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Immunology

The C1q subunit of the first component of complement binds to laminin: A mechanism for the deposition and retention of immune complexes in basement membrane

(connective tissue/glomerulonephritis/vasculitis)

JOHN F. BOHNSACK*, ANDREA J. TENNER*, GORDON W. LAURIE†, HYNDA K. KLEINMAN†, GEORGE R. MARTIN†, AND ERIC J. BROWN*

*Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases; and †Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20205

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ABSTRACT **The C1q subunit of complement component C1 is known to bind to immune complexes, which often are deposited in basement membrane. We investigated the possibility that this deposition is a result of binding to laminin, a large basement membrane glycoprotein. C1q showed saturable binding to immobilized laminin; this binding was increased at reduced ionic strength. Intact C1 did not bind laminin. A ternary complex was formed by laminin, C1q, and aggregated IgG. This complex formation was dependent on and proportional to the amount of C1q bound to the aggregated IgG. Binding of laminin to C1q occurred with a K_d of 2 nM and was stronger than the binding of C1q to fibronectin. Preliminary data, including electron micrographs of rotary-shadowed preparations, suggest that laminin binds to the collagen-like tail of C1q. Electron microscopy localized the site of interaction with C1q to a short arm of laminin. Since laminin is found only in basement membranes, the interaction between laminin and C1q could be involved in the deposition and retention of immune complexes in these structures.**

Basement membrane is a thin matrix that lines epithelia and endothelia in addition to surrounding muscle cells and the whole nervous system. Basement membranes are frequent sites of immune-complex deposition, which could be due to an affinity of immune complexes for some as yet unidentified basement membrane component. Laminin, a major and specific component of basement membrane, is a large glycoprotein ($M_r \approx 10^6$). It was originally isolated from the basement membrane of a mouse tumor (1) but has since been found by immunocytological techniques in all basement membranes investigated (2). Purified laminin, which binds to basement membrane collagen (type IV) and basement membrane heparan sulfate (3), can mediate the attachment of a variety of cell types to type IV (basement membrane) collagen substrates (4). Fibronectin is another connective tissue glycoprotein that is also associated with basement membranes, though in lesser amounts. Unlike laminin, fibronectin is also present in fibrous connective tissues (2). Fibronectin is also found in high concentrations in the circulation (≧300 μg/ml), whereas there is little or no circulating laminin (<1 μg/ml). Fibronectin binds to diverse substances including collagens I–IV, glycosaminoglycans, and receptors on certain cell surfaces (5).

The binding of immune complexes to a basement membrane component may occur via C1q, since C1q binds to immune complexes and its structure includes a collagenous tail (6). C1q exists in plasma as a subunit of the complex macromolecule C1, the first component of complement. C1 consists of C1q and the tetrameric complex $C1r_2s_2$ of the proenzymes C1r and C1s. In the presence of Ca^{2+}, C1r and C1s bind to the collagenous region to form hemolytically active C1 (7). Binding of C1 to the Fc portion of IgG or IgM in an antigen–antibody complex, which occurs via the globular domains of C1q (8), initiates the classical pathway of complement activation through conversion of C1r and C1s to their active-enzyme forms, $C1\bar{r}$ and $C1\bar{s}$. After activation, $C1\bar{r}$ and $C1\bar{s}$ are displaced from C1q through the action of C1-inhibitor (9), leaving C1q bound to the immune complex with its collagen-like tail exposed for interaction with other molecules. Fibronectin has been shown to bind directly to C1q (10, 11) and to C1q-bearing immune complexes *in vitro* (12).

In this report, we demonstrate a high-affinity interaction between C1q and laminin. Further, C1q can mediate the binding of aggregated IgG, a model immune complex, to laminin. Addition of excess purified $C1\bar{r}_2\bar{s}_2$ competitively inhibits this interaction of laminin with C1q-bearing immune complexes, suggesting that laminin interacts with the collagen-like region of C1q.

MATERIALS AND METHODS

Protein Purification and Iodination. Laminin (13), C1q (14), human IgG (15), and fibronectin (16) were purified as described. IgG was aggregated by heat treatment at 63°C for 30 min and the large aggregates (≧1.8×10^6 daltons) (aggIgG) obtained from the void volume of a Bio-Gel A-5m column were stored in 0.145 M NaCl/5 mM sodium phoaphate, pH 7.2 (P_i/NaCl) at 4°C until used. $C1\bar{r}_2\bar{s}_2$ was obtained by depleting human C1 of C1q (17); this preparation contained about 430 μg of $C1\bar{r}_2\bar{s}_2$ and 7 μg of C1q per ml, as determined by hemolytic titration. Bovine serum albumin (BSA) and human serum albumin (HSA) were purchased from Sigma.

C1q was radiolabeled with $Na^{125}I$ (specific activity of labeled C1q, 5.4×10^5 cpm/μg) as described (14). Laminin, fibronectin, and aggIgG were iodinated with $Na^{125}I$ to specific activities of 7.7×10^5, 5.4×10^5, and 3.2×10^6 cpm/μg, respectively, by using Iodobeads (Pierce).

Preparation of Protein-Coated Surfaces. Wells of Immulon II Remov-a-well strips (Dynatech, Alexandria, VA) were coated overnight at 4°C with protein in 0.1 M $NaHCO_3$, pH 9.5 (coating buffer; 100 μl per well). Laminin- or BSA-coated plates were prepared using the protein at 50 μg/ml in coating buffer; for aggIgG-coated wells, the protein concentration was 90 μg/ml. Immediately before use, the wells were washed three times with P_i/NaCl containing 0.05% Tween 20 (Fisher) (P_i/NaCl/Tween). For experiments done at low ionic strength, the wells were washed with 0.1 M sodi-

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Abbreviations: C1, first component of complement, consisting of subunits C1q, C1r, and C1s; aggIgG, aggregated IgG; BSA, bovine serum albumin; HSA, human serum albumin; ^{125}I-, ^{125}I-labeled.

um acetate, pH 7.4, containing 0.05% Tween 20 (NaOAc/Tween). In both cases, the wells were incubated in wash buffer containing 1% BSA for 30 min at room temperature to quench their remaining nonspecific binding sites.

Binding Assays. Radiolabeled aggIgG, C1q, or laminin, diluted in either physiologic (P_i/NaCl; conductance = 8.5 mS) or low ionic strength buffer (NaOAc; conductance = 3.7 mS), were incubated in Immulon II wells coated with various proteins for 2 hr at room temperature in the presence of 1% BSA. To quantitate association, wells were washed extensively after the incubation, and bound radioactivity in individual wells was determined in a gamma counter. In some experiments, unlabeled C1q was substituted for radiolabeled C1q, and the amount of C1q bound after 2 hr was determined by ELISA. This ELISA assay is capable of detecting from 10 to 150 ng of human C1q per ml (15).

Each binding curve was determined in duplicate in all experiments, and data are presented as the mean of duplicates (which always agreed within 10%) of representative experiments. Further details of individual experiments are given in the text.

Electron Microscopy. Rotary-shadowed replicas of aggregates of C1q and laminin were examined in the electron microscope according to the method of Shotton *et al.* (18). Briefly, equal volumes of C1q and laminin at 30 μg/ml in P_i/NaCl were mixed together, dialyzed overnight at 4°C vs. 0.155 M ammonium acetate at pH 7.4, sprayed in 50% glycerol onto freshly cleaved mica discs, and rotary-shadowed with platinum at 2 mPa in an Edwards 306A coater. The replica was examined in a JEOL 100C electron microscope.

RESULTS

Binding of C1q to Laminin. Several methods were used to study possible interactions between C1q and laminin. In some studies, wells were coated with laminin and incubated with either labeled or unlabeled C1q. Using an ELISA to measure binding, we found that C1q showed saturable binding to laminin and no binding to uncoated wells under conditions of physiologic ionic strength (Fig. 1*a*). Similar results were obtained with ^{125}I-labeled C1q (^{125}I-C1q) (Fig. 1*b*). The binding of ^{125}I-C1q was measured both in P_i/NaCl and in NaOAc buffer. Binding of the labeled ligand to laminin was observed under both conditions but was about 5-fold greater at the lower ionic strength.

Electron microscopic examination of mixtures of C1q and laminin after rotary shadowing showed complexes between the two. Under these conditions, the globular heads of C1q could be well visualized, but the stalk was not well defined. The majority of interactions clearly involved a short arm of laminin, while it is less clear which domain of the C1q molecule was involved in the interaction (Fig. 2 and Table 1).

Binding of Laminin to aggIgG via C1q. To test the ability of IgG-bearing immune complexes to bind to laminin and the ability of C1q to enhance this interaction, ^{125}I-aggIgG was incubated in wells coated with laminin or albumin. Under these conditions, little or no label bound to the wells (Fig. 3). However, in the presence of C1q, there was a concentration-dependent binding of aggIgG to laminin that appeared to

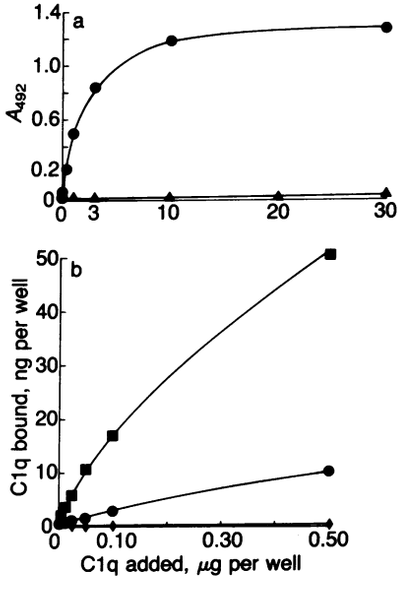

FIG. 1. Soluble C1q binds to solid-phase laminin. (*a*) C1q binding to laminin-coated wells (●) at physiologic ionic strength (P_i/NaCl/Tween/1% BSA) was determined by ELISA (15) at various inputs of C1q. Uncoated plates to which C1q was added (▲) had the same A_{492} as wells without C1q. (*b*) ^{125}I-C1q bound to laminin-coated wells was measured after a 2-hr incubation in P_i/NaCl/Tween/1% BSA (●) or NaOAc/Tween/1% BSA (■). Binding of C1q to laminin-coated wells occurred at physiologic ionic strength (P_i/NaCl), but was markedly increased at low ionic strength (NaOAc). No significant binding of C1q to uncoated plates was detected (◆).

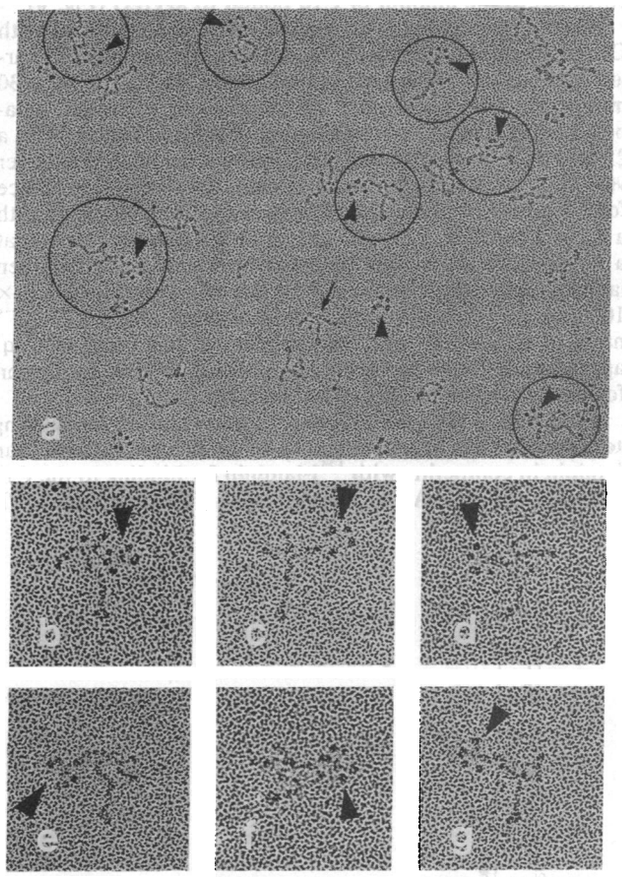

FIG. 2. Electron micrographs of C1q–laminin aggregates. Equal amounts of laminin (30 μg/ml) and C1q (30 μg/ml) in 50% glycerol were sprayed onto mica discs and rotary-shadowed. The replicas were examined in the electron microscope. (*a*) Representative field at lower power showing aggregates (circled) of C1q bound to laminin; within each circle, the bound C1q is indicated by an arrowhead. Near the center of the field is a free laminin molecule (arrow) and beside it is a free C1q molecule (arrowhead). The binding of C1q to laminin usually occurs through a short arm of laminin (see Table 1). (×64,000.) (*b–g*) Higher-power electron micrographs of binding of C1q (arrowhead) to the short arm of laminin. The portion of C1q that binds laminin is not clear; often the heads may be seen but not the tail. In *d* and *g*, the tail seems to be bound to laminin. (×120,000.)

Table 1. C1q binding site on laminin, as determined by electron microscopy

Site of interaction on laminin*	% complexes containing interaction†
Long arm	7
Short arm‡	93
Top short arm	16
Lateral short arm	43

*As diagrammed in ref. 24.
†Forty-two C1q–laminin aggregates were counted.
‡Denotes total of "top" and "lateral" short arms in addition to short arms that could not be identified clearly as either.

reach saturation. The presence of C1q caused no increase in the binding of ^{125}I-aggIgG to albumin-coated wells.

The binding of ^{125}I-laminin to aggIgG-coated wells was then measured. Because it was known that ionic strength affects the C1q–IgG interaction, these studies were carried out in NaOAc buffer. In a parallel experiment, we measured the binding of C1q to the immobilized aggIgG and found that concentrations of C1q $\geqq$ 1 μg per well caused maximal binding to the antibody aggregate. The binding of ^{125}I-laminin to the aggIgG-coated wells was dependent on C1q and was proportional to the amount of C1q bound to aggIgG (Fig. 4).

Next, wells coated with aggIgG were preincubated with C1q to produce C1q-bearing model immune complexes. Various amounts of unlabeled laminin were added to the wells 30 min prior to the addition of ^{125}I-laminin. As expected, unlabeled laminin inhibited the binding of labeled ligand, with a 25-fold excess of unlabeled laminin reducing binding by over 90% (Fig. 5). A Scatchard plot of these data yielded evidence for a high-affinity interaction between C1q and laminin with a K_d of 2 nM (Fig. 5 *Inset*). This analysis also suggested that a second, much weaker interaction could occur between laminin and some component of the mixture. Since 3.8×10^{-5} nmol of laminin bound at saturation, and 1.9×10^{-4} nmol of C1q were bound to the plate at this input of C1q, approximately one molecule of laminin bound at saturation for every five molecules of bound C1q.

Competition Between Fibronectin and Laminin for Binding to C1q. Fibronectin was found to be much less effective than laminin in competing with ^{125}I-laminin for binding to the C1q complex (Fig. 5). A 200:1 molar ratio of fibronectin to ^{125}I-laminin was required to cause a 50% reduction in laminin binding to the C1q complex. Under similar conditions, a 50% reduction of ^{125}I-fibronectin binding to C1q–aggIgG required only a 1.5:1 molar ratio of laminin to fibronectin (not shown). These results suggest that the affinity of C1q bound to an immune complex is lower for fibronectin than for laminin. We also directly compared the binding of labeled laminin and fibronectin to aggIgG containing various amounts of C1q. This study showed (Fig. 6) that the binding of fibronectin was increased by added C1q in a manner similar to laminin. However, the binding of laminin increased 6-fold, whereas only a 2-fold increase in fibronectin binding was seen under the same conditions, and maximal binding on a molar basis was greater for laminin than for fibronectin, even though the input of fibronectin was more than five

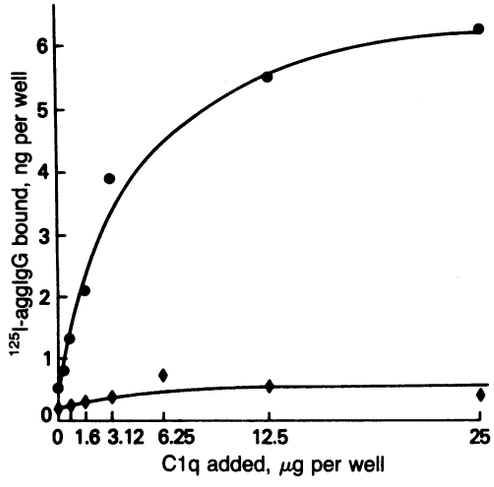

FIG. 3. C1q mediates binding of aggIgG to laminin-coated wells. In each well, 88 ng of ^{125}I-aggIgG in P_i/NaCl/Tween/1% BSA was incubated in laminin- (●) or BSA-coated (♦) wells. Bound ^{125}I-aggIgG was determined after a 2-hr incubation by measuring radioactivity in washed, individual wells with a gamma counter. Addition of C1q to the incubation mixtures enhanced binding of aggIgG to wells coated with laminin but not to wells coated with BSA.

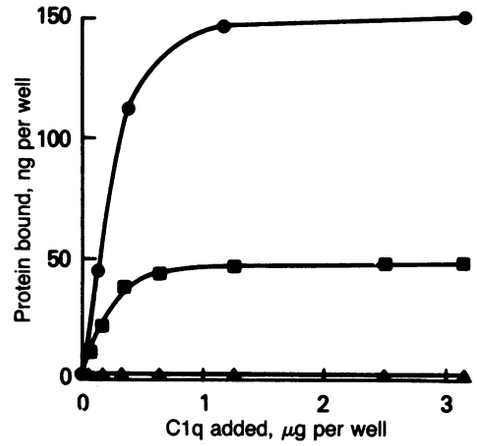

FIG. 4. Laminin binds to solid-phase aggIgG via C1q. Various concentrations of C1q were incubated in NaOAc/Tween/BSA for 2 hr in wells coated with aggIgG; they were then washed four times, leaving bound C1q-bearing model immune complexes. Then, 400 ng of ^{125}I-laminin in NaOAc/Tween/BSA was added to each well and incubated for 2 hr before the bound radioactivity was determined (■). ^{125}I-laminin did not bind to uncoated plates even when C1q was preincubated in the uncoated plates (▲). The amount of C1q bound to the aggIgG-coated plate (●) was determined in a separate experiment in which labeled C1q was used.

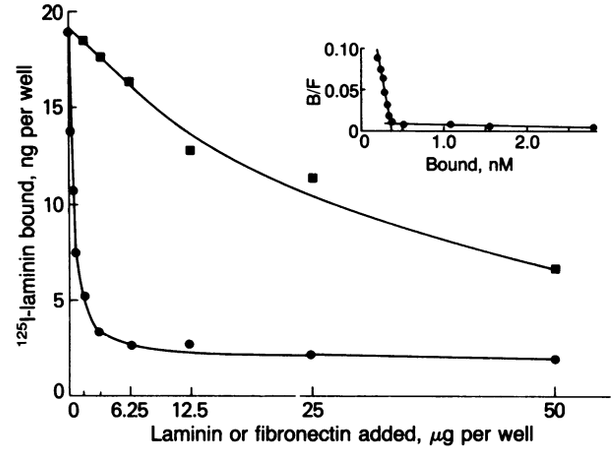

FIG. 5. Inhibition of binding of laminin to C1q by excess laminin or fibronectin. After 250 ng of C1q had been incubated for 2 hr on solid-phase aggIgG, unbound C1q was removed by washing. Then, unlabeled laminin (●) or fibronectin (■) at various concentrations was incubated in these wells for 30 min. Finally, 245 ng of ^{125}I-laminin was added to each well, and the incubation was continued for 2 hr more. (*Inset*) Scatchard plot of laminin binding to C1q-bearing immobilized model immune complexes. The apparent K_d of soluble laminin and C1q under these conditions is 2 nM (r = 0.98). B/F, bound/free.

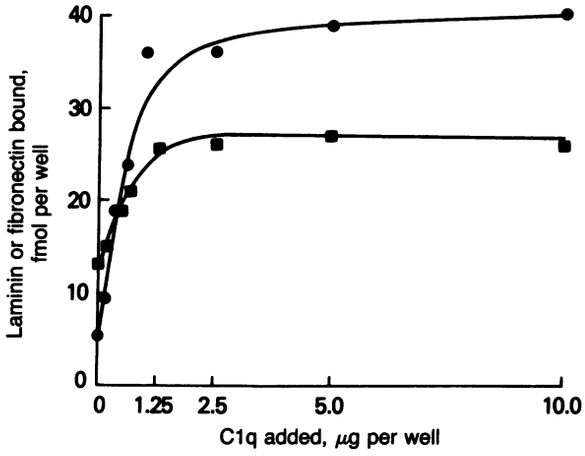

FIG. 6. Comparison of binding of ^{125}I-fibronectin and ^{125}I-laminin to C1q-bearing immobilized model immune complexes. As described in the legend to Fig. 4, various concentrations of C1q were incubated in aggIgG-coated wells for 2 hr before unbound C1q was removed by washing. Then, 0.42 fmol of ^{125}I-laminin (●) or 2.3 fmol of ^{125}I-fibronectin (■) in 100 μl of buffer was added. Bound radiolabeled protein was determined after a 2-hr incubation.

times that of laminin. Thus, the binding of laminin to C1q is stronger than the binding of fibronectin under these circumstances.

Because the possibility existed that the BSA used in the binding buffers might be contaminated with small amounts of plasma bovine fibronectin or its proteolytic cleavage products, and because the polyclonal antibodies used in our fibronectin ELISA do not recognize bovine fibronectin, we performed the same experiments with 1% HSA in the buffer. The binding curves of laminin and fibronectin binding to C1q–aggIgG were not affected whether the buffer contained BSA or HSA. The preparation of HSA contained no plasma fibronectin or immunoreactive fibronectin fragments, as determined by ELISA. Thus, the poorer binding of fibronectin to C1q–aggIgG could not be explained by competition for C1q binding by fibronectin or fragments of fibronectin in either the BSA or HSA.

Inhibition by $C1\overline{r_2s_2}$. The effect of the $C1\overline{r_2s_2}$ complex on the binding of laminin to C1q was tested. Wells were coated with aggIgG and then were incubated with sufficient C1q to allow about 50% maximal binding. Unbound C1q was removed and various amounts of the $C1\overline{r_2s_2}$ complex were added to the wells. Next, ^{125}I-laminin (1 μg per well) was added, and the preparation was incubated for an additional 2 hr. The prior addition of $C1\overline{r_2s_2}$ almost completely inhibited laminin binding (Fig. 7). In other experiments (data not shown), we found that $C1\overline{r_2s_2}$ did not displace labeled C1q from IgG-coated wells. These findings suggest that $C1\overline{r_2s_2}$ and laminin compete for binding to the same region of C1q, presumably the collagenous tail of the molecule.

DISCUSSION

In this paper, we present evidence for a strong interaction between laminin, a major and specific component of basement membranes, and the C1q subunit of the first component of complement. The possibility of such an interaction was suggested by the presence of C1q and immune complexes in basement membrane in certain disorders. C1q and immune complexes interact and could localize to basement membrane by binding to a component of the extracellular matrix. Since laminin is a multidomain molecule with binding sites for type IV collagen, heparan sulfate, gangliosides, sulfated sugars, and cell surface receptors, it seemed a likely candidate for binding either immune complexes or C1q.

To look for such interactions, we used purified proteins

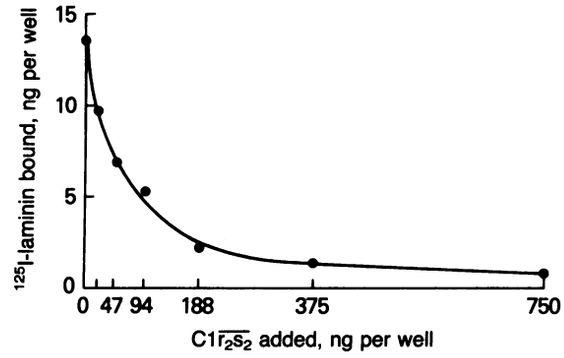

FIG. 7. $C1\overline{r_2s_2}$ competitively inhibits binding of laminin to C1q-bearing model immune complexes. In each well, 250 ng of C1q was incubated with solid-phase aggIgG for 2 hr, and unbound C1q was removed by washing. Various concentrations of $C1\overline{r_2s_2}$ in 50 μl of NaOAc/Tween/BSA then was incubated in these wells for 30 min before addition of 100 ng of ^{125}I-laminin in 50 μl of buffer and an additional 2-hr incubation.

and measured the binding of C1q to laminin-coated surfaces by the use of radioactive ligands and immunoassays. These studies showed saturable binding of C1q to laminin, which was enhanced under conditions of low ionic strength. In related studies, we studied the interaction of laminin and C1q with aggIgG. As expected, C1q bound to the aggIgG. Although no direct interaction was found between laminin and aggIgG, C1q mediated the binding of laminin to aggIgG. Using appropriate conditions of preincubation of C1q with immobilized laminin or aggIgG, we found that binding of the third ligand was proportional to the amount of bound C1q. Scatchard analysis of the data indicated that binding of laminin to C1q occurred through a single, high-affinity interaction.

Electron microscopic examination of rotary-shadowed replicas of C1q–laminin aggregates revealed that the majority of C1q–laminin interactions take place through a short arm of laminin, the site previously shown to be responsible for binding of type IV collagen (19). The location of the interacting site on C1q was not as well defined. However, since the globular heads of C1q are bound to aggIgG (8), the collagenous tail of C1q would be available for binding. Further, the $C1\overline{r_2s_2}$ complex, which binds to the collagenous tail of C1q (7), completely blocked the binding of laminin. Taken together, these data strongly suggest that laminin binds to the collagen-like tail of C1q, which is exposed only after $C\overline{1r}$ and $C\overline{1s}$ are removed from intact activated $C\overline{1}$ by C1-inhibitor.

Previous studies have shown an interaction between C1q and fibronectin, particularly under conditions of low ionic strength or after denaturation of C1q (10–12). Although only a weak interaction was found between the proteins in solution (11), it was suggested that this interaction is enhanced when either reactant is bound to a substratum. Like the fibronectin–C1q interaction, a low ionic strength buffer enhanced the interaction between C1q and laminin. C1q–fibronectin and C1q–laminin interactions are also similar in that $C1\overline{r_2s_2}$ competitively inhibited the formation of both complexes (11), suggesting that neither fibronectin nor laminin has a significant interaction with intact C1. In our experiments, fibronectin did not completely inhibit binding of laminin to C1q, whereas laminin quite readily inhibited binding of fibronectin. For our experiments, Scatchard analysis of laminin binding to the C1q-bearing model immune complexes revealed a K_d of 2 nM. Using a buffer of identical ionic composition, Bing *et al.* (10) derived a K_d of 82 nM for the interaction of fluid-phase C1q with solid-phase fibronectin. Although there are significant differences in experimental design, this 40-fold difference in K_d is consistent with the

observation that C1q binds better to laminin than to fibronectin.

Although the presence of antibody and complement components has been well demonstrated in glomerular basement membrane lesions in clinical and experimental glomerulonephritis, the factors leading to immune-complex deposition remain unresolved. C1q has been shown to exist in the glomeruli of patients with lupus nephritis in almost three-fourths of the kidney biopsy specimens studied (20, 21). C1q is typically present in the glomeruli of patients with type I membranoproliferative glomerulonephritis and is occasionally seen in acute glomerulonephritis, but it is not present in type II membranoproproliferative glomerulonephritis (22, 23). A lupus syndrome with immune-complex formation may also affect patients with C1q deficiency. Thus, it is clear that C1q is not the sole determinant of basement membrane immune-complex deposition. Nonetheless, the immune complex–C1q–laminin interaction may be a major factor in the pathophysiology of some immune-complex diseases.

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