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Direct Interaction between Human Cytomegalovirus Glycoprotein B and Cellular Annexin II

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Cellular annexin II has been shown to specifically bind human cytomegalovirus (HCMV) and be a component of highly purified virions. In this report, we characterize the interaction of annexin II with HCMV. We found that the binding of annexin II to the HCMV envelope occurs partially through the calcium-dependent phospholipid-binding ability of annexin II since some annexin II was dissociated from virions with chelating agents. However, a substantial proportion of virion-associated annexin II was resistant to chelation, which suggested a calcium-independent interaction between annexin II and an HCMV envelope component. The search for a nonphospholipid component to account for this binding led to the discovery that HCMV glycoprotein B (gpUL55) (gB) can physically interact with annexin II. We present three lines of evidence to support the conclusion that HCMV gB can bind host cell annexin II.

The betaherpesvirus human cytomegalovirus (HCMV) is a pathogen that is responsible for serious disease and death among immunocompromised populations and is the leading cause of infectious birth defects in the United States (2). As with most other herpesviruses, the mechanism whereby HCMV gains access to the interior of host cells to begin its replicative cycle has not been completely elucidated. It is apparent that HCMV entry into host cells is a complex process that likely involves sequential viral-glycoprotein–host cell receptor interactions (9). Given the fact that there is no HCMV vaccine and limited antiviral therapies, information learned about the early events in HCMV infection will lend itself toward developing effective prophylactic treatments.

HCMV binding to cell surface heparan sulfate proteoglycans (HSPGs) is the initial interaction between virus and host cell (11, 20), yet HSPGs alone are insufficient to render a cell permissive to infection (10). This initial heparin-dissociable virus binding is rapidly converted to a more stable binding state (11), possibly through engagement of a proteinaceous receptor. A number of cellular molecules have been proposed to serve as receptors for HCMV that would function downstream of the initial HSPG-virus interaction. These proteins include CD13 (23), a 92.5-kDa phosphoprotein (17, 18), and a 34-kDa protein (1, 21, 25). Recently, a 34-kDa protein from human endothelial cells, identified as annexin II, was found to specifically bind HCMV virions, suggesting that cell surface annexin II may function as a receptor for HCMV on endothelial cells (33). Annexin II very likely corresponds to the 34-kDa protein described earlier from virus overlay experiments due to its similar molecular weight and its distribution on a wide range of human cell types (10, 33).

Annexin II was also demonstrated to be present on the envelope in purified HCMV virions at about 1,000 copies per virion as determined by immunocytochemical analysis (32). Since binding of purified annexin II to HCMV was largely inhibited in the presence of chelating agents (33), it was assumed that binding of annexin II to the HCMV envelope was dependent on calcium and occurred as a result of the calcium-dependent phospholipid-binding ability characteristic of the entire family of annexin proteins, including annexin II. We confirm in this report that not only is annexin II present in HCMV virions but a majority of the virus-associated annexin II molecules are insensitive to calcium chelation. This result is not expected if annexin II is binding only viral-envelope phospholipids. The presence of EDTA- and EGTA-resistant annexin II in HCMV envelopes indicates that annexin II may be interacting with another, nonphospholipid viral component. We wished to determine if annexin II had a ligand on HCMV in addition to envelope phospholipids.

Glycoprotein B (gpUL55) (gB) of HCMV is the major constituent of the viral envelope. Monoclonal antibody studies have shown that HCMV gB plays important roles in HCMV entry, cell-cell spread of internal virions, and fusion of infected cells (19, 29, 30). In addition, gB is one envelope protein capable of heparin binding (11). Given the abundance of gB in the viral envelope and its multifunctional role in HCMV infection, we tested the ability of gB to interact with annexin II. Our results indicate that gB does form a physical association with host cell annexin II and that this interaction is not dependent on the presence of calcium.

HCMV strain AD169 was propagated, titers were determined in immortalized fibroblasts (8), and virions were purified as previously described (21). Polyclonal anti-annexin II serum was kindly provided by K. Hajjar (Cornell University Medical College) or produced in New Zealand White rabbits immunized with purified, recombinant human annexin II. To test the calcium dependence of HCMV-associated annexin II, AD169 virions were subjected to 20 mM EDTA–20 mM EGTA treatment for 1 h at 4°C. Virions were subsequently pelleted through a 20% sorbitol cushion in a Sorvall SC5C centrifuge using a SS-34 rotor at 40,000 × *g* for 30 min. Virion pellets were resuspended in a volume of 20% sorbitol equivalent to the supernatant volume, and fractions of each were analyzed via sodium dodecyl sulfate (SDS)–12.5% polyacrylamide gel electrophoresis (PAGE), electrotransferred to nitrocellulose, and immunoblotted with polyclonal anti-annexin II serum. Phosphorimaging (Fig. 1A) and densitometry (Fig. 1B) of immunoblots were performed with a Bio-Rad model GS-525

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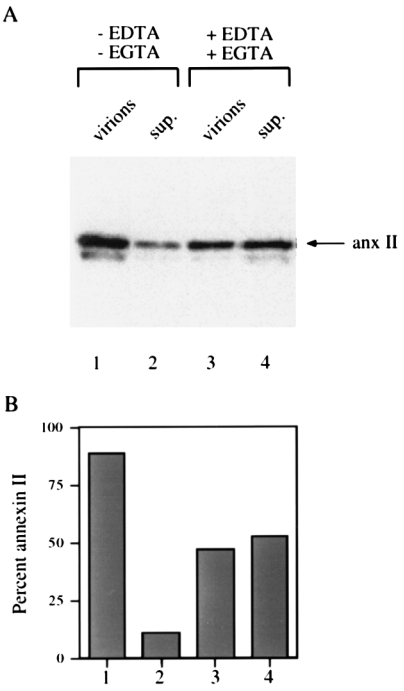

FIG. 1. (A) Chelator-induced release of annexin II from HCMV. HCMV virions were control treated in TBS (−EDTA −EGTA) or treated with TBS containing 20 mM EDTA and 20 mM EGTA (+EDTA +EGTA) for 1 h at 4°C. Virions were separated from supernatants (sup.) by centrifugation through a 20% sorbitol cushion, and fractions were resolved by SDS–12.5% PAGE under reducing conditions, electrotransferred to nitrocellulose, and immunoblotted with polyclonal anti-annexin II serum. The annexin II signal is indicated (anx II). (B) The immunoblot in panel A was phosphorimaged and analyzed with Bio-Rad's Molecular Analyst software to quantitate the percentage of annexin II distributed between the virion and supernatant fractions for each treatment.

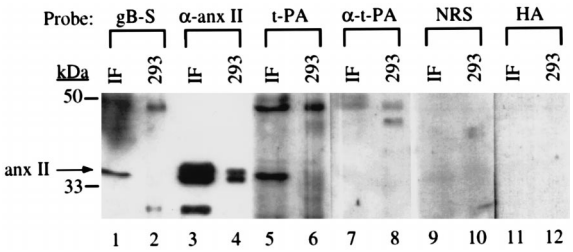

FIG. 2. Immunoblot of IF and 293 membrane proteins probed with various compounds. Membrane proteins were resolved by SDS–10% PAGE under reducing conditions and electrotransferred onto a nitrocellulose sheet. The blot was cut into strips and probed with baculovirus-produced gB-S, anti-annexin II antibody (α-anx II), t-PA, anti-t-PA (α-t-PA) antibody, normal rabbit serum (NRS), and baculovirus-produced influenza virus HA, followed by anti-gB (27-78), anti-t-PA, or anti-HA and the appropriate secondary antibody conjugated to horseradish peroxidase. The annexin II signal is indicated.

Molecular Imager and Bio-Rad Molecular Analyst software as per manufacturer's instructions. In control-treated virions, 11% of the annexin II was found in the supernatant fraction (Fig. 1B, lane 2) while 89% of the annexin II remained associated with virions (Fig. 1B, lane 1). The 11% of annexin II found in the supernatant fraction of control-treated virions likely represents spontaneous dissociation or virion degradation. In virions treated with the chelators EDTA and EGTA, 42% of HCMV-associated annexin II was dissociated from the envelope of HCMV into the supernatant fraction (Fig. 1B, lane 4) when the value (53%) was adjusted for spontaneous dissociation. Virions treated with high concentrations of EDTA and EGTA retained 47% of the virion-associated annexin II (Fig. 1B, lane 3). Either this population of chelator-resistant annexin II is binding to HCMV via a calcium-independent interaction or it is not available to the action of the chelating agents. The latter scenario is unlikely since immunocytochemical analysis revealed that almost all of the annexin II present on HCMV was localized to the virion envelope, not to an internal structure (32). Additionally, annexin II has been reported to be resistant to EDTA dissociation from other biological membranes, although the nature of the EDTA-resistant interaction between annexin II and membranes has not been characterized (14, 28). Furthermore, the original virus overlay experiments identifying annexin II (then known as a 30- to 34-kDa protein) as an HCMV-binding protein were performed with buffers containing EDTA (1, 21). All of these factors suggest that annexin II may be binding to HCMV through a calcium-independent, and potentially phospholipid-independent, interaction.

To test the idea that HCMV gB may be the viral ligand for annexin II, membrane proteins from annexin II-abundant, permissive human fibroblasts (IF) (8) and annexin II-poor, nonpermissive human epithelial 293 cells were subjected to SDS-PAGE followed by ligand blotting. Membranes were prepared from IF and 293 cells by a hypotonic lysis protocol, and protein content was quantitated by the Bio-Rad Protein Assay. Equal amounts (75 μg) of membrane protein from IF and 293 cells were analyzed in the experiments. As previously reported (15), 293 cells had reduced levels of annexin II per microgram of membrane protein compared to IF cells as evidenced by direct immunoblotting with a polyclonal anti-annexin II serum (Fig. 2, lanes 3 and 4). A truncated form of HCMV gB (gB-S) produced in a baculovirus expression system (7) recognized a protein with an electrophoretic mobility similar to that of annexin II in IF cells, while this protein was not detected in 293 cells (Fig. 2, lanes 1 and 2). Strikingly, the recognition pattern of tissue plasminogen activator (t-PA) (provided by B. Schwartz, University of Wisconsin—Madison), a known physiological ligand for annexin II (15), was nearly identical to that of gB-S (Fig. 2, lanes 1, 2, 5, and 6). Both gB-S and t-PA preferentially recognized the more slowly migrating component of the characteristic annexin II doublet (Fig. 2, lanes 1, 3, and 5). Whether this doublet pattern represents full-length and proteolytic forms of annexin II or phosphorylated versus nonphosphorylated forms remains to be determined. No proteins of the molecular size of annexin II were detected with anti-t-PA antibodies (provided by B. Schwartz), normal rabbit serum, or a recombinant soluble form of another viral glycoprotein, hemagglutinin (HA) from influenza virus (provided by V. Hinshaw, University of Wisconsin—Madison). The use of HA is a particularly germane control since this viral glycoprotein was also produced in a baculovirus expression system, indicating that production in insect cells was not mediating the effect of binding. Also of note is the difference in signal strength resulting from the use of membranes from two cell lines expressing different levels of annexin II. Since the levels of annexin II in 293 cells are much reduced compared to the IF cell line, the fact that in both gB-S- and t-PA-probed lanes a signal is detectable only in the IF lanes further indicates that the protein species being bound is annexin II.

Proteins associated with one another are generally able to be coimmunoprecipitated provided appropriate lysis conditions are employed. We next conducted immune-precipitation–immune-blotting experiments to further examine interaction be-

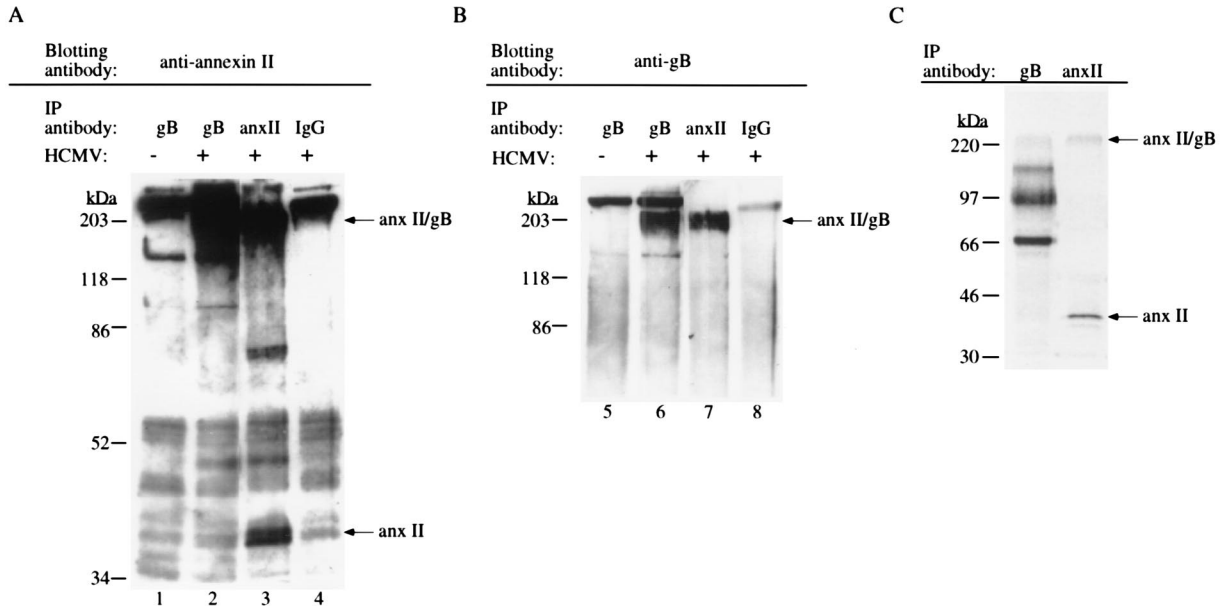

FIG. 3. Coimmunoprecipitation of HCMV-infected immortalized fibroblast lysates. Infected (+) or mock-infected (−) lysates were immunoprecipitated (IP) with a mixture of anti-gB antibodies, affinity-purified anti-annexin II (anxII) antibodies, or bovine immunoglobulin G (IgG). Immunoprecipitates were subjected to SDS–10% PAGE under nonreducing conditions, electrotransferred to nitrocellulose, and immunoblotted. (A) Immunoprecipitates were blotted with anti-annexin II, followed by protein A-conjugated horseradish peroxidase (HRP). This blot was stripped in 100 mM β-mercaptoethanol–2% SDS–62.5 mM Tris (pH 6.7) for 30 min at 50°C and then immunoblotted with 27-78 (anti-gB) followed by protein A-conjugated HRP (B). Monomeric annexin II and the combined annexin II-gB signal (anx II/gB) are indicated. (C) ^{35}S-labeled HCMV virions were subjected to immunoprecipitations with anti-annexin II and anti-gB antibodies and resolved through SDS–10% PAGE under nonreducing conditions. The phosphorimage of the dried gel is shown.

tween gB and annexin II. At 72 to 96 h postinfection, IF cultures infected with HCMV strain AD169 at a multiplicity of infection of 1 were harvested and subjected to immune precipitation analysis employing either anti-gB or anti-annexin II antibodies. For these experiments, cells were lysed in RIPA buffer (1% Nonidet P-40, 0.5% deoxycholic acid, 0.1% SDS, 150 mM NaCl, 1 mM EDTA, 50 mM Tris [pH 7.5]) plus protease inhibitors (2 μg each of antipain, aprotinin, chymostatin, leupeptin, and pepstatin A per ml) for 30 min on ice and residual insoluble material was removed by centrifugation. Clarified cell lysates were adjusted to a volume of 1 ml with TNE (0.15 M NaCl, 5 mM EDTA, 50 mM Tris [pH 7.4]) and precleared for 1 h with a formalin-killed staphylococcal/protein G suspension (Sigma). For precipitations, either an antibody cocktail consisting of monoclonal anti-gB antibodies 27-78, 7-17, and 9-3 (4, 24) or affinity-purified anti-annexin II antibodies were added and the mixtures were incubated at 4°C. Immobilized protein A (Pierce) was added and allowed to collect immune complexes for 1 h at room temperature. Protein A pellets were washed at least five times with TNE plus 0.1% Triton X-100 and once with Tris-buffered saline (20 mM Tris [pH 7.4], 150 mM NaCl). Pellets were resuspended in SDS-PAGE sample buffer lacking β-mercaptoethanol, boiled for 3 min, resolved through SDS–10% PAGE, and electrotransferred onto nitrocellulose membranes. The composition of the immunoprecipitates was confirmed by immunoblotting with anti-annexin II (Fig. 3A) or anti-gB (Fig. 3B). As shown in Fig. 3, anti-gB antibodies immunoprecipitated gB as well as annexin II (lanes 2 and 6) from HCMV-infected fibroblasts. Similarly, annexin II antibodies immunoprecipitated annexin II and gB (lanes 3 and 7). In these unreduced samples, annexin II coimmunoprecipitated with anti-gB antibodies was detected as a species with highly retarded electrophoretic mobility on immunoblots that comigrated with gB (lanes 2 and 6). Immunoprecipitations with anti-annexin II antibodies resulted in detection of the monomeric form of annexin II (38 kDa; lane 3) as well as the slower-migrating species; this high-molecular-weight annexin II species also comigrated with gB (lanes 3 and 7). These reciprocal coimmunoprecipitation patterns, as well as the detection of a form of annexin II with greatly reduced electrophoretic mobility in the presence of gB, strongly suggest that gB and annexin II form a complex in the context of HCMV infection. Interestingly, the complex formed by annexin II and gB is resistant to the conditions employed to remove antibody-bound antigens, namely 2% SDS and boiling.

In order to determine if gB and annexin II were interacting within the virion envelope, immunoprecipitations were carried out on purified ^{35}S-labeled HCMV virions. HCMV-infected IF cells were labeled with 50 μCi of [^{35}S]methionine-[^{35}S]cysteine (NEN) per ml at 5 days postinfection, and virions were isolated 10 days postinfection as previously described (21). Virions were solubilized and lysates were immunoprecipitated as described above for HCMV-infected cells. Immune complexes were resolved through SDS–10% PAGE, after which the gel was dried and phosphorimaged (Fig. 3C). Similarly to the experiments employing HCMV-infected cells, immunoprecipitation of HCMV virions with anti-annexin II and anti-gB antibodies resulted in the coimmunoprecipitation of a high-molecular-weight complex (Fig. 3C, anx II/gB) that was resistant to treatment with 2% SDS sample buffer and boiling. These results demonstrate that gB and annexin II are interacting within the viral envelope.

To further demonstrate an interaction between annexin II and HCMV gB, recombinant gB-S was utilized in affinity chromatography experiments. Recombinant gB-S lacks both transmembrane and cytoplasmic sequences to facilitate secretion and was engineered to contain a carboxy-terminal histidine tag (His_6) for purification via metal-chelate chromatography (7). A

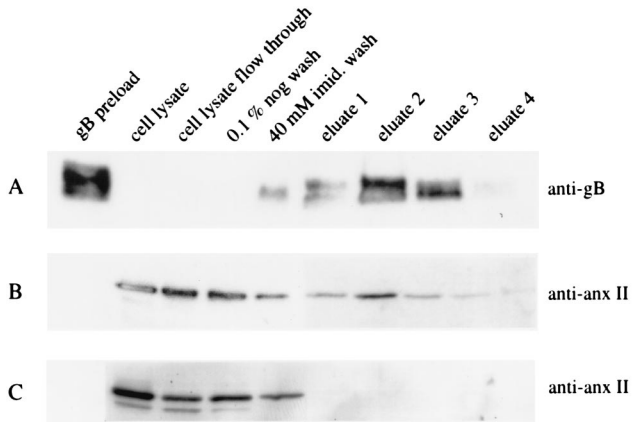

FIG. 4. Immobilized gB-S binds annexin II from a cell lysate. Secretory gB (gB-S) was immobilized by virtue of a carboxy-terminal His_6 tag (7) on Ni^{2+}-NTA affinity resin and was exposed to IF cell lysates. Recombinant gB-S and any associated cellular proteins were eluted from the matrix, and fractions were resolved in parallel by SDS–12.5% PAGE under reducing conditions. (A) Column fractions were immunoblotted with anti-gB antibody 27-78. (B) Duplicate column fractions were immunoblotted with anti-annexin II (anti-anx II) serum. (C) Fractions from a control experiment in which a cell lysate was applied to Ni^{2+}-NTA resin that lacked gB-S were immunoblotted with anti-annexin II serum. gB-S collected from baculovirus-infected insect cell medium (gB preload) was applied to a Ni^{2+} column. IF cell lysates were prepared in 1% nog (cell lysate), diluted to 0.1% nog, and applied to the column twice, after which an aliquot of the unbound fraction was taken (cell lysate flowthrough). The column was washed with 0.1% nog (0.1% nog wash) followed by 40 mM imidazole (imid.), and then proteins were eluted with 500 mM imidazole (eluates 1 to 4).

cellular lysate prepared from IF cells in Tris-buffered saline containing 1% *n*-octylglucoside (Boehringer Mannheim) was diluted to 0.1% *n*-octylglucoside and applied to gB-S immobilized on an agarose-nitrilotriacetic acid (NTA)-Ni^{2+} matrix. The affinity matrix was stringently washed with 40 mM imidazole in phosphate-buffered saline (pH 8.6), and gB-S and any associated proteins were eluted with 500 mM imidazole–500 mM $NaPO_4$–300 mM NaCl–10% glycerol, pH 6.0. As shown in Fig. 4A and B, annexin II was retained on the gB-S column and was coeluted under conditions that removed gB-S. In contrast, annexin II was not retained on a column lacking gB-S (Fig. 4C). These results, again, strongly imply that gB and annexin II are capable of forming an association, given that annexin II was bound by immobilized gB-S in the presence of a complete complement of detergent-solubilized cellular proteins.

This is the first report of a cellular binding protein (annexin II) for HCMV gB, a multifunctional glycoprotein that is the immunodominant and most abundant constituent of the HCMV envelope. As was previously suggested, it is likely that HCMV acquires annexin II concomitantly with the cellular lipid bilayer during virion envelopment (32). This proposal is supported by the fact that annexin II is found abundantly on endosomal membranes (14, 16), which are a proposed site for final HCMV envelopment (27).

Since annexin II is ascribed membrane trafficking and fusion functions (3, 5, 6, 12, 13, 31), and given the biological roles assigned to HCMV gB, it is attractive to hypothesize that the annexin II-gB complex may be involved in one of the many membrane fusion events required during HCMV infection and dissemination such as fusion at cell surface, egress of virions, envelopment, cell-cell spread, and release of particles from host cells. Since annexin II is also an intracellular protein, found in the cytoplasm (34), in association with the cytoskeleton (22, 26), and on endosomal membranes (14, 16), it is possible that gB and annexin II are associating intracellularly. Studies to address the kinetics, compartmentalization, and function of the interaction between cellular annexin II and HCMV gB are under way.

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REFERENCES

1. **Adlish, J. D., R. S. Lahijani, and S. C. St. Jeor.** 1990. Identification of a putative cell receptor for human cytomegalovirus. Virology **176:**337–345.
2. **Alford, C. A., and W. J. Britt.** 1993. Cytomegalovirus, p. 227–255. Human herpesviruses. Raven Press, New York, N.Y.
3. **Ali, S. M., M. J. Geisow, and R. D. Burgoyne.** 1989. A role for calpactin in calcium-dependent exocytosis in adrenal chromaffin cells. Nature **340:**313–315.
4. **Britt, W. J.** 1984. Neutralizing antibodies detect a disulfide-linked glycoprotein complex within the envelope of human cytomegalovirus. Virology **135:** 369–378.
5. **Burgoyne, R. D.** 1988. Calpactin in exocytosis. Nature **331:**20.
6. **Burgoyne, R. D., and M. J. Clague.** 1994. Annexins in the endocytic pathway. Trends Biochem. Sci. **19:**231–232.
7. **Carlson, C., W. J. Britt, and T. Compton.** Expression, purification and characterization of a soluble form of human cytomegalovirus glycoprotein B. Virology, in press.
8. **Compton, T.** 1993. An immortalized human fibroblast cell line is permissive for human cytomegalovirus infection. J. Virol. **67:**3644–3648.
9. **Compton, T.** 1995. Towards a definition of the HCMV entry pathway. Scand. J. Infect. Dis. **99:**30–32.
10. **Compton, T.** Unpublished observations.
11. **Compton, T., D. M. Nowlin, and N. R. Cooper.** 1993. Initiation of human cytomegalovirus infection requires initial interaction with cell surface heparan sulfate. Virology **193:**834–841.
12. **Creutz, C. E.** 1992. The annexins and exocytosis. Science **258:**924–931.
13. **Drust, D. S., and C. E. Creutz.** 1988. Aggregation of chromaffin granules by calpactin at micromolar levels of calcium. Nature **331:**88–91.
14. **Emans, N., J.-P. Gorvel, C. Walter, V. Gerke, R. Kellner, and G. Griffiths.** 1993. Annexin II is a major component of fusogenic endosomal vesicles. J. Cell Biol. **120:**1357–1369.
15. **Hajjar, K. A., A. T. Jacovina, and J. Chacko.** 1994. An endothelial cell receptor for plasminogen/tissue plasminogen activator. I. Identity with annexin II. J. Biol. Chem. **269:**21191–21197.
16. **Harder, T., and V. Gerke.** 1994. The annexin II(2)p11(2) complex is the major protein component of the Triton X-100-insoluble low-density fraction prepared from MDCK cells in the presence of Ca2+. Biochim. Biophys. Acta **1223:**375–382.
17. **Keay, S., and B. Baldwin.** 1992. The human fibroblast receptor for gp86 of human cytomegalovirus is a phosphorylated glycoprotein. J. Virol. **66:**4834–4838.
18. **Keay, S., T. C. Merigan, and L. Rasmussen.** 1989. Identification of cell surface receptors for the 86-kilodalton glycoprotein of human cytomegalovirus. Proc. Natl. Acad. Sci. USA **86:**10100–10103.
19. **Navarro, D., P. Paz, S. Tugizov, K. Topp, J. La Vail, and L. Pereira.** 1993. Glycoprotein B of human cytomegalovirus promotes virion penetration into cells, transmission of infection from cell to cell, and fusion of infected cells. Virology **197:**143–158.
20. **Neyts, J., R. Snoeck, D. Schols, J. Balzarini, J. D. Esko, S. A. Van, and C. E. De.** 1992. Sulfated polymers inhibit the interaction of human cytomegalovirus with cell surface heparan sulfate. Virology **189:**48–58.
21. **Nowlin, D. M., N. R. Cooper, and T. Compton.** 1991. Expression of a human cytomegalovirus receptor correlates with infectibility of cells. J. Virol. **65:** 3114–3121.
22. **Osborn, M., N. Johnsson, J. Wehland, and K. Weber.** 1988. The submembranous location of p11 and its interaction with the p36 substrate of pp60 src kinase in situ. Exp. Cell Res. **175:**81–96.
23. **Söderberg, C., T. D. Giugni, J. A. Zaia, S. Larsson, J. M. Wahlberg, and E. Möller.** 1993. CD13 (human aminopeptidase N) mediates human cytomegalovirus infection. J. Virol. **67:**6576–6585.
24. **Spaete, R. R., R. M. Thayer, W. S. Probert, F. R. Masiarz, S. H. Chamberlain, L. Rasmussen, T. C. Merigan, and C. Pachl.** 1988. Human cytomegalovirus strain Towne glycoprotein B is processed by proteolytic cleavage. Virology **167:**207–225.
25. **Taylor, H. P., and N. R. Cooper.** 1990. The human cytomegalovirus receptor on fibroblasts is a 30-kilodalton membrane protein. J. Virol. **64:**2484–2490.
26. **Thiel, C., M. Osborn, and V. Gerke.** 1992. The tight association of the tyrosine kinase substrate annexin II with the submembranous cytoskeleton depends on intact p11- and Ca(2+)-binding sites. J. Cell Sci. **103:**733–742.
27. **Tooze, J., M. Hollinshead, B. Reis, K. Radsak, and H. Kern.** 1993. Progeny vaccinia and human cytomegalovirus particles utilize early endosomal cisternae for their envelopes. Eur. J. Cell Biol. **60:**163–178.

28. **Tressler, R. J., T. V. Updyke, T. Yeatman, and G. L. Nicolson.** 1993. Extracellular annexin II is associated with divalent cation-dependent tumor cell-endothelial cell adhesion of metastatic RAW117 large-cell lymphoma cells. J. Cell. Biochem. **53:**265–276.
29. **Tugizov, S., D. Navarro, P. Paz, Y. L. Wang, I. Qadri, and L. Pereira.** 1994. Function of human cytomegalovirus glycoprotein B: syncytium formation in cells constitutively expressing gB is blocked by virus-neutralizing antibodies. Virology **201:**263–276.
30. **Tugizov, S., Y. Wang, I. Qadri, D. Navarro, E. Maidji, and L. Pereira.** 1995. Mutated forms of human cytomegalovirus glycoprotein B are impaired in inducing syncytium formation. Virology **209:**580–591.
31. **Wilton, J. C., G. M. Matthews, R. D. Burgoyne, C. O. Mills, and J. K. Chipman.** 1994. Fluorescent choleretic and cholestatic bile salts take different paths across the hepatocyte: transcytosis of glycolithocholate leads to an extensive redistribution of annexin II. J. Cell Biol. **127:**401–410.
32. **Wright, J. F., A. Kurosky, E. L. Pryzdial, and S. Wasi.** 1995. Host cellular annexin II is associated with cytomegalovirus particles isolated from cultured human fibroblasts. J. Virol. **69:**4784–4791.
33. **Wright, J. F., A. Kurosky, and S. Wasi.** 1994. An endothelial cell-surface form of annexin II binds human cytomegalovirus. Biochem. Biophys. Res. Commun. **198:**983–989.
34. **Zokas, L., and J. R. J. Glenney.** 1987. The calpactin light chain is tightly linked to the cytoskeletal form of calpactin I: studies using monoclonal antibodies to calpactin subunits. J. Cell Biol. **105:**2111–2121.