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C3b Receptors in glomerular disease*

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SUMMARY

Using two indicator systems—sheep erythrocytes or fluoresceinated *S. typhi* coated with C3b—the presence of a receptor for C3b (but not C3d) in the normal human glomerulus is confirmed. No receptor could be detected in other species tested (mouse, rat, guinea-pig, rabbit and rhesus monkey). Binding of indicator particles was reduced or lost in diseases associated with glomerular capillary deposition of C3, but not in those with mesangial deposition alone. However in some cases the receptor was lost in the absence of detectable C3 deposition. No receptors were detected in proliferating cells in glomerular crescents.

INTRODUCTION

Gelfand, Frank & Green (1975) and Gelfand *et al.* (1976) have demonstrated a receptor for the activated third component of complement, C3b, on cells in the normal human glomerulus. These findings have been confirmed by Matre & Tonder (1976) and by Sobel, Gabay & Lagrue (1976) although the latter have suggested that the receptor is specific for the product of the reaction of C3b with C3b inactivator (KAF), i.e. C3d. The presence of such a receptor could provide a specific mechanism for the localization of complement-coated immune complexes in the glomerulus, and explain why the kidney is so often singled out in diseases associated with circulating immune complexes.

In this paper we confirm the presence of a receptor specific for C3b in the normal human glomerulus. In order to determine the role of the receptor in the pathogenesis of nephritis we have examined the state of the receptor, as determined by its capacity to bind indicator cells coated with C3b, in a large series of renal biopsies. In particular we have examined the relationship between glomerular deposition of immunoglobulin and complement and the degree of binding of indicator cells.

MATERIALS AND METHODS

Two systems have been used to demonstrate the receptor. In the first, sheep red cells (E) coated with a rabbit anti-sheep red cell haemolysin (A) (Wellcome Reagents) have been coated with complement (C) using guinea-pig (gp), human (h), or mouse (m) serum. $EAC142_{gp}$ and $EAC1423b_h$ were prepared by standard methods (Lachmann, Hobart & Aston, 1973). $EAC142_h$ were prepared by incubating 200 μl of a human serum genetically deficient in C3 with 5 ml 1% EA for 5 min at 37°C; $EAC4_h$ were prepared from these cells by decaying off C1 and C2 (90 min at 37°C). $EAC142_{gp}3b_h$ were prepared by incubating 10 minimal haemolytic doses of purified human C3 with $EAC142_{gp}$ for 15 min at 37°C. $EAC1423b_m$ were prepared by incubating 5 ml 1% EA with 0·5 ml AKR (C5-deficient) mouse serum for 30 min at 37°C. In an attempt to detect receptors for the Fc piece of immunoglobulin EA were also prepared using purified anti-ox red cell rabbit IgG and IgM (gift of Dr Christopher Spry) and ox red cells. EAC3d were generated by incubating $EAC4_{gp}3b_h$ or $EAC1423b_m$ with purified human C3b inactivator (gift of Professor P. Lachmann) before incubating them with the kidney sections.

The second indicator system used was *S. typhi* fluoresceinated and coated with human C3b. *S. typhi* type 0901 were grown in 200 ml Oxoid No. 2 broth (Oxoid Ltd, London) at 37°C on a shaking tray overnight. They were heat killed (60°C for 20 min), washed and resuspended in 5 ml complement-fixation diluent (CFD).

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For fluorescein conjugation, a modification of the method of Gelfand, Fauci & Green (1976) was used. One millilitre of the *S. typhi* suspension was diluted 1 in 5 in CFD and stirred for 30 min at room temperature with 1 mg fluorescein isothiocyanate (FITC) dissolved in 2 ml 0·1 M Na_2HPO_4. Excess FITC was removed by washing in CFD, and the sedimented *S. typhi* resuspended in 1 ml normal human serum and incubated for 30 min at 37°C. They were then washed and resuspended to 1 ml in CFD.

Normal kidneys. Of seven kidneys examined, two were blocks of normal tissue removed at nephrectomy for carcinoma of the kidney and the remainder unused cadaver donor kidneys. One of the latter was used to provide normal standard sections to which all abnormal kidneys were compared. Animal kidneys (mouse, rat, guinea-pig, rabbit, rhesus monkey) were obtained from normal laboratory stock immediately after a lethal dose of i.v. Nembutal.

Renal biopsies. Percutaneous renal biopsies were obtained by routine methods from a series of patients with various types of glomerular disease, and with acute tubular necrosis (see Table 2 for diagnoses and numbers of cases).

Detection of receptors. All samples were snap-frozen and stored in liquid nitrogen. Frozen sections 4 μ thick were incubated with the indicator cells for 15 min at room temperature, and washed in CFD. Sections with EAC were viewed wet under phase contrast, while sections with *S. typhi* C3b were mounted in buffered glycerol and examined in a u.v. microscope (Leitz Orthoplan). *S. typhi* C3b were used for the evaluation of receptors in abnormal kidneys.

All glomeruli present in the sections were evaluated independently by two observers. Adherence of indicator cells was scored on a scale from 0 to +++, the normal (control) kidneys scoring +++.

Immunofluorescence. Frozen sections were stained with fluoresceinated antisera to C3 and IgG, IgA and IgM using previously described techniques (Evans *et al.*, 1973). C3 deposition was recorded as present (trace or more) or absent.

RESULTS

Normal kidneys

The receptor was present in all seven normal kidneys examined. Only indicator cells bearing C3b were bound (Table 1) with a uniform distribution (Fig. 1) suggesting that the receptor is located on the visceral epithelial cell rather than the mesangial or endothelial cell. The appearances did not suggest receptors were available on parietal epithelial cells.

TABLE 1. Degree of glomerular binding occurring with indicator cells coated with various complement components, graded on a scale of 0 to +++

Indicator cells	Binding
EA	0
EAC14	0
EAC4	0
EAC142	0
EAC1423b	+++
EAC43b	+++
S. typhi C3b	+++

Similar results were obtained using all sources of complement, including human, mouse, rabbit and guinea-pig serum. On the other hand there was no binding in kidney sections from all other species tested (mouse, rabbit, guinea-pig, rat and rhesus monkey) even when using homologous serum as a complement source.

There was no binding of EA alone, indicating that the glomerulus lacks a receptor for the Fc piece of IgG, as occurs on cells of the lymphoreticulophagocytic system which bear C3b receptors.

Experiments using C3b inactivator (KAF)

In order to determine whether the receptor is specific for C3b or also recognizes the later breakdown product, C3d, as suggested by Sobel *et al.* (1976), EAC3b were preincubated with purified human KAF

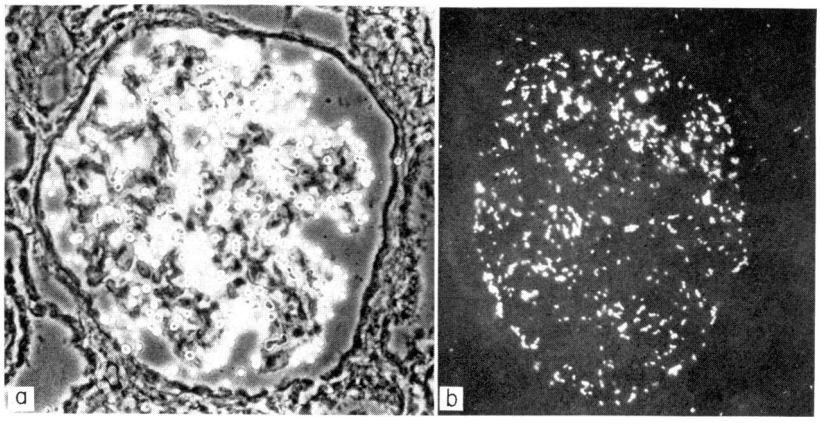

FIG. 1(a). Normal glomerulus incubated with EAC142$_{gp}$3b$_h$ (Phase contrast × 220). (b) Normal glomerulus incubated with *S. typhi* C3b (u.v. × 220).

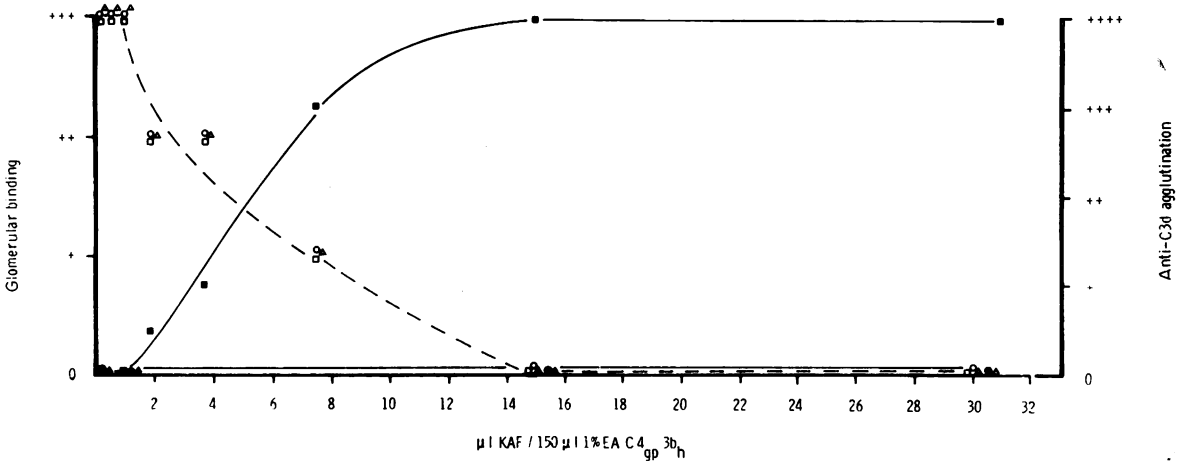

FIG. 2. Comparison of degree of glomerular binding (open symbols) of EAC4$_{gp}$3$_h$ and degree of agglutination (closed symbols) with anti-C3d antiserum of the same cells following preincubation with purified human KAF for 30 (△, ▲), 60 (○, ●) or 180 (□, ■) min. There is a concentration-dependent fall-off in subsequent glomerular binding, similar at all pre-incubation periods but only with prolonged incubation (180 min) does agglutination with C3d antiserum become positive.

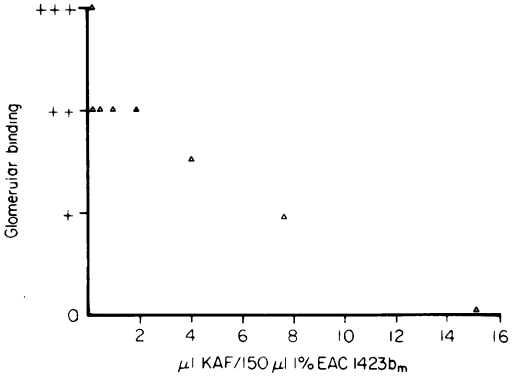

FIG. 3. Results of preincubation of EAC1423b$_m$ with purified human KAF for 30 min. The results are similar to those obtained with EAC4$_{gp}$3b$_h$.

for 30, 60 or 180 min at 37°C (Fig. 2). All three preincubation periods gave a similar concentration-dependent decrease in glomerular binding. However it is worth emphasizing that only with the longest (180 min) preincubation period with KAF did agglutination of these cells occur; complete loss of subsequent glomerular binding was observed after preincubation for 30 or 60 min, although these periods were apparently insufficient to generate detectable C3d on the cells.

Binding of EA1423b$_m$ was also inhibited by preincubation with KAF for 30 min (Fig. 3).

These experiments confirm that the receptor is specific for C3b.

Renal biopsies

Table 2 shows the results of examining a series of 111 renal biopsies from 105 patients. We have compared the degree of binding of the *S. typhi* C3b indicator cells (a measure of the state of the receptor) with C3 deposition as judged by immunofluorescence.

TABLE 2. Comparison of degree of glomerular binding of *S. typhi* C3b with presence or absence of *in vivo* deposition of C3 (as detected by immunofluorescence). Degree of glomerular binding is graded 0, +, ++ or +++, and the number of cases in each category showing detectable C3 is shown in brackets

		Glomerular binding			
	No. of biopsies	0	+	++	+++
Mesangiocapillary glomerulonephritis	20	15 (15)	3 (3)	1 (1)	1 (0)
Acute glomerulonephritis	12	8 (6)	1 (1)	3 (3)	0
Membranous glomerulonephritis	11	5 (3)	1 (1)	5 (2)	0
SLE	6	1 (1)	1 (1)	1 (0)	3 (2)
Goodpasture's syndrome	8	7 (2)	0	0	1 (0)
Rapidly progressive glomerulonephritis	7	5 (2)	2 (0)	0	0
Minimal change kidney	8	1 (0)	0	2 (0)	5 (0)
IgA disease	15	0	2 (2)	6 (4)	7 (6)
Focal glomerulonephritis without IgA deposition	2	0	0	0	2 (0)
Henoch–Schonlein purpura	4	0	0	0	4 (3)
Focal glomerulosclerosis	6	0	1 (1)	3 (0)	2 (1)
Amyloidosis	6	3 (1)	2 (1)	0	1 (0)
Acute tubular necrosis	6	0	0	3 (0)	3 (0)

In those thirty-nine biopsies in which parietal deposition of C3 was demonstrated by immunofluorescence (mesangiocapillary glomerulonephritis, acute glomerulonephritis, membranous glomerulonephritis, Goodpasture's syndrome and rapidly progressive glomerulonephritis) the receptors were absent in twenty-eight and reduced in all the remaining eleven biopsies. By contrast in fifteen biopsies where C3 deposition was present but limited to the mesangial regions (IgA disease, Henoch–Schonlein purpura) the binding was normal in nine but reduced (though still detectable) in the remaining six.

An unexpected finding was that the receptor was sometimes completely undetectable in cases in which C3 deposition was not detectable by immunofluorescence e.g. of seven cases of Goodpasture's syndrome with absent receptors, C3 was not detected in five.

One biopsy from a patient with mesangiocapillary glomerulonephritis and one from a case of Goodpasture's syndrome showed normal receptors. These were both follow-up biopsies in patients who had had substantial recovery of renal function and in whom receptors had been completely absent in biopsies taken on presentation.

All glomerular crescents examined showed a complete absence of receptors.

DISCUSSION

The points of major interest arising from these experiments are: the confirmation of a receptor for C3b, but not C3d; its absence in other species; the loss of the receptor in diseases associated with parietal deposition of C3 *in vivo*; our failure to detect C3b binding by crescentic cells; and the absence of the receptor in certain biopsies in which C3 deposition was not demonstrable.

We, like Gelfand *et al.* (1975) and Sobel (personal communication), have as yet failed to demonstrate a C3b receptor in other species. This is puzzling, especially in the case of the subhuman primate which otherwise shows a close similarity in distribution of C3b receptors.

The pattern of binding of both *S. typhi* C3b and C3b-coated erythrocytes is strongly suggestive that the receptor is located on the visceral epithelial cell, in accordance with the findings of Gelfand *et al.* (1976) using scanning electron microscopy. This conclusion is further supported by our observations on diseased glomeruli: the receptors are generally normal, or only slightly reduced, in diseases associated with C3 deposition only in the mesangium (as in IgA disease) but lost in diseases showing parietal deposition of C3 (as in mesangiocapillary glomerulonephritis). These conclusions are also in accord with our findings in SLE. In five patients with focal disease (attributed by Germuth & Rodriguez (1973) to mesangial deposits) the receptors were normal, whereas in two patients with diffuse involvement (attributed to capillary complex deposition (Germuth & Rodriguez, 1973)) the receptors were markedly reduced. Our findings are at variance with those of Sobel *et al.* (1976) who concluded that the receptor was on mesangial cells.

A finding of particular interest and importance is that the receptors may be absent in cases in which no C3 deposition is detectable by immunofluorescence. A number of explanations may be advanced for this phenomenon: firstly, immunofluorescence failed to detect small amounts of C3b sufficient to block the receptors *in vivo*. Secondly, that receptors may be lost due to epithelial cell damage, for example secondary to another mechanism such as anti-glomerular basement membrane antibody. (Similarly it is not surprising to find receptors absent in sclerosed glomeruli). Thirdly, receptors could be blocked by leakage of C3 across the capillary wall; proteases released from cells might then lead to generation of C3b with binding to receptors. The last possibility seems unlikely in view of the finding that receptor activity was frequently normal in patients with IgA nephropathy and heavy non-selective proteinuria.

In the light of recent suggestions that crescents may be formed at least partly by macrophages (Kondo, Shigematsu & Kobayashi, 1972; Atkins *et al.*, 1976) it is of interest that we could not identify C3b binding by crescentic cells. We have similarly failed (unpublished observations) to demonstrate C3b receptors in crescentic cells in experimental nephrotoxic nephritis in rabbits. Our data therefore support the classical view (Morita, Suzuki & Churg, 1973; Heptinstall, 1974) that crescents are predominantly the result of proliferation of epithelial cells of Bowman's capsule. However the possibility of blockade of receptors on these cells by amounts of C3 not detectable by immunofluorescence again cannot be excluded.

Of interest is the finding that receptors returned to normal in two follow-up biopsies, one of mesangiocapillary glomerulonephritis and one of Goodpasture's syndrome, in both cases being associated with recovery of renal function. Thus regeneration of receptors may occur *in vivo*.

The findings presented in this paper suggest that the C3b receptor may be important in the pathogenesis of renal diseases associated with circulating immune complexes.

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