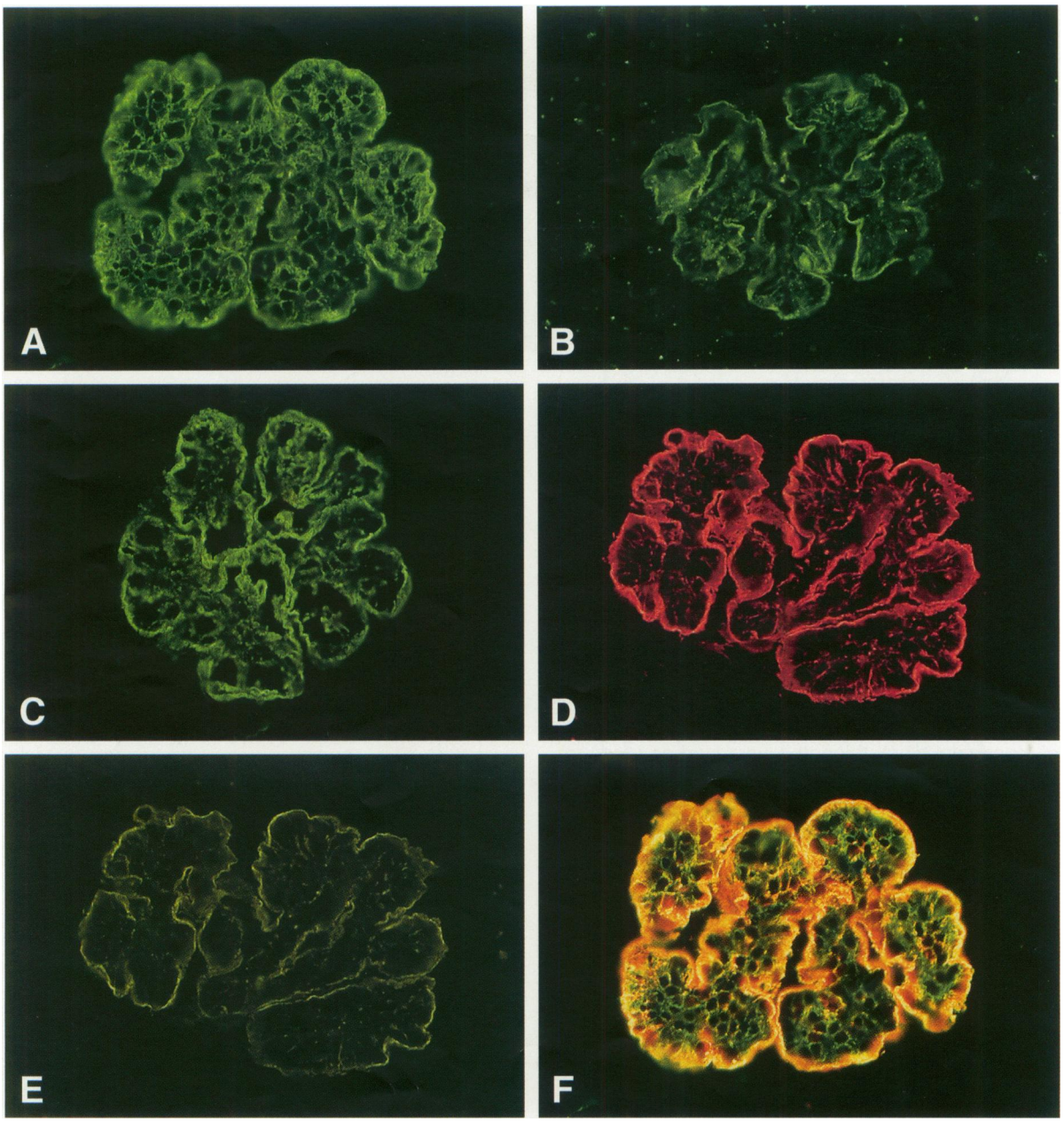

Figure 1. *Indirect immunofluorescence performed on frozen kidney sections from piglets with MPGN type II. There is a strong positive reaction for complement proteins along the glomerular capillary walls and in the mesangium.* **A:** *MPGN, 30-day-old piglet, PAb C3, FITC (295×).* **B:** *MPGN, 22-day-old piglet, PAb C5, FITC (295×).* **C:** *MPGN, 25-day-old piglet, MAb C6, FITC (295×).* **D:** *MPGN, 30-day-old piglet, MAb TCC, TRITC (295×).* **E:** *Same glomerulus as shown in* **D**, *PAb vitronectin, FITC (295×).* **F:** *MPGN, 30-day-old piglet. Same glomerulus as shown in* **A**. *Double labeling with PAb C3-FITC and MAb TCC-TRITC. C3 deposition dominates in the mesangium (295×).*

proteins in general are highly conserved between the human and porcine species, consistent with previous observations,[13] and that they may be used successfully in *in vitro* immunological techniques and immunofluorescence studies of complement activation in porcine tissues.

In the MPGN piglets, the glomerular TCC deposits were associated with a strong co-staining for vitronectin. Although small amounts of vitronectin have been found in membrane-inserted TCC ("SC5b-9-(m)"),[14] our observations indicate that fluid-phase TCC (SC5b-9) has been trapped in the glomeruli from the circulation. This assumption is supported by the observation of extensive systemic complement activation as monitored in plasma. It seems less probable that a local renal complement activation could be capable of causing the extensive plasma C3 depletion and TCC formation observed in MPGN

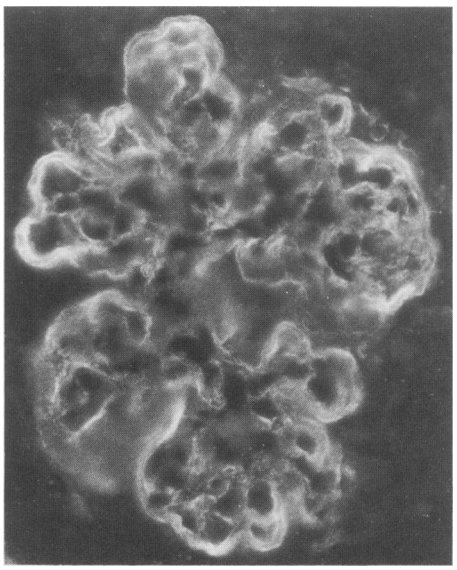

Figure 2. *MPGN, 25-day-old piglet, PAb C3. Observe the double contours of the glomerular capillary walls. FITC (510×).*

piglets. The glomerular inflammatory reaction in the present porcine MPGN was recognized as pronounced mesangial cell proliferation and accumulation of polymorphonucleated neutrophilic cells.[1] These inflammatory reactions may have been elicited by local release of C5a and inflammatory effects of TCC[15,16] mediated by a certain *in situ* activation in addition to the systemic activation.

The principal histological glomerular lesion in porcine MPGN was glomerular basement membrane thickening and mesangial cell proliferation. In addition, three patterns of histological glomerular lesions could be observed, designated exudative, crescentic, and classical lesions.[1] However, no difference in the immunofluorescence pattern could be observed between these morphological patterns. The observation that some of the piglets with MPGN did not reveal any exudative glomerular lesions[1] may indicate that both mechanisms of local complement activation with formation of C5b-9(m), and trapping of circulating SC5b-9 could occur in porcine MPGN.

Urinary TCC excretion is generally considered a result of active glomerular immune deposit formation.[17–20] TCC was not detected in the urine from healthy piglets, but was up to 0.3 AU/ml in MPGN, which amounted 2% of the corresponding plasma TCC. Urinary C3 was maximally 0.02% in healthy and 0.21% in MPGN piglets. Because MPGN piglets showed low plasma C3, urinary C3 amounted up to 5% of the corresponding plasma C3 in some piglets. The measured levels of urinary TCC were considerably lower than previously reported in human MPGN.[20] The relatively modest urinary TCC levels compared with the massive amounts of TCC in plasma and glomeruli support the assumption of glomerular trapping of systemic activated complement as the major source of the deposits in porcine MPGN. The biological effects of fluid-phase TCC are not fully understood. Extensive studies of human nonimmune and immune complex-mediated glomerular diseases have demonstrated glomerular deposition of TCC, often co-stained with vitronectin in injured glomeruli.[21–24] The biological effects of glomerular deposits of TCC have also been studied in different experimental animal models of immune complex-mediated glomerulonephritis.[16] Such studies have proved that complement is able to mediate glomerulonephritis by inflammatory cell-dependent and noninflammatory cell-independent effects on glomerular permeability. However, despite numerous available data on TCC deposition in glomerular disease, the pathogenetic role of such deposits remain unclear.[25]

One healthy littermate of MPGN piglets was killed at 84 days of age. Histological examination of the kidneys of this case revealed a diffuse mesangial glomerulopathy, a lesion commonly observed in Norwegian slaughtered swine (Jansen JH, personal observation). In contrast to the MPGN piglets, immunofluorescence staining of glomeruli from this piglet revealed mesangial deposits containing mainly IgM and IgA with co-deposition of IgG, most probably representing mesangial immune complex deposits. The demonstration by immunofluorescence of mesangial TCC formation as detected by the C9 neoepitope-specific MAb aE11 was associated with positive staining for both C4 and properdin, but complete absence of vitronectin. Thus, the observed mesangial complement deposition in this piglet may have been caused by activation both via the classical and alternative pathways.[26,27] The observed absence of glomerular vitronectin in this piglet strongly suggests that the TCC had been formed *in situ* in the membrane form (C5b-9[m]).

The more sensitive immunofluorescence technique on frozen renal sections confirmed the observations of negative reaction for glomerular immune complex deposits and positive reaction for glomerular C3 in MPGN piglets as previously observed in formalin-fixed, paraffin-embedded, trypsinated sections using the peroxidase-anti-peroxidase technique.[1] The described linear fluorescence for complement in MPGN piglets with formation of double contours along the glomerular capillary walls, seems to indicate that complement is deposited on either side of the thickened capillary wall. The intramembranous dense deposits were ob-

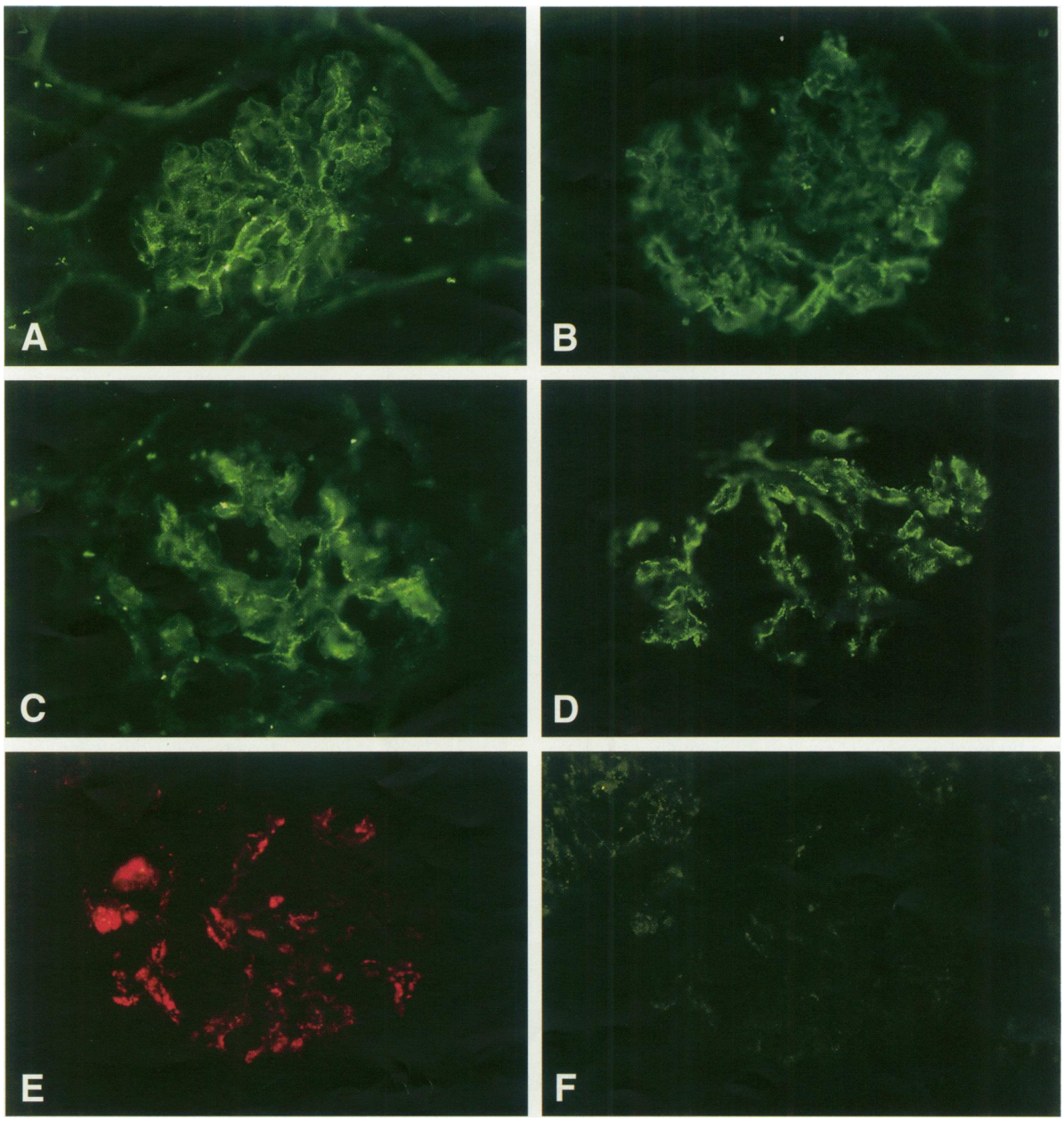

Figure 3. *Control section from an 84-day-old healthy littermate of glomerulonephritic piglets. This piglet developed a mesangial glomerulopathy with mesangial and paramesangial immune complex deposits.* **A:** *Mesangial and paramesangial deposits of IgG, PAb IgG, FITC (295×).* **B:** *Mesangial and paramesangial deposits of IgM, PAb IgM, FITC (295×).* **C:** *Mesangial and paramesangial deposits of IgA, PAb IgA, FITC (295×).* **D:** *Mesangial and paramesangial deposits of C3, PAb C3, FITC (295×).* **E:** *Mesangial and paramesangial deposits of TCC, MAb TCC, TRITC (295×).* **F:** *Same glomerulus as shown in* **E**. *Negative reaction for vitronectin, PAb vitronectin, FITC (295×).*

served ultrastructurally as a homogenous, intensively electron-dense material.[1] Thus, it seems likely that these dense deposits do not represent complement material.

The disease described is a porcine analogue to human MPGN type II. The piglets show systemic complement activation as revealed by low plasma C3 and high plasma TCC concentrations. At birth, even piglets that remained healthy showed low C3 concentrations (8.5% of adult values). This fact made differentiation between healthy and MPGN piglets difficult at this stage. After 1 week, the C3 concentration had approached adult levels in healthy piglets, but stayed low in MPGN piglets. Simultaneously, TCC had increased to a steady-state, high level in diseased piglets. Consequently, from 1 week of age, all MPGN piglets revealed a C3 concentration below 18% and/or a TCC concentration

Table 2. *Plasma Concentrations of C3 and TCC in MPGN Piglets, Healthy Littermates (healthy), and Healthy, Unrelated Piglets (controls) from 0 to 7 Weeks of Age*

Age (weeks)	Status	*n*	C3 (%)median (range)	TCC (AU/ml)median (range)
0	Healthy	15	8.5 (0.9–25.8)	1.0 (0.4–2.8)
0	MPGN	4	1.9 (1.2–3.0)*	4.0 (2.0–5.5)*
1	Healthy	17	50.0 (18.4–90.3)	1.3 (0.5–6.6)
1	MPGN	6	4.5 (3.5–7.7)†	21.0 (9.2–22.9)†
2	Healthy	40	54.3 (18.7–130.6)	2.0 (0.4–4.4)
2	MPGN	17	4.8 (2.4–22.1)†	14.6 (6.3–32.2)†
3	Healthy	32	68.3 (22.0–179.3)	2.1 (0.4–6.9)
3	MPGN	13	5.3 (2.2–17.1)†	15.5 (6.1–25.0)†
3	Controls	15	89.4 (53.6–107.4) n.s.	0.8 (0.4–1.0)†
4	Healthy	27	69.7 (25.5–157.4)	1.8 (0.6–2.8)
4	MPGN	11	4.6 (3.0–11.4)†	13.6 (5.2–35.8)†
5	Healthy	22	66.3 (27.9–102.2)	1.7 (0.7–3.3)
5	MPGN	6	6.8 (3.9–7.9)†	11.6 (4.7–21.6)†
6	Healthy	15	75.0 (37.8–109.7)	2.1 (0.6–3.5)
6	MPGN	5	6.5 (3.5–7.9)*	7.9 (5.0–10.8)†
7	Healthy	9	67.0 (22.9–102.9)	1.6 (1.1–2.5)
7	MPGN	2	7.9 (7.6–8.2)‡	6.8 (4.7–8.8)‡

The difference between age-matched healthy and MPGN piglets or healthy and control piglets was evaluated by the Mann–Whitney two sample test: * $P < 0.01$; † $P < 0.001$; ‡ $P < 0.05$; n.s. = not significant ($P = 0.18$).

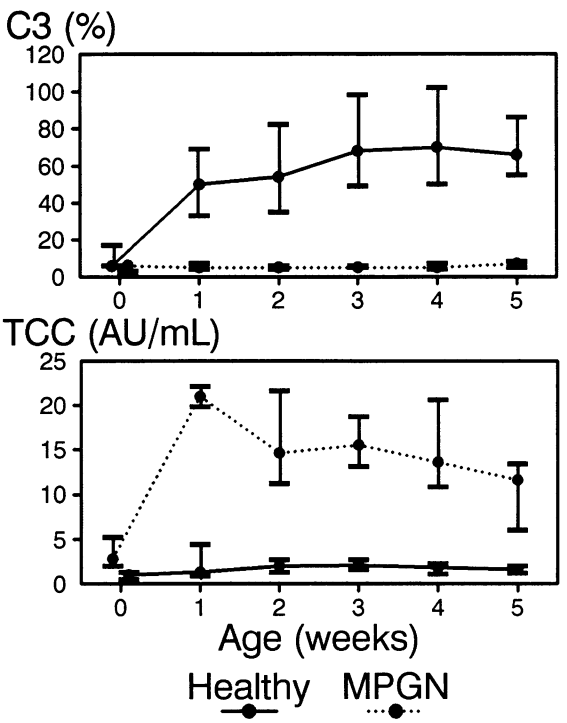

Figure 4. *The plasma concentrations of C3 and TCC in piglets with MPGN and their healthy littermates followed from birth until the age of 5 weeks. The closed circles represent the median concentration, and the bars represent the 25 to 75 percentile range.*

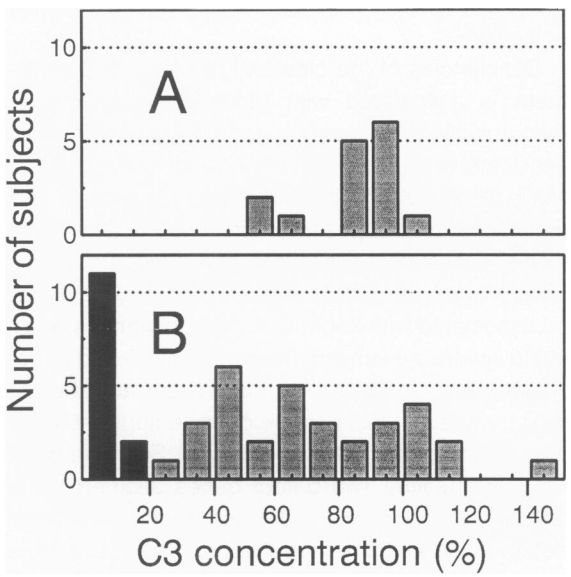

Figure 5. *The distribution of plasma C3 concentrations of 3-week-old piglets. Histogram A represents 15 piglets from families with no known cases of MPGN. Histogram B represents 45 piglets that all were offspring from carriers of the glomerulonephritic trait. The grey bars represent healthy piglets, the closed bars represent MPGN piglets.*

above 7 AU/ml, whereas such values were not seen in any healthy piglet. Thus, the diagnosis of MPGN could be made with 100% sensitivity and specificity at 1 week of age, 2 to 3 weeks before recognizable clinical disease.

Carriers of the glomerulonephritic trait seemed to be healthy. We have sought for parameters of carrier identification. The plasma C3 from healthy littermates were lower, but not significantly so, than in the controls, and the TCC concentrations of the healthy littermates were significantly higher than in the controls. Parents that were obligate carriers as observed by segregation of affected piglets in the offspring did not necessarily present low C3. TCC concentration of healthy littermates at 3 weeks was 2.1 AU/ml compared to 0.8 AU/ml in the controls, suggesting that carrier piglets show elevated TCC in addition to low C3. However, there was no correlation between C3 and TCC in healthy piglets. Interassay variations may at least partially explain the difference in TCC and C3

between healthy piglets and controls. It is thus questionable whether carriers of the glomerulonephritic trait may be identified from measuring plasma C3 and TCC concentrations.

The median C3 concentration of healthy piglets was only 8.5% at birth. Even though two-thirds of these piglets were expected to be carriers of the glomerulonephritic trait and thus might show constitutionally low C3, it is evident as seen in Figure 4 that piglets have low C3 levels at birth. This is in contrast to the situation in human newborns in which C3 values are reported to be about 90% of adult values.[28] From this observation, it is reasonable to presume that even other complement components are low in newborn piglets and increase rapidly during the first week of living. Thus, relatively low TCC in newborn MPGN piglets may be a consequence of limited amounts of available terminal components. Alternatively, low C3 per se might limit terminal pathway activation.

Deficiencies of the classical pathway of complement is associated with glomerulonephritides in man, mainly of the immune complex type. We could not detect any glomerular deposits of immunoglobulins in the kidneys from these piglets, despite the fact that mesangial deposits of immunoglobulins were clearly detected in the kidney of a piglet that developed mesangial glomerulopathy. MPGN type II may be associated with nephritic factors in humans, leading to severe systemic consumption of C3 and deposition in the glomeruli. The early onset and the hereditary basis of this porcine disease suggest that it may not be of the autoimmune type. Because carriers were healthy, two defect genes seemed to be necessary to develop the disease. Thus, an inherited defect of a complement regulatory protein is a more probable mechanism for the disease than an inherited activating factor.

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