Ebola Virus Glycoproteins Induce Global Surface Protein Down-Modulation and Loss of Cell Adherence

Graham Simmons, Rouven J. Wool-Lewis, Frédéric Baribaud, Robert C. Netter, and Paul Bates*

Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6076

Received 21 August 2001/Accepted 7 November 2001

The Ebola virus envelope glycoprotein (GP) derived from the pathogenic Zaire subtype mediates cell rounding and detachment from the extracellular matrix in 293T cells. In this study we provide evidence that GPs from the other pathogenic subtypes, Sudan and Côte d’Ivoire, as well as from Reston, a strain thought to be nonpathogenic in humans, also induced cell rounding, albeit at lower levels than Zaire GP. Sequential removal of regions of potential O-linked glycosylation at the C terminus of GP1 led to a step-wise reduction in cell detachment without obviously affecting GP function, suggesting that such modifications are involved in inducing the detachment phenotype. While causing cell rounding and detachment in 293T cells, Ebola virus GP did not cause an increase in cell death. Indeed, following transient expression of GP, cells were able to readhere and continue to divide. Also, the rounding effect was not limited to 293T cells. Replication-deficient adenovirus vectors expressing Ebola virus GP induced the loss of cell adhesion in a range of cell lines and primary cell types, including those with proposed relevance to Ebola virus infection in vivo, such as endothelial cells and macrophages. In both transfected 293T and adenovirus-infected Vero cells, a reduction in cell surface expression of adhesion molecules such as integrin β1 concurrent with the loss of cell adhesion was observed. A number of other cell surface molecules, however, including major histocompatibility complex class I and the epidermal growth factor receptor, were also down-modulated, suggesting a global mechanism for surface molecule down-regulation.
accessibility using NetOGlyc, version 2.0 (http://www.cbs.dtu.dk/services/NetOGlyc) (18).

Production and assaying of adenovirus vectors. Bacterial recombination to produce adenovirus vectors with E1 and E3 deleted was carried out as previously described (23). Briefly, pAD-TrackCMV containing the coding sequences of Ebola GP, EboR GP, and ASLV-A EnvA and an empty vector were cotransfected into 293T cells, baby hamster kidney (BHK) cells, and murine MC57 cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% bovine calf serum. Stable MC57 cell lines bearing the human coxsackievirus/adeno-virus receptor (hCAR) (7) were made following transfection of the hCAR coding sequence (kindly provided by J. Bergelson) in pcDNA3(+)neo using Lipofectamine 2000 (Invitrogen) in accordance with the manufacturer's instructions and selection with Geneticin G-418 sulfate (Invitrogen). Human microglial (U87) cells, African green monkey kidney cells (Vero), and cat kidney cells (CCC) were maintained in DMEM supplemented with 10% fetal calf serum (FCS). Human promonocyte cell line U937 was maintained in RPMI 1640 medium supplemented with 10% FCS. Serum-free, nonadherent 293H cells (Invitrogen) were maintained in 293 SFM II (Invitrogen). Human umbilical vein endothelial cells (HUVEC; Clonetics) were maintained in EGM medium (Clonetics). Human pulmonary artery endothelial cells (HPAEC; Clonetics) were maintained in EGM-2 medium (Clonetics). Coronary artery smooth muscle cells (CASM; Clonetics) were maintained in SmGM-2 medium (Clonetics). Blood monocyte-derived macrophages were prepared by plastic adherence as described previously (38) and were maintained in RPMI 1640 supplemented with 10% human serum.

Vectors. cDNAs encoding the Ebola GP, EboS GP, and EboR GP and EboC-GP were kindly provided by Anthony Sanchez (Centers for Disease Control and Prevention) and their coding sequences were subcloned together with that of the avian sarcoma and leukosis virus type A (ASLV-A) envelope, EnvA, into mammalian expression plasmid pCB6 as well as pAD-TrackCMV (23). EboZ, EboS, and EboR GP cDNAs lacking a stop codon were also subcloned via PCR amplification and pCDNA6/neo together with that of the hCAR coding sequence (kindly provided by J. Bergelson) in pcDNA3(+)neo using Lipofectamine 2000 (Invitrogen) to produce full-length GP bearing a V5-His tag at the C terminus. EboZ GP mutants lacking various lengths of the mucin-like domain at the C terminus of GP (see Fig. 3A) were made by overlapping extension PCR using flanking primers to the 5′ and 3′ ends of cDNA for EboZ GP (with the addition of a V5-His tag) as well as a pair of internal primers. The internal primers consisted of 311-5′-GTCTTTCACATGTTGTTAATCCTCTTCTGAAAG3′-346 and its reverse complement to produce mutΔ1, 311-5′-GTCTTTCACATGTTGTTAATCCTCTTCTGAAAG3′-346 and its reverse complement to produce mutΔ1234, 341-5′-CTGAAGCACTCAAGG3′-436 and its reverse complement to produce mutΔ23, and 341-5′-CTGAAGCACTCAAGG3′-463 and its reverse complement to produce mutΔ234. Regions of O-linked glycosylation were predicted (see Fig. 3A) based on sequence context and surface

FIG. 1. Ebola virus GP expression induces cell rounding. 293T cells were transfected with 20 μg of pAD-TrackCMV containing the coding sequence for EboZ GP (A), EboS GP (B), EboR GP (C), EboC-GP (D), or ASLV-A EnvA (E). GFP expression was observed at 48 h posttransfection using a Nikon TE300 inverted microscope. Fields represent findings from multiple experiments.
Results

GP-induced cell rounding is common to all subspecies of Ebola virus. 293T cells were transiently transfected with the EboZ GP coding sequence in pAD-TrackCMV coexpressing GFP under the control of a separate cytomegalovirus (CMV) promoter. GP-transfected cells underwent cell rounding and detachment from the tissue culture plastic as previously described (Fig. 1) (10, 39, 50). In addition, reduced adherence to plates coated with extracellular matrix components (e.g., fibronectin and vitronectin) was seen (data not shown). Rounding was observed within 18 h posttransfection and reached a peak at 48 h. GPs from the other African strains, EboS and EboR, were also found to cause 293T cell rounding, although less dramatically than EboZ GP (Fig. 1). A less defined effect was observed with the “nonpathogenic” strain, EboR (Fig. 2A). Mock-transfected cells or cells transfected with a control viral envelope GP, the ASLV-A envelope (EnvA), did not demonstrate the rounding phenotype. EboS has a lower fatality rate than EboZ, while EboR appears to be nonpathogenic in humans (2); thus the extent of cell rounding appears to correlate with pathogenesis. Although variations in the processing efficiency of GP are apparent, the differences in cell rounding were not merely a reflection of levels of expression, as EboZ, EboS, and EboR GPs bearing V5 epitope tags on their C termini were expressed at broadly similar levels (Fig. 2B), despite dramatic differences in the numbers of floating cells induced (Fig. 2A).

The rounding phenotype maps to multiple O-linked glycosylation sites on GP1. Deletion of an O-linked glycosylation-rich, mucin-like domain in the C-terminal region of GP1 results in the loss of the rounding phenotype (50). Tellingly, insertion of the mucin domain into the envelope GP from MLV also produces a protein capable of inducing cell rounding (50). It is not clear, however, if the carbohydrate moieties or the protein itself is responsible for the phenotype. Interestingly, the membrane-bound mucin GP, episialin, induces a cell rounding and detachment phenotype very similar to that induced by Ebola virus GP despite little primary sequence homology between the two proteins (40, 46). Thus, to further map the determinants involved in the rounding phenotype, EboZ GP mutants containing serial deletions in the mucin-like domain were made (Fig. 3A); the associated mutations were based on four clusters of potential O-link glycosylated serines/threonines in the C-terminal portion of GP. Forty-eight hours posttransfection, levels of total cellular expression of EboZ GP and the mutants varied by less than twofold, as judged by SDS-PAGE and immunoblotting for the V5 epitope tag on the C terminus of GP2 (Fig. 3B). These levels correlated with cell surface expression, as determined by flow cytometry using KZ52, a human Fab fragment directed against GP (data not shown). Protein folding of the mutants was likely unaffected by the deletions, as coexpression of mutΔ1234, lacking all of the
predicted C-terminal O-linked glycosylation sites, with plasmids encoding MLV Gag/Pol and LacZ produced infectious MLV pseudotype particles with titers equivalent to those of wild-type EboZ GP (titers of \(9.2 \times 10^4\), \(5.3 \times 10^2\), and \(1.2 \times 10^5\) FFU/ml for particles with no envelope and particles with envelopes consisting of EboZ GP, EboZ GP with a C-terminal V5-His tag, and mut\(\Delta1234\) with a C-terminal V5-His tag, respectively). Thus, the regions of EboZ GP involved in binding to cellular receptors expressed on 293T cells, as well as those mediating membrane fusion, lie outside the region removed in mut\(\Delta1234\).

Sequential removal of segments of the mucin-like domain led to a successive reduction in cell detachment induced in 293T cells. Removal of two predicted glycosylation sites in mut\(\Delta1\) reduced the levels of floating cells by approximately 50%. The loss of a further five potential sites reduced rounding by another 50% and so forth until removal of all of the predicted type O glycosylation sites from the C terminus of GP1 virtually restored the numbers of floating cells to levels characteristic of mock-transfected cells. Retention of the first two O-linked glycosylation sites in mut\(\Delta123\) and mut\(\Delta234\) led to reductions of approximately 65 and 90%, respectively. Thus, the overall length of the domain in EboZ GP rather than specific determinants appears to contribute to the rounding-and-detachment phenotype (Fig. 3B). EboR GP also contains a mucin-like domain; thus, the distribution or geometry of the O-linked sugars may affect the loss of adhesion observed.

**GP-induced cell rounding is not confined to 293T cells.** Adenovirus vectors expressing Ebola virus GP together with GFP under the control of a separate CMV promoter were used
to efficiently transduce a range of cell lines and primary cell types. In 293T cells transduced with EboZ GP encoded by adenovirus (Ad/EboZ-GP), levels of GP surface expression were approximately fivefold higher than those obtained by transient transfection, as determined by flow cytometry (data not shown). Ad/EboZ-GP caused cell rounding and detachment in glioma cell line U87 (Fig. 4A), as well as several other human adherent cell lines, including HeLa, NP2, HOS, and 293T (data not shown). Interestingly, introduction of GP into a nonadherent variant of 293 cells (293H) caused the cells to lose their ability to form large aggregates (Fig. 4A). Thus, Ebola virus GP appears to be able to affect cell-to-cell interactions as well as cell-to-substrate attachment. In each case EboR GP also demonstrated a lesser effect than EboZ GP but one that was visible, with later time points giving increased cell detachment for EboR GP. Although Ad/EboZ-GP was able to mediate cell rounding in many human and nonhuman cell types, little effect was noted in promonocytic cell line U937, which had been differentiated to macrophage-like cells by 2 days of prior treatment with phorbol ester PMA (Fig. 4A). Interestingly, in PMA-differentiated U937 cells, but not in any other cell type tested, large multinucleated syncytia were noted upon GP expression, particularly in Ad/EboR-GP-infected cells.

While experimental infection with EboZ is almost invariably fatal in nonhuman primates, it is initially nonpathogenic in adult rodents such as mice and guinea pigs, although Ebola virus rapidly becomes pathogenic upon serial passage in these hosts (34). The cell detachment phenotype, however, was also observed in a range of nonhuman cell types, including African green monkey kidney cells (Vero), as well as feline (CCC), hamster (BHK), and murine cells (MC57) (Fig. 4B). Although Ad/EboZ-GP was the most potent, EboR GP also induced rounding in all cell types tested. While EboZ GP-induced cell rounding and detachment were clearly visible after 18 h in some cell types, including CCC cells, other cell lines such as...
Vero required 48 h to fully display the phenotype. These differences may reflect the intrinsic adherent properties of different cell types, as cells such as Vero require more-stringent protocols to remove them from the extracellular matrix using trypsin-EDTA.

Ad/Ebo-GP induced rounding in primary cell types. The finding that Ad/Ebo-GP did not mediate cell rounding in PMA-treated U937 cells (Fig. 4A), a model of macrophages, which represent the major cell target for filoviruses in vivo (5, 15, 33), prompted us to investigate the effects of Ebola virus GP in primary macrophages. In contrast to what was found in the experiments with U937 cells, transduction by Ad/EboZ-GP but not ASLV EnvA encoded by adenovirus (Ad/ASLV-EnvA) induced cell detachment of primary blood monocyte-derived macrophages from three independent donors in at least 50% of transduced cells as judged by GFP coexpression (Fig. 4C). Induction of cellular detachment in macrophages by GP may thus play a major role in viral pathogenesis.

In addition to macrophages, several other primary cell types, including HUVEC and HPAEC, as well as a smooth muscle cell type (CASMCl, also demonstrated the rounding phenotype very strongly upon transduction with Ad/EboZ-GP (Fig. 4C). The levels of endothelial cell infection in vivo and the extent to which direct viral cytopathic damage of the endothelium impacts Ebola virus pathogenesis are at present controversial (4). The efficiency with which Ad/EboZ-GP induced rounding in endothelial cells, however, suggested that even if only a very low percentage of cells within an intact endothelial monolayer were infected with Ebola virus and hence expressing GP, cell detachment may impact greatly endothelium integrity and thus may play a role in the massive hemorrhage associated with filovirus infection. Indeed, transduction of intact endothelial cell monolayers with Ad/Ebo-Z-GP at low MOIs resulting in 1% GFP+ cells led to an increase in monolayer permeability (data not shown).

GP cytotoxicity. Over 90% of floating cells taken from a transiently transduced culture were strongly positive for EboZ GP by indirect immunofluorescence (Fig. 5), while trypan blue exclusion indicated that over 95% of these cells were viable. Upon being replated in a fresh culture dish, the cells adhered to the surface within 1 to 2 days (Fig. 5). Throughout the readherence over 95% of the cells remained viable. Likewise, cell viability remained constant at around 95% for 5 to 80 h, as assayed by 7AAD staining (data not shown). In addition, early apoptosis markers such as annexin V were absent from the 7AAD-negative population, suggesting no enhancement of apoptosis in these cells (data not shown). Thus, GP does not appear to be acutely toxic nor does it irreversibly trigger cell death. This does not rule out, however, the possibility that long-term expression of GP may indeed induce cytotoxicity. Indeed, loss of cell-to-cell contact can lead to induction of apoptosis in a mechanism termed anoikis (17). Stable 293 cell lines expressing the Ebola virus GPs, however, could be easily established.

GP modulates expression of adhesion and other cell surface molecules. The observation that EboZ GP expression caused cells to detach from the culture dish suggested that loss of functional ligands, such as the integrins, to extracellular matrix components may be occurring. Indeed, down-regulation of a number of integrin subunits, particularly $\beta_1$ (CD29) and $\alpha_v$ (CD51) (Fig. 6A), expressed in 293T cells was observed upon EboZ GP, but not EnvA, expression. Consistent with the levels of detachment observed, EboR GP expression resulted in a detectable, but less dramatic, reduction in the cell surface presence of integrin subunits. Down-regulation of other cell surface markers, however, including the epidermal growth factor receptor (EGFR), the transferrin receptor, and major histocompatibility complex class I (MHC-I), was also observed with both EboZ GP and EboR GP, suggesting that the mechanism of loss of integrins from the cell surface may not be specific but rather global.

A similar pattern of global cell surface marker down-regulation was seen in Ad/Ebo-GP-transduced Vero cells (Fig. 6B) and HPAEC (data not shown). In both cases down-modulation of $\beta_1$ and $\alpha_v$-integrin was accompanied by a reduction in surface expression of EGFR and MHC-I. Interestingly, the down-regulation of surface markers on HUVEC transduced with Ebola virus GP showed a more specific pattern, with MHC-I and adhesion molecule PECAM-1 (CD31) showing strong down-modulation with EboZ GP, but not $\beta_1$ (Fig. 6C). Levels of $\alpha_v$-integrin were modestly reduced. Ad/Ebo-GP also induced strong down-regulation of MHC-I, but not of PECAM. Thus, adhesion molecules other than the integrins may be involved in GP-induced cell detachment in some cell types.

DISCUSSION

Expression of recombinant envelope GP from all four sub-species of Ebola virus induced variable degrees of rounding and detachment of human cells from the tissue culture surface, although expression via an adenovirus vector was required to observe significant rounding with GP from the nonpathogenic strain, EboR. Thus, intrinsic differences in the abilities of GPs from distinct strains to induce rounding and detachment exist, and these abilities follow the same order as mortality rates in humans and other primates, i.e., EboZ $\gg$ EboS $\gg$ EboR. The relative differences in the rounding phenotypes induced by

FIG. 5. Ebola virus GP does not induce cell death. Floating cells (3 $\times$ 10^6 per well) from 293T cells 48 h posttransfection were replated into fresh six-well plates. At 24-h intervals floating cells were counted ( ● ) and then pooled with versine-detached adherent cells, and viable ( ● ) and nonviable ( ■ ) cell numbers were estimated using trypan blue exclusion. Cells were then adhered to poly-d-lysine-coated plates, and the percentage of Ebola virus GP-positive cells ( △ ) was quantified using indirect immunofluorescence staining with KZ52. Values represent the geometric means of triplicate samples ± standard errors. Data are representative of three independent experiments.
EboZ GP and EboR GP in simian and human cells appeared to be similar (Fig. 4) despite a report that Ad/EboR-GP caused endothelial damage in cynomolgus monkey carotid arteries but not human saphenous veins (50). Simian endothelial cells were not tested in the present study; thus it is possible that the simian, but not the human, endothelium is more sensitive to the cell rounding induced by EboR GP than the simian cell lines tested. However, these results suggest the lack of a simple correlation between EboZ and EboR pathogenesis in humans and nonhuman primates, as suggested by Yang et al. (50). It is likely that GP from both subspecies can induce endothelial damage given sufficiently high GP expression. Importantly, both strains also induced detachment in primary macrophages, the major in vivo target for Ebola virus replication, particularly during the early stages of disease.

The detachment phenotype induced by a particular GP correlated with levels of GP expression, with increasing cellular levels of both EboZ and EboS GPs leading to increased detachment (Fig. 2A). Due to a transcriptional editing event required for GP expression, only 20% of transcripts are associated with GP production during virus infection (35, 42). The fact that reducing the levels of cellular GP reduces the rounding phenotype in 293T cells suggests that sGP may be a mechanism for controlling levels of GP and thus blunting its cell-rounding effects. Indeed, mutation of the transcriptional editing site to give only membrane-bound GP increases the cytopathogenicity of virus in tissue culture (44). It remains unclear, however, whether the cytopathicity and pathogenesis seen with wild-type Ebola virus are due to the remaining levels of GP expression or other viral factors. Ad/Ebo-GP transduction of endothelial cells, however, gives levels of GP expression similar to those resulting from wild-type Ebola virus (50) infection, suggesting that the levels of membrane-bound GP expressed in the context of transcriptional editing remain sufficient to induce cell rounding. It will be interesting to see if recombinant viruses bearing envelopes, such as mut1234, which do not induce cell rounding are cytotoxic in cell culture and pathogenic in animals.

The fact that Ebola virus GP did not directly cause cell death but rather induced a loss of adherence, contrary to a previous report (50), suggested that GP may modulate the surface expression or activation of cell adhesion molecules. Integrin-mediated adhesion plays many roles in cell adhesion, signaling, and immune regulation (1, 8, 9, 22, 27, 45); thus, these molecules were initially tested. Indeed, integrin molecules, especially β1- and αv-integrin subunits, were down-regulated from the surfaces of several, but not all, cell types by Ebola virus GP. Several viruses, including human CMV, down-regulate or bind to integrin subunits and can induce cell rounding and detachment (37). Moreover, similar effects can be induced by antibodies to β1-integrin (30). Thus, loss of integrins from the cell surface may play a major role in the induction of cell rounding by Ebola virus GP. EboR GP did not affect the cell surface integrin levels as strongly as EboZ GP, which correlates well with the morphological changes observed with GP expression. In addition, other adhesion molecules such as PECAM-1 (Fig. 6) and VCAM (data not shown) were down-regulated on endothelial cells by Ad/EboZ-GP while β1-integrin was not, suggesting that integrin down-regulation may not to be solely responsible for cell detachment, although loss of other integrin subunits from endothelial cells may occur. Many other nonadhesion molecules, such as MHC-I and EGFR as well as CD55, a GPI-linked protein (data not shown), are also down-modulated, suggesting that a broad mechanism is involved in surface molecule down-regulation. Thus, cell surface marker down-regulation is unlikely to be directly due to virus-cellular receptor interactions as suggested previously (39).

The mucin-like domain in Ebola virus GP is required for the rounding phenotype (Fig. 3) (50). Mucins are a diverse family of GPs epitomized by significant O-linked carbohydrate modification. Interestingly, mucins such as epsilasin induce strikingly similar decreases in cell attachment (11, 46). The presence of a mucin-like domain is conserved among all filoviruses, suggesting functional importance; however, this domain is unlikely to contain elements involved in binding cellular receptors required for entry as the domain is entirely dispensable for efficient infection of 293T cells. In addition, its removal does not adversely affect protein folding and conformation, suggesting that it forms an entirely separate domain. No particular motif in the mucin-like domain appears to be responsible for the rounding phenotype; rather the entire domain contributes to rounding (Fig. 3). Thus, the overall level of glycosylation and/or charge may play a role. Alternatively, the mucin-like domain may act as a nonspecific lectin, binding to many glycosylated proteins and thus causing their retention in the export machinery. Indeed, the majority of cell associated GPs appear endoglycosidase H sensitive, suggesting that they are retained in the endoplasmic reticulum or early Golgi (44). Volchkov and colleagues suggest that the large degree of N- and especially O-linked glycosylation required on GP may "exhaust" the cellular machinery for posttranslational modification of proteins (44). The expression of GP truncated before the membrane-spanning domain to produce a secreted form of GP (secGP) containing all of the glycosylation sites of full-length GP does not cause rounding, however (39). Likewise, sGP expression does not induce cell rounding, nor is coexpression of sGP together with GP able to reduce the levels of cell detachment induced by GP (data not shown). Thus, membrane-anchored expression appears to be required for the rounding phenotype. Also, over 90% of floating cells were strongly positive for GP expression (Fig. 5), suggesting that surface GP expression is unlikely to induce detachment in nontransfected neighboring cells.

The loss of cell adhesion as well as the specific loss of MHC-I and adhesion molecules involved in stabilizing cell-to-cell interactions is likely to negatively impact both the stimulation of an anti-Ebola virus CD8 T-cell response and the ability of T cells to kill infected cells. Indeed, the more specific down-modulation of MHC-I in HUVEC may suggest that such host defense molecules are the intended target of the down-regulation. One of the most striking aspects of Ebola virus pathology is lymphocyte depletion and suppression of T-cell proliferation as well as an apparent lack of inflammatory infiltrates in target organs. T cells have been implicated as the primary mediators of clearance in nonfatal cases of filovirus infection (3), and perfusion of Ebola virus-infected nude mice with cytotoxic T cells specific for Ebola virus epitopes within 2 days of infection prevents death (47). Ad/EboZ-GP was able to induce MHC-I down-regulation in macrophages in addition to cell lines and endothelial cells (data not shown). Cells of the
monocyte/macrophage lineage provide the initial target for Ebola virus replication in vivo, and many types of tissue macrophages (e.g., Kupffer cells in the liver) are infected throughout the course of disease. These cells play an important role in both innate and adaptive immune responses, particularly as antigen-presenting cells, forming conjugates to activate T cells. Thus, disruption of the ability of these cells to productively interact with T cells may adversely affect the immune response to Ebola virus, contributing to its highly pathogenic properties.

The integrins also play a critical role in the trafficking of leukocytes through the endothelium to sites of infection. Specifically, the initial tethering and rolling of leukocytes along the endothelium, followed by the arrest and final diapedesis into sites of infection, all require regulated integrin function (9). In response to direct infection by many RNA and DNA viruses or to exposure to inflammatory cytokines, endothelial cells up-regulate immunomodulatory genes such as intercellular adhesion molecule 1 and MHC genes (21). This activated state is necessary for the diapedesis of leukocytes through the endothelium and into the tissue. Infection of endothelial cells with replication-competent EboZ, however, inhibits this antiviral state from being induced, even following superinfection with measles virus (19, 20). This inhibition is at least partially due to Ebola virus protein VP35, which acts as a potent alpha interferon antagonist (6). However, the down-modulation of PECAM-1 and MHC-I in endothelial cells by GP alone indicates that GP also plays a major role in this process.

In summary, expression of Ebola virus GP mediates a loss of...
cellular adhesion, which can be at least partially attributed to the down-modulation of many cell surface markers including adhesion molecules by an as yet unidentified mechanism. While loss of cell adhesion, particularly in endothelial cells, may contribute to the hemorrhagic disease characteristic of Ebola virus infection, it is likely that a major role of GP may be in modulating the host’s ability to mount an effective immune response. Loss of adherence, as well as a reduction in MHC-I surface levels, may thus play a major role in the highly pathogenic course of Ebola virus infection. The precise role of cell surface levels in modulating the host’s ability to mount an effective immune response is not fully elucidated without the identification of the natural host(s) of filoviruses.

ACKNOWLEDGMENTS

We thank Dennis Burton for providing KZ52 and Bert Vogelstein for pAD-Track and pAD-Easy plasmids. We also thank the members of the Bates laboratory for useful discussions, Robert Doms for critical reading of the manuscript, and Jacqueline Reeves and Stephen Eck for advice on adenovirus production. G.S. is the recipient of a long-term EMBO fellowship. This work was supported by grants to P.B. from the National Institutes of Health.

REFERENCES
