Published in final edited form as:
Cell Microbiol. 2008 July ; 10(7): 1546–1558. doi:10.1111/j.1462-5822.2008.01149.x.

Differential regulation of the attachment of KSHV infected human B cells to ECM by KSHV encoded gB and cellular αV integrins

Ossie F. Dyson, **Telisha L. Oxendine**, **Khalief E. Hamden**, **Patrick W. Ford**, and **Shaw M. Akula***

Department of Microbiology & Immunology, Brody School of Medicine at East Carolina University, Greenville, NC 27834.

Summary

Kaposi's sarcoma-associated herpesvirus (KSHV) has two modes replication: latent and lytic replication. Reactivation from latency is dictated, in part, by the cell cycle. Herein, we have attempted to delineate the importance of cell cycle in KSHV pathogenesis by exploring the expression pattern of cell surface receptors during different phases of the cell cycle. αV integrin expression is augmented during S phase in fibroblasts, epithelial, and KSHV infected cells. Using a Matrigel system, we pioneer the concept that KSHV infected primary effusion lymphoma (PEL) cells can attach to extracellular matrix proteins. This attachment is mediated primarily via αV integrins or virally encoded gB, and occurs preferentially in cells from S phase or cells from S phase actively supporting a lytic infection, respectively. Such an ability of infected B cells to attach to endothelial cells may also aid in the dissemination of infection. The keystone of this work is that for the first time, we describe the ability of KSHV infected B cells to preferentially use cellular (αV) or viral (gB) receptors to specifically bind cells, depending upon the stage of the cell cycle and infection.

Keywords

KSHV; HHV-8; Cellular attachment; gB; αV; integrins; Matrigel; reactivation

Introduction

Kaposi's sarcoma-associated herpesvirus (KSHV), otherwise known as human herpesvirus-8 (HHV-8), is the most recently characterized of the human herpesviruses. KSHV is a member of the γ-2 herpesvirus family (genus *Rhadinovirus*) (Chang *et al.*, 1994). KSHV primarily causes all four forms of Kaposi's sarcoma (KS). It is also a lymphoproliferative agent that has been etiologically linked to two other malignant lymphomas called: primary effusion lymphoma (PEL), also known as body cavity-based lymphoma (Cesarman *et al.*, 1995), and multicentric Castleman disease (Soulier *et al.*, 1995).

KSHV has two modes of infection, as is common in the herpesvirus family: (1) lytic replication, which generates infectious progeny virions that destroy the host cell, and (2) latent replication, in which the viral genome persists in its host cell but with dramatically restricted gene expression and without cell destruction (Zhong *et al.*, 1996). *In vivo*, only less than 3% of infected cells in KS lesions or in KSHV positive lymphomas display evidence of lytic KSHV gene expression (Renne *et al.*, 1996). Host cell conditions play a crucial role in regulating both KSHV lytic and latent infection of target cells (Bryan *et al.*, 2006; Mercader *et al.*, 2000).

*Corresponding author: Shaw M. Akula, Department of Microbiology & Immunology, Brody School of Medicine, East Carolina University, Greenville, North Carolina, USA 27834. Phone: (252)744-2702 Fax: (252) 744-3104 Email: akulas@ecu.edu

Recent studies have demonstrated cell cycle progression to have a role in reactivation of KSHV infection (Bryan *et al.*, 2006; McAllister *et al.*, 2005). A direct correlation between cell cycle progression, KSHV reactivation, and cell surface topology has also been observed (Whitman *et al.*, 2007). This study demonstrated that the KSHV infected cells in G0/1 phase had finger-like projections on their surface; while cells in S phase had a relatively smooth cell surface. Herein, we attempted to analyze if the cell cycle had any effect on the expression of cellular receptors utilized by KSHV for its pathogenesis. We provide several lines of evidence to substantiate the diverse role played by virus encoded gB and cellular αV integrins in mediating attachment of KSHV infected cells to the extracellular matrix (ECM) proteins expressed on the target cells.

Results

Expression levels of αV integrins are augmented during S phase of the cell cycle

Cell cycle has a specific and significant impact on cell surface topology (Whitman *et al.*, 2007). Based on the above findings, we hypothesized cell cycle progression to alter expression of receptors on the target cells. To study the effect of cell cycle on the expression of cellular receptors in human foreskin fibroblast (HFF; a fibroblast), 293 (epithelial cells), and GFP-BCBL-1 (KSHV infected human B cells) cells, quantitative real time PCR (qRT-PCR) was used for the analysis. Cells were sorted in different phases of cell cycle by the conventional approach of serum starving (Krek and DeCaprio, 1995). As such, the cells were separated into three groups each. The groups consisted of (1) cells that were synchronized in G0/1 phase by serum starving for 24h; (2) cells incubated with 10% fetal bovine serum (FBS) for 8h following serum starvation, and (3) cells incubated with 10% FBS for 16h following serum starvation (S phase). The expression of αV, β1, dendritic cell specific intercellular adhesion molecule 3 grabbing nonintegrin (DC-SIGN), 1,4-N-acetylhexosaminyl transferase (GlcNAcT-I), vascular endothelial growth factor receptor-1 (VEGFR-1), and VEGFR-2 were analyzed in this study using specific primers. These receptor molecules (except GlcNAcT-1) were selected as they have been shown by previous studies to play a direct or indirect role in KSHV infection and pathogenesis (Rappocciolo *et al.*, 2006; Hamden *et al.*, 2004; Akula *et al.*, 2002; Akula *et al.*, 2001a; Birkmann *et al.*, 2001). GlcNAcT-1 is an enzyme that initiates the heparan sulfate (HS) biosynthetic pathway and HS, incidentally, is the binding receptor for KSHV (Akula *et al.*, 2001a). TK1, β-actin, and KSHV ORF8 were used as controls. β-actin was the internal control; TK1 was used as a determinant of cell cycle as its expression is greatly increased during S phase and nearly absent during G0/1 phase (Mao *et al.*, 2005); and the expression of ORF8 indicated an active KSHV replication (Jenner *et al.*, 2001). As expected, TK1 expression was significantly elevated in HFF, GFP-BCBL-1, and 293 cells obtained from S phase of the cell cycle (Fig. 1A-C). ORF8 was expressed only by GFP-BCBL-1 cells indicating that they were infected and supported an active replication (Fig. 1B). Interestingly, αV integrins were the only receptors to consistently demonstrate an increased expression during S phase in all the cell types tested (Fig. 1A-C). β1 integrin, GlcNAcT-1, and VEGFR-1 expression were significantly elevated only in HFF cells during S phase (Fig. 1A). The above findings were further confirmed by monitoring the expression levels of αV protein by Western blotting (Fig. 1D). Overall, our results demonstrated a statistically significant increase in the level of αV expression as the cell progressed from G0/1 to S phase.

KSHV infected PEL cells obtained from S phase attach to ECM proteins

Apart from their role as virus entry receptors, integrins also regulate cellular attachment (Dyson *et al.*, 2007; Prieto *et al.*, 1993). Hence, we analyzed if the cell cycle progression-based elevated levels of αV integrins altered the ability of KSHV infected human B cells (GFP-BCBL-1 and BCP-1 suspension cells), to adhere to the ECM. Before, proceeding with the experiment, we analyzed expression of αV integrins in cells from G0/1 and S phases obtained by sorting using

a FACScan flow cytometer as per earlier protocols (Bryan *et al.*, 2006). This was done to authenticate our results obtained by synchronizing cells in different phases of cells by serum starvation method (Fig. 1). The majority of GFP-BCBL-1 and BCP-1 cells were in G0/1 phase followed by G2/M and S phase of cell cycle (Fig. 2A, B; a representative plot for GFP-BCBL-1 cells). The cells from G0/1 and S phase were sorted into separate tubes. These cells were analyzed for the expression of αV by qRT-PCR. There was significantly higher number of GFP-BCBL-1 and BCP-1 cells positive for αV expression in S phase of cell cycle when compared to those obtained from G0/1 phase (Fig. 2C). These results corroborate our qRT-PCR data from cells synchronized by serum starvation method (Fig. 1).

The role of αV integrins in mediating cell attachment was analyzed on a Matrigel. A Matrigel consists of several ECM proteins which are used to form a soluble basement membrane complex (Akula *et al.*, 2005). Cells expressing appropriate integrins will bind Matrigel as integrins interact with ECM proteins. GFP-BCBL-1 and BCP-1 cells were sorted into G0/1 and S phase using a flow cytometer. The sorted cells were layered on a Matrigel and incubated at 37°C. After 3h post-seeding, wells were vigorously washed to remove unadsorbed cells, and the adhered cells were counted under a microscope. Our results showed GFP-BCBL-1 and BCP-1 cells in S phase significantly attached to the Matrigel (Fig. 2D). On the contrary, there were very few cells from G0/1 phase that attached to the Matrigel (Fig. 2E). This is the first form of proof provided to show that KSHV infected suspension cells bind to ECM proteins; preferentially when they are in S phase.

αV integrins mediate attachment of uninduced PEL cells to ECM proteins

Integrins control a variety of cellular functions that include cell survival, gene expression, cell cycle, actin dynamics, cell migration, angiogenesis, including cell attachment (Martin *et al.*, 2002). The RGD amino acids are the minimal region of many ECM proteins required for the interactions with a subset of host cell surface integrins (Plow *et al.*, 2000). Hence, we tested the ability of RGD peptides to inhibit the attachment of flow cytometer-sorted PEL (GFP-BCBL-1 and BCP-1) cells in S phase to Matrigel. GRGDSPL peptides significantly inhibited attachment of GFP-BCBL-1 and BCP-1 cells (sorted from S phase) to Matrigel when compared to the GRADSPL peptides (Fig. 3A). We observed a dose-dependent inhibition of GFP-BCBL-1 and BCP-1 cell attachment by GRGDSPL peptides (Fig. 3A). These results implicated a possible role for RGD-binding integrins in mediating PEL cell attachment to Matrigel.

Next, we tested the ability of integrin-specific antibodies to neutralize the ability of flow cytometer-sorted GFP-BCBL-1 and BCP-1 cells in S phase to attach to a Matrigel. Antibodies to αV integrins significantly lowered the attachment of GFP-BCBL-1 and BCP-1 cells on a Matrigel (Fig. 3B). Antibodies to β1 integrins also lowered the attachment of PEL cells, but to a lesser extent. However, antibodies to β2 and β4 integrins did not significantly alter the ability of GFP-BCBL-1 and BCP-1 to attach to ECM proteins (Fig. 3B). Pre-immune IgGs did not alter the ability of the above PEL cells to attach on a Matrigel (data not shown). Our results demonstrated a specific and predominant role for αV integrins in the attachment of PEL cells to ECM proteins in a Matrigel system. Interestingly, αV integrins are RGD binding integrins (Plow *et al.*, 2000).

KSHV gB expression is enhanced in TPA-induced PEL cells in S phase

KSHV is found predominantly in a latent state. *In vitro*, a lytic infection can be induced by treating the cells with phorbol ester (TPA). In an uninduced state, only a small percentage of PEL cells support a KSHV lytic replication (Renne *et al.*, 1996). Next, BCBL-1 and BCP-1 cells were synchronized in S phase, uninduced or induced with TPA for 48h, fixed in acetone, and the expression of gB analyzed by IFA. Only a small portion of uninduced cells in S phase exhibited gB expression, suggestive of a lytic replication (data not shown; Fig. 4A). However,

the number of gB expressing cells increased dramatically under TPA induced conditions (Fig. 4A). These results clearly demonstrate a significant increase in gB expression in the TPA-induced BCBL-1 and BCP-1 cells derived from S phase.

KSHV gB mediates attachment of PEL cells supporting a lytic infection to ECs

PEL cells obtained from S phase that are supporting an active lytic infection express cellular αV and virus encoded gB; both of which can mediate cellular attachment. Integrin αV mediates cell attachment by binding to ECM proteins expressed on the target cells while, gB can mediate attachment by interacting with integrins expressed on the target cells. Hence, we attempted to analyze the functions of these receptor molecules in mediating attachment of PEL cells supporting a lytic infection to the ECs. In order to analyze the differential roles of αV and gB in cell attachment, we used BCBL-1 and BCP-1 cells that were flow cytometer-sorted in S phase and left uninduced or induced with TPA to support a lytic infection. Specific function blocking antibodies against αV and gB were used to analyze their ability to neutralize attachment of the above cells to HMVEC-d cells cultured in 96 well plates. Antibodies to αV significantly lowered the ability of uninduced BCBL-1 and BCP-1 cells in S phase to adhere to HMVEC-d cells when compared to antibodies to gB (Fig. 4B). On the contrary, when compared to cells that were incubated with medium (Fig. 4C, D), anti-gB antibodies (Fig. 4C, E) significantly inhibited the ability of BCBL-1 cells obtained from S phase and induced with TPA (supporting a lytic infection) from attaching to the surface of HMVEC-d cells. Monoclonal anti-αV antibodies (Fig. 4C, F) inhibited attachment of the above cells only to a lesser extent; while, pre-immune IgGs (Fig. 4G) did not alter the ability of cells supporting a lytic infection to bind HMVEC-d cells. Similar results were observed in BCP-1 cells that were supporting a lytic infection (Fig. 4C). Our results clearly implicate a greater role for gB (than αV) in mediating attachment of cells obtained from S phase and supporting an active KSHV lytic replication.

RGD domain of gB plays a crucial role in mediating attachment of PEL cells supporting a lytic infection to ECs

To confirm if the RGD domain in gB plays a role in cell attachment, we tested the ability of rabbit antibodies directed against RGD-containing peptides from within gB (RGDgB-N1) and rabbit antibodies against peptides lacking the RGD motif from within gB (RGDgB-C) in blocking PEL cells obtained from S phase and supporting a lytic infection from attaching to ECs. When compared to cells that were incubated with medium and pre-immune IgGs, anti-RGDgB-N1 antibodies significantly inhibited the ability of BCBL-1 and BCP-1 cells obtained from S phase and supporting a lytic infection from attaching to the surface of HMVEC-d cells (Fig. 5A). In contrast, anti-gB-C antibodies did not significantly alter the ability of cells to bind HMVEC-d cells (Fig. 5A). Further, we analyzed the ability of soluble gBΔTM (possesses the RGD motif) and gBΔTM-RGA (lacks the RGD motif) to competitively block the attachment of cells mediated by gB. HMVEC-d cells were incubated with serum free RPMI, gBΔTM in serum free RPMI, or gBΔTM-RGA in serum free RPMI at 37°C. After 1h incubation, the cells were washed in RPMI and layered with BCBL-1 and BCP-1 cells that were obtained from S phase and TPA-induced. KSHV encoded gBΔTM lowered the ability of BCBL-1 and BCP-1 cells supporting a lytic replication to bind to HMVEC-d cells (Fig. 5B). KSHV encoded gBΔTM-RGA did not significantly influence the attachment of cells implicating a crucial and specific role for the gB interactions with the RGD binding integrins in the attachment of cells to HMVEC-d cells.

Differential role for αV and gB in regulating cellular attachment

In uninduced KSHV infected BCBL-1 and BCP-1 cells obtained from S phase, αV integrins seem to mediate cell attachment (Fig. 4B). However, in TPA-induced BCBL-1 and BCP-1

cells (also in S phase), gB seems to play a predominant role in cell attachment (Fig. 4C). We hypothesized this phenomenon to be due to the intrinsic variation in the expression pattern of the receptor molecules. To test our hypothesis, we monitored the expression pattern of these receptor molecules by qRT-PCR under three different conditions: (1) G0/1 phase, (2) S phase, and (3) S phase and TPA induced. The qRT-PCR data suggests a significant increase in the expression of gB in cells from S phase that are TPA-induced when compared to those in S phase (Fig. 5C). Overall, the rank order of gB expression in cells from different phases was as follows: G0/1 < S phase < S phase + TPA-induced. In contrast, although αV expression was elevated in cells from S phase when compared to cells from G0/1 phase, we did not observe significant differences in the expression levels in cells obtained from S phase and S phase that are TPA-induced (Fig. 5C). The rank order of αV expression in cells from different phases was as follows: G0/1 < S phase = S phase + TPA-induced. The above results from qRT-PCR were further confirmed by IFA (Fig. 5D). BCBL-1 cells supporting a latent infection did not express gB while they expressed elevated levels of αV. On the contrary, BCBL-1 cells supporting a lytic infection expressed higher levels of gB as well as αV, to a lesser extent (refer to Fig. 6); interestingly, αV expression on the cell surface co-localized with gB expression under such circumstances. Co-localization indicates relative proximity of both these receptor molecules. This would possibly result in the binding of gB to αV on the cell surface and neutralize its function. Similar results were observed in BCP-1 cells (data not shown). However, both these experiments did not measure the flux in the levels of αV with respect to gB expression. Hence, we sorted TPA-induced BCBL-1 cells in S phase to yield two different cell populations: cells positive for gB and those negative for the expression of gB (Fig. 6A, B). The gB positive cells supported a lytic infection while the gB negative cells supported a latent infection (Akula et al., 2001a). RNA was extracted from the two different cell populations and qRT-PCR performed to monitor the expression of αV. Our results demonstrated about a three fold drop in the expression of αV in cells expressing gB when compared to cells negative for gB (Fig. 6C). Similar results were observed in BCP-1 cells with respect to αV expression (data not shown). The results point to the fact that there is a down-modulation of αV concomitant with an upregulation of gB expression. Taken together, this sharp rise in the expression levels of gB over αV may contribute for an increase in the role of gB (rather than αV) in promoting attachment of cells undergoing a lytic infection.

Discussion

The life cycle of many viruses depicts one of a wily opportunist. In particular, KSHV remains dormant while a cell proceeds with its normal functions until, finally, something prompts the cell to let its guard down. It is at this time when the virus takes full advantage of the situation. KSHV exerts its influence via hijacking cellular machinery for its gene expression and replication. KSHV, like other herpesviruses, has two modes of infection. During latency, very few genes are expressed. Most of the time, KSHV remains in this state, as roughly 1-3% of cells undergo lytic replication (Chen and Lagunoff, 2005). However, during lytic infection, a different and more numerous set of genes are expressed in a well orchestrated manner; eventually leading to possibly cell death and virus spread. Recent reports from our lab have looked into the intricate relationship between KSHV reactivation and the role of the cell cycle. Our results demonstrated a direct link between KSHV reactivation, cell cycle, and cell surface topology (Whitman *et al.*, 2007; Bryan *et al.*, 2006). In the present study, we focused further on the manner by which cell cycle progression, reactivation, and receptor expressions may alter KSHV associated pathogenesis.

Cells from S phase of the cell cycle are conducive for KSHV reactivation (Bryan *et al.*, 2006; McAllister *et al.*, 2005). Among the several receptor proteins that may have roles in KSHV pathogenesis, αV integrins were found to be augmented during S phase in three cell types (HFF, BCBL-1, and 293 cells) of different lineage. αV integrins were shown to definitely

have a significant part to play in mediating cellular attachment to ECM proteins. Integrins are key receptors involved in cellular attachment (Dyson *et al.*, 2007; Spessotto *et al.*, 2003; Spessotto *et al.*, 2001). The number of BCBL-1 cells derived from S phase that attached to a Matrigel was significantly more than those in G0/1 phase (Fig. 2E). RGD peptides and anti-αV antibodies both disrupted cellular attachment, thus confirming the role for αV integrins in mediating attachment of uninduced KSHV infected cells (Fig. 3). These results corroborated our earlier findings in that αV and β1 integrins played a role in cellular attachment and angiogenesis, respectively (Dyson *et al.*, 2007). It is easy to forget that B-lymphocytes have the ability to attach *in vivo*; probably because they are commonly referred to as suspension cells. However, the role of B cell attachment has been described in numerous reports (Spessotto *et al.*, 2003; Spessotto *et al.*, 2001). B cell adhesion is involved primarily in directing lymphocytes towards lymphoid organs and inflammation sites (Yago *et al.*, 1997). Adhesion is part of the process of B-cell migration across the endothelium which is initiated through the rolling of B cells across the ECs and subsequent activation (Carlos and Harlan, 1994). B cell binding to ECM proteins also leads to their close proximity to survival factors released by ECs that provide survival signals (Murti *et al.*, 1996). Survival signals promote cell division (Brunet *et al.*, 2001). Taken together, it makes sense for cells predominantly in S phase over G0/1 phase of the cell cycle to attach to ECs. Under the conditions tested KSHV infection of target cells (HFF cells) seem to be independent of cell cycle (data not shown). This could be due to the cell type used in the assay and the involvement of other factors that may influence infection.

The receptor expression profile changes as the cells progress from one phase to another. On the same lines, the expression of KSHV encoded gB increased by significant magnitudes over αV when PEL cells progressed from supporting a latent to an actively lytic replication (Fig. 5C and 6). Accordingly, we determined gB to play a major role when compared to αV in mediating attachment of cells that were supporting a lytic replication (Fig. 4B, C). Based on our results, we propose a model that may well explain the differential role for αV and gB in mediating attachment of KSHV infected B cells to the ECs (Fig. 7). This model takes into account the following changes that occur on the cell surface with respect to cell cycle progression: (1) the cells in G0/1 phase have a Medusa-head like cell surface and the cells in S phase have a smooth cell surface as observed under an electron-microscope (Whitman *et al.*, 2007); (2) The cells in resting stage (G0/1) primarily support a latent KSHV infection, while the cells in S phase provide optimal conditions for a productive reactivation of a latent infection; in other words, a lytic infection (Bryan *et al.*, 2006;McAllister *et al.*, 2005); and (3) The present studies provide evidence that αV expression is cell cycle dependent and is not altered by TPA-induced reactivation. On the contrary, gB expression is cell cycle dependent and is significantly enhanced upon KSHV lytic infection. Under normal and uninduced conditions, S phase cells express elevated levels of αV when compared to cells in G0/1 phase. Hence, αV integrins mediate attachment of PEL cells primarily in S phase by interacting with ECM proteins (like laminin) expressed on the target cells. However, as the cells in S phase start to support a KSHV lytic infection, these cells express elevated levels of gB on their surface that can also mediate attachment by interacting with RGD binding integrins expressed on the target cells. Apart from the fact that there is a sudden increase in the levels of gB over αV in cells supporting reactivation (Fig. 6), there is a possibility for gB to bind directly to αV integrins on the cells and neutralize their function (Fig. 5D). At this point, we hypothesize that such an attachment of cells supporting a lytic infection to the ECs will serve to prevent apoptosis and increase survivability for enhanced KSHV replication; both of which are essential for a productive reactivation (Bryan *et al.*, 2006).

This is the first report that delineates a role for two different receptor molecules (cellular and viral) in mediating attachment of B cells in different stages of the cell cycle and KSHV infection. It is a classic example of the manner in which KSHV hijacks cellular processes and manipulates them for its own benefit. Thus, our results have provided new insight into studying

virus-target cell interactions with a special emphasis on KSHV reactivation, cell cycle, and surface topology. The study also raises several interesting questions that may lead to a better understanding of the whole process of KSHV reactivation: (1) what is the role of actin fibers/microtubules in the change in surface topology and receptor expression as the cells progress from one phase of the cell cycle to the other?; (2) what is the role of gB in promoting survivability in KSHV infected cells?; and (3) does gB also promote migration in a fraction of these adhered B cells? Taken together, the present studies will serve as a step taken in the correct direction towards the understanding of KSHV reactivation.

Experimental procedures

Cells

BCBL-1, BCBL-1 cells harboring recombinant KSHV expressing green fluorescent protein (GFP-BCBL-1), BCP-1, 293, HFF, and dermal microvascular endothelial cells (HMVEC-d; CC-2543, Clonetics) were used in this study. BCBL-1, GFP-BCBL-1, and BCP-1 cells were grown in phenol red-free RPMI medium (Invitrogen, Carlsbad, CA, USA) containing 10% charcoal stripped FBS (Atlanta Biologicals Inc., Lawrenceville, GA, USA), L-glutamine, and antibiotics (Akula *et al.*, 2004). 293 and HFF cells were propagated in DMEM containing 10% charcoal stripped FBS as per earlier protocols (Ford *et al.*, 2004; Hamden *et al.*, 2004). HMVEC-d cells were propagated in EGM™ MV-microvascular endothelial cell (EC) medium (Clonetics) as per standard protocols (Dyson *et al.*, 2007). The passage numbers for HMVEC-d cells used in this study ranged between 5 and 9.

Peptides and antibodies

GRGDSPL and GRADSPL peptides purchased from Sigma (St. Louis, MO) were used in this study. Function-blocking antibodies to various integrins were used in this study. Mouse monoclonal anti-human integrin β2 [CD18] (clone R3.3) was purchased from Biosource, Camarillo, CA. Mouse monoclonal anti-human integrin β1 [CD29] (clone 6S6) and rat monoclonal anti-human integrin β4 [CD104] (clone 439-9B) were purchased from Chemicon international (Temecula, CA). Mouse monoclonal anti-human integrin αV (clone 313.6F8) purchased from Calbiochem (San Diego, CA) was used in this study. Rabbit anti-gB antibodies, rabbit anti-RGDgB-N1 antibodies, and rabbit anti-gB-C antibodies were also used in this study (Akula *et al.*, 2002).

Quantitative real-time PCR (qRT-PCR)

The qRT-PCR was performed using the synthesized cDNA in a 25μl reaction volume to analyze the expression of ORF8, thymidine kinase 1 (TK1), β-actin, VEGFR-1, VEGFR-2, DC-SIGN, β1, GlcNAcT-I, αV as per earlier protocols (Whitman *et al.*, 2007; Bryan *et al.*, 2006; Rouahi *et al.*, 2006; Nair *et al.*, 2005; Yang *et al.*, 2004; Li *et al.*, 2002).

Western blotting

All the buffers used in this project were made with water that was endotoxin and pyrogen free. Equal amounts (25μg) of protein was used in Western blotting experiments (Dyson *et al.*, 2007). The Western blots were routinely probed first with rabbit anti-αV antibody (Millipore, Temecula, CA). These blots were then "stripped' for reprobing with mouse anti-actin antibodies (Clone AC-74; Sigma) as per standard protocols. The bands were scanned and the band intensities were assessed using the ImageQuaNT software program (Molecular Dynamics).

Sorting of cells in different phases of the cell cycle

In this study we used two different methods to sort cells in different phases of cell cycle and they were serum starvation method and by flow cytometry. We synchronized BCBL-1 cells in G0/1 and S phase of cell cycle as per standard protocols (Krek and DeCaprio, 1995). In serum starvation method, cells were synchronized at G0/1 by 24h of incubation in serum-free RPMI 1640 or DMEM supplemented with antibiotics and 2mM glutamine. Cells were synchronized at S phase by culturing the above (those synchronized in G0/1 phase) cells in RPMI 1640 or DMEM supplemented with 10% FBS, antibiotics and 2mM glutamine for 16h. For major part of this study we used PEL cells (BCBL-1 and BCP-1) that were sorted in different phases of cell cycle by a flow cytometer. Briefly, 5×10^6 cells cultured in RPMI medium containing 10% FBS was incubated with 10μg/ml concentration of Hoechst dye 33342 (Sigma) at 37°C for 45min. These cells were analyzed by using a FACScan flow cytometer (Becton Dickinson) to discriminate cells in different stages of the cell cycle as per earlier protocols (Bryan *et al.*, 2006).

Adhesion assay

The ability of KSHV infected GFP-BCBL-1 and BCP-1 cells to attach to ECM were analyzed on a tumor-derived basement membrane matrix (Matrigel; Discovery labware, Bedford, MA). Ninety-six-well culture dishes were coated with 80μl/well of Matrigel on ice, then allowed to polymerize for 30 min at 37°C. Target cells from different phases of the cell cycle were seeded at a concentration of 5,000 cells/well onto the Matrigel. After 3h post seeding, the wells were washed vigorously to remove any unbound cells and the adherent cells were counted under a fluorescent microscope and an inverted microscope for GFP-BCBL-1 and BCP-1 cells, respectively. Overall, this assay was used to test the ability of RGD peptides and integrin antibodies to block attachment of cells to Matrigel.

Immunofluorescence assay (IFA)

Flow cytometer-sorted cells from S phase that were left uninduced or TPA-induced for 48h were analyzed for the expression of gB by IFA as per earlier protocols (Akula *et al.*, 2001b). Briefly, target cells were washed twice in phosphate buffered saline (PBS), spotted on glass slides, air-dried, and then fixed in ice-cold acetone for 10 min. The fixed cells were washed in PBS and reacted with rabbit anti-gB antibodies followed by goat anti-rabbit antibodies conjugated to fluorescence isothiocyanate (FITC) (Sigma) for 30 min at room temperature. Stained cells were washed with PBS and mounted in anti-fade reagent (Molecular Probes, Eugene, Oreg.) before examining under a fluorescent microscope (Nikon Eclipse E600) with appropriate filters. In another set of experiments, we analyzed if αV co-localized with gB on the surface of KSHV infected cells. This was done by using flow cytometer-sorted PEL cells from S phase. These cells were also TPA induced for 48h. The cells were fixed in 0.1% paraformaldehyde at +4°C for 10 min. The cells were washed twice in PBS at 1000 rpm, +4° C for 10 min and further incubated with rabbit anti-gB antibodies and mouse monoclonal anti-human integrin αV antibodies for 30 min on ice with constant shaking. The cells were washed twice in PBS and further incubated with goat anti-rabbit FITC and goat anti-mouse tetramethyl rhodamine isothio-cyanate (TRITC, Sigma) for 30 min on ice as before. These cells were washed twice as before, spotted on a glass slide, a coverslip placed on it, and viewed under a fluorescent microscope.

Purifying Soluble KSHV-gBΔTM and gBΔTM-RGA

Expression and purification of the KSHV-gBΔTM (2106bp; encoding aa 1 to 702 lacking the transmembrane and cytoplasmic domains) and KSHV-gBΔTM-RGA proteins from the infected High-5 cells were done using nickel columns (PharMingen) as per procedures described before (Wang *et al.*, 2003).

Flow cytometry to sort gB positive and negative cells

Cells in S phase were treated with 20ng/ml of TPA. After 36h post TPA treatment, these cells were washed twice in cold PBS and stained with rabbit anti-gB antibodies for 30 min on ice. The cells were washed thrice in cold PBS and further incubated with a predetermined concentration of anti-rabbit antibodies conjugated to fluorescence isothiocyanate (FITC) (Sigma). After 30 min of incubation, these cells were washed as before and sorted for cells that were positive and negative for gB, respectively, using a FACScan flow cytometer (Becton Dickinson) as per standard lab protocols (Bryan et al., 2006). RNA was extracted from the cells and qRT-PCR performed to analyze the expression of αV as per standard protocols.

Cell-to-cell attachment assay

HMVEC-d cells cultured in 96 well plates were used for this study. TPA-induced BCBL-1 and BCP-1 cells in S phase were seeded at a concentration of 1,000 cells/well allowing 2h at 37° C for them to adhere to HMVEC-d cells. At the end of 2h incubation, the cells were washed three times in phenol red-free RPMI to get rid of unattached cells. After the final wash, wells were analyzed for the number of PEL cells attached to HMVEC-d cells by counting them. This experimental design was used to test the ability of anti-gB (30μg/ml), anti-RGDgB-N1 (30μg/ml), anti-gB-C (30μg/ml), and anti-αV antibodies (20μg/ml) to inhibit attachment of BCBL-1 and BCP-1 cells to HMVEC-d cells. Briefly, the above PEL cells were incubated with specific antibodies at +4°C in 100μl volume. After 1h incubation, cells were washed once in 13ml of RPMI containing 2% FBS and layered on HMVEC-d cells and tested for their ability to adhere. In another set of experiments, HMVEC-d cells were incubated with serum free RPMI, 25ng/ml of gBΔTM in serum free RPMI, or 25ng/ml of gBΔTM-RGA in serum free RPMI at 37°C. After 1h incubation, the cells were washed in RPMI and further layered with PEL cells and tested for their ability to adhere as per standard protocols.

Acknowledgements

This work was supported in part by NIH/NIBIB grant R21EB006483 to SMA. We also thank Mr. Huxley for critically reading this manuscript.

References

Akula SM, Pramod NP, Wang FZ, Chandran B. Human herpesvirus 8 envelope-associated glycoprotein B interacts with heparan sulfate-like moieties. Virology 2001a;284:235–249. [PubMed: 11384223]

Akula SM, Wang FZ, Vieira J, Chandran B. Human herpesvirus 8 interaction with target cells involves heparan sulfate. Virology 2001b;282:245–255. [PubMed: 11289807]

Akula SM, Pramod NP, Wang FZ, Chandran B. Integrin alpha3beta1 (CD 49c/29) is a cellular receptor for Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) entry into the target cells. Cell 2002;108:407–419. [PubMed: 11853674]

Akula SM, Naranatt PP, Walia NS, Wang FZ, Fegley B, Chandran B. Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) infection of human fibroblast cells occurs through endocytosis. J Virol 2003;77:7978–7990. [PubMed: 12829837]

Akula SM, Ford PW, Whitman AG, Hamden KE, Shelton JG, McCubrey JA. Raf promotes human herpesvirus-8 (HHV-8/KSHV) infection. Oncogene 2004;23:5227–5241. [PubMed: 15122343]

Akula SM, Ford PW, Whitman AG, Hamden KE, Bryan BA, Cook PP, McCubrey JA. B-Raf-dependent expression of vascular endothelial growth factor-A in Kaposi sarcoma-associated herpesvirus-infected human B cells. Blood 2005;105:4516–4522. [PubMed: 15705790]

Birkmann A, Mahr K, Ensser A, Yaguboglu S, Titgemeyer F, Fleckenstein B, Neipel F. Cell surface heparan sulfate is a receptor for human herpesvirus 8 and interacts with envelope glycoprotein K8.1. J Virol 2001;75:11583–11593. [PubMed: 11689640]

Brunet A, Datta SR, Greenberg ME. Transcription-dependent and -independent control of neuronal survival by the PI3K-Akt signaling pathway. Curr Opin Neurobiol 2001;11:297–305. [PubMed: 11399427]

Bryan BA, Dyson OF, Akula SM. Identifying cellular genes crucial for the reactivation of Kaposi's sarcoma-associated herpesvirus latency. J Gen Virol 2006;87:519–529. [PubMed: 16476973]

Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. Blood 1994;84:2068–2101. [PubMed: 7522621]

Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N Engl J Med 1995;332:1186–1191. [PubMed: 7700311]

Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 1994;266:1865–1869. [PubMed: 7997879]

Chen L, Lagunoff M. Establishment and maintenance of Kaposi's sarcoma-associated herpesvirus latency in B cells. J Virol 2005;79:14383–14391. [PubMed: 16254372]

Dyson OF, Bryan BA, Lambert PJ, Ford PW, Akula SM. beta1 Integrins Mediate Tubule Formation Induced by Supernatants Derived from KSHV-Infected Cells. Intervirology 2007;50:245–253. [PubMed: 17460413]

Ford PW, Hamden KE, Whitman AG, McCubrey JA, Akula SM. Vascular Endothelial Growth Factor Augments Human Herpesvirus-8 (HHV-8/KSHV) Infection. Cancer Biol Ther 2004;3

Giancotti FG, Ruoslahti E. Integrin signaling. Science 1999;285:1028–1032. [PubMed: 10446041]

Hamden KE, Ford PW, Whitman AG, Dyson OF, Cheng SY, McCubrey JA, Akula SM. Raf-induced vascular endothelial growth factor augments Kaposi's sarcoma-associated herpesvirus infection. J Virol 2004;78:13381–13390. [PubMed: 15542692]

Jenner RG, Alba MM, Boshoff C, Kellam P. Kaposi's sarcoma-associated herpesvirus latent and lytic gene expression as revealed by DNA arrays. J Virol 2001;75:891–902. [PubMed: 11134302]

Krek W, DeCaprio JA. Cell synchronization. Methods Enzymol 1995;254:114–124. [PubMed: 8531680]

Li J, Shworak NW, Simons M. Increased responsiveness of hypoxic endothelial cells to FGF2 is mediated by HIF-1alpha-dependent regulation of enzymes involved in synthesis of heparan sulfate FGF2-binding sites. J Cell Sci 2002;115:1951–1959. [PubMed: 11956326]

Mao Y, Wu J, Skog S, Eriksson S, Zhao Y, Zhou J, He Q. Expression of cell proliferating genes in patients with non-small cell lung cancer by immunohistochemistry and cDNA profiling. Oncol Rep 2005;13:837–846. [PubMed: 15809747]

Martin KH, Slack JK, Boerner SA, Martin CC, Parsons JT. Integrin connections map: to infinity and beyond. Science 2002;296:1652–1653. [PubMed: 12040184]

McAllister SC, Hansen SG, Messaoudi I, Nikolich-Zugich J, Moses AV. Increased efficiency of phorbol ester-induced lytic reactivation of Kaposi's sarcoma-associated herpesvirus during S phase. J Virol 2005;79:2626–2630. [PubMed: 15681463]

Mercader M, Taddeo B, Panella JR, Chandran B, Nickoloff BJ, Foreman KE. Induction of HHV-8 lytic cycle replication by inflammatory cytokines produced by HIV-1-infected T cells. Am J Pathol 2000;156:1961–1971. [PubMed: 10854219]

Murti KG, Brown PS, Kumagai M, Campana D. Molecular interactions between human B-cell progenitors and the bone marrow microenvironment. Exp Cell Res 1996;226:47–58. [PubMed: 8660938]

Nair MP, Mahajan SD, Schwartz SA, Reynolds J, Whitney R, Bernstein Z, et al. Cocaine modulates dendritic cell-specific C type intercellular adhesion molecule-3-grabbing nonintegrin expression by dendritic cells in HIV-1 patients. J Immunol 2005;174:6617–6626. [PubMed: 15905500]

Plow EF, Haas TA, Zhang L, Loftus J, Smith JW. Ligand binding to integrins. J Biol Chem 2000;275:21785–21788. [PubMed: 10801897]

Prieto AL, Edelman GM, Crossin KL. Multiple integrins mediate cell attachment to cytotactin/tenascin. Proc Natl Acad Sci U S A 1993;90:10154–10158. [PubMed: 7694284]

Rappocciolo G, Jenkins FJ, Hensler HR, Piazza P, Jais M, Borowski L, et al. DC-SIGN is a receptor for human herpesvirus 8 on dendritic cells and macrophages. J Immunol 2006;176:1741–1749. [PubMed: 16424204]

Renne R, Zhong W, Herndier B, McGrath M, Abbey N, Kedes D, Ganem D. Lytic growth of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in culture. Nat Med 1996;2:342–346. [PubMed: 8612236]

Rouahi M, Champion E, Hardouin P, Anselme K. Quantitative kinetic analysis of gene expression during human osteoblastic adhesion on orthopaedic materials. Biomaterials 2006;27:2829–2844. [PubMed: 16427124]

Soulier J, Grollet L, Oksenhendler E, Cacoub P, Cazals-Hatem D, Babinet P, et al. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. Blood 1995;86:1276–1280. [PubMed: 7632932]

Spessotto P, Yin Z, Magro G, Deutzmann R, Chiu A, Colombatti A, Perris R. Laminin isoforms 8 and 10 are primary components of the subendothelial basement membrane promoting interaction with neoplastic lymphocytes. Cancer Res 2001;61:339–347. [PubMed: 11196184]

Spessotto P, Cervi M, Mucignat MT, Mungiguerra G, Sartoretto I, Doliana R, Colombatti A. beta 1 Integrin-dependent cell adhesion to EMILIN-1 is mediated by the gC1q domain. J Biol Chem 2003;278:6160–6167. [PubMed: 12456677]

Wang FZ, Akula SM, Sharma-Walia N, Zeng L, Chandran B. Human herpesvirus 8 envelope glycoprotein B mediates cell adhesion via its RGD sequence. J Virol 2003;77:3131–3147. [PubMed: 12584338]

Whitman AG, Dyson OF, Lambert PJ, Oxendine TL, Ford PW, Akula SM. Changes occurring on the cell surface during KSHV reactivation. J Electron Microsc (Tokyo) 2007;56:27–36. [PubMed: 17392397]

Yago T, Tsukuda M, Tajima H, Nishi T, Kurata-Miura K, Ohkubo J, Minami M. Analysis of initial attachment of B cells to endothelial cells under flow conditions. J Immunol 1997;158:707–714. [PubMed: 8992986]

Yang L, Dan HC, Sun M, Liu Q, Sun XM, Feldman RI, et al. Akt/protein kinase B signaling inhibitor-2, a selective small molecule inhibitor of Akt signaling with antitumor activity in cancer cells overexpressing Akt. Cancer Res 2004;64:4394–4399. [PubMed: 15231645]

Zhong W, Wang H, Herndier B, Ganem D. Restricted expression of Kaposi sarcoma-associated herpesvirus (human herpesvirus 8) genes in Kaposi sarcoma. Proc Natl Acad Sci U S A 1996;93:6641–6646. [PubMed: 8692871]

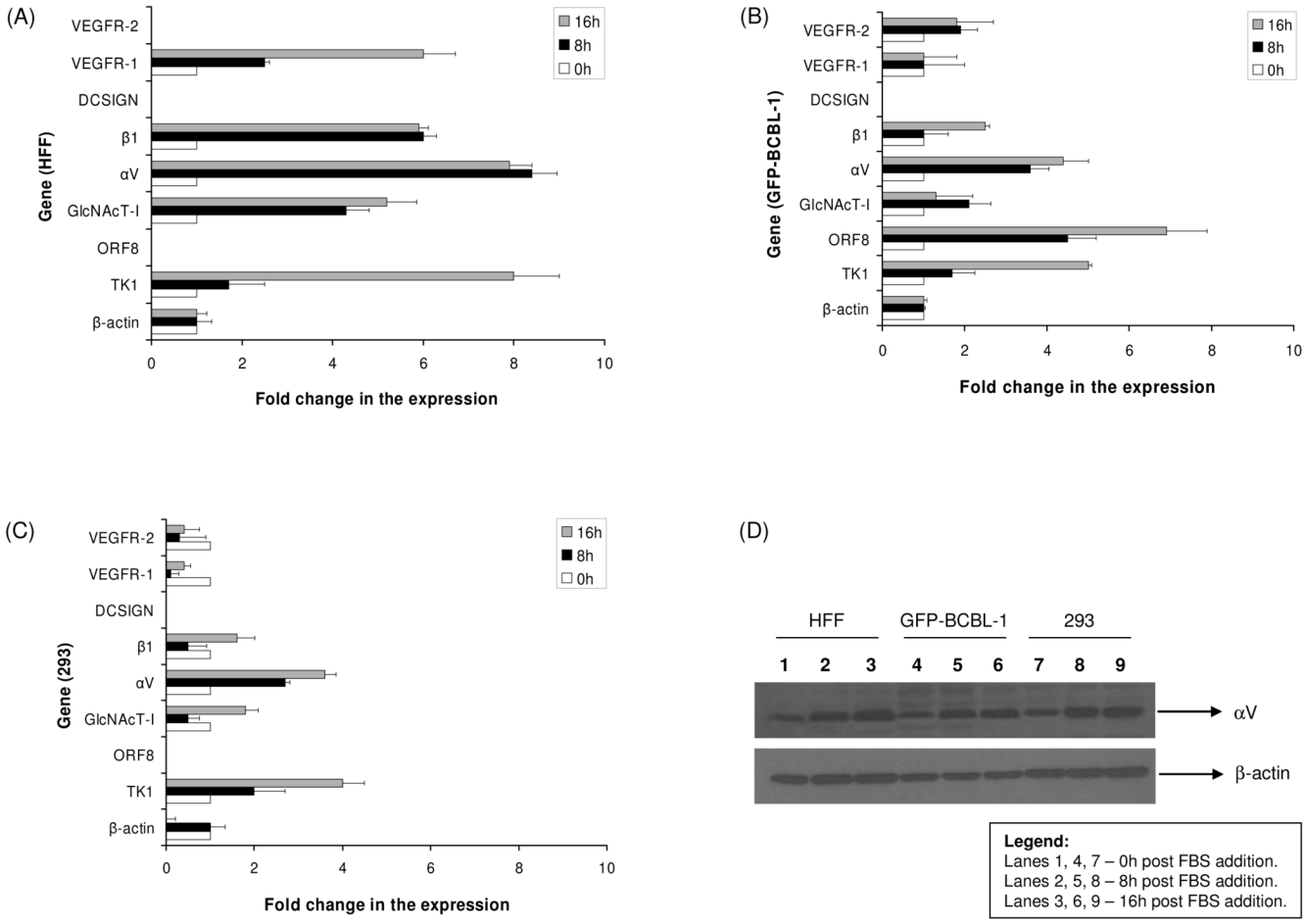

Figure 1. An elevated level of αV expression was observed in KSHV infected GFP-BCBL-1 cells derived from S phase

(A) HFF, BCBL-1, and 293 cells were serum starved for 24h. These cells were incubated in respective medium containing 10% FBS. The cells were collected at 0, 8, and 16h post FBS addition, lysed, RNA extracted, and qRT-PCR performed. The fold increase in the expression of different genes is represented by a bar graph and was calculated based on the graph generated by qRT-PCR of a known concentration of the respective genes. Expression of a gene at 0h was considered as 1 fold. The lowest limit of detection in the standard samples was 6 to 60 copies for a gene. Data presented represent the average ± SD of three experiments. **(B)** Western blot analysis of αV integrins and β-actin expression in the above cells was performed using cell lysates obtained at different time points post FBS addition as per standard protocols using proteins resolved in a 7.5% SDS-PAGE and respective antibodies.

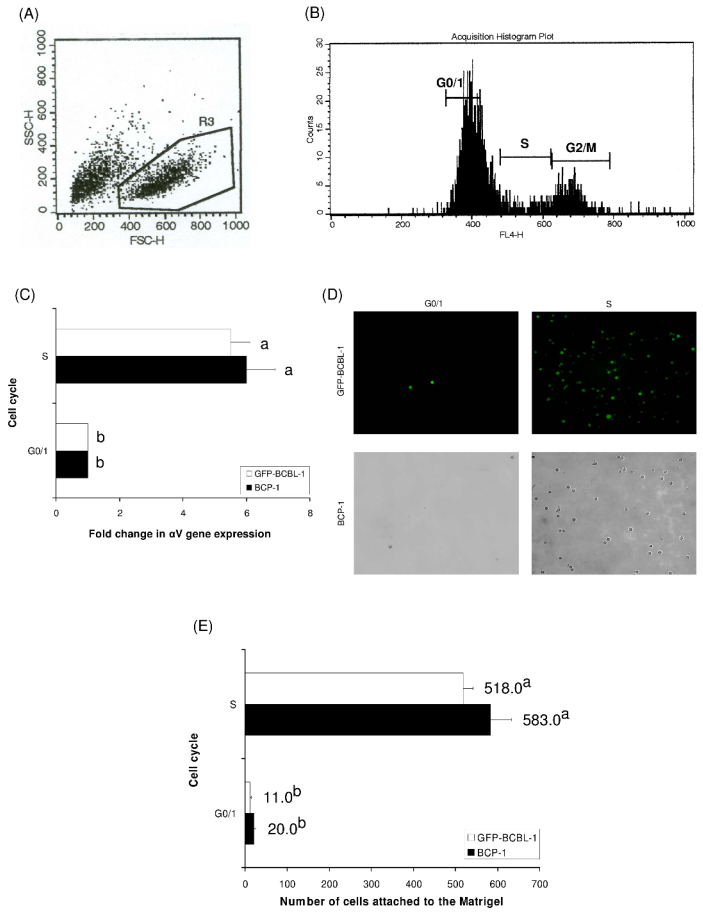

Figure 2. FACScan flow cytometer-sorted PEL cells in S phase express elevated levels of αV

(A) Forward- and side-scatter plot of GFP-BCBL-1 cells stained with Hoechst dye 33342. Cells gated in R3 were sorted. **(B)** Cells in R3 were sorted to obtain cells from the G0/1 and S phases of the cell cycle, respectively. Both panels A and B are representative flow cytometry plots. **(C)** These sorted cells at different phases were lysed, RNA extracted, and qRT-PCR performed. The fold increase in the expression of genes is represented by a bar graph. Expression of a gene at G0/1 phase was considered as 1 fold. Data presented represent the average±SD of three experiments. Columns with different superscripts are statistically significant ($P<0.05$) by least significant difference (LSD). **(D) PEL cells derived from S phase attach to ECM proteins.** Flow cytometer-sorted PEL cells from S phase were seeded onto a Matrigel. The sorted cells were incubated at 37°C, allowing enough time for attachment. After 3h incubation, cells were washed to remove any unbound cells and viewed under an inverted fluorescent (GFP-BCBL-1) and light microscope (BCP-1), respectively. Representative micrographs of BCBL-1 and BCP-1 cells from G0/1 and S phase attached on a Matrigel is shown (Magnification: 40X; panels for BCP-1 cells have been magnified for better clarity). **(E)** Data represents the number of attached PEL cells counted over 5 random fields. Each bar denotes the average ± SD of three experiments. Average values on columns with different superscripts are statistically significant ($P < 0.05$) by LSD.

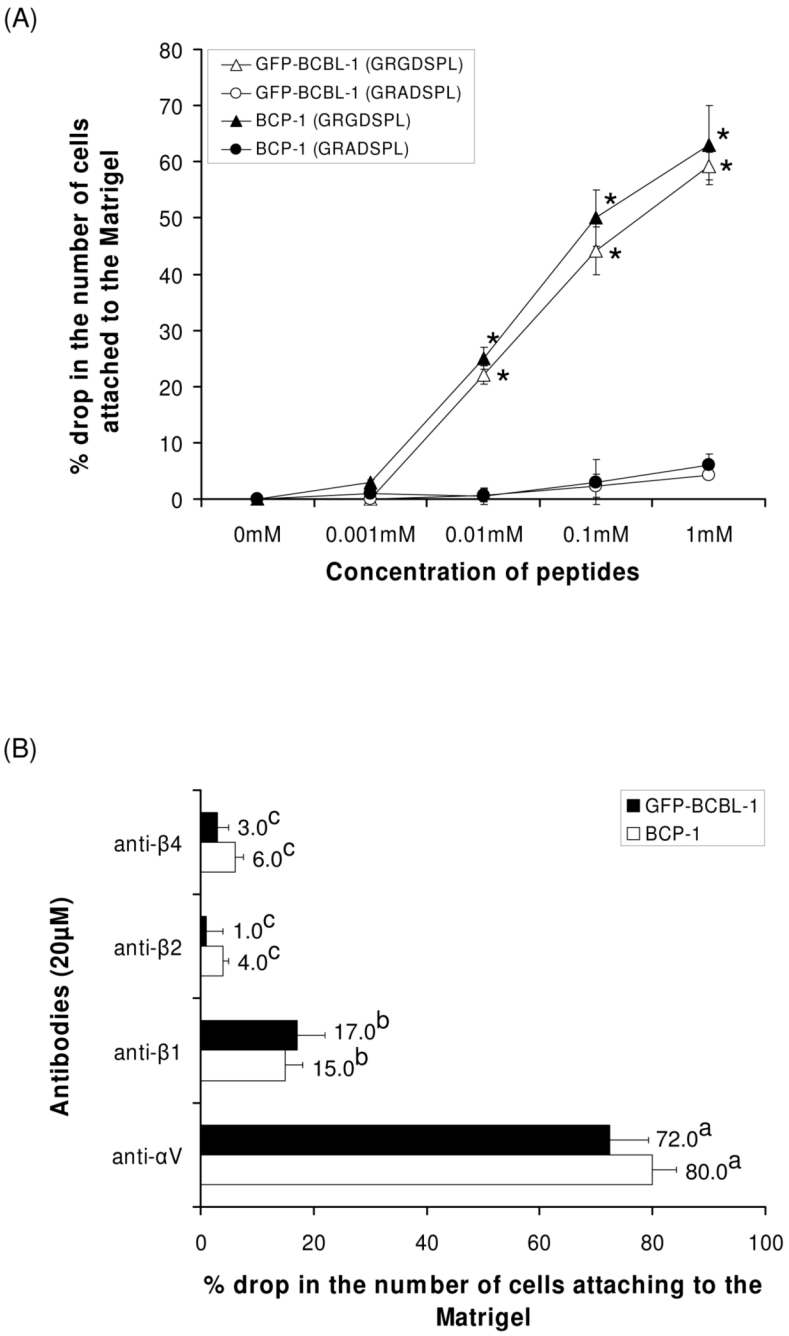

Figure 3. Uninduced PEL cell attachment to ECM proteins is regulated by αV integrins

(A) RGD peptides block PEL cell attachment. Briefly, cells in S phase were incubated with different concentrations of GRGDSPL or GRADSPL peptides at 37°C. After 1h, these cells were seeded onto a Matrigel and incubated at 37°C. At the end of 3h, cells were washed to remove any unbound cells and viewed under an inverted microscope to count for the number of attached cells. Data represent the percentage drop in the number of cells attached at a given peptide concentration. Each point on the graph represents the average ± SD of three experiments. The points with an asterisk mark indicate the value to be statistically significant ($p < 0.05$) by LSD. **(B)** Attachment of PEL cells to ECM proteins is blocked by function blocking anti-αV antibodies. Cells in S phase were treated with antibodies targeting αV, β1,

β2, or β4 integrins for 1h at 37°C. These cells were layered on a Matrigel and incubated for an additional 3h. After washing, the number of attached cells was counted under an inverted microscope. Data represent the percentage drop in the number of cells attached. Each point on the graph represents the average ± SD of three experiments. Average values on columns with different superscripts are statistically significant ($P < 0.05$) by LSD.

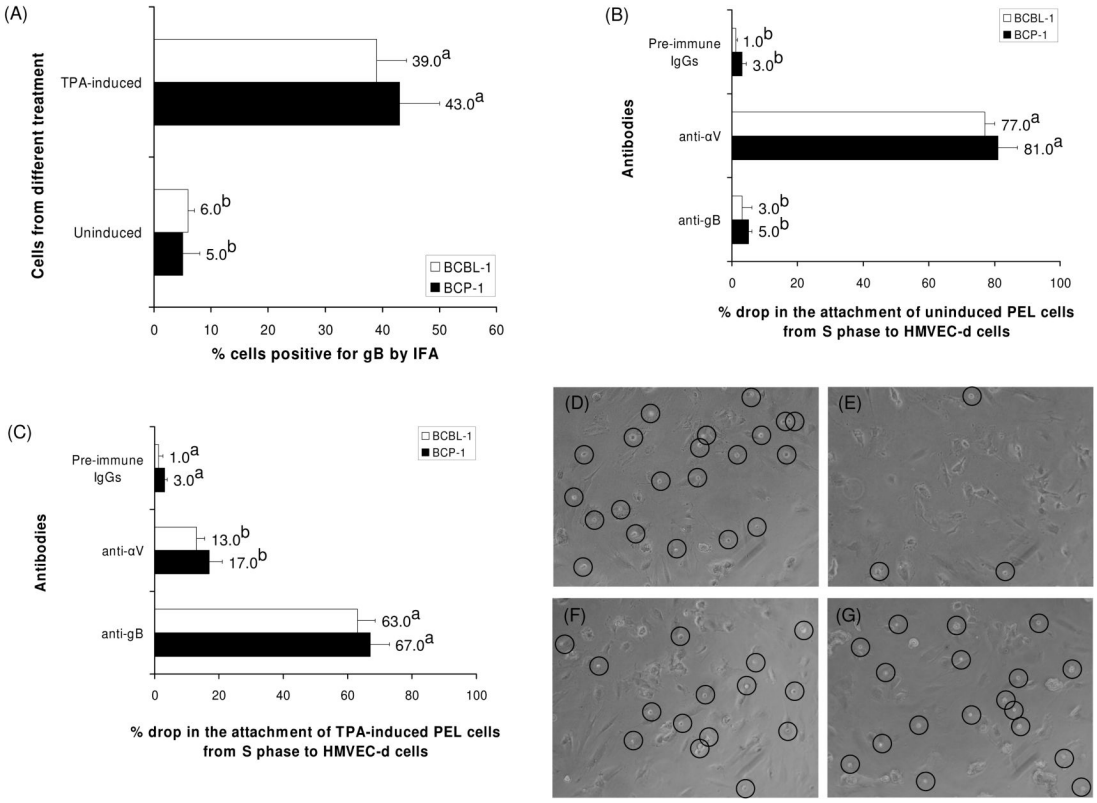

Figure 4. TPA-induced PEL cells in S phase display an increase in KSHV gB expression

(A) Data represent the percentage of uninduced or TPA-induced BCBL-1 and BCP-1 cells that are positive for gB expression. Briefly, target cells were synchronized in S phase, uninduced or TPA-induced for 48h, fixed in acetone, and the expression of gB analyzed by IFA. Each bar denotes the average ± SD of three experiments. Average values on columns with different superscripts are statistically significant ($P < 0.05$) by LSD. **(B-G) TPA-induced PEL cells attach to ECs via KSHV gB.** Flow cytometer-sorted PEL cells in S phase were either uninduced **(B)** or induced with 20ng/ml of TPA and incubated for 48h **(C)**. These cells were incubated with RPMI or RPMI containing specific antibodies at 37°C. After 1h incubation, the cells were added to wells with HMVEC-d cells. After 2h, cells were washed using phenol red-free RPMI to remove any unbound cells. PEL cells attached to HMVEC-d cells were counted under a microscope. Each bar denotes the average ± SD of three experiments. Average values on columns with different superscripts are statistically significant ($P < 0.05$) by LSD. Representative micrographs **(D-G)** showing the effects of incubating PEL cells with RPMI alone **(D)**, anti-gB antibodies **(E)**, anti-αV **(F)**, and pre-immune IgGs **(G)** on attachment to HMVEC-d cells is shown. BCBL-1 cells attached to target cells are identified by black circles.

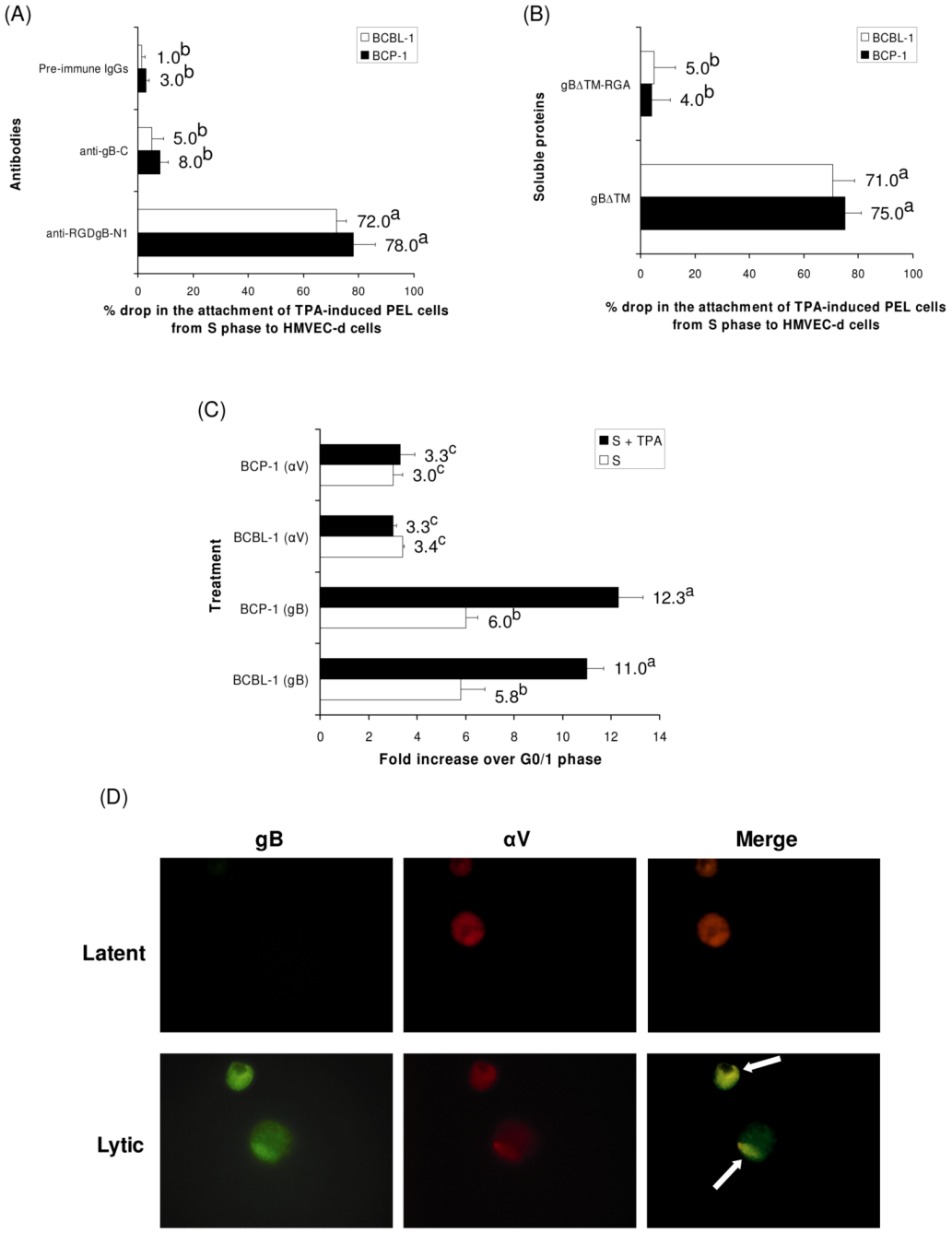

Figure 5. RGD motif in the KSHV encoded gB is essential for the attachment of TPA-induced PEL cells in S phase to ECs

(A) Flow cytometer-sorted PEL cells in S phase were induced with 20ng/ml of TPA and incubated for 48h. These cells were incubated with RPMI containing specific antibodies at 37° C. After 1h incubation, the cells were added to wells with HMVEC-d cells. After 2h, cells were washed using phenol red-free RPMI to remove any unbound cells. PEL cells attached to HMVEC-d cells were counted under a microscope and the % drop in attachment represented as a bar graph. **(B)** HMVEC-d cells were incubated with RPMI, RPMI containing gBΔTM, or RPMI containing gBΔTM-RGA at 37°C. After 1h, these cells were used to test the ability of TPA-induced PEL cells in S phase to attach as per standard protocols. Each bar denotes the

average ± SD of three experiments. Average values on columns with different superscripts are statistically significant ($P < 0.05$) by LSD. **(C, D) αV and gB regulate cellular attachment under different conditions. (C)** Flow cytometer-sorted PEL cells were used in this study. Target cells from G0/1, S, or in S phase and TPA-induced for 48h were lysed, and RNA extracted. The expression of αV integrins and gB was analyzed via qRT-PCR using specific primers. Data represent the fold change from G0/1 phase. Each bar denotes the average ± SD of three experiments. Average values on columns with different superscripts are statistically significant ($P < 0.05$) by LSD. **(D)** Fluorescent microscopy to analyze the expression pattern of gB and αV in BCBL-1 cells. The above cells were fixed in 0.1% paraformaldehyde at +4° C for 10 min. The fixed cells were incubated with rabbit anti-gB antibodies and mouse monoclonal anti-human integrin αV antibodies for 30 min on ice. These cells were washed in PBS and further incubated with goat anti-rabbit FITC and goat anti-mouse tetramethyl rhodamine isothio-cyanate for 30 min on ice. The above stained cells were examined under a fluorescent microscope. Cells expressing gB were considered to be supporting a lytic infection; while those negative for gB were considered to be supporting a latent KSHV infection. Arrowheads indicate the areas of co-localization between gB and αV expression. Magnification, 100X.

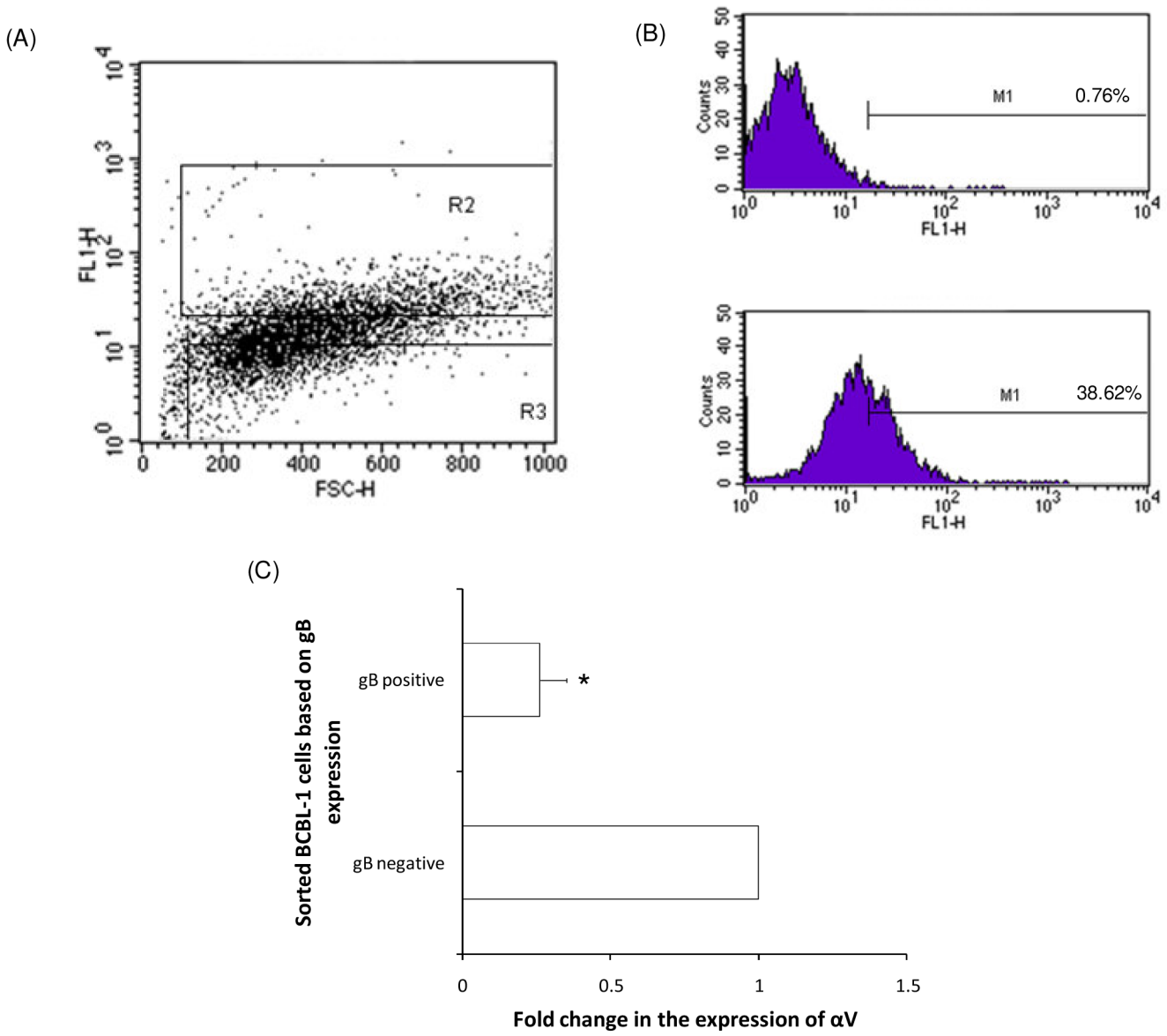

Figure 6. PEL cells supporting a lytic infection express elevated levels of gB and a significantly lower levels of αV

BCBL-1 cells in S phase were TPA stimulated for 36h. These cells were stained for the expression of gB using specific antibodies. The stained cells were sorted using a FACScan flow cytometer (Becton Dickinson) to obtain gB positive and gB negative cells. **(A)** A representative forward- and side-scatter plot of these stained cells is provided. **(B)** Cells in R2 and R3 were sorted to obtain cells that were positive and negative for gB expression, respectively. **(C)** RNA was extracted from the sorted cells and qRT-PCR performed to analyze the expression of αV. Expression of αV gene by gB negative cells was considered as 1 fold. Each bar denotes the average ± SD of three experiments. The column with an asterisk mark indicates the value to be statistically significant ($P<0.05$) by LSD.

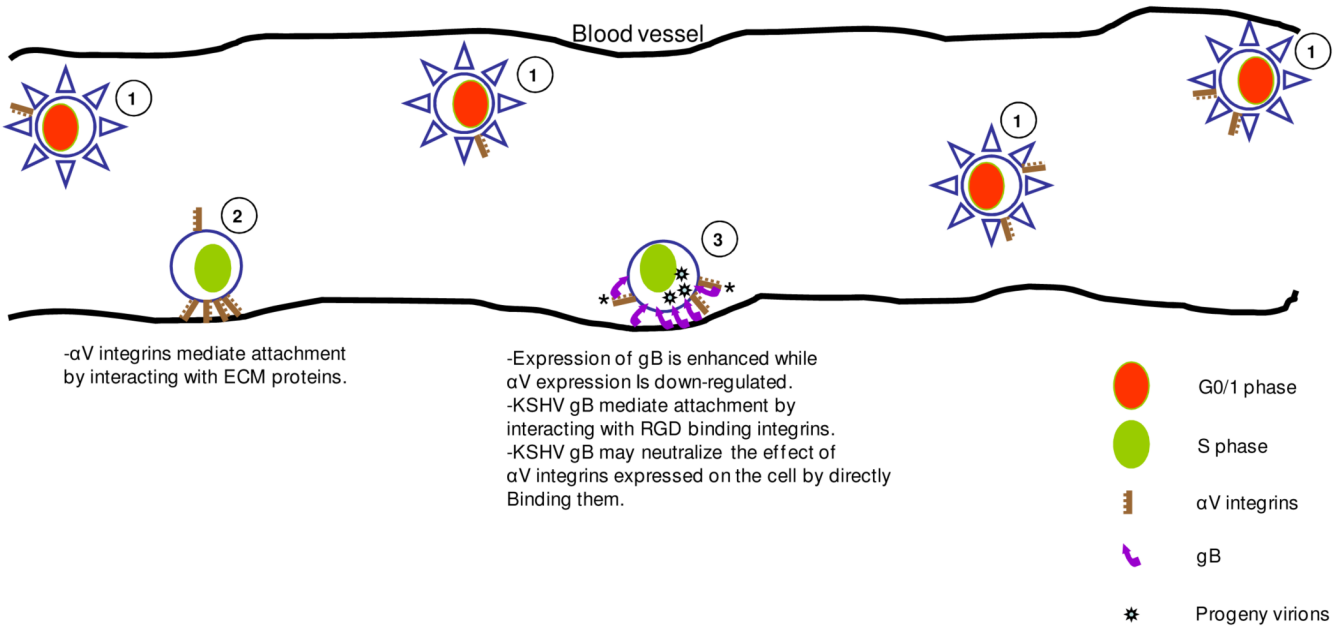

Figure 7. A schematic model describing roles for αV and gB in the attachment of KSHV infected BCBL-1 cells during different phases of the cell cycle and infection

KSHV infected B cells in G0/1 phase do not attach to the ECM (1). They attach to ECM primarily when they are in S phase via αV integrins (2). However, it is gB that mediates attachment in cells from S phase that are supporting a lytic infection (3). This is partly because of the fact that the expression of gB is enhanced by several folds in cells supporting lytic infection and the possibility that gB can neutralize the effects of the limited number of αV expressed on the same cells by directly binding to them (*).