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# The Human Fibroblast Receptor for gp86 of Human Cytomegalovirus Is a Phosphorylated Glycoprotein

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**A human embryonic lung (HEL) cell receptor for gp86 of human cytomegalovirus that functions in virus-cell fusion was further characterized. Anti-idiotype antibodies that mimic gp86 were used to immunoprecipitate the 92.5-kDa fibroblast membrane receptor for gp86, which was preincubated with various endoglycosidases. The receptor, which has a pI ranging from 5.3 to 5.6, appears to be a glycoprotein with primarily N-linked sugar residues, some of which have high concentrations of mannose and some of which are complex oligosaccharides. Western blots (immunoblots) of electrophoretically transferred receptor incubated with various biotinylated lectins confirmed the presence of sugar moieties, including *N*-acetylglucosamine, glucose or mannose, and galactose, but not fucose or *N*-acetylgalactosamine. This gp86 receptor from uninfected HEL cells also incorporated radiolabeled phosphate from orthophosphoric acid, indicating that it is a constitutively phosphorylated receptor.**

Herpesviruses, including human cytomegalovirus (HCMV), infect cells by a series of processes including attachment, fusion of viral envelope with the cell membrane, and transportation of the capsid to the nuclear membrane. The mechanisms by which each stage of infection takes place are unknown. At least three cellular receptors for HCMV have been identified: class I human leukocyte antigen (HLA) molecules, which may serve as cell surface receptors for HCMV to which β-2 microglobulin is bound (8); a 92.5-kDa fibroblast membrane protein, which appears to be a specific receptor for gp86 on the virion envelope (14); and 30-kDa (26) and 32- and 34-kDa (2) proteins to which HCMV binds. By attempting to block specific phases of HCMV infectivity with the anti-idiotype antibodies that we used to identify the 92.5-kDa fibroblast membrane receptor for gp86 (14, 15), we recently showed that both gp86 and its receptor appear to be involved in the process of HCMV fusion with the cell membrane but do not appear to be primary mediators of HCMV attachment (13).

The membrane proteins involved in fusion of enveloped viruses with eucaryotic cells are of particular importance because of their role in viral infectivity. Moreover, these proteins are also of interest because an understanding of the mechanism by which they mediate or allow virus-cell fusion may help us to also understand intracellular and intercellular membrane fusion events (11, 28).

Although specific fusion proteins on the surface of several enveloped virions have been identified, most of these viruses can fuse with liposomes in the absence of specific cell membrane receptors (11, 16, 28). This appears to be particularly true for viruses that enter cells through a low-pH-dependent mechanism of entry following endocytosis. However, certain viruses, particularly those that undergo pH-independent fusion directly at the cell membrane, appear to require specific host factors for viral fusion (28). In the case of human immunodeficiency virus 1 (HIV-1), a specific segment of the CD4 receptor appears to be required for viral fusion and entry (5, 9).

We have now further characterized the 92.5-kDa receptor for gp86 of HCMV receptor in an effort to learn more about its possible mechanism of action. This report presents evidence, based on endoglycosidase sensitivity and lectin binding, that the receptor is a glycoprotein. In addition, because certain cell membrane receptors such as CR2 (the C3d Epstein-Barr virus receptor) and CD4 (the HIV-1 receptor) appear to be activated by phosphorylation (1, 6), we attempted to determine by [$^{32}$P]orthophosphoric acid incorporation whether the 92.5-kDa receptor is also phosphorylated.

## MATERIALS AND METHODS

**Cell culture.** Human embryonic lung (HEL-299) fibroblasts were obtained from the American Type Culture Collection (CCL-137) and maintained in culture as previously described (13, 14). CCRF-SB cells were obtained from the American Type Culture Collection (CCL-120) and maintained in RPMI 1640 medium with 10% fetal calf serum.

**Antibodies.** An anti-idiotype antibody (4-3-5) that mimics gp86 of HCMV was generated, characterized, and affinity purified as described elsewhere (15). BALB/c immunoglobulin M (IgM) control antibody was obtained from Southern Biotechnology Associates, Birmingham, Ala., and affinity purified by the same method as the anti-idiotype antibody. Anti-HLA class I heavy-chain determinant was obtained from Pel Freez, Brown Deer, Wis.

**Radiolabeling of cell membrane proteins.** Confluent HEL monolayers were labeled with $^{32}$P by a modification of a procedure described elsewhere (21). Approximately $10^7$ cells were preincubated at 37°C for 1 h in phosphate-free buffer (10 mM Tris acetate, 150 mM NaCl, 5 mM KCl, 1.8 mM glucose, 5 mM $MgCl_2$, and 2 mM glutamine [pH 7.4]). This buffer was removed, phosphate-free buffer containing 1 mCi of [$^{32}$P]orthophosphoric acid (New England Nuclear, Wilmington, Del.) was added to each flask, and the mixture was incubated at 37°C for an additional 3 h. Following $^{32}$P labeling, the cells were rinsed three times in phosphate buffer (137 mM NaCl, 2.6 mM KCl, 1.4 mM $KH_2PO_4$, 8.6 mM $NaH_2PO_4$, pH 7.2), scraped into 10 ml of the phosphate buffer, and pelleted. The phosphate buffer was removed, and the pellet was lysed on ice in 100 μl of buffer containing 25 mM NaCl, 5 mM $MgCl_2$, 25 mM Tris HCl (pH 7.4), 2% Nonidet P-40, and 1 mM phenylmethylsulfonyl fluoride.

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Following centrifugation at 1,500 rpm (200 × *g*) for 20 min, the supernatant was removed and further centrifuged at 40,000 rpm (125,000 × *g*) for 70 min at 4°C. The supernatant was again removed and stored at −70°C.

**Immunoprecipitation.** $^{32}$P-labeled solubilized HEL cell membrane proteins were incubated with affinity-purified 4-3-5 or IgM control antibody (200 μg/$10^7$ cells) at 37°C for 2 h and then further incubated with 200 μl of rat anti-mouse IgM-Sepharose (Zymed Laboratories, South San Francisco, Calif.) at 37°C for 1.5 h. Beads were pelleted, rinsed, resuspended in denaturing sample buffer, and boiled for 3 min, as previously described (14).

**Endoglycosidase treatment.** Immunoprecipitated cell membrane proteins were diluted 1:5 in the appropriate buffer for each endoglycosidase according to the manufacturer's instructions (Boehringer Mannheim, Indianapolis, Ind.) in a 37°C water bath overnight; controls were run in the same buffer without the addition of enzyme. Preparations treated with endoglycosidase D and *O*-glycanase were pretreated with neuraminidase for 5 h at 37°C and acetone precipitated on ice. After endoglycosidase treatment, protein from all tubes was acetone precipitated and then resuspended in denaturing sample buffer and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

**PAGE.** One-dimensional SDS-PAGE was performed with 7.5% polyacrylamide gels under denaturing conditions or 10% polyacrylamide gels under nondenaturing conditions, as previously described (14). Two-dimensional SDS-PAGE was performed by a modification of O'Farrell's method (20), using 10% polyacrylamide nondenaturing gels in the second dimension.

**Western blots (immunoblots).** For lectin-binding studies, receptor was purified by electroelution from 10% native polyacrylamide gels with a Hoefer (San Francisco, Calif.) electroelution apparatus according to the manufacturer's instructions, run on a 10% native polyacrylamide gel, and blotted onto nitrocellulose under native conditions, as previously described (14). The nitrocellulose membranes were then blocked with 2% polyvinylpyrrolidone-40 (Sigma, St. Louis, Mo.) in phosphate-buffered saline (PBS) overnight at 4°C, rinsed with PBS, and further incubated with various biotinylated lectins (Vector Laboratories, Burlingame, Calif.) at 37°C for 2 h with rocking. After additional rinses in PBS, the membranes were further incubated with horseradish peroxidase-labeled avidin (Vector Laboratories) at 37°C for 2 h, rinsed, and developed with α-chloronaphthol (Sigma).

For isoelectric point determination, cell membrane proteins were separated by two-dimensional SDS-PAGE and transferred to nitrocellulose membranes under nondenaturing conditions. The blotted proteins were then incubated with 4-3-5 or control BALB/c IgM antibody at 37°C for 2 h, rinsed with PBS, and further incubated with horseradish peroxidase-conjugated goat anti-mouse IgM (Zymed Laboratories) at 37°C for 2 h. Following final rinses with PBS, the blots were developed with α-chloronaphthol.

## RESULTS

**Effect of endoglycosidase treatment on the 92.5-kDa receptor.** Radiolabeled receptor was immunoprecipitated by 4-3-5 from solubilized HEL cell membrane proteins and subsequently treated with endoglycosidase F (to remove both high-mannose and complex N-linked oligosaccharides), endoglycosidase H (to remove high-mannose N-linked oligosaccharides), or neuraminidase (to remove sialic acid residues). Following neuraminidase treatment, some preparations of receptor were further treated with endoglycosidase D (to remove complex N-linked oligosaccharides) or *O*-glycanase (to remove O-linked oligosaccharides). Analysis by SDS-PAGE showed that receptor pretreated with endoglycosidase F was changed in its apparent molecular mass from 92.5 kDa (Fig. 1, lane Ctr) to 88.5 kDa (lane F), suggesting that some N-linked oligosaccharide species are present. Following incubation with endoglycosidase H, the apparent molecular mass decreased to 90.5 kDa (lane H), and following incubation with endoglycosidase D, it decreased to approximately 89.5 kDa (lane D), indicating that some of the N-linked species were high-mannose oligosaccharides but that many were complex in nature. Neuraminidase treatment alone resulted in a small change to approximately 91.5 kDa (lane N), indicating the presence of some sialic acid residues; *O*-glycanase treatment resulted in virtually no change in migration of the receptor (lane O), indicating the general absence of O-linked oligosaccharides.

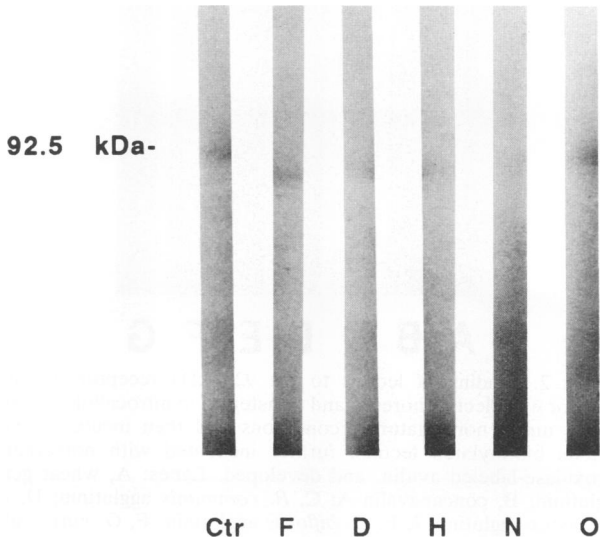


FIG. 1. Endoglycosidase treatment of the 92.5-kDa receptor. Radiolabeled receptor was immunoprecipitated and treated with various endoglycosidases, and its subsequent apparent molecular weight was determined by SDS-PAGE and autoradiography. Lanes: Ctr, untreated control; F, endoglycosidase F; D, endoglycosidase D; H, endoglycosidase H; N, neuraminidase; O, *O*-glycanase.

**Identification of specific oligosaccharide species with biotinylated lectins.** To confirm the presence of oligosaccharides, lectin-binding studies were performed. Evidence for the binding of wheat germ agglutinin (*Triticum vulgaris* agglutinin) to the receptor was consistently obtained, suggesting the presence of [D-GlcNAc]$_2$ (Fig. 2, lane A), concanavalin A (α-D-Man or α-D-Glc) (lane B), and *Ricinis communis* agglutinin I (β-D-Gal or D-GalNAc) (lane C). In contrast, binding was consistently absent for *Ulex europaeus* agglutinin I (fucose) (Fig. 2, lane D), *Dolichos biflorus* and *Glycine max* agglutinins (D-GalNAc) (lanes E and F), and *Arachis hypogaea* agglutinin (β-D-Gal[1,3]-D-GalNAc) (lane G), suggesting the general absence of fucose and galactosamine residues in this receptor.

Preincubation of immunoprecipitated receptor with endoglycosidase F eliminated binding of the lectins (Fig. 3, results for concanavalin A), suggesting that the lectins were indeed binding to sugar moieties on the receptor.

**pI determination.** The isoelectric point of the 92.5-kDa

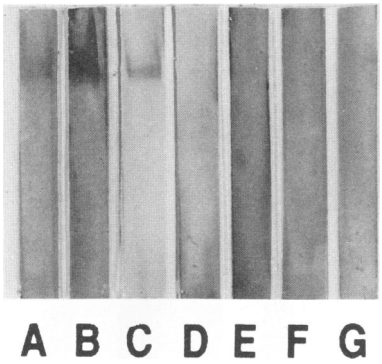


FIG. 2. Binding of lectins to the 92.5-kDa receptor. Purified receptor was electrophoresed and transferred to nitrocellulose membranes under nondenaturing conditions and then incubated with various biotinylated lectins, further incubated with horseradish peroxidase-labeled avidin, and developed. Lanes: A, wheat germ agglutinin; B, concanavalin A; C, *R. communis* agglutinin; D, *U. europaeus* agglutinin I; E, *D. biflorus* agglutinin; F, *G. max* agglutinin; G, *A. hypogaea* agglutinin.

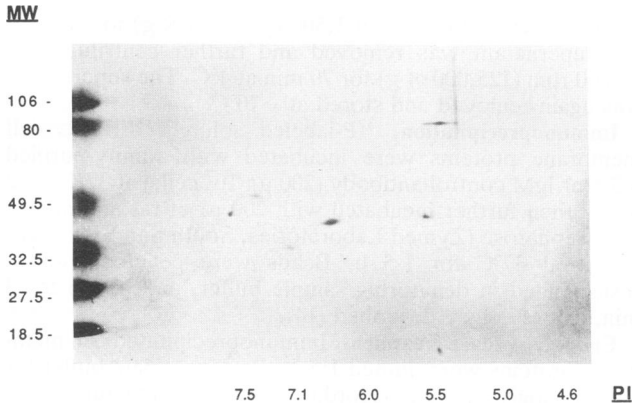


FIG. 4. pI determination for the 92.5-kDa receptor. Solubilized HEL cell membrane proteins were separated by two-dimensional PAGE, transferred to a nitrocellulose membrane, incubated with 4-3-5, further incubated with horseradish peroxidase-conjugated goat anti-mouse IgM, and developed. Molecular weights (in thousands) are indicated on the left.

receptor was determined by two-dimensional PAGE of the receptor, which was followed by Western blotting with the 4-3-5 monoclonal antibody. As shown in Fig. 4, the isoelectric point of the glycosylated receptor ranged from 5.3 to 5.6. A control two-dimensional Western blot which was incubated with an unrelated IgM BALB/c antibody that did not bind to the 92.5-kDa species was also run (data not shown).

**Incorporation of [$^{32}$P]orthophosphoric acid.** To determine whether the receptor represents a phosphorylated receptor, we performed initial experiments in uninfected HEL cells grown in the presence of [$^{32}$P]orthophosphoric acid. Following 3 h of incubation, cells were rinsed, solubilized cell membrane preparations were made, and receptor was immunoprecipitated with 4-3-5 or an unrelated BALB/c IgM antibody as a negative control. For a positive control for the phosphorylation procedure, a constitutively phosphorylated HLA class I heavy chain was immunoprecipitated from a human B-lymphoblastoid cell line (SB) by a monoclonal antibody against a common HLA class I determinant (data not shown). Our results indicated that the receptor is in fact phosphorylated and that it is constitutively phosphorylated in the absence of added exogenous stimuli (Fig. 5).

## DISCUSSION

Although many viral fusion proteins have been identified (11, 16, 28), little is known about the cell membrane components involved in the fusion process. To further characterize the fibroblast membrane receptor for gp86 of HCMV, we used glycosidic enzymes and lectins to determine whether the receptor is a glycoprotein and, if so, which types of sugar moieties are involved in its structure. This type of analysis has previously been used to characterize certain virion glycoproteins, including those of HCMV and simian cytomegalovirus (4). As noted in those studies, this method has the advantage of not requiring the large amounts of material necessary for biochemical analysis of carbohydrates, the results are not affected by cell metabolism the way that the incorporation of radiolabeled sugar moieties can be, and the results are consistent with the specificities of the lectins and endoglycosidases (4).

Enveloped viruses can undergo pH-dependent or pH-independent fusion with the cell membrane. Examples of enveloped viruses that undergo pH-independent fusion in-

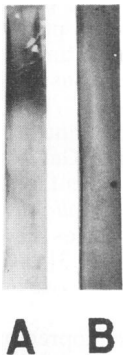


FIG. 3. Elimination of lectin binding by endoglycosidase F pretreatment. Purified receptor was pretreated with buffer alone (A) or endoglycosidase F (B) and subsequently incubated with biotinylated concanavalin A and developed as for Fig. 2.

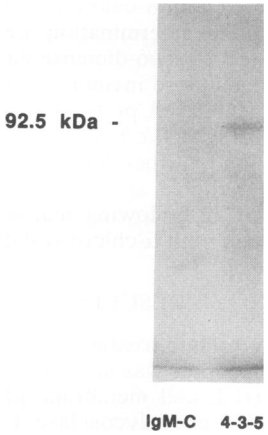


FIG. 5. Phosphorylation of the 92.5-kDa receptor. HEL cells were incubated with [$^{32}$P]orthophosphoric acid, and then receptor was immunoprecipitated with 4-3-5 or control IgM monoclonal antibody.

clude members of the paramyxovirus family (such as Sendai virus and measles virus) (18), murine coronavirus (25), herpes simplex virus (23), and HIV-1 (17, 24). While the pH dependence of viral fusion may be a property of the viral fusion protein, it appears that specific host factors may also be necessary for pH-independent viral fusion to occur (28). Studies in which fusion of Sendai virus or measles virus to cells can be inhibited by preincubating the cells with N-terminal segments of viral fusion proteins suggest the presence of specific cell receptors for those proteins (11, 22), and quantitative studies of Sendai virus fusion also indicate that specific cell receptors which are required for virus-cell fusion exist (10, 19). In addition, several reports indicate that specific monoclonal antibodies against CD4 do not inhibit adsorption of HIV-1 but do inhibit virus-cell fusion and viral infectivity, and mapping studies suggest that amino acids between positions 178 and 292 (or approximately the third domain of CD4) are necessary for HIV-1 fusion with the cell membrane (5, 9). We recently inhibited HCMV fusion with HEL fibroblasts, but not adsorption of HCMV to the same cells, by preincubating the cells with monoclonal anti-idiotype antibodies that antigenically mimicked an epitope on gp86 (13). This suggests that the 92.5-kDa receptor to which these antibodies specifically bind is likely involved in the HCMV-cell fusion process.

To date, the best-characterized cell membrane protein shown to be involved in virus fusion is the HIV-1 receptor, CD4. Like CD4, which is a 55-kDa cell membrane glycoprotein (7), the 92.5-kDa human fibroblast fusion protein receptor for HCMV is a glycoprotein. Also like CD4 (1), it is constitutively phosphorylated. It is interesting that stimulation of $CD4^+$ cells with phorbol myristate acetate or specific antigen results in a rapid increase in the level of CD4 phosphorylation, followed by dephosphorylation, suggesting that phosphorylation may be linked to signal transduction. The highly conserved cytoplasmic domain appears to be physically associated with an internal membrane tyrosine kinase ($p56^{lck}$), and ligand binding appears to be associated with a tyrosine phosphorylation signal to as-yet-unidentified substrates (27). It appears, however, that tyrosine protein kinase signalling does not result from HIV-1 gp120 or HIV-1 virion binding to CD4 (12), even at 37°C for up to 15 min, making it unlikely to be involved in the fusion process. Experiments are in progress to determine whether the HCMV receptor requires protein kinase signalling for viral fusion to occur.

Several studies (reviewed in reference 11) suggest that the fusion of cell membranes with each other during endocytosis and exocytosis, processes which bear similarity to virus-cell fusion, however, requires ATP hydrolysis. It is unknown whether protein phosphorylation plays a direct role in membrane fusion, but it is intriguing that two protein kinase activities have been attributed to clathrin-coated vesicles (3, 11). It is also interesting that a trypsin-sensitive component on vesicle membranes which is necessary for fusion has been identified, suggesting that a specific vesicle protein is required for fusion. Additional studies of both viral fusion protein receptors and cell membrane fusion proteins are necessary to understand the extent to which these processes are similar.

In addition to its role in fusion of cytomegalovirus with the cell membrane, the 92.5-kDa HCMV gp86 fibroblast membrane receptor, like other cell surface receptors for viral glycoproteins, is likely to have a specific cellular function which, like that of CD4, may require protein phosphorylation for signal transduction. Available data about this receptor suggest that, also like CD4, it is similar in several respects to growth factor receptors. This receptor is a phosphorylated glycoprotein located at the surface of fibroblast membranes (14); the fact that it is constitutively phosphorylated suggests that it may be a kinase capable of autophosphorylation, like class 1A (e.g., epidermal growth factor receptor), 1B (e.g., insulin receptors), or 1C (e.g., platelet-derived growth factor receptor) receptors (reviewed in reference 29), all of which are known to possess tyrosine kinase activity. Its broad appearance on native gels, which changes to a narrow band on denaturing gels (14), suggests that, like CD4 and growth factor receptors, it may also have intramolecular disulfide bonding; there is, however, no evidence to suggest that the receptor has any intermolecular disulfide bonding as class 1B growth factor receptors do. Experiments are in progress to determine whether the gp86 receptor also possesses kinase activity, and if so, which cellular components may be phosphorylated as a result of its activation.

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## REFERENCES

1. **Acres, R. B., P. J. Conlon, D. Y. Mochizuki, and B. Gallis.** 1986. Rapid phosphorylation and modulation of the T4 antigen on cloned helper T cells induced by phorbol myristate acetate or antigen. J. Biol. Chem. **261:**16210–16214.
2. **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.
3. **Bar-Zvi, D., and D. Branton.** 1986. Clathrin-coated vesicles contain two protein kinase activities. Phosphorylation of clathrin beta-light chain by casein kinase II. J. Biol. Chem. **261:**9614–9621.
4. **Benko, D. M., and W. Gibson.** 1986. Primate cytomegalovirus glycoproteins: lectin-binding properties and sensitivities to glycosidases. J. Virol. **59:**703–713.
5. **Celada, F., C. Cambiaggi, J. Maccari, S. Burastero, T. Gregory, E. Patzer, J. Porter, C. McDanal, and T. Matthews.** 1990. Antibody raised against soluble CD4-rgp120 complex recognizes the CD4 moiety and blocks membrane fusion without inhibiting CD4-gp120 binding. J. Exp. Med. **172:**1143–1150.
6. **Changelian, P. S., and D. T. Fearon.** 1986. Tissue-specific phosphorylation of complement receptors CR1 and CR2. J. Exp. Med. **163:**101–115.
7. **Dialynas, D. P., D. B. Wilde, P. Marrack, et al.** 1983. Characterization of the murine antigenic determinant L3T4a, recognized by monoclonal antibody Gk1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen reactivity. Immunol. Rev. **74:**29–55.
8. **Grundy, J. E., J. A. McKeating, P. J. Ward, A. R. Sanderson, and P. D. Griffiths.** 1987. β-2 microglobulin enhances the infectivity of cytomegalovirus and when bound to the virus enables class I HLA molecules to be used as a virus receptor. J. Gen. Virol. **68:**793–803.
9. **Healey, D., L. Dianda, J. P. Moore, J. S. McDougal, M. J. Moore, P. Estress, D. Buck, P. D. Kwong, P. C. L. Beverley, and Q. J. Sattentau.** 1990. Novel anti-CD4 monoclonal antibodies separate human immunodeficiency virus infection and fusion of CD4+ cells from virus binding. J. Exp. Med. **172:**1233–1242.
10. **Hoekstra, D., and K. Klappe.** 1986. Sendai virus-erythrocyte membrane interaction: quantitative and kinetic analysis of viral binding, dissociation, and fusion. J. Virol. **58:**87–95.
11. **Hoekstra, D., and J. W. Kok.** 1989. Entry mechanisms of enveloped viruses. Implications for fusion of intracellular mem-

branes. Biosci. Rep. **9:**273–305.
12. **Horak, I. D., M. Popovic, E. M. Horak, P. J. Lucas, R. E. Gress, C. H. June, and J. B. Bolen.** 1990. No T-cell tyrosine protein kinase signalling or calcium mobilization after CD4 association with HIV-1 or HIV-1 gp120. Nature (London) **348:**557–560.
13. **Keay, S., and B. Baldwin.** 1991. Anti-idiotype antibodies that mimic gp86 of human cytomegalovirus inhibit viral fusion but not attachment. J. Virol. **65:**5124–5128.
14. **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.
15. **Keay, S., L. Rasmussen, and T. C. Merigan.** 1988. Syngeneic monoclonal anti-idiotype antibodies that bear the internal image of a human cytomegalovirus neutralization epitope. J. Immunol. **140:**944–948.
16. **Marsh, M., and A. Helenius.** 1989. Virus entry into animal cells. Adv. Virus Res. **36:**107–150.
17. **McClure, M. O., M. Marsh, and R. A. Weiss.** 1988. Human immunodeficiency virus infection of CD4-bearing cells occurs by a pH-independent mechanism. EMBO J. **7:**513–518.
18. **Morrison, T.** 1988. Structure, function, and intracellular processing of paramyxovirus membrane proteins. Virus Res. **10:** 113–136.
19. **Nir, S., K. Klappe, and D. Hoekstra.** 1986. Mass action analysis of kinetics and extent of fusion between Sendai virus and phospholipid vesicles. Biochemistry **25:**8261–8266.
20. **O'Farrell, P. H.** 1975. High resolution two dimensional electrophoresis of proteins. J. Biol. Chem. **250:**4007–4021.
21. **Pober, J. S., B. C. Guild, and J. L. Strominger.** 1978. Phosphorylation *in vivo* and *in vitro* of human histocompatibility antigens (HLA-A and HLA-B) in the carboxy-terminal intracellular domain. Proc. Natl. Acad. Sci. USA **75:**6002–6006.
22. **Richardson, C. D., and P. W. Choppin.** 1983. Oligopeptides that specifically inhibit membrane fusion by paramyxoviruses: studies on the site of action. Virology **131:**518–532.
23. **Spear, P., M. Wittels, A. Fuller, D. WuDunn, and R. Johnson.** 1989. Herpes simplex virus: pathway of entry into cells, p. 163–175. *In* R. Compans, A. Helenius, and M. Oldstone (ed.), Cell biology of virus entry, replication and pathogenesis, vol. 90. Alan R. Liss, Inc., New York.
24. **Stein, B. S., S. D. Gowda, J. D. Lifson, R. C. Penhallow, K. G. Bensch, et al.** 1987. pH-independent HIV entry into CD4-positive T cells via virus envelope fusion to the plasma membrane. Cell **49:**659–668.
25. **Sturman, L. S., C. S. Ricard, and K. V. Holmes.** 1985. Proteolytic cleavage of the E2 glycoprotein of murine coronavirus: activation of cell-fusing activity of virions by trypsin and separation of two different 90K cleavage fragments. J. Virol. **56:**904–911.
26. **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.
27. **Veillette, A., A. Ninan, L. Caron, and D. Davidson.** 1991. The lymphocyte-specific tyrosine protein kinase $p56^{lck}$. Semin. Immunol. **3:**143–152.
28. **White, J. M.** 1990. Viral and cellular membrane fusion proteins. Annu. Rev. Physiol. **52:**675–697.
29. **White, M. F.** 1991. Structure and function of tyrosine kinase receptors. J. Bioenerg. Biomembr. **23:**63–82.