A RECEPTOR FOR THE THIRD COMPONENT OF COMPLEMENT IN THE HUMAN RENAL GLOMERULUS

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In the course of studying the nature of mononuclear cellular infiltrates in tissue sections of human kidney it was noted that indicator sheep erythrocytes densely coated with the third component of complement (C3) specifically adhered to all of the glomeruli in the tissue sections. The deposition of complement (C) within the glomerulus is a feature of many immunologically related renal diseases (1, 2), yet the precise mechanism by which C is deposited remains unexplained. We feel that this observation, suggesting the presence of a receptor for C, is, therefore, of particular interest.

Materials and Methods

Buffers consisted of veronal-buffered saline with added Ca++, Mg++, and 0.15 % gelatin (VBS) and a low ionic strength buffer of dextrose in VBS with added Ca++, Mg++, and gelatin (0.065 %) (DVBS) (3).

Sheep erythrocytes (E) were sensitized with highly purified rabbit IgM anti-Forssman antibody as previously described (4). In experiments with erythrocytes exposed to fresh mouse serum, 5 x 10^6 cells/ml were incubated with a 1/10 dilution of CDF, mouse serum for 30 min at 37°C to form EAC. These cells were then thoroughly washed.

Cellular intermediates in the C system were prepared by either of two methods using components of guinea pig or human C. In each case C4, C2, and C3 were either all of guinea pig or all of human origin. Either EA or EAC4 (max <5 min) were exposed to partially purified guinea pig C1 (1,000 site-forming units (SFU)/cell) to form EAC1 or EAC14, respectively, and then were washed with DVBS (3).

Washed EAC1 were mixed with partially purified human or guinea pig C4 (Cordis Laboratories, Miami, Fla.), 100 SFU/cell, and incubated at 37°C for 30 min. The mixture was brought to 30°C. Human or guinea pig C2 (100 SFU/cell) and human or guinea pig C3 (Cordis Laboratories) (2-200 SFU/cell) were added. These mixtures were incubated at 30°C for 1 h and then washed twice in DVBS.

EAC14 were mixed with 100 SFU/cell of C2 (human or guinea pig). After 10 min at 30°C, C3 was added (100-1,000 SFU/cell). The mixtures were reincubated at 30°C for 1 h. EA which had been exposed to C1, C4, C2, and C3 by either method were coated with C3b as evidenced by a positive immune adherence reaction and by their agglutination by an anti-C3b antiserum.

To cleave cell-bound C3b these cells were exposed to a source of the C3b inactivator, either whole serum heated at 56°C for 30 min or the partially purified human C3b inactivator (Cordis Laboratories). Equal volumes of EAC1423b (1.5 x 10^6 cells/ml in DVBS) and undiluted heated serum or undiluted partially purified inactivator were combined. After 1-2 h at 37°C the cells were washed and resuspended to a density of 1.5 x 10^6 cells/ml. These cells were tested by immune adherence and were only used when this reaction was negative. Representative samples of these cells had surface C3d as indicated by agglutination with anti-C3d antibody. Attempts were made...
to block the adherence of C3b-coated erythrocytes with partially purified C3b. EAC14 prepared with guinea pig reagents were mixed with excess partially purified guinea pig C2 and C3 and incubated for 2 h at 37°C. Under these conditions erythrocyte-bound C3 convertase is formed and cleaves C3 into its two major fragments, C3a and C3b. A portion of the C3b attaches to EAC14 to form the cell intermediate EAC1423b. The majority of the C3b remains in the supernatant fluid as fluid-phase C3b. The resulting supernate was used as the source of guinea pig C3b. As a control in which no C3b is generated, partially purified C2 and C3 were mixed and incubated in the absence of EAC142.

To determine the degree of adherence between the erythrocyte reagents and tissue sections, 8 μm frozen sections of unfixed tissue were cut with a cryostat and allowed to air dry. The sections were layered with the various indicator erythrocyte reagents (1.5 x 10⁶ cells/ml) and incubated in a moist chamber at room temperature for 15 min. The slides were then washed in phosphate-buffered saline to remove nonadherent erythrocytes. The resultant preparations were fixed for 15 min in Perfix (Applied Bioscience, Patterson, N. J.), stained with Giemsa, and examined by light microscopy (5, 6).

Results

Sheep erythrocytes (SRBC) showed no binding to human renal tissue sections. Similarly, SRBC coated with only IgM anti-Forssman antibody showed no adherence (Fig. 1 a). In contrast, SRBC coated with IgM anti-Forssman antibody which were then incubated in fresh mouse serum as a source of C, showed specific glomerular localization (Figs. 1 b and c). Each of 12 autopsy specimens examined was positive and demonstrated binding to virtually all glomeruli present in the section. No binding was observed to interstitial structures. Autopsy specimens were obtained between 3 and 36 h after death and were frozen in O. C. T. embedding compound (Ames Co., Div. of Miles Lab., Inc., Elkhart, Ind.) at -40°C for subsequent evaluation. The ages of the patients at the time of death ranged from 1 day to 82 yr. None of the patients died as a result of renal disease.

EA treated with mouse serum incubated at 56°C for 30 min showed no renal glomerular adherence, suggesting that the presence of activated C on the reagent erythrocyte was required for adherence. To verify that the heat-labile serum component was indeed C, a series of erythrocyte reagents were prepared with partially purified human or guinea pig C components and their adherence was examined. In these experiments no binding was observed with erythrocytes coated with IgM antibody and the first (C1) and 4th (C4) components of C. However, the addition to the EAC14 cells of C2 plus C3 of guinea pig or human origin led to specific glomerular localization (Table I) of these cells. Indicator cells prepared under these conditions have large amounts of C3 on their surface and this is an essential requirement for binding.

The next experiments were performed to determine which fragment of C3 was required for glomerular binding. EA reacted with purified C1, C4, C2, and C3 are known to have deposited C3b on their surface (EAC1423b) (7). In the presence of heated serum (a source of C3 inactivator) or partially purified C3 inactivator, C3b is cleaved to C3c and C3d; the latter remains bound to the indicator cell surface (8, 9). EAC1423b-coated cells were exposed to either heated (56°C, 1/2 h) serum or partially purified human C3 inactivator for 1–2 h at 37°C. As shown in Table II, these cells (EAC1423d) demonstrated no specific glomerular localization, although such cells could be shown to be heavily coated with C3d using specific anti-C3d antibody.
Characterization of C Components Required for Glomerular Binding

| Indicator cells       | Binding |
|-----------------------|---------|
| E                     | 0       |
| IgMEAC                | 0       |
| IgMEAC1               | 0       |
| IgMEAC14              | 0       |
| IgMEAC1423            | +++     |

Indicator SRBC coated with IgM anti-Forssman antibody were prepared with partially purified guinea pig C components after which they were layered on frozen sections of human kidney and their binding to glomeruli evaluated.

Determination of the C3 Fragment Required for Binding

| Preincubation of Indicator IgMEAC1423b with: | Preincubation of kidney sections | Binding to glomeruli |
|---------------------------------------------|---------------------------------|---------------------|
| None                                        | None                            | +++                 |
| Heated serum                                | None                            | 0                   |
| C3 inactivator                              | None                            | 0                   |
| None                                        | Soluble C2 plus C3              | + +                 |
| None                                        | Soluble C3b                     | 0                   |

IgMEAC1423b were incubated with heated serum (56°C for 30 min) or partially purified C3 inactivator. These reagents were layered on frozen sections of kidney which were untreated or preincubated with either soluble C2 and C3 (containing little C3b) or C3b. The binding of the erythrocyte reagents to the glomeruli was then evaluated.

These results suggest that C3b is required for glomerular localization of the indicator erythrocytes. To further demonstrate the specificity of the glomerular C3 receptor for C3b, an attempt was made to inhibit the adherence of C3b-coated inciator SRBC by prior incubation of the kidney section with soluble C3b. As shown in Table II, prior incubation for 1/2 h with soluble C2 + C3 (containing little C3b) failed to block subsequent binding of C3b-coated cells. However, prior incubation with a soluble C3b almost completely blocked subsequent binding of EAC or C3b-coated indicator cells.

The species specificity of the required C was explored. C obtained from all species tested including mouse, human, and guinea pig was effective in mediating this reaction. We next investigated the pathway requirement for C3 deposition. In the experiments reported above, EAC1423b were formed by classical pathway activation. The adherence of indicator particles coated with C3b via activation of the alternate pathway was also demonstrated using a technique.

Fig. 1. (a) Portion of a glomerulus incubated with IgMEA cells. No adherent indicator IgMEA erythrocytes are present. × 100. (b) Portion of a glomerulus treated with IgMEAC1423b; interstitial areas including tubules are negative. Adherent indicator erythrocytes are present selectively over glomeruli. A similar result was observed when whole mouse serum was used as a source of C. × 100. (c) A portion of a glomerulus and surrounding tissue treated with IgMEAC1423b. Adherent indicator erythrocytes are present over glomerulus on the left. Tubule on right shows no adherent indicator erythrocytes. × 400.
not reported in detail here (10). In brief, fluorescent bacteria were incubated in normal human serum as a source of alternate pathway components. Under these conditions, the bacteria spontaneously activate C3 via the alternate pathway and the C3b can be shown to coat the bacterial cell wall. Such fluoresceinated C3b-coated bacteria adhered to the glomeruli. Moreover, fluoresceinated bacteria incubated in heat-inactivated serum did not. Thus, C3b-coated indicator cells adhered selectively to the glomerulus whether activated via the classical or the alternate pathway. Preliminary attempts to demonstrate a C3 receptor in the glomeruli of rats, mice, guinea pigs, rhesus monkeys, rabbits, and dogs using similar reagents and techniques were unsuccessful.

Discussion

In this communication we have demonstrated that indicator erythrocytes bearing the activated third component of complement (C3b) are bound selectively to human renal glomeruli in tissue sections. Indicator cells not containing C3b are not bound. In addition, prior incubation of the tissue section with soluble C3b almost completely blocks subsequent adherence of indicator cells containing C3b. Binding of SRBC coated densely with C3b was observed in all apparently undiseased kidney autopsy specimens examined, including that from a 1-day old child. C3b-coated SRBC have also been shown to bind to splenic B-cell germinal centers in tissue sections (5), however glomerular localization of C3b-coated SRBC in tissue sections requires far more erythrocyte-bound C3b than does splenic germinal center localization (M. C. Gelfand, M. M. Frank, and I. Green, unpublished observations). The exact location of the C3 receptor within the human glomerulus is not yet known, however, preliminary observations suggest a localization on the surface of the endothelial cell (Raymond Nagle, unpublished observation).

The importance of the demonstration of such a receptor for C in the human glomerulus lies in its potential elucidation of the pathogenesis of immune complex deposition in glomeruli. A feature of immune complex glomerulonephritis is the deposition of C-contained complexes in the glomerulus leading to C-mediated immune injury (1, 2). The present finding suggests that a possible mechanism for this is the selective fixation of C3b-bearing immune complexes to the glomerular C receptor. Thus, antigen-antibody complexes containing C3b activated via the classical pathway (analogous to EAC) or complexes containing C3b produced by alternate pathway activation (analogous to C3-coated fluorescent bacteria) may be selectively localized within the glomerulus rather than in other capillary beds.

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