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Immunology

Identification of the zinc-dependent endothelial cell binding protein for high molecular weight kininogen and factor XII: Identity with the receptor that binds to the globular "heads" of C1q (gC1q-R)

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ABSTRACT **High molecular weight kininogen (HK) and factor XII are known to bind to human umbilical vein endothelial cells (HUVEC) in a zinc-dependent and saturable manner indicating that HUVEC express specific binding site(s) for those proteins. However, identification and immunochemical characterization of the putative receptor site(s) has not been previously accomplished. In this report, we have identified a cell surface glycoprotein that is a likely candidate for the HK binding site on HUVECs. When solubilized HUVEC membranes were subjected to an HK-affinity column in the presence or absence of 50 μM $ZnCl_2$ and the bound membrane proteins eluted, a single major protein peak was obtained only in the presence of zinc. SDS/PAGE analysis and silver staining of the protein peak revealed this protein to be 33 kDa and partial sequence analysis matched the NH_2 terminus of gC1q-R, a membrane glycoprotein that binds to the globular "heads" of C1q. Two other minor proteins of ≈70 kDa and 45 kDa were also obtained. Upon analysis by Western blotting, the 33-kDa band was found to react with several monoclonal antibodies (mAbs) recognizing different epitopes on gC1q-R. Ligand and dot blot analyses revealed zinc-dependent binding of biotinylated HK as well as biotinylated factor XII to the isolated 33-kDa HUVEC molecule as well as recombinant gC1q-R. In addition, binding of ^{125}I-HK to HUVEC cells was inhibited by selected monoclonal anti-gC1q-R antibodies. C1q, however, did not inhibit ^{125}I-HK binding to HUVEC nor did those monoclonals known to inhibit C1q binding to gC1q-R. Taken together, the data suggest that HK (and factor XII) bind to HUVECs via a 33-kDa cell surface glycoprotein that appears to be identical to gC1q-R but interact with a site on gC1q-R distinct from that which binds C1q.**

High molecular weight kininogen (HK) is a plasma protein that is cleaved by plasma kallikrein to liberate the vasoactive peptide bradykinin (1, 2). It possess six domains, the first three homologous to cysteine protease inhibitors (2 and 3 retain inhibitory activity) a fourth domain including the bradykinin moiety, and domains 5 and 6, which interact with surfaces (domain 5) and with prekallikrein or factor XII (domain 6). HK also functions as a coagulation cofactor (3, 4) by virtue of the interactions mediated by domains 5 and 6 (5–7). In addition, HK has been shown to bind to vascular endothelial cells in a zinc-dependent reaction (8, 9) requiring interaction with kininogen domains 3 and 5 (10, 11). The isolated heavy and light chains derived from kinin-free kininogen can each interact with endothelial cells (12) because they contain domains 3 and 5, respectively, although the two sites may act cooperatively in the intact protein (13).

We have also shown that the initiating protein of the cascade, factor XII or Hageman factor, can also bind to endothelial cells with a K_m (144 nM) and zinc requirement (50 μM) virtually identical to that of HK (14). Furthermore, HK and factor XII compete for binding (K_i of 98–152 nM), which suggests interaction with a common receptor (14).

In the present report, we describe the isolation and partial characterization of the putative high molecular weight kininogen receptor from human umbilical vein endothelial cells (HUVEC). This molecule is a 33-kDa membrane protein that binds to HK or factor XII in a zinc-dependent reaction. Immunochemical and partial sequence analyses show this protein to be identical to the receptor that binds to the globular "heads" of C1q (gC1q-R) described by Ghebrehiwet *et al.* (15).

MATERIALS AND METHODS

Endothelial Cell Culture. HUVECs were isolated according to Jaffe (16) and cultured in gelatin-coated dishes in Medium 199 (GIBCO) with 20% fetal bovine serum (FBS), 20% NU-serum (Collaborative Research), 100 μg/ml penicillin, 100 μg/ml streptomycin, and 2.5 μg/ml amphotericin B. For cell-binding studies, the cells were subcultured into gelatin-coated 96-well strip plates in 0.2 ml culture medium and the cells were washed 3× in phosphate-buffered saline (PBS) to remove bound serum proteins before each experiment. The cells were identified as endothelial cells by their distinct cobblestone morphology and interaction with antiserum to Von Willebrand's factor (16). All cells were used at the third cell passage.

Preparation of the HK-Affinity Column. Purified HK (Enzyme Research Laboratories, South Bend, IN) was coupled with 3M Emphaze Biosupport Medium AB1 (Pierce) according to the manufacturer's directions. Briefly, 10 mg of HK (6 ml) was dialyzed against 0.1 M carbonate buffer, pH 9.0, and sodium citrate was added to a final concentration of 0.6 M. To this, 0.25 g of dry beads were added and mixed for 2 hr at room temperature. This mixture was poured into a column (1 × 8 cm) and washed with 10 vol of PBS. The eluate and the washings were collected to determine the coupling efficiency, calculated to be 70%. Then, 6 ml of 3 M ethanolamine, pH 9.0, was added to the column, the column was closed at both ends, and mixed end-over-end for 2.5 hr at room temperature. The

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Abbreviations: HK, high molecular weight kininogen; HUVEC, human umbilical vein endothelial cells; gC1q-R, a membrane glycoprotein that binds to the globular "heads" of C1q; MF, mature form; TF truncated form.

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column was then washed sequentially with 10 vol each of PBS, 1 M NaCl, and PBS.

Solubilization of Membrane Proteins. The confluent HUVEC monolayers were transferred to a serum-free medium (endothelial-SFM) for 24 hr, washed 3× in warm Hepes buffered saline (HBS) (10 mM Hepes/137 mM NaCl/4 mM KCl/11 mM glucose), and the cells were gently scraped into cold homogenization buffer [10 mM Hepes/150 mM NaCl/2 mM phenylmethylsulfonyl fluoride (PMSF)/1 μM aprotinin/1 μM pepstatin/1 mM EDTA/0.1% soya bean trypsin inhibitor (SBTI)] on ice using a Teflon spatula. The cell suspension was sonicated using a Heat Systems/Ultrasonics (model W225R) sonicator continuously for 2 min (output control, 4.0%; duty cycle, 60) on ice and the sonicate centrifuged at 45,000 × *g* for 1 hr. The pellet, containing the membrane fraction, was resuspended in homogenization buffer containing 1% Triton X-100 and kept at 4°C overnight with gentle mixing. This suspension was centrifuged at 45,000 × *g* for 1 hr. Alternatively, membranes were first prepared by freeze-thawing, the membranes pellets were washed and membrane protein solution was prepared using the above buffer containing 1% Emulphogene BC720 as described (15). The supernatant, containing solubilized membrane components, was collected and dialyzed against dialysis buffer (homogenization buffer without SBTI and EDTA) containing 0.1% Triton X-100. This membrane lysate was applied to the HK− affinity column and the flow through was collected. The column was washed with dialysis buffer, eluted with 0.1 M glycine·HCl, pH 2.5, and 0.5 ml fractions were collected. The column was then washed and reequilibrated with dialysis buffer containing 50 μM zinc chloride. The initial effluent was also made to 50 μM in zinc and was applied to the column. The column was again washed and eluted with 0.1 M glycine·HCl (pH 2.5). Both the eluates were neutralized to pH 7.0 by adding 30 μl of 1 M Tris·HCl (pH 9.0) to each tube. Elution was monitored by protein determination and dot-blot using biotinylated HK.

Biotinylation of Proteins. Biotinylation of proteins was performed using NHS-LC-biotin (556.58 kDa) according to the following procedure. Proteins to be labeled (0.5–1 mg/ml) were first dialyzed against 2 × 1 liter of 0.2 M $NaHCO_3$ buffer (pH 8.3). Labeling was initiated by addition of 50 μg of a 1 mg/ml solution of biotin freshly dissolved in dimethyl sulfoxide (DMSO). The reaction was allowed to proceed for 4 hr at room temperature with constant but gentle tumble mixing after which the reaction was stopped and the protein dialyzed first against 1 liter of the initial dialysis buffer, then against 2 × 1 liter PBS, pH 7.5. The degree of biotinylation was verified by ELISA using wells coated with various dilutions of the labeled protein and probing with alkaline phosphatase-conjugated streptavidin (AP-STRAV) or ExtrAvidin (AP-XTRAV).

Iodination of HK. HK was iodinated with $Na^{125}I$ by Iodobeads (Pierce) according to manufacturer's recommendations used previously (17). Following iodination, ^{125}I-labeled HK was separated on a desalting column (Econo-Pac 10DG, Bio-Rad). Specific labeling, determined by trichloroacetic acid precipitation following addition of bovine serum albumin as carrier protein, ranged from 2 to 2.5 × 10^9 cpm/mg protein. HK thus labeled did not lose its clotting activity, which was 12.5 units/mg.

Electrophoresis and Western Blot Analysis. SDS/10% polyacrylamide gel was performed using the buffer system of Laemmli (18). After electrophoresis, the gels were stained with Coomassie brilliant blue or silver stain, destained, and dried. For Western blot analysis, the separated proteins were electrotransferred to nitrocellulose membranes and were probed with mAbs or biotinylated ligands. Bound probes were visualized by alkaline phosphatase-conjugated secondary antibodies or avidin.

Dot Blot Analyses. A grid pattern containing several 1 × 1 cm squares was drawn with a soft pencil on a sheet of nitrocellulose membrane (Schleicher & Schuell). Dot blot analyses were performed by spotting 2–5 μg of proteins onto each square and air dried for 5 min at room temperature. The membrane was then incubated (1 hr, 37°C) in TBS-BSA (20 mM Tris-buffered saline, pH 7.5, containing 150 mM NaCl and 1% BSA) to block the free sites, washed 3× in TBST (TBS + 0.05% Tween 20), and each protein spot reacted with 2–3 μl of a predetermined dilution of the biotinylated ligand in question. After 1 hr at room temperature, the membrane was washed and the bound ligand probed by sequential reaction (1 hr each) first with AP-STRAV followed by a substrate solution containing 0.3 mg/ml nitroblue tetrazolium (NBT) and 0.15 mg/ml 5-bromo-4-chloroindolyl phosphate (BCIP) in color development buffer (100 mM Tris·HCl, pH 9.5/100 mM NaCl/5 mM $MgCl_2$). Before addition to the color development solution, the NBT and BCIP are kept as concentrated stock solutions in 70% and 100% *N,N*-dimethylformamide, respectively. After full color development was achieved, the reaction was stopped by first rinsing the residual precipitates from the membrane with TBS and followed by washing with TBS-E (TBS + 1 mM EDTA) for 15 min.

Proteins and Protein Determinations. C1q was purified according to previously described, well-established procedures (19). Protein concentrations were determined by the method of Bradford (20); for proteins containing non-ionic detergents, the detergent-compatible, bicinchoninic acid-dependent protein assay (21) was used.

mAbs. The production and characterization of a panel of anti-gC1q-R mAbs has been recently completed and will be described in detail elsewhere (B.G., E.I.B.P., and K.B.M.R. unpublished work). The four mAbs used in this study are IgG1k isotypes and were purified from serum-free culture supernatants by 2× 40% ammonium sulfate precipitation. mAbs 46.23 and 60.11 are directed against epitopes within the N-terminal stretch of gC1q-R corresponding to residues 76–93, and mAb 25.15 and 74.5.2 recognize epitopes in a stretch of the molecule corresponding to residues 204–218.

Binding of ^{125}I-HK to HUVEC and Inhibition by C1q or Anti-gC1q-R. All binding studies were performed at 37°C. HUVECs were washed four times with 4 × 10^{-7} M PMSF-treated binding buffer (137 mM NaCl/4 mM KCl/11 mM glucose/10 mM Hepes/1 mM $CaCl_2$/50 μM $ZnCl_2$/0.5 mg/ml BSA) and incubated in the same buffer containing labeled HK. Following 1 hr incubation, the unbound material was aspirated, the cells were washed four times, the wells were separated, and the bound radioactivity was determined. For inhibition studies, the cells were preincubated with either protein competitor or various mAbs to gC1q-R for 30 min. Each measurement was in triplicate and the experiments were repeated two times. The mean and standard deviation was determined and inhibition compared with the binding of ^{125}I-HK in the presence of nonimmune mouse IgG.

Expression in *Escherichia coli* and Purification of Recombinant gC1q-R. The construction of an expression plasmid pGex-2T containing an insert encoding the mature form (MF, residues 74–282) of gC1q-R, as well as the expression, purification, and characterization of the resulting recombinant gC1q-R has been described in detail in a previous publication (15). In addition, a truncated form (TF) of gC1q-R lacking the first 22 amino acid residues present in the MF has been generated and the conditions used for the transformation of the plasmid containing the correct TF insert into the *E. coli* strain BL 21, overexpression, purification to homogeneity, and characterization of the purified protein has also been described in detail elsewhere (22). The homogeneity of MF and TF was verified by both SDS/PAGE (Fig. 1) under reducing conditions as well as by Western blot analysis using mAbs reactive with both forms of the recombinant proteins.

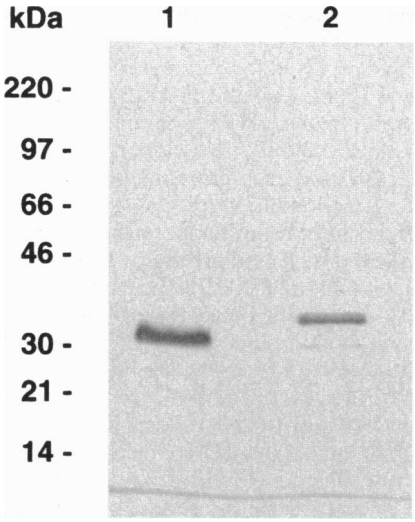

FIG. 1. SDS/PAGE analyses of gC1q-R. The homogeneity of the MF (MF, lane 2) and the truncated form (TF, lane 1) of gC1q-R were analyzed by a 9% (wt/wt) SDS/polyacrylamide gel run under reducing conditions. The bands were stained with Coomassie brilliant blue.

RESULTS

Isolation of a Zinc-Dependent HK Binding Protein. A solubilized endothelial cell membrane preparation was passed over an HK-affinity column in the presence or absence of zinc ion as described in methods. An aliquot of each eluate fraction (with or without zinc) was spotted onto nitrocellulose membrane and blotted with biotinylated HK, developed with AP-STRAV, followed by reaction with NBT/BCIP. The results, shown in Fig. 2, aligned to represent comparable fractions, indicate a prominent increase in HK binding after elution in the presence of zinc. Fractions 8 to 15 were then pooled and concentrated and analyzed by SDS/PAGE using a silver stain or Coomassie brilliant blue as shown in Fig. 3. In the absence of zinc, weak bands are identified at 70 kDa and 45 kDa with the silver stain. Although these protein bands become somewhat more prominent when elution is done in the presence of zinc, the main feature was the appearance of a new prominent band at 33-kDa that was visible with Coomassie blue. In addition, ligand blot experiments (data not shown) demonstrated that, whereas biotinylated HK bound to the 33-kDa band, no discernible binding was seen to either the 70- or the 45-kDa bands. Based on this information, the 33-kDa protein was subjected for N-terminal amino acid sequence analysis in the following manner.

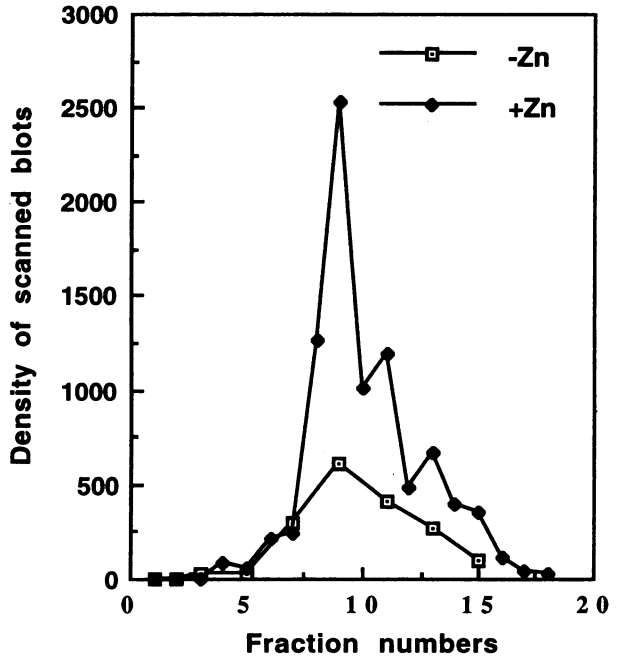

FIG. 2. Elution profile of proteins obtained from the HK–3M Emphaze column. Solubilized endothelial cell membrane preparations were applied to the HK-affinity column in the presence and absence of zinc. The bound protein was eluted with glycine·HCl (0.1 M, pH 2.5) and 0.5 ml fractions were collected into tubes containing 30 μl each of 1 M Tris·HCl, pH 9.0. An aliquot of each fraction was spotted onto the nitrocellulose membrane and probed with biotinylated HK. The intensity of the color was scanned and the values plotted.

An identical gel was electrotransferred to an Immobilon-P^{SQ} membrane and stained with Coomassie blue; the band was cut out, transferred to a 1.5-ml Eppendorf tube, washed with distilled water, dried, and subjected to gas phase hydrolysis (100°C, 24 hr). The protein was then extracted with 30% methanol in 0.05 M HCl and derivatized using a Waters Pico-Tag system. The initial yield was 4.38 pmol and, as shown in Table 1, the NH_2 terminal sequence of this HUVEC membrane isolated HK-binding protein was identical to the known NH_2 terminus of gC1q-R (15). We next performed a Western blot using anti-gC1q-R monoclonal antibody 60.11 to further assess the identity of these two proteins. mAb 60.11 was able to recognize the 33-kDa HUVEC-derived membrane binding protein (Fig. 3*C*) but not the bands at 45 kDa or 70 kDa.

Binding of HK and Factor XII to Purified vs. Recombinant gC1q-R. HUVEC membrane purified or recombinant (MF) gC1q-R (rgC1q-R) at 1.0–2.0 μgs were applied to nitrocellulose membranes and blotted with biotinylated HK or factor XII in the presence or absence of 50 μM zinc. Sufficient *o*-phenanthroline was added to bind the zinc in the purified (but not recombinant) gC1q-R to allow zinc-independent binding to be assayed. The results, shown in Fig. 4, demonstrate binding of HK or factor XII to either purified or rgC1q-R in the presence of zinc and markedly diminished binding in its absence or when 2 mM Ca^{2+} or Mg^{2+} was used instead of Zn^{2+} (not shown). Additional controls included biotinylated IgG that did not bind to gC1q-R and substituting prekallikrein for gC1q-R to which biotinylated HK bound as

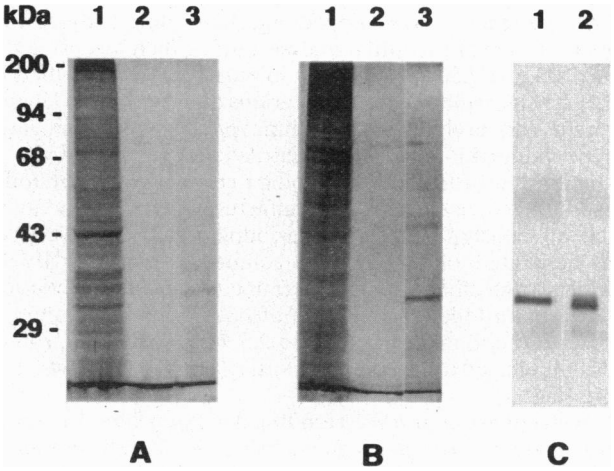

FIG. 3. SDS/PAGE analysis of proteins eluted from HK column. Fractions that reacted with HK were pooled and subjected to a 10% SDS/polyacrylamide gel (*A* and *B*), as well as Western blot analysis (*C*). In *A*, the proteins were stained with Coomassie blue, whereas in *B* they were stained with silver stain. In both *A* and *B*, lane 1 is the starting endothelial membrane preparation, lane 2, the eluate without zinc and lane 3 the eluate with zinc. (*C*) A Western blot of the zinc eluate (lane 3 in *A* or *B*) using anti-gC1q-R 60.11 under reducing (lane 1) and nonreducing (lane 2) conditions.

Table 1. Comparison of the NH_2-terminal sequence of HK-binding protein and gC1q-R

Protein	NH_2-terminal sequence
HK-binding protein	LHTDGDKAFVDFL
gC1q-R	LHTDGDKAFVDFL

The gC1q-R sequence information was reproduced from ref. 15.

expected (5). Furthermore, Fig. 5 shows that while excess unlabeled HK almost totally (90%) reverses the ability of factor XII to bind to gC1q-R as assessed by scanning, factor XII only partially (46%) reverses HK binding. This difference may be due to relative affinity of the two ligands for the gC1q-R molecule.

In other experiments (not shown), the binding of HK to TF was shown to be similar to that of MF indicating that the major binding site for HK lies outside the deleted region of gC1q-R.

Inhibition of Binding of HK to Endothelial Cells. HUVECs were incubated for 30 min with HK (8.7×10^{-8} M), C1q (10.8×10^{-8} M), or anti-gC1q-R mAbs 74.5.2 and 60.11. Then, in the presence of 50 μM zinc, ^{125}I-HK was added and incubated for 60 min at 37°C, conditions known to saturate the binding sites (11). For each condition, the percentage inhibition of ^{125}I-HK binding was determined. The results of a representative experiment are shown in Fig. 6. Whereas a 10 molar excess of nonradiolabeled HK inhibited subsequent ^{125}I-HK binding, a comparable concentration of C1q did not, indicating that the binding site on gC1q-R for C1q and for HK do not overlap. This is consistent with the observed binding of HK to TF, which lacks the C1q binding site. Furthermore, while mAb 60.11 did not efficiently inhibit ^{125}I-HK binding to HUVEC, mAb 74.5.2, did. Other mAbs recognizing epitopes in different regions of the molecule were also tested for their inhibitory activity. Of these only 25.15, which is similar to 74.5.2, was able to inhibit ^{125}I-HK binding to HUVEC, and 46.23 was only moderately inhibitory.

DISCUSSION

The proteins of the contact activation cascade namely, HK, factor XII, prekallikrein, and factor XI, have been shown to assemble along the surface of cells, including platelets (23–25), endothelial cells (13, 26), and neutrophils (27). In each instance, we have begun to define the nature of the cell binding proteins responsible for this interaction. Our focus has been the endothelial cell because it is the site of action of the product of these interactions, namely, bradykinin.

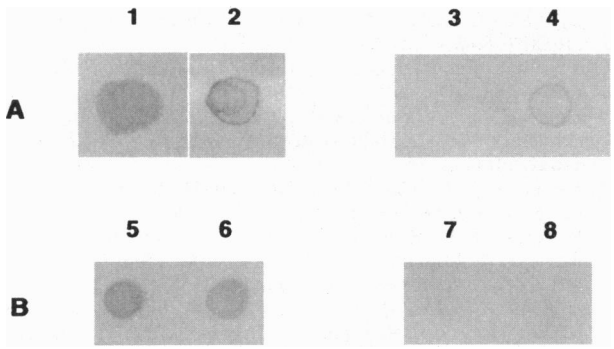

FIG. 4. Dot blot analysis of HK and factor XII binding to the purified endothelial membrane preparation and gC1q-R. The proteins were spotted onto the nitrocellulose membrane [lanes 1, 3, 5, and 7, recombinant protein (2 μg); lanes 2, 4, 6, and 8, purified protein (1 μg)] and probed with biotinylated HK (*A*) or biotinylated factor XII (*B*) in the presence (lanes 1, 2, 5, and 6) or absence (lanes 3, 4, 7, and 8) of 50 μM zinc.

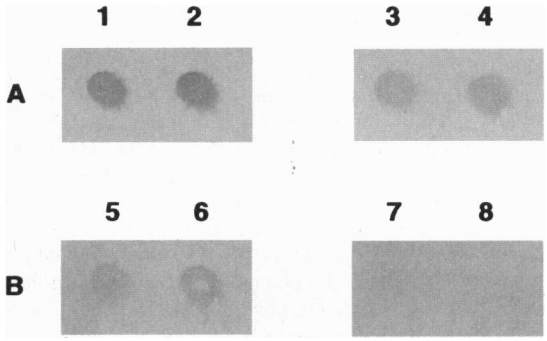

FIG. 5. Dot blot analysis showing competitive displacement of HK and factor XII binding to gC1q-R. gC1q-R (2 μg) were spotted and probed with (*A*) biotinylated HK in the absence (1 and 2) or presence (3 and 4) of 100 M excess of unlabeled factor XII and (*B*) biotinylated factor XII in the absence (5 and 6) and presence (7 and 8) of 100 M excess of unlabeled HK. Binding was done for 1 hr at room temperature in the presence of 50 μM zinc.

By ligand affinity chromatography using HK-3M Emphaze, we have isolated a prominent binding protein of 33 kDa in the presence of zinc plus two proteins of 45 and 70 kDa (based on SDS/PAGE) in the presence or absence of zinc. Although the two minor proteins that stain weakly with Coomassie blue appear to be associated with the prominent 33-kDa band, ligand blot experiments showed that only the 33-kDa protein was able to bind HK. Based on this information, we proceeded to characterize the 33-kDa molecule. The 33-kDa protein appears to be identical to gC1q-R, a ubiquitously distributed cell membrane protein that binds to the globular heads of C1q. J. Dedio *et al.*[¶] also presented data in which this same protein was isolated from an endothelial cell membrane preparation;

[¶]J. Dedio, R. Kellner, and W. Müller-Esterl, Fourteenth International Symposium on Kinins, September 1995, Denver.

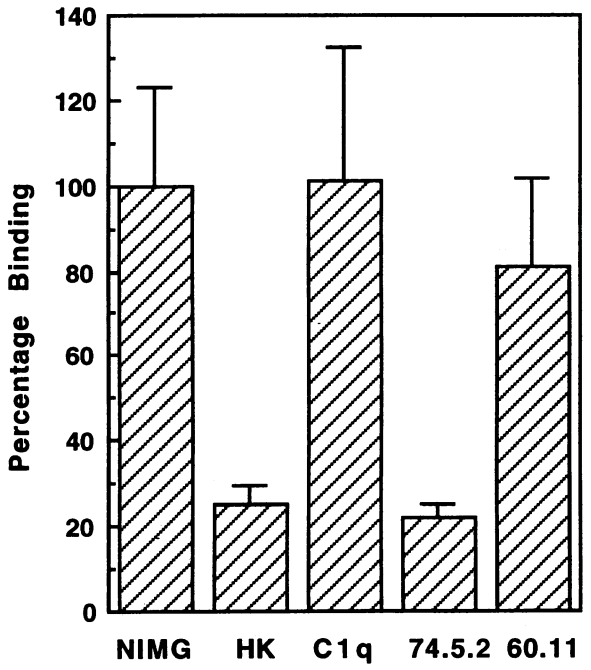

FIG. 6. Inhibition of ^{125}I-HK binding to HUVEC. HUVECs were incubated with HK (8.7×10^{-8} M), C1q (10.9×10^{-8} M), mAbs 74.5.2 and 60.11(12.5 μg/ml), or nonimmune mouse IgG1 (NIMG, 12.5 μg/ml). After 30 min, ^{125}I-HK (1 μg/ml) was added and further incubated for 1 hr at 37°C. Each bar is a mean (± SD) of two experiments, each performed in triplicate.

however, zinc dependence of the interaction with HK was not addressed (37).

There are two types of highly acidic, widely codistributed noncrossreacting cell surface molecules that bind to specific regions of the C1q molecule: (*i*) a 60-kDa, calreticulin-like molecule that binds to the collagen-like "stalks" (cC1q-R) (for review, see ref. 28) and (*ii*) a 33-kDa glycoprotein that expresses preferential affinity for the globular heads (gC1q-R) (15). Both receptor molecules are present on the surface of lymphocytes (15, 28), platelets (29), endothelial cells (30), neutrophils (31), and mast cells (32). The 33-kDa molecule is an unusually acidic, single chain glycoprotein with an apparent molecular mass of 33 kDa (SDS/PAGE) both under reducing and nonreducing conditions, but migrates as a tetramer when analyzed by gel filtration under nondissociating conditions (15). The "native" gC1q-R molecule purified from Raji cells has been shown to inhibit complement activation by preventing the binding of C1q to antibody-coated sheep erythrocytes suggesting that the binding site for gC1q-R and the binding site for immune complexes may be located at the same position on the C1q globular heads (15). The full-length cDNA encoding the 33-kDa gC1q-R has been isolated and found to encode a pre-pro-protein of 282 residues. The N terminus of the mature protein isolated from Raji cells begins at residue 74 of the predicted pre-pro-sequence and is preceded by an unusually long polypeptide (73 residues) that is presumed to contain a signal peptide as well as an unconventional transmembrane domain (15). The MF (residues 74–282), as well as a TF lacking the first 22 amino acid residues have been overexpressed in *E. coli*, purified to homogeneity (Fig. 1), and were used in our studies.

The data obtained thus far with neutrophils differs from that reported herein. HK is thought to interact with neutrophils by binding to MAC-1 or the C3bi receptor since antibodies to this receptor inhibit the interaction (33). Direct isolation of the receptor from a membrane preparation was not attempted. Nevertheless, it is of interest that in two instances, an adhesion molecule which interacts with a complement-derived protein also may interact with a protein of the kinin-forming cascade. By immunofluorescence, factor XII also appears to bind to neutrophils, but its specific interaction has not been studied (34). Prekallikrein is also found on the neutrophil surface but it is assumed to be attached to HK (34) rather than to a membrane binding protein. Endothelial cells do not possess MAC-1, however neutrophils do have cell surface gC1q-R (31).

Although the platelet-binding protein for HK is uncertain, the interaction is likely to be quite complex. First, there is zinc-dependent binding to both unactivated and activated platelets (8, 24); however, the latter interaction appears to be markedly augmented (24). Interaction of HK with thrombospondin has been demonstrated (35) although zinc dependence was not evident. Nevertheless, thrombospondin is present in platelet α granules and is prominent on the surface of activated platelets, therefore binding of HK to it may have physiologic relevance. HK also inhibits thrombin-dependent platelet aggregation (36) but does not bind to thrombin. Thus, interaction with either the GP1b-IX complex or the G protein linked thrombin receptor (or both) appears likely. Nevertheless direct interaction with these putative binding sites has not been demonstrated.

The data thus far suggest that the binding of proteins of the contact activation/kinin forming cascade to cells may vary depending on the particular cell type. In general, the suspected binding proteins are adhesion molecules and some appear to bind complement proteins. The gC1q-R is the first to be isolated and shown to be responsible for the binding seen with an intact cell preparation. Using cloned and expressed gC1q-R, we have demonstrated binding to both factor XII and HK that is zinc dependent. In addition, antibodies to gC1q-R can inhibit zinc-dependent binding of HK to endothelial cells. Yet C1q itself did not inhibit binding of HK to endothelial cells, and those mAbs (B.G., P. Eggleton, L. E. Leigh, A. M. Hofman, B.-L. Lim, K.B.M.R., and E.I.B.P., unpublished work) that inhibit C1q binding to gC1q-R such as 60.11, had no effect upon the interaction of HK with gC1q-R. Antibody 60.11 as well as 46.23 are directed against gC1q-R epitopes contained in the N-terminal residues 76–93, which contains a major C1q binding site (15) as well as a site for binding vitronectin or the ternary complex, vitronectin-thrombin-antithrombin (28). On the other hand, mAb 74.5.2, which inhibits ^{125}I-HK binding to HUVEC, is directed against epitopes that are proximal to the C terminus of the molecule (residues 204–218). Thus, the gC1q-R site for HK (or factor XII, since HK and factor XII compete for the same site) appears to differ from that of C1q.

Because factor XII has been shown to autoactivate along the surface of endothelial cells (12), we propose that bradykinin formation can be initiated along the vascular inner lining where it can then interact with the bradykinin B-2 receptor. Vasodilatation and increased vascular permeability result. The gC1q-R may serve to concentrate plasma components along the surface and thereby facilitate their interaction. It is also possible that endothelial cells are activated or otherwise transduce a signal upon interaction of ligands with the gC1q-R. The other proteins we isolated at 45 kDa and 70 kDa have not been identified; they may represent weaker zinc-independent sites or may interact in some way with gC1q-R. Studies are in progress to examine these possibilities.

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