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Demonstration and characterization of C3 receptors on rat glomerular epithelial cells

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Demonstration and characterization of C3 receptors on rat glomerular epithelial cells. Receptors for C3 have been demonstrated on the glomerular podocyte in humans. There is conflicting evidence regarding the presence of C3 receptors on rat glomerular cells. Even when shown to be present, the ligand specificity of the receptor has not been determined. Decapsulated rat glomeruli obtained from male Sprague–Dawley rats weighing 50 to 100 g were placed in enriched culture media. On days four to eight, cells of epithelial morphology were observed growing out of glomeruli. Receptors for C3 were detected by rosette formation of sheep erythrocytes (E) coated with antibody (A) and complement (EAC) around the glomerular epithelial cells in culture. The EACs were prepared by incubating antibody–coated sheep erythrocytes with C5-deficient mouse serum or with individual components of complement. Results indicate the presence of two types of C3 receptors on glomerular epithelial cells—CR1 for C3b and CR2 for C3d. The functional roles of these receptors remain to be elucidated.

Human glomeruli have been shown to possess receptors for the C3b and C3bi component of complement [1, 2]. The C3b receptor has been localized to the visceral epithelial cell [3]. The existence of these receptors has implied that the glomerular epithelial cells may somehow be involved in the uptake and elimination of immune reactants which may localize to the glomerulus. The presence of these receptors on glomeruli of vertebrate species other than man is controversial. A number of investigators have not been able to find C3 receptors on glomeruli of a variety of animals including the rat [1, 4]. Studies of glomerular cells in culture have yielded conflicting results. Whereas Kreisberg, Hoover and Karnovsky found C3 receptors on rat glomerular epithelial cells [5], Foidart and associates failed to do so [6]. We report the results of our studies of the presence and ligand specificity of C3 receptors on rodent glomerular cells in primary culture.

Methods

Rat glomeruli were grown in culture by the method of Kreisberg, Hoover and Karnovsky [5]. Male Sprague–Dawley rats (Harlan Sprague–Dawley, Wisconsin, USA) weighing approximately 50 to 100 g were anesthetized by intraperitoneal injection of sodium pentobarbital, 35 mg/kg body weight, supplemented by ether, as needed. The kidneys were perfused via the aorta using Hank's balanced salt solution (BSS).[1] (Grand Island Biological Co., Grand Island, New York, USA) at a pressure of about 120 mm Hg in order to flush out formed elements of blood from the renal circulation. The kidneys were removed, bisected, and the cortex dissected out under sterile conditions. The cortex was minced and serially passed through sieves of mesh size 60, 150, and 200, respectively (W.S. Tyler Inc., Mentor, Ohio, USA), while being generously rinsed with Hank's BSS. The glomeruli were collected from the top of the 200-mesh sieve and washed repeatedly with Hank's BSS by centrifugation at 100 ×g for 10 minutes. When examined by phase contrast microscopy and transmission electron microscopy, most of the glomeruli were decapsulated and tubular fragments were rare. The isolated glomeruli were then placed in the culture medium in multiple well culture dish (Corning, New York, USA), at 37°C, in an atmosphere of 95% air and 5% CO_2. The culture medium was a composite of two media: (1) RPMI 1640 (KC Biological, Lenexa, Kansas, USA) containing 15 mM Hepes, 20% fetal calf serum (heat inactivated), 0.66 U/ml of insulin and antibiotics—penicillin 100 U/ml and streptomycin 100 μg/ml, and, (2) conditioned medium from 3T3 fibroblasts (American Type Culture Collection, Rockville, Maryland, USA) in log phase of growth comprising Dulbecco's modified Eagle's medium and 10% fetal calf serum. The conditioned medium from fibroblasts was filtered with 0.22 μ filter (Nalge, Rochester, New York, USA) before use. The nutrient medium was changed every third day.

Morphologic studies

Cultures were examined and photographed by phase contrast microscopy (Nikon Diaphot, Tokyo, Japan).

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[1] Abbreviations used in this paper: Hank's BSS, Hank's balanced salt solution; CR1, a type of C3 receptor that binds C3b and C4b; CR2, a type of C3 receptor that binds C3bi and C3d; CR3, a type of C3 receptor that binds C3bi, non-C3d; E, sheep erythrocytes; A, rabbit anti-sheep E IgM antibody; C, ligands of complement such as C3b, C3d; GVBE, gelatin veronal buffer with 0.01 M EDTA; GVB, gelatin veronal buffer; GEC, glomerular epithelial cells.

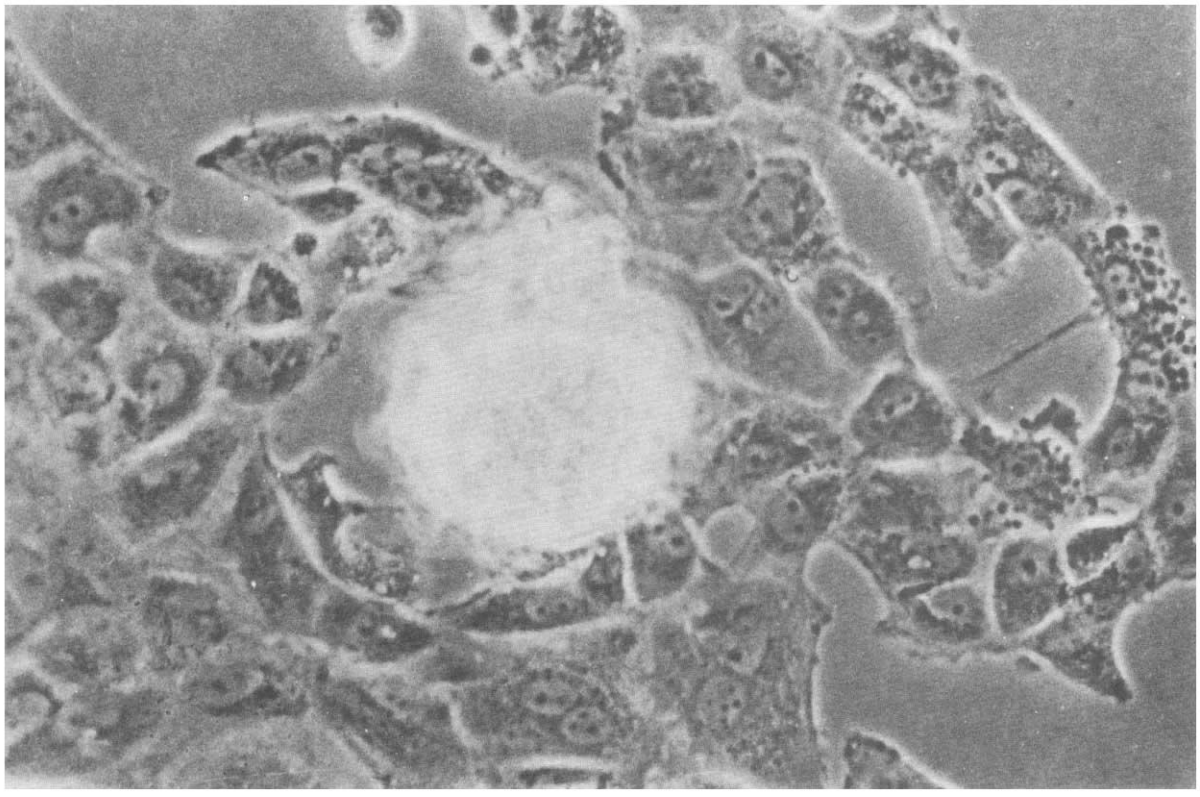

Fig. 1. *Epithelial cells growing out of glomerulus on day six of primary culture.* The glomerulus is in the center. The epithelial cells are tightly packed. The cells have large nuclei, prominent nucleoli and granular cytoplasm (×360, phase contrast).

Scanning and transmission electron microscopy were performed on cultures which were fixed for one hour in 2% wt/vol paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 [7]. The cultures were then washed and stored in buffer.

For transmission electron microscopy, the glomeruli were planted on plastic cover slips and after they were embedded in plastic, the specimens were immersed in liquid nitrogen and snapped off of the cover slips. The sections were re-embedded in plastic and oriented so that the thin sections were cut perpendicular to the original surface. Other cultures were harvested from culture flasks following fixation, centrifuged, and the button was embedded in plastic. Thin sections, cut with diamond knives and mounted on 100- and 200-mesh grids, were stained with uranyl acetate and lead citrate, and examined in an electron microscope (Philips 301).

Scanning electron microscopy was performed on cultures grown on coverslips. Following fixation, the cultures were osmicated, rapidly dehydrated in alcohol, and changed to Freon 113 (DuPont, Wilmington, Delaware, USA) as the transitional fluid. The cultures were critical point dried (Balzers CPD 020), coated with gold (Pelco sputter coater 900C) and examined in a scanning electron microscope at 15 KV (JEOL 35 smc, JEOL Ltd., Tokyo, Japan).

Nonspecific esterase reaction was employed to detect possible presence of macrophages in primary culture. Immunofluorescence using antibody to Factor VIII antigen (Dako Labs, California, USA) was performed to detect the presence of endothelial cells in culture.

Assays for C3 receptors

In humans, individual receptors for distinct ligands of C3 have been described: CR1 binds C3b and C4b, CR2 binds C3bi and C3d, and, CR3 binds C3bi, non-C3d [8]. Receptors for C3 ligands on rat cells in vivo have not been described so far. Assays were carried out in order to determine the presence of CR1 and CR2 receptors for different ligands of C3 on rat glomerular cells in culture. These receptors were detected by preparing sheep erythrocytes (E) coated with rabbit anti-sheep erythrocyte IgM antibody (A) and different ligands of C3 (C). Thus EAC14b and EAC14b23b were prepared for the detection of CR1 and EAC(C5 deficient), and EAC3d for CR2. Established, standard protocols of the assay were employed and rosette formation with the coated sheep RBC (EAC) was used as the index of the presence of C3 receptors [9].

Preparation of EA

Ten ml of sheep erythrocytes (E) in Alsever's solution (Cordis, Miami, Florida, USA) were washed three times with 0.9 N saline at 450 ×g at 4°C for 10 minutes. The buffy coat was discarded and the sheep E were suspended in GVBE at 5% vol/vol. The sheep E were used within two weeks of purchase and discarded if lysis was seen. The rabbit anti-sheep E IgM antibody (A) was diluted at 1:60 in 0.01 M GVBE as recommended by the manufacturer (Cordis) and warmed to 37°C.

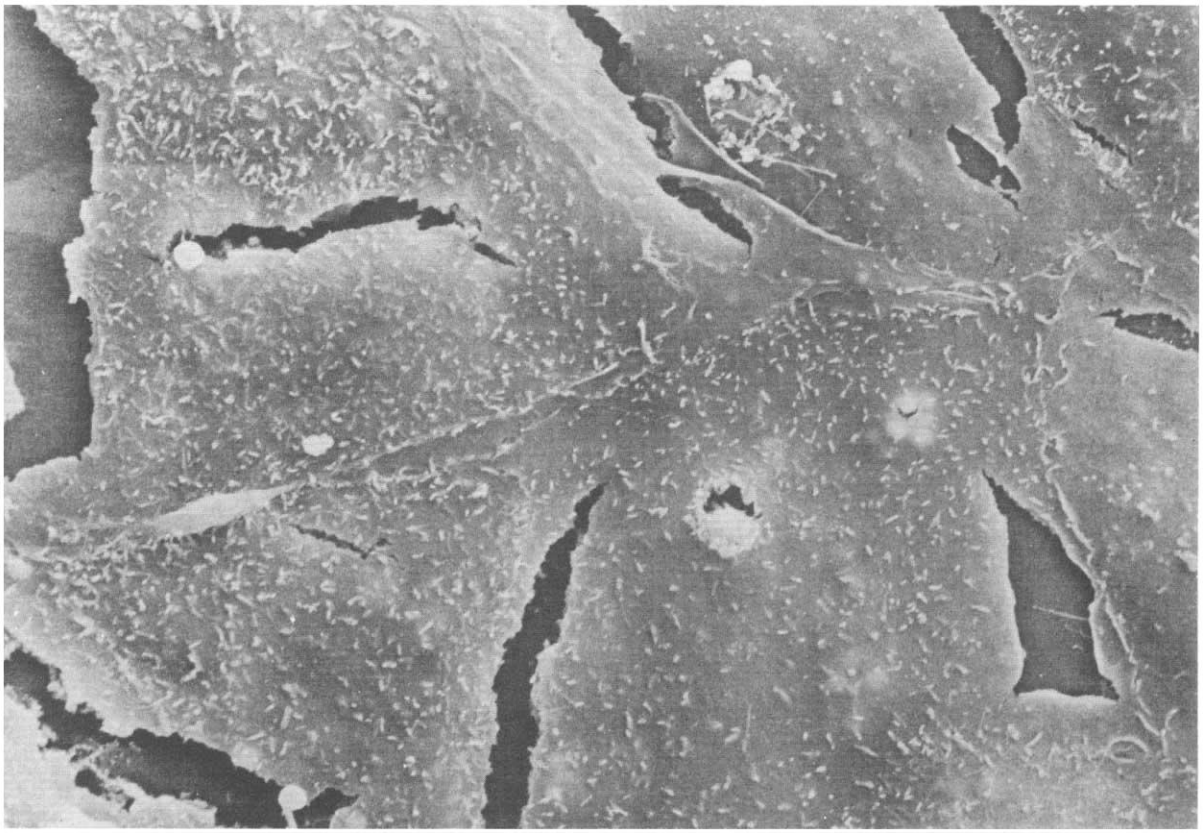

Fig. 2. *Scanning electron micrograph of glomerular epithelial cells on day seven of primary culture.* The cells are flat and closely opposed with numerous microvilli on the luminal surface of the cells (×1800).

Equal volumes (6 ml) of the 5% sheep E suspension and 1:60 dilution of rabbit A were incubated together in a waterbath at 37°C for 30 minutes with gentle agitation, and transferred to an ice bath for 30 minutes. The EA were washed in ice–cold 0.01 M GVBE once, in ice–cold gelatin veronal buffer (GVB) twice and resuspended in GVB at a concentration of 2.5% (vol/vol). The EA were warmed to 37°C in a water bath.

Preparation of EA coated with complement (EAC)

Human complement C1, C2, C3 and C4 were commercially purchased in the form of lyophilized powder (Cordis). They were reconstituted with 5 ml of cold distilled water and contained 50,000 CH50 units of C1 and 5,000 CH50 units of C2, C3 and C4 respectively.

Assays for CR1

CR1 was detected by using EAC14b and EAC14b23b which were prepared as detailed below.

EAC14b [9]. Ten ml of EA suspended at 2.5% (vol/vol) in GVB and warmed to 37°C were mixed with 1.0 ml of C1 and incubated at 37°C for 15 minutes. The EAC1 were washed twice in GVB warmed to 37°C, using a room temperature centrifuge at 275 ×g for 10 minutes. The EAC1 were resuspended at 2.5% (vol/vol) in GVB warmed to 37°C and incubated with 1 ml of C4 at 37°C for 15 minutes. The GVB was maintained at 37°C when using C1 to prevent loss of C1 that occurs below this temperature. The EAC14b were diluted in 50 ml of ice cold GVB and washed twice by centrifugation at 275 ×g for 10 minutes in a room temperature centrifuge. The EAC14b were resuspended at 1% in PBS-BSA (vol/vol) and stored at 0° to 4°C to be used within two days.

EAC14b23b (modified method of Burkholder et al [4]). EAC14b as prepared above were mixed with 1.0 ml each of reconstituted human C2 and C3 in a single step and incubated at 37°C for 60 minutes. The EAC14b23b were washed x2 in GVB at 275 ×g for 10 minutes and resuspended at 1% in PBS-BSA.

Assays for CR2

CR2 was detected by employing EAC(C5 deficient) and EAC3d which were prepared as follows.

EAC(C5 deficient) [10]. Blood collected from the C5-deficient mice (strain DBA-2J, Jackson Laboratories, Bal Harbor, Maine, USA) was centrifuged at 300 ×g at 4°C for 10 minutes. The serum was aliquoted in 0.19 ml volume and stored at −70°C. The EA were incubated in equal volumes of 1.90 ml with 1:10 dilution of the C5-deficient mouse serum (0.19 ml serum diluted in 1.71 ml of GVB), at 37°C for 30 minutes. The EAC(C5 def) were centrifuged at a low speed and washed twice in GVB. The EAC(C5 def) in 0.01 ml were resuspended in 1.99 ml of Hank's BSS (0.5%) and stored at 0 to 4°C to be used within one week. Due to the presence of C3 inactivators in the mouse serum, mostly C3bi or C3d, rather than C3b, is deposited on the EAC prepared by this method.

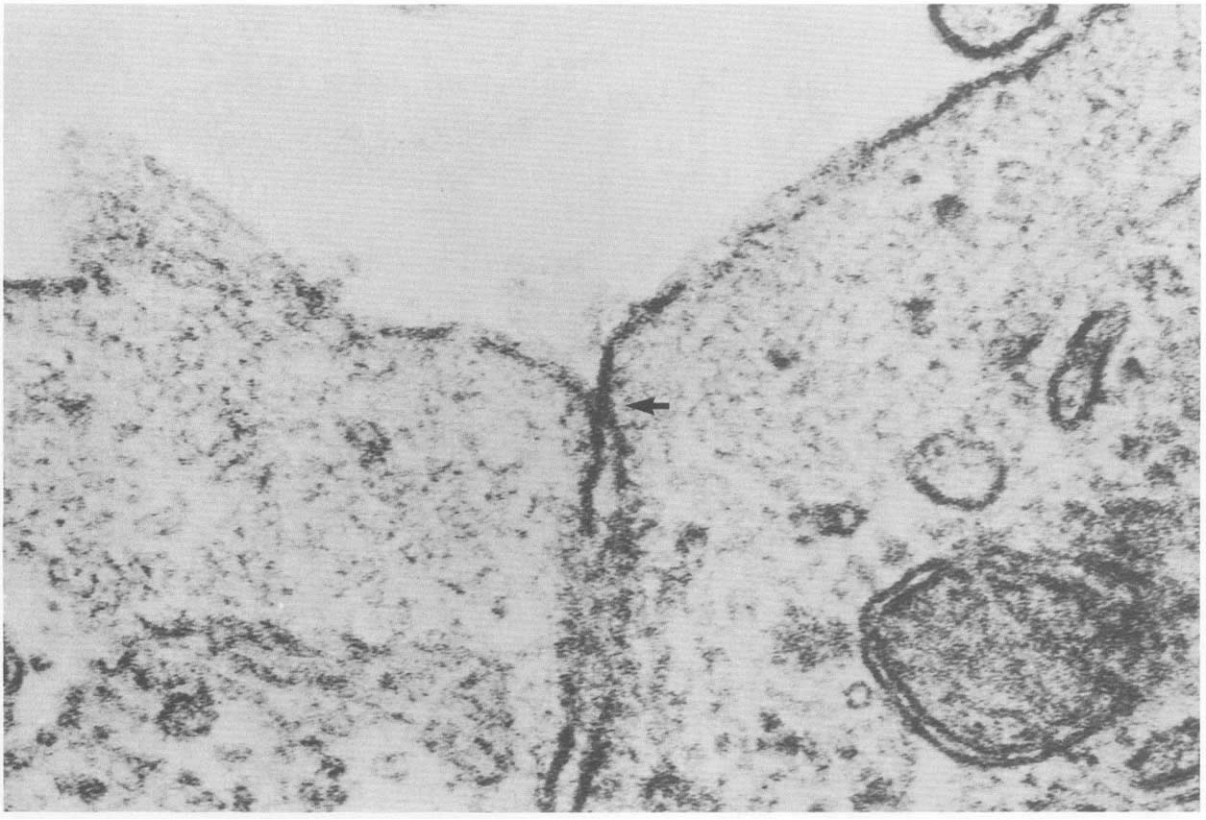

Fig. 3. *Transmission electron micrograph of glomerular epithelial cells in primary culture.* Note the suggestion of a zonula occludens (tight junction) close to the apical surface of the cells (arrow) (×170,000).

EAC3d. The EAC prepared with C5-deficient mouse serum may carry C3bi predominantly [8]. As C3bi can bind both CR2 and CR3, in order to detect CR2, EAC have to be prepared containing C3d only, the specific ligand for CR2. To ensure that the C3 present on EAC was in the form of C3d, the EAC(C5 def) were further treated with trypsin [9]. Three ml of EAC(C5 def) were incubated with 30 μl of trypsin (100 μg/ml) at 37°C for 30 minutes, giving a final concentration of 1 μg/ml of trypsin. The EAC3d were washed three times with GVB and resuspended at 1% in PBS-BSA. The EAC3d were stored at 4°C and used within two days. The EAC3d bind only to CR2 type of C3 receptor.

The EAC rosette assay using rat glomerular epithelial cells

The assay was performed with glomerular cells in primary culture on days four to eight. The medium was aspirated and the cell layer was washed twice in Hank's BSS. The EAC bound with various ligands—EAC14b, EAC14b23b, EAC(C5 def) and EAC3d were layered over the cells and incubated for 60 minutes at 37°C in a moist chamber. Following incubation, the cell layer was washed at least three times with PBS. The rosettes were observed by both phase contrast microscopy and scanning electron microscopy. A rosette was defined as binding of at least three RBC to a cell. Each rosette assay was performed on at least four individual primary glomerular culture lines. In every primary culture, each individual C3 ligand was incubated with a minimum of three wells ($N = 12$ for each C3 ligand).

The EAC rosette assays were performed employing a number of controls. Human kidney biopsy material (glomeruli), human erythrocytes (group 0), and human mononuclear cells in peripheral blood formed rosettes with EAC14b and EAC1423b and were employed as "positive" controls for assays with these ligands, as they are known to possess CR1 receptor [8]. The human mononuclear cells were obtained by ficoll gradient separation (Pharmacia, Piscataway, New Jersey, USA). The rosette formation with the human erythrocytes was not as numerous as with the other two positive controls, so that the latter were more frequently used. Since these indicator cells did not form rosettes with the Daudi lymphoblasts, they did not have C3d on their surfaces. The Daudi lymphoblasts in culture formed rosettes with EAC3bi and EAC3d, but not with CR1 ligands. Therefore they were used as "positive" control for CR2. The Daudi cells are known to possess only CR2 receptors and not CR1 [9]. The mouse peritoneal macrophages formed rosettes with EAC14b, EAC1423b and EAC(C5 def) but not with EAC3d; that is, they possessed CR1 and CR3 receptors and not CR2. Thus, they were used as "negative" control for CR2 receptor (Gordon D. Ross, personal communication, [11]). These observations also demonstrated that the EAC3d indicator cells do not have residual reactive C3b, C4b or C3bi on their surfaces. As whole human C1 (in the absence of serum) and not purified C1q was used in the preparation of the ligands of CR1,

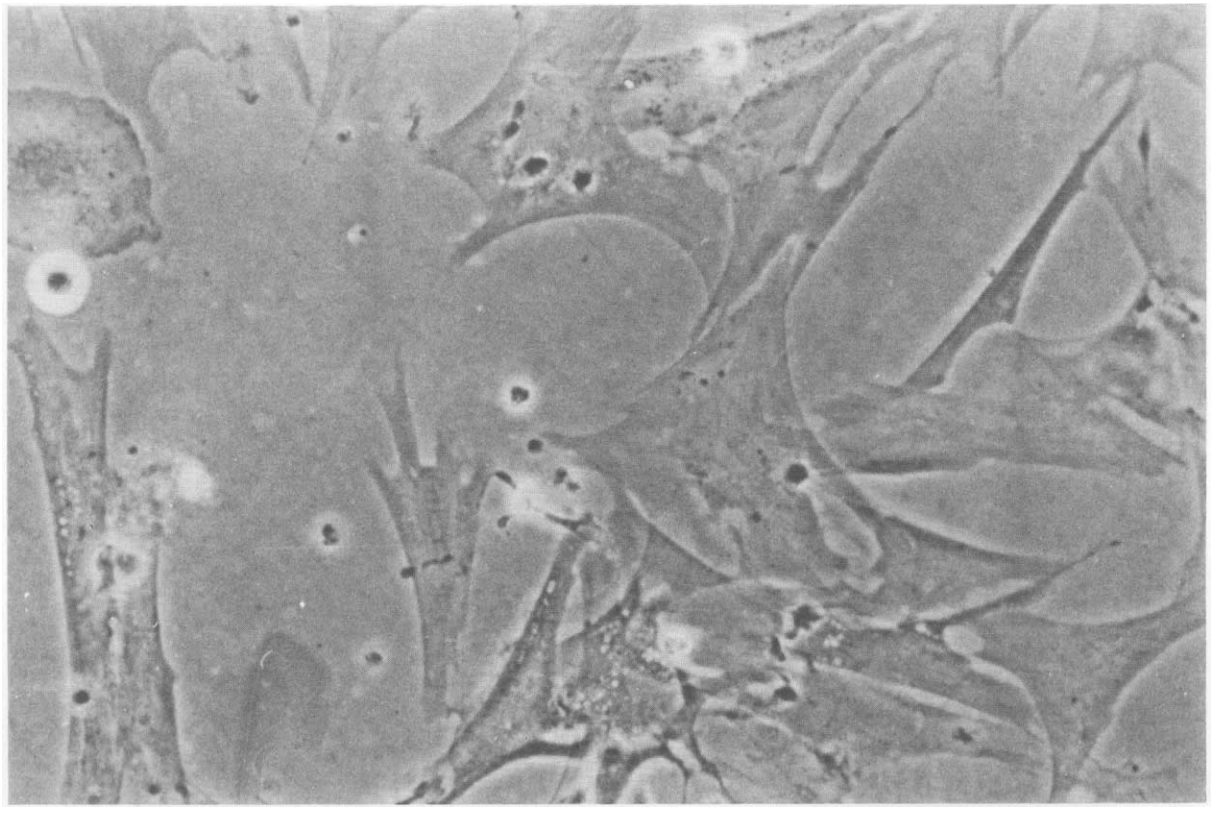

Fig. 4. *Mesangial-like cells during week four of primary glomerular culture.* Note the stellate shape, loose arrangement with abundant intercellular spaces (×360, phase contrast).

it is very unlikely that binding of the C1 containing ligands was to a C1q receptor. Also, the method of preparation of EAC14b and EAC1423b differed considerably from that of EC1q employed by others in the detection of C1q receptor. To study whether EAC rosette formation was a nonspecific property of in vitro cell culture conditions, a non-epithelial cell line, the mouse 3T3-fibroblasts in culture was employed as control.

The glomerular cells were incubated with sheep erythrocytes (E) alone in every experiment. Also, assay for C3 receptors was performed under conditions of saturation of potential IgM binding sites on glomerular cells. This was done to distinguish between rosette formation due to the presence of potential IgM binding sites and that due to the C3 receptor. The glomerular cells in primary culture were first incubated with 1:60, 1:120 dilutions of rabbit anti-sheep erythrocyte IgM antibody at 37°C for 60 minutes. The cells were then washed twice with Hank's BSS and EA, EAC14b, EAC1423b, EC(C5 def) and EAC3d assays performed in the usual manner. The experiments were performed on three separate series of primary glomerular cultures, on days five, six and seven.

Results

Identification of glomerular cells in culture

Two types of cells were seen to grow from the glomeruli at different times following planting of glomeruli in the culture medium. On days four to five, large, polygonal, tightly packed cells with large nuclei and granular cytoplasm were seen growing out of the parent glomerulus, as observed by phase contrast microscopy (Fig. 1). By scanning electron microscopy, these cells measured approximately 60 μ in diameter and had a centrally located nucleus. They grew in sheets with closely apposed cell borders and the luminal surface was covered with numerous microvilli (Fig. 2). The cells were seen to grow in a monolayer by transmission electron microscopy and there was a complex pattern of lateral projections at the cell borders which interdigitated with those of the adjacent cell. Close to the apical surface the cells were joined at a junction suggestive of a short zonula occludens (tight junction) (Fig. 3). The cytoplasm was very attentuated at the periphery, and it contained few organelles. In the perikaryon, there were well developed Golgi bodies, rough endoplasmic reticulum and frequent, large secondary lysosomes which contained granular and membranous debris. By morphology these cells appeared epithelial and will be referred to as glomerular epithelial cells (GEC).

During the first week of primary culture, some cells which stained positively with antibody to Factor VIII antigen were seen, indicating the presence of endothelial cells. When nonspecific esterase reaction was performed, a few cells reacted positively indicating the presence of monocyte–macrophages also. However, endothelial cells and macrophages accounted for a minority of the cell population.

During the second week of culture, the GEC were progressively replaced by loosely arranged, stellate cells which became

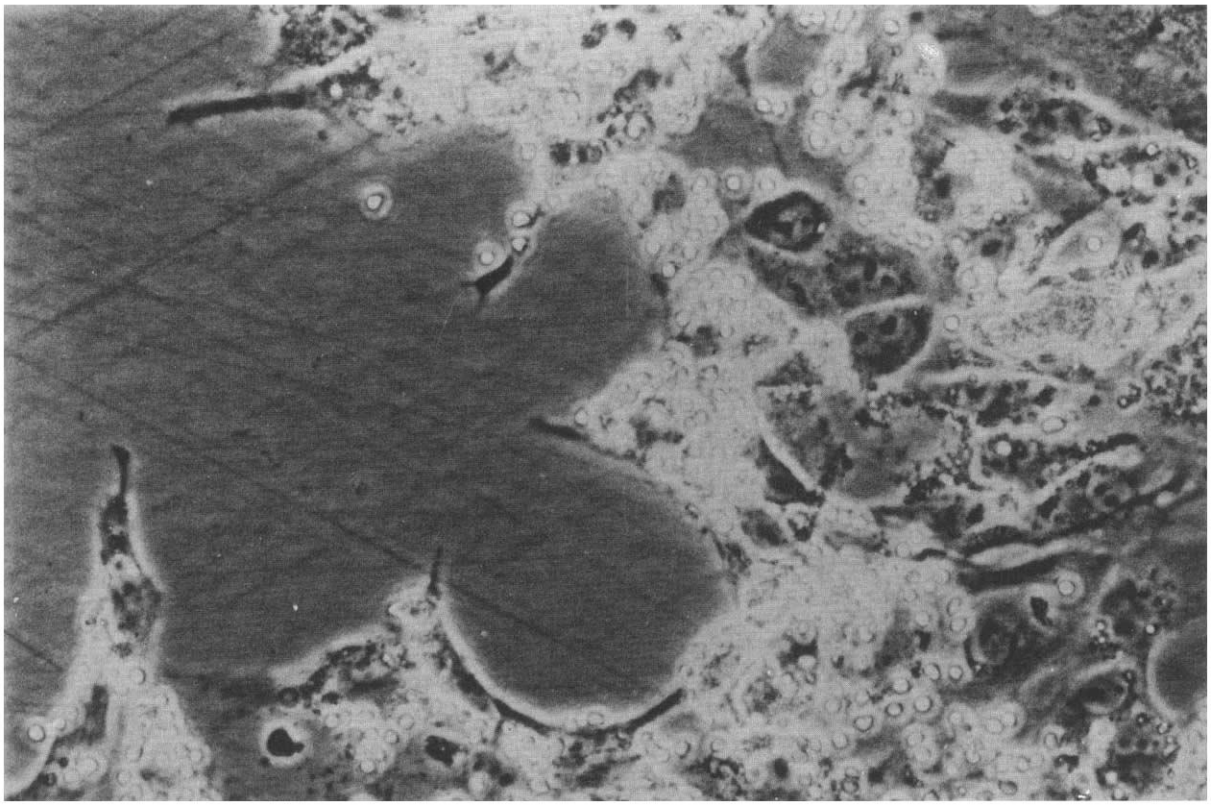

Fig. 5. *Glomerular epithelial cells forming rosettes with EAC14b indicating the presence of CR1 type of receptors.* Sheep RBCs coated with IgM antibody and complement (14b) sharply delineate the margins of the colony of GEC (×364, phase contrast).

the predominant cell type by the third week (Fig. 4). These have been referred to as the mesangial-like cells [5]. In order to conduct experiments on the GEC only, the EAC rosette assays were performed only between days four to eight.

Detection of CR1 and CR2 types of C3 receptors on GEC using specific ligands

Demonstration of CR1. Both EAC14b and EAC14b23b formed rosettes around GEC in primary culture (Fig. 5). The evidence for the presence of CR1 was obtained using two distinct ligands—C3b and C4b. The sheep erythrocytes carrying C3b also formed rosettes on human glomeruli on renal biopsy (Fig. 6); whereas EAC14b and EAC14b23b formed rosettes with human mononuclear cells. Human glomeruli and mononuclear cells are both known to have CR1 and were employed as "positive" controls [8]. However, when rat renal cortical tissue was incubated with EAC14b and EAC14b23b no rosette formation was seen over the glomeruli or any other structures.

When examined by phase contrast microscopy, the binding of C3b- or C4b-carrying sheep RBC to GEC was not uniform in a given colony of cells. In general, in a given colony about 60% of cells of epithelial morphology formed rosettes. The cells towards the periphery of the GEC colony appeared to bind the EAC more frequently than those towards the center, close to the glomerulus. The sheep RBC appeared to surround borders of individual GEC (Fig. 7). When examined by scanning electron microscopy, each GEC bound 15 to 30 sheep RBC which were generally distributed around the surface of the cell (Fig. 8). No rosette formation was seen with cell fragments or debris (Fig. 8) implying that an intact cell was necessary for binding to the complement receptor.

Demonstration of CR2. The EAC(C5 def) and EAC3d (Fig. 9) formed rosettes with the GEC in primary culture, thus providing evidence for the presence of CR2. Confirmation of ability of EAC3d to bind only to CR2 type of receptor was obtained using two controls. First, Daudi lymphoblasts which are known to possess only CR2 receptor formed rosettes with EAC3d but not with CR1 ligands. Second, mouse peritoneal macrophages which possess CR1 and CR3 receptors but not CR2, failed to react with EAC3d; however, rosette formation was seen with EAC14b, EAC1423b (ligands for CR1) and with EAC(C5 def), that is, EAC3bi (a ligand for CR3). The distribution and pattern of binding of ligand carrying sheep erythrocytes to the GEC were indistinguishable between CR1 and CR2. The glomeruli on biopsy of rat renal cortex did not bind with EAC(C5 def) or with EAC3d.

The IgM blocking experiment. When the GEC were incubated with EA-IgM alone, adherence of RBCs was seen. To demonstrate rosette formation due to C3 receptors as distinct from that due to IgM binding sites, the latter were presaturated with 1:60 and 1:120 dilutions of the rabbit anti-sheep E IgM antibody and rosette assays performed with EA, EAC14b, EAC1423b, EAC(C5 def) and EAC3d. Whereas EA failed to bind to GEC following preincubation with IgM, rosette formation by EAC14b, EAC1423b (for CR1) and EAC(C5 def),

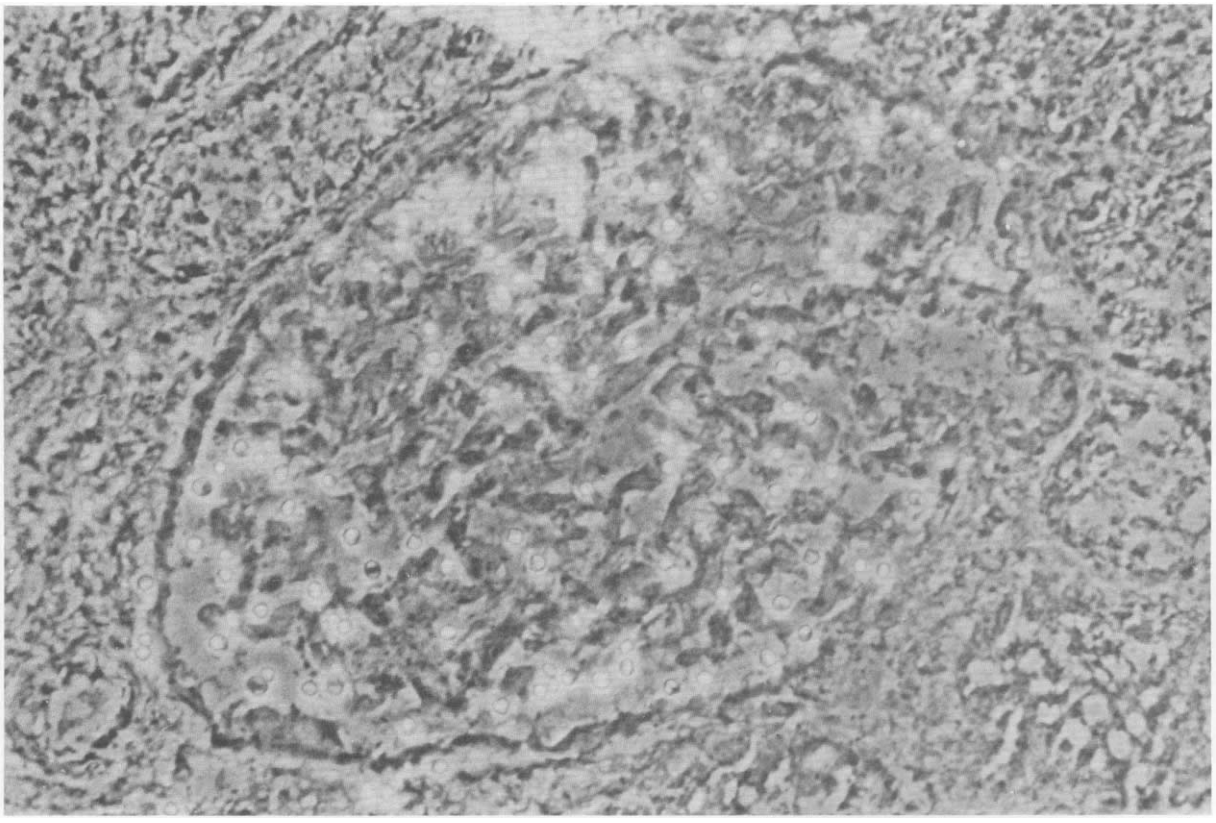

Fig. 6. *The EAC1423b have formed rosettes with a normal human glomerulus in a frozen section.* The EAC did not bind to the tubulo-interstitium. (×364, phase contrast).

EAC3d (for CR2) was seen. These results were reproduced on days five, six and seven of three separate lines of primary glomerular cultures.

Free sheep erythrocytes did not bind GEC in any experiment. In order to exclude rosette formation as a nonspecific property of in vitro cell culture, a non-epithelial cell line, mouse 3T3 fibroblasts in culture were tested with sheep erythrocytes alone (E) and with EAC14b, EAC14b23b, EAC(C5 def) and EAC3d. No rosettes were detected with any of these particles.

Discussion

We have demonstrated the presence of CR1 and CR2 receptors on glomerular epithelial cells in culture. As the rodent glomerulus is a composite of several cell types, glomerular cells in culture require precise identification. Four cell types can potentially grow out of isolated, decapsulated glomeruli in culture: mesangial cells, Ia positive monocytes [12], endothelial cells, and visceral epithelial cells. Evidence that the cells which we have employed to detect C3 receptors are glomerular visceral epithelial cells includes the following: (i) the polygonal shape of the cells, tightly packed arrangement with cobblestone appearance of the cell monolayer; the presence of copious microvilli and tight junctions are well accepted morphological criteria for epithelia in culture [13]. The mesangial cells [5], endothelial cells [14], and monocytes [15] do not share these morphological features when studied in culture. (ii) The epithelial cells clearly proliferate and increase in number as duration of culture is prolonged whereas monocytes are not known to replicate in in vitro culture conditions [16]. (iii) The epithelial and mesangial cells are the predominant cell type at different times in glomerular culture [5, 6]. Our observations confirm that the cells are mostly epithelial during the first week, whereas they are largely overgrown by mesangial cells by the third week. This type of cell growth is in accordance with the mosaic theory of growth of explanted tissue [17]. Our experiments with C3 receptors were conducted only between days four to eight when the cell type was predominantly epithelial. (iv) Whereas the human GEC possess C3 receptors there are important differences in the expression of this receptor on other glomerular cell types [18]. The mesangial cells do not possess these receptors [5, 6, 18]. Receptors for C3 have not been described with unstimulated endothelial cells in culture, and the endothelial cells are further characterized by the presence of factor VIII antigen on their surface. The other glomerular cell type which possesses C3 receptors is the monocyte and the GEC can be distinguished from these cells on the basis of morphology negative nonspecific esterase reaction and ability to proliferate in culture. Even though monocytes and endothelial cells were present in primary culture, numerically they formed a minority of the total cell population and could not account for the number of cells displaying rosettes (around 60% of total cell number).

The existence of C3 receptors on cultured rat glomerular epithelial cells has been controversial [5, 6]. Our findings confirm and extend the observations of Kreisberg, Hoover and Karnovsky who were the first to demonstrate C3 receptors on

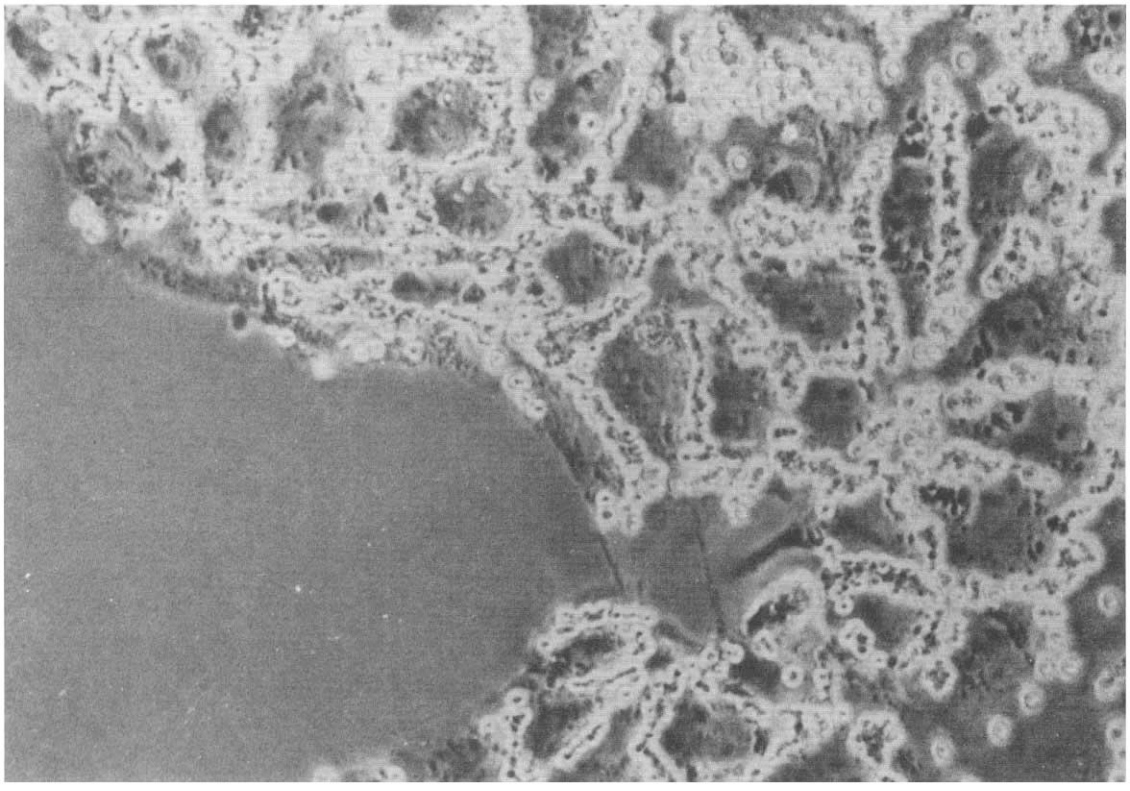

Fig. 7. *The C3 ligand carrying sheep RBC surround each individual glomerular epithelial cell at the edge of a colony.* The receptors appear to be generally distributed along the cell borders (×352, phase contrast).

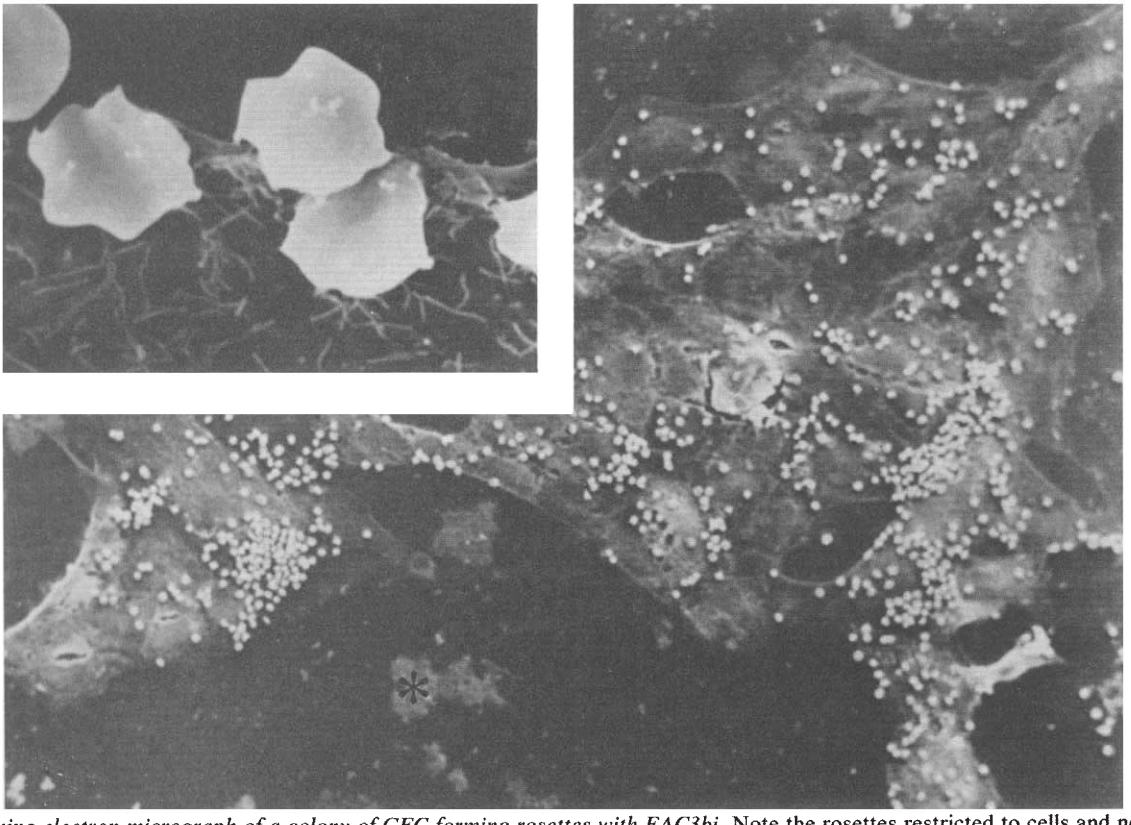

Fig. 8. *Scanning electron micrograph of a colony of GEC forming rosettes with EAC3bi.* Note the rosettes restricted to cells and not binding to cell debris (asterisks). The number of RBCs binding to each cell varies between 15 to 30 (×235). The inset shows higher magnification of an area of an individual GEC binding C3 bound RBC. Note the microvilli (×3500).

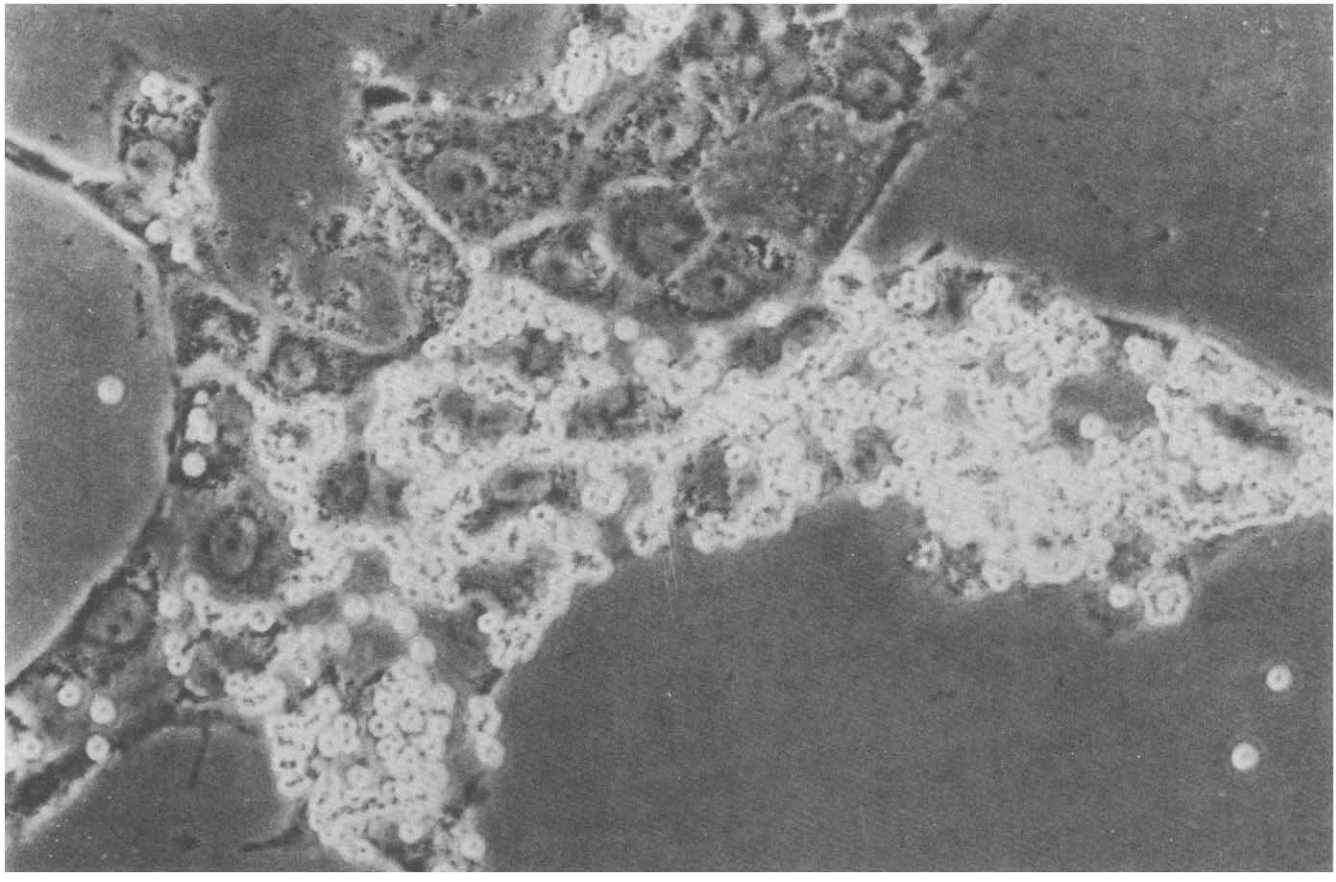

Fig. 9. *The EAC3d have formed rosettes with GEC on day five of primary culture (×400, phase contrast).*

rat GEC in culture [5]. They described rosette formation with EAC which were prepared using C5-deficient mouse serum as the source of C3. As the form of C3 deposited on EAC by this method maybe C3bi and/or C3d, and as C3bi can bind with both CR2 and CR3 types of C3 receptors, the specific receptor detected by these investigators is not clear. Thus, while C3 receptors were shown to be present on rat GEC in culture, the nature and ligand specificity of these receptors remained unknown. Foidart et al were unable to detect C3 receptors on either of the two types of rat glomerular cells in culture [6]. They employed human or mouse serum as a source of C3. The reasons for their inability to detect C3 receptors on cultured rat GEC are not clear. The ability to demonstrate the C3 receptors can depend upon the source of complement employed in the assay. Striker, Killen and Farin have reported that C3b receptors can be detected on human GEC in culture when fetal calf serum is used as the source of complement, but not when the complement source is pooled human serum [18]. The inability of human serum to be used as a source of C3 ligands has been attributed to the presence of C3 fragments which have been shown to inhibit EAC rosette formation in other cell systems [19].

By employing established protocols that deposit specific ligands C4b and C3b on the indicator sheep erythrocytes, we were able to demonstrate the presence of CR1. These ligands have been used to detect CR1 on human glomeruli and human mononuclear cells, both of which possess that receptor [8]. In addition, rosette formation with EAC3d demonstrated the CR2 receptor, which specifically binds the C3d fragment. Employment of appropriate "positive" control for CR2 (Daudi lymphoblasts) and "negative" control (mouse peritoneal macrophages) further confirmed the ability of ligands prepared (EAC3d) to detect CR2. Adherence of EA-IgM to GEC was observed and could represent a mixed agglutination reaction rather than presence of receptor for IgM. Shared antigens between sheep RBC against which the IgM antibody is directed and rat GEC could lead to the same results as if Fc receptors for IgM were present. Pre-incubation of GEC with IgM resulted in abolition of the adherence of EA-IgM to GEC indicating the binding sites are saturable and C3 receptors demonstrable following saturation.

Despite our own results and the reports of Kreisberg, Hoover and Karnovsky [5], it has not been possible to similarly demonstrate the receptors on intact rat glomeruli [1, 4]. We were also unable to detect the receptors in glomeruli in frozen sections of rat kidney biopsy. It appears that conditions that exist in in vitro cell culture unmask or enhance the expression of C3 receptors or may remove some in vivo inhibitory influence. Factors peculiar to in vitro culture may also have resulted in the patchy distribution of the receptors in a given colony of GEC. The distribution was more uniform when the individual cells were examined, the receptors being circumferentially located along the plasma membrane of the GEC. This is consistent with the distribution of the CR1 on individual human GEC described by Kazatchkine et al [20]. C3 receptor expression is not a nonspecific property of culture conditions unre-

lated to the cell type. For example, C3 receptors are not present on 3T3 fibroblasts in culture.

The presence of CR1 on cultured GEC implies that they are capable of immune adherence. Because of their anatomic location, it appears unlikely that GEC may participate in trapping immune complexes circulating in glomerular capillaries. The complement receptors may aid in the removal of previously deposited, adjacent subepithelial immune complexes. Several other functions have been attributed to CR1 in other cell systems, some of which may be relevant to GEC. The CR1 is known to serve as a cofactor in the degradation of C3b to C3bi by factor I [21], C3bi to C3dg [22] and promote decay of bound C4b [23]. It may thus perform an autoregulatory function on events that follow the occupation of CR1 on GEC.

In an attempt to determine whether the density of C3 receptors can relate to disease predisposition or severity, several studies have tried to correlate the presence of C3-containing immune complexes on human renal biopsies with the density of C3 receptor, with conflicting results [20, 24–27]. Some authors have found decreased C3 receptor activity in diseases associated with subepithelial immune complex deposition, such as membranous nephropathy [24–26]. Conceivably, the decreased density of C3 receptors on GEC could predispose the glomerulus to the development of subepithelial immune deposits because of a diminished ability of the GEC to carry out receptor-specific endocytosis.

While there has been controversy in the literature regarding the existence of C3 receptors in rodent glomeruli, our results demonstrate the presence of both CR1 and CR2 receptors on epithelial cells growing from rat glomeruli in primary culture. Despite the difficulty demonstrating the C3 receptors in vivo, the rodent GEC may be capable of expressing these functional structures under appropriate in vivo conditions.

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