Biochemical and Biophysical Research Communications 377 (2008) 93–97

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Viral hijacking of human receptors through heterodimerization

Henry F. Vischer *, Saskia Nijmeijer, Martine J. Smit, Rob Leurs

Leiden/Amsterdam Center for Drug Research (LACDR), Division of Medicinal Chemistry, Faculty of Sciences, VU University Amsterdam, De Boelelaan 1083, 1081 HV, Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 10 September 2008
Available online 26 September 2008

Keywords:
Epstein-Barr virus
Herpesvirus
GPCR
Heterodimerization
Chemokines

ABSTRACT

Epstein-Barr virus (EBV) is a human herpesvirus that primarily infects B lymphocytes and is associated with tumor development. Like other herpesviruses, EBV has pirated and modified host genes encoding important regulatory cellular proteins to take over cellular control after infection. One of these genes (BILF1) encodes a G protein-coupled receptor (GPCR). It is currently accepted that GPCRs exist and function as dimers. B lymphocyte migration and functioning is regulated by chemokines acting on their cognate receptors. In this study, we show that BILF1 heterodimerizes with various chemokine receptors using BRET, trFRET and co-immunoprecipitation. Importantly, heterodimerization of BILF1 with chemokine receptors may alter the responsiveness of B lymphocytes to chemokines thereby altering homing and homeostasis of infected B lymphocytes and might be essential for EBV dissemination and/or involved in EBV-induced pathogenesis.

Epstein-Barr virus (EBV) is one of the most successful pathogens, since more than 90% of the human population is infected with this orally transmitted γ-herpesvirus. Following primary infection, EBV persists lifelong in a latent form in memory B cells. Differentiation of these memory B cells into antibody-secreting cells (ASCs) induces the transcription of viral lytic genes, resulting in the production and shedding of infectious progeny into the saliva [1]. One of these viral lytic genes is the G protein-coupled receptor (GPCR) BILF1 [2–4]. GPCRs are cell membrane-associated proteins that play a central role in the communication between cells by activating intracellular signaling in response to specific extracellular stimuli. Not surprisingly, EBV as well as other herpes- and poxviruses have pirated GPCRs to modulate cellular functioning for their own benefits [5].

GPCRs have always been considered to exist and function as monomeric entities. However, increasing evidence indicates that GPCRs physically interact with each other. Importantly, heterodimerization of two different GPCRs can alter the functional properties of the individual partners, including ligand binding, signaling and trafficking [6]. Appearance of BILF1 on the surface of EBV-infected ASCs and the novel concept of GPCR dimerization, prompted us to hypothesize that BILF1 might hijack cellular communication through heterodimerization with human GPCRs. Like other leukocytes, migration of B cells to and within specific tissues is coordinated by the differential expression of chemokine receptor subtypes on their cell surface, which confers responsiveness to local gradients of cognate chemokines [7]. In addition, EBV infection upregulates the expression of the human EBI2 receptor [8]. Hence, a subset of human chemokine receptors and EBI2 might be potential candidates for BILF1 heterodimerization.

Materials and methods

DNA constructs. DNA encoding for triple HA-tagged human chemokine receptors were purchased from Missouri S&T cDNA Resource Center. EBI2 receptor DNA was purchased from Origene. BILF1 and US28 were previously described [2,9]. Receptors were tagged at the N-terminus using PCR-based methods. Fusion proteins for bioluminesence energy transfer (BRET) studies were generated by fusing the GPCRs in frame with *Renilla* luciferase (Rluc) or enhanced yellow fluorescence protein (eYFP) as previously described [10]. DNA encoding the chimeric G protein $G\alpha_{qi5}$ was a gift from Dr. Conklin (University of California, San Francisco) [11]. All generated constructs were verified by sequencing prior to use.

Cell culture and transfection. HEK293T cells were cultured and transiently transfected with indicated amounts of cDNA using 25-kDa linear polyethylenimine (Polysciences) as previously described [12]. Total DNA in gene-dosing experiments was kept constant by addition of empty plasmid.

Inositol phosphate (InsP) accumulation. HEK293T cells (10^6) were cotransfected with 625 ng wild type or tagged BILF1 and 625 ng $G\alpha_{qi5}$ DNA. Forty-eight hours after transfection, InsP accumulation was quantified as previously described [13].

α-bungarotoxin (BTX)-binding. Whole cell ligand binding was performed 48 h after transfection using ~0.25 nM [^{125}I]-BTX (Amersham) or 0.6 μM rhodamine-conjugated BTX (Sigma–Aldrich), essentially as previously described [9,14]. Alternatively,

* Corresponding author. Fax: +31 20 5987610.
E-mail address: vischer@few.vu.nl (H.F. Vischer).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.09.082

radioligand binding assays were performed on membrane fractions, as described previously [12].

BRET. HEK293T cells were cultured and transfected in white-bottomed 96-well plates. GPCR-eYFP expression was quantified 48 h after transfection using a Victor^2 plate reader (Perkin–Elmer) by measuring fluorescence at 535 nm upon excitation at 498 nm. Next, BRET and GPCR-Rluc expression was determined by measuring fluorescence at 535 nm and luminescence at 460 nm, respectively, in the presence of 5 μM coelenterazine h (Promega). BRET ratio is defined as the difference in emission ratio (535/460 nm) between cells expressing both GPCR-Rluc and GPCR-eYFP and cells expressing GPCR-Rluc alone.

Time-resolved fluorescence resonance energy transfer (trFRET). HEK293T cells were transfected with 250 ng FLAG-BILF1 and 0–500 ng HA-GPCR DNA/10^6 cells. TrFRET was assessed on a Novostar plate reader (BMG LabTechnologies) as previously described with minor modifications [10]. Following incubation with either both Eu^{3+}-labeled anti-HA and XL665-labeled anti-FLAG antibodies (AB), or Eu^{3+}-labeled ABs alone (CisBio International), the cells were washed and resuspended in PBS containing 0.3 M KF (10^7 cells/ml). Specific trFRET is expressed as Δ665 as previously described [15].

Co-immunoprecipitation (Co-IP) experiments. HEK293T (~8x10^6) were transfected with 20 ng FLAG-BILF1 and/or 50 ng HA-GPCR DNA/10^6 cells. Twenty-four hours after transfection, cell lysates were prepared and incubated with anti-FLAG M2 affinity gel (Sigma–Aldrich) for 1.5 h according to the manufacturer's instructions. Resins were washed three times with Tris-buffered saline (TBS) supplemented with 0.1% Triton X-100 before resuspending the pellet in 2× SDS–PAGE sample buffer. Immunocomplexes were resolved by 12% SDS–PAGE, and transferred to PVDF membranes (AppliChem). After blocking in TBS supplemented with 0.1% Tween and 5% low-fat milk, samples were subjected to immunoblotting using anti-FLAG M2 (Sigma–Aldrich) or anti-HA (Roche) ABs, followed by horseradish peroxidase (HRP)-conjugated secondary ABs (Bio-Rad and Dako) and visualization using an ECL reagent (GE Healthcare).

Results

Characterization of BILF1 and human GPCR constructs

BILF1 was tagged at the N- and C-terminus to allow detection of dimerization by means of BRET, trFRET and Co-IP (Fig. 1A). Since BILF1 is still an orphan GPCR, an α-bungarotoxin (BTX)-binding site (BBS) was introduced immediately after the N-terminal methionine [14]. BBS-BILF1-Rluc and BBS-BILF1-eYFP are expressed at the cell surface at comparable levels as BBS-BILF1 (Fig. 1B and 1C). Constitutive signaling of BILF1 to phospholipase C in cells co-expressing the chimeric G protein $G\alpha_{qi5}$ is not affected by the various N- and C-terminal modifications (Fig. 1D) [2,4,11]. Confocal microscopy imaging using rhodamine-conjugated BTX confirmed that BBS-BILF1-Rluc and BBS-BILF1-eYFP are expressed on the cell membrane, and colocalize with human GPCR-eYFP proteins (data not shown).

BRET screening for BILF1 heterodimers

Saturation BRET experiments were carried out to assess close proximity (<10 nm) between BILF1 and other GPCR proteins. To this end, cells were cotransfected with 50 ng BBS-BILF1-Rluc (~500 fmol/mg membrane protein; Fig. 2) and 0–2000 ng GPCR-eYFP DNA per 10^6 cells. Specific close proximity between BRET donor and acceptor pairs is reflected by a hyperbolic BRET ratio (BRETr), which reaches an asymptote with increasing eYFP/Rluc ratios [16]. Interestingly, BILF1 appears to be in close proximity with most tested candidate GPCRs (Fig. 2), potentially forming dimers with approximately equal propensities as revealed by the observed $BRET_{50}$ values (Table 1) [16]. Nonspecific close proximity due to random collision was observed in cells cotransfected with BBS-BILF1-Rluc and HCMV-encoded US28-eYFP, and is reflected by a quasi-linear increase in BRETr with increasing eYFP/Rluc ratios and a significantly lower $BRET_{50}$ value (Table 1).

BILF1 heterodimerization at the cell surface

To demonstrate that the identified BILF1 homo and heterodimers are localized at the cell surface, trFRET experiments were carried out on intact cells using Eu^{3+}- and XL665-conjugated ABs to detect N-terminally HA-tagged GPCRs and FLAG-tagged BILF1, respectively (Fig. 3A) [15]. Transfection of 250 ng FLAG-BILF1/10^6 cells, resulted in submaximal FLAG-BILF1 protein detection, ensuring that XL665-labeled FLAG-BILF1 is not a limiting factor for trFRET (Fig. 3B). HA-tagged receptor cell surface expression was measured as Eu^{3+} fluorescence, yielding saturable expression levels with increasing concentrations of cotransfected HA-GPCR DNA (0–500 ng/10^6 cells). Typically, HA-BILF1 and 3HA-CXCR4 are expressed at high cell surface levels, whereas 3HA-CCR9, 3HA-CCR10, and 3HA-CXCR3 are expressed at considerable lower levels (Fig. 3B, E, and H). In contrast, 3HA-CCR6, 3HA-CCR7, HA-EBI2, and 3HA-CXCR5 were undetectable (not shown). Similar HA- and FLAG-tagged GPCR detection was observed using ELISA and immunoblotting (not shown). Importantly, FLAG-BILF1 cell surface expression was not significantly affected by increasing HA-CRs protein levels (not shown). Significant emission was measured at 665 nm (i.e. F2) when dimers were labeled with both Eu^{3+}- and XL665-conjugated ABs, whereas marginal leakthrough was observed at 665 nm (i.e. F1) when cells were labeled with only Eu^{3+}-conjugated ABs (Fig. 3A and C). Specific FRET (i.e. Δ665) increases linearly with increasing 3HA-GPCR surface expression, if

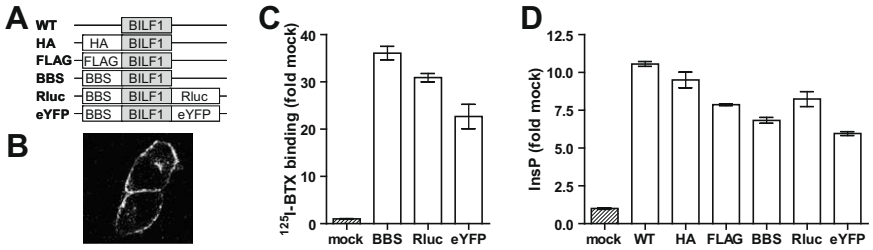

Fig. 1. N- and C-terminal modifications of BILF1 have minor effect on BILF1-mediated InsP accumulation. (A) BILF1 [WT] was tagged at the N-terminus with a BTX-binding site (BBS), a HA-epitope (HA), or FLAG-epitope (FLAG). BBS-BILF1 was fused in frame with Rluc and eYFP sequences after deletion of the stopcodon. (B) BBS-BILF1-Rluc labeled with rhodamine-conjugated BTX on the surface of transiently transfected HEK293T cells. (C) ^{125}I-BTX binding on intact HEK293T cells expressing BBS-BILF1, BBS-BILF1-Rluc or BBS-BILF1-eYFP. (D) Constitutive accumulation of InsP upon cotransfection of BILF1 constructs with the chimeric G protein $G\alpha_{qi5}$. Data are means ± SEM of representative experiments performed at least 3 times.

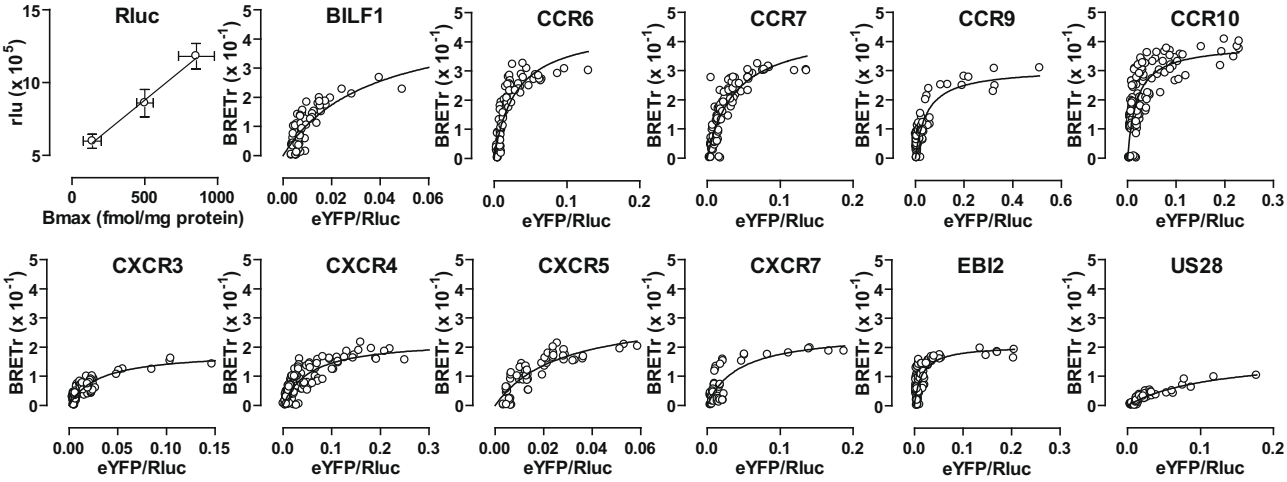

Fig. 2. Detection of BILF1 dimers in living cells by saturation BRET. Bioluminescence increases linearly with BBS-BILF1-Rluc protein expression (Rluc). HEK293T cells were cotransfected with constant amount of BBS-BILF1-Rluc and increasing amount of GPCR-eYFP DNA. BRET ratio (BRETr) is defined as [BRET/Rluc] in the presence of GPCR-eYFP minus [BRET/Rluc] in absence of GPCR-eYFP. BRETr was plotted as function of increasing GPCR-eYFP/BBS-BILF1-Rluc expression. Data were obtained from three to eight independent experiments each performed in triplicate. Curves were fitted using nonlinear regression, assuming a single binding site.

Table 1
Heterodimerization between the EBV-encoded GPCR BILF1 measured by saturation BRET and trFRET

Cotransfected GPCR	BRET $BRET_{50}$ value	trFRET FRET slope
BILF1	0.035 ± 0.009	1.139 ± 0.049
CCR6	0.030 ± 0.006	ND
CCR7	0.038 ± 0.006	ND
CCR9	0.047 ± 0.005	0.641 ± 0.153
CCR10	0.019 ± 0.002	0.705 ± 0.105
CXCR3	0.031 ± 0.003	0.572 ± 0.105
CXCR4	0.055 ± 0.006	0.676 ± 0.036
CXCR5	0.027 ± 0.006	ND
CXCR7	0.043 ± 0.011	ND
EBI2	0.015 ± 0.003	ND
US28	0.124 ± 0.030	ND

BRET was determined as function of increasing GPCR-eYFP and BBS-BILF1-Rluc ratios. $BRET_{50}$ values (±SE) were determined from nonlinear regression curves, assuming one binding site, fitted to pooled data from at least three independent experiments. FRET (i.e. Δ665) was determined as function of increasing HA-GPCR and constant FLAG-BILF1 cell surface expression. FRET slope values (±SE) were determined from linear regression curves, fitted to pooled data from at least three independent experiments. Both $BRET_{50}$ and FRET slope values represents the dimerization propensity between BILF1 and host GPCRs. ND, not determined.

these receptors are in close proximity with FLAG-BILF1. Significant Δ665 was observed between FLAG-BILF1 and HA-BILF1 (Fig. 3D), or 3HA-CXCR4 (Fig. 3J). On the other hand, much less Δ665 was measured between FLAG-BILF1 and 3HA-CCR9 (Fig. 3F), 3HA-CCR10 (Fig. 3G), or 3HA-CXCR3 (Fig. 3I), which is a direct consequence of the low cell surface expression of these HA-tagged chemokine receptors. Nonetheless, linear regression analysis revealed that FLAG-BILF1 dimerizes with equal propensities with all tested HA-GPCRs (Table 1).

Co-immunoprecipitation of BILF1 heterodimers

Since BRET and trFRET only revealed close-proximity (<10 nm) between GPCRs, direct physical interactions between FLAG-BILF1 and HA-GPCRs was evaluated by means of Co-IP using anti-FLAG beads. Co-IP of HA-BILF1, 3HA-CCR9, 3HA-CCR10, 3HA-CXCR3, and 3HA-CXCR4 monomers at ~35 kDa was revealed in SDS–PAGE by anti-HA immunoreactivity in anti-FLAG immunoprecipitated fractions of cells coexpressing FLAG-BILF1 (Fig. 4). No anti-HA reactive bands were observed in anti-FLAG immunoprecipitated fractions derived from mixing cells that expresss either FLAG-BILF1 or HA-GPCRs prior to immunoprecipitation, ruling out the formation of aggregates during solubilization in detergent. To confirm appropriate expression of HA-GPCR in both mixed and cotransfected cells, samples of cell lysates were immunoblotted with anti-HA ABs. Weak anti-HA immunoreactivity (~35 kDa) was observed in both mixed and cotransfected samples, which is a consequence of transfecting low DNA quantities to obtain lowest detectable receptor expression levels.

Discussion

Nowadays, it has become widely accepted that GPCR proteins have the capacity to form dimers and even higher-order multimers [6]. In the present study, we provide for the first time evidence on the physical interaction between a pathogen-derived GPCR and host cell GPCR proteins.

Hitherto, BILF1 expression has only been analyzed and detected in EBV-positive cell lines *in vitro*, and is in particular upregulated during reactivation of lytic replication in these latently infected cells [2–4,8]. *In vivo*, expression of EBV lytic genes is induced by the terminal differentiation of latently infected memory B cells into ASCs. We therefore hypothesized that human GPCRs endogenously expressed by these B cells are likely candidates to interact with BILF1.

Interestingly, BILF1 is in close proximity with all tested chemokine receptors (i.e. CCR6, CCR7, CCR9, CCR10, CXCR3, CXCR4, CXCR5, CXCR7), EBI2, and itself as revealed by saturation BRET. To confirm that these are indeed forming heterodimers and are present as dimer on the cell surface co-immunoprecipitation and antibody-based trFRET, respectively, were performed. In contrast to the GPCR-eYFP constructs, the N-terminally HA-tagged CCR6, CCR7, CXCR5, CXCR7, and EBI2 constructs were not detectable by Eu^{3+}-ABs, ELISA or immunoblotting, suggesting that this N-terminal modification impairs protein expression, as also observed for the mouse cytomegalovirus-encoded chemokine receptor M33 [17].

Direct physical interaction of BILF1 with itself, CCR9, CCR10, CXCR3, and CXCR4 was confirmed by co-immunoprecipitation experiments, whereas trFRET analysis revealed the presence of

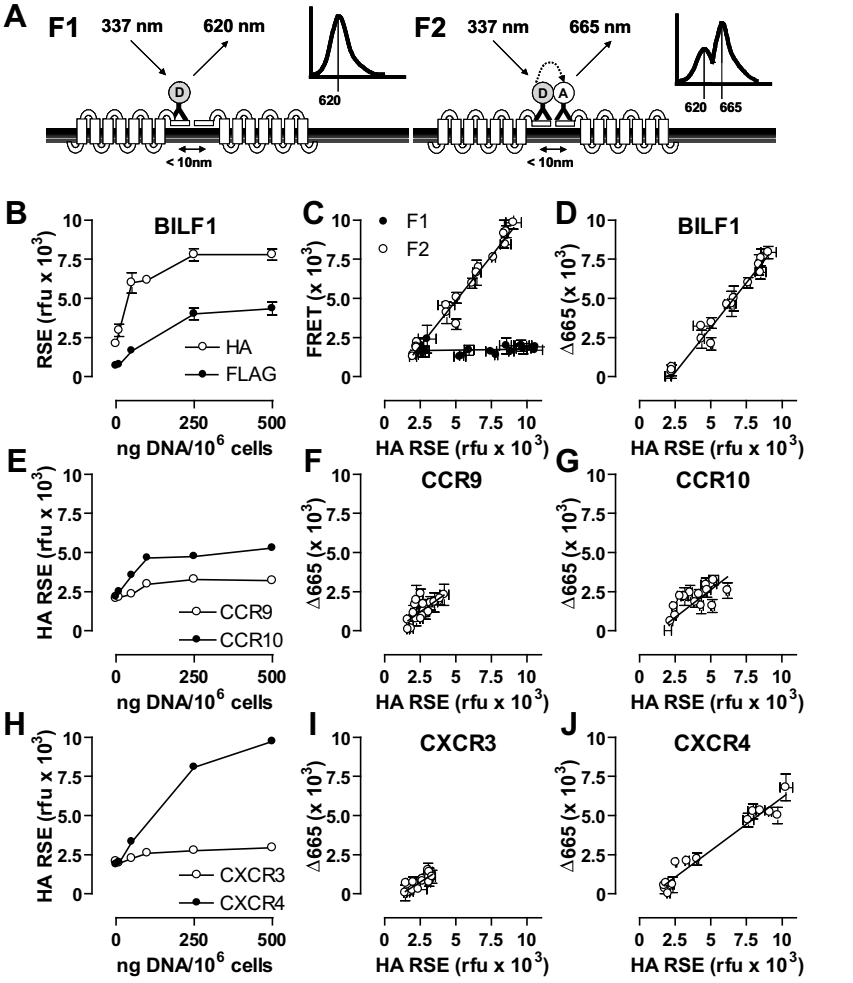

Fig. 3. Detection of BILF1 dimers in living cells by trFRET. (A) Cell surface expressed HA-GPCRs are labeled with Eu^{3+}-ABs, whereas FLAG-BILF1 is labeled with XL665-ABs. Excitation with light of 337 nm causes Eu^{3+} emission at 620 nm. Leakthrough emission from Eu^{3+} excitation is quantified at 665 nm in the absence of XL665-ABs, i.e. F1. If XL665-ABs are in close proximity, resonance energy transfer occurs and light is emitted at 665 nm, i.e. F2. (B) FLAG-BILF1 and HA-BILF1 receptor surface expression (RSE) was determined using XL665- and Eu^{3+}-ABs, respectively, on intact cells transfected with increasing DNA concentrations. (C) F1 (o) and F2 (●) emission determined on cells expressing a constant level of FLAG-BILF1 and increasing HA-BILF1 RSE. (D) Specific FRET between FLAG-BILF1 and HA-BILF1 was calculated as Δ665 = F2–F1 and plotted as function of increasing HA-BILF1 RSE. (E, H) HA-CR RSE (F, G, I, J) Δ665 in cells coexpressing FLAG-BILF1 and increasing RSE HA-CR. Data are means ± SEM from three independent experiments each performed in triplicate.

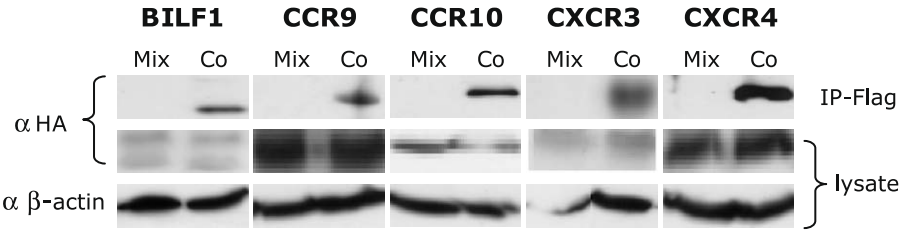

Fig. 4. Detection of BILF1 dimers using Co-IP. HEK293T were transfected with FLAG-BILF1 and/or HA-CR. Prior to solubilization cells expressing FLAG-BILF1 were mixed (1:1) with HA-CR-expressing cells (i.e. mix), whereas cells coexpressing these receptors were mixed (1:1) with control cells (i.e. co). Lysates were immunoprecipitated with anti-FLAG-beads, resolved by SDS–PAGE and immunoblotted with anti-HA antibodies (top). Lysates were immunoblotted with anti-HA (middle) or anti-β-actin antibodies (bottom). Immunoblots are shown from representative experiments that were performed three times.

these BILF1 homo and heterodimers on the cell surface. Even-though, the specific FRET (Δ665) signal was marginal for CCR9, CCR10, and CXCR4, due to low HA-GPCR expression levels, linear regression analysis revealed that BILF1 heterodimerizes with these receptors with an equal propensity as with CXCR4.

In conclusion, BILF1 physically interacts with various host chemokine receptors on the cell surface of human cells, acting as a GPCR scavenger. The obvious question that arises is what is the functional consequence of this act of receptor hijacking? Recently, negative binding cooperativity of both cognate chemokines and small drug-like compounds was shown between CCR2/CCR5 and CCR2/CXCR4 chemokine receptor heterodimers [18–20]. Hitherto, BILF1 is still an orphan GPCR. Consequently, in the absence of a ligand for BILF1 such a binding cooperativity cannot be investigated. Moreover, BILF1 signals in a ligand-independent manner and might affect the partnering receptor in the heterodimer by being in an active confor-

mation. Alternatively, BILF1 signaling might also affect host cell receptor signaling further downstream. Currently, we are investigating the functional consequence of BILF1 heterodimerization with human chemokine receptors. Importantly, hijacking of human GPCRs by virally encoded GPCRs will likely affect their normal functioning and redirect them to be beneficial to herpesvirus survival or might even turn them into malignant mediators of herpesvirus-associated pathologies. In fact, these heterodimeric complexes between host- and pathogen-derived GPCRs might represent the hitherto unappreciated key mediators in herpesvirus-induced diseases and may serve as novel therapeutic targets in the intervention of herpesvirus-associated pathologies.

Acknowledgments

H.F.V. and M.J.S. were supported by a Veni and Vidi grant, respectively, from The Netherlands Organization for Scientific Research. We thank Dr. J.P. Pin (IGF, Montpellier, France) for helpful discussions, and T.M. Hulscher and L. Bosch (LACDR, Division of Medicinal Chemistry, Faculty of Sciences, VU University Amsterdam) for technical assistance.

References

[1] L.L. Laichalk, D.A. Thorley-Lawson, Terminal differentiation into plasma cells initiates the replicative cycle of Epstein-Barr virus in vivo, J. Virol. 79 (2005) 1296–1307.
[2] P.S. Beisser, D. Verzijl, Y.K. Gruijthuijsen, E. Beuken, M.J. Smit, R. Leurs, C.A. Bruggeman, C. Vink, The Epstein-Barr Virus BILF1 gene encodes a G Protein-coupled receptor that inhibits phosphorylation of RNA-dependent protein kinase, J. Virol. 79 (2005) 441–449.
[3] S.J. Kaptein, M. Losen, P. Martinez-Martinez, H. Lavreysen, A. Vanheel, M.H. De Baets, C.A. Bruggeman, C. Vink, P.S. Beisser, Generation of polyclonal antibodies directed against G protein-coupled receptors using electroporation-aided DNA immunization, J. Pharmacol. Toxicol. Methods (2007).
[4] S.J. Paulsen, M.M. Rosenkilde, J. Eugen-Olsen, T.N. Kledal, Epstein-Barr virus-encoded BILF1 is a constitutively active G protein-coupled receptor, J. Virol. 79 (2005) 536–546.
[5] H.F. Vischer, C. Vink, M.J. Smit, A viral conspiracy: hijacking the chemokine system through virally encoded pirated chemokine receptors, Curr. Top. Microbiol. Immunol. 303 (2006) 121–154.
[6] G. Milligan, G-protein-coupled receptor heterodimers: pharmacology, function and relevance to drug discovery, Drug Discov. Today 11 (2006) 541–549.
[7] E.J. Kunkel, E.C. Butcher, Plasma-cell homing, Nat. Rev. Immunol. 3 (2003) 822–829.
[8] M.M. Rosenkilde, T. Benned-Jensen, H. Andersen, P.J. Holst, T.N. Kledal, H.R. Luttichau, J.K. Larsen, C. Geisler, J.P. Christensen, T.W. Schwartz, Molecular pharmacological phenotyping of EBI2 – an orphan 7TM receptor with constitutive activity, J. Biol. Chem. (2006).
[9] P. Casarosa, M. Waldhoer, P.J. LiWang, H.F. Vischer, T. Kledal, H. Timmerman, T.W. Schwartz, M.J. Smit, R. Leurs, CC and CX3C chemokines differentially interact with the N terminus of the human cytomegalovirus-encoded US28 receptor, J. Biol. Chem. 280 (2005) 3275–3285.
[10] R.M. van Rijn, P.L. Chazot, F.C. Shenton, K. Sansuk, R.A. Bakker, R. Leurs, Oligomerization of recombinant and endogenously expressed human histamine H(4) receptors, Mol. Pharmacol. 70 (2006) 604–615.
[11] P. Coward, S.D. Chan, H.G. Wada, G.M. Humphries, B.R. Conklin, Chimeric G proteins allow a high-throughput signaling assay of Gi-coupled receptors, Anal. Biochem. 270 (1999) 242–248.
[12] D. Verzijl, S. Storelli, D.J. Scholten, L. Bosch, T.A. Reinhart, D.N. Streblow, C.P. Tensen, C.P. Fitzsimons, G.J. Zaman, J.E. Pease, I.J. de Esch, M.J. Smit, R. Leurs, Noncompetitive antagonism and inverse agonism as mechanism of action of nonpeptidergic antagonists at primate and rodent CXCR3 chemokine receptors, J. Pharmacol. Exp. Ther. 325 (2008) 544–555.
[13] J.W. Hulshof, H.F. Vischer, M.H. Verheij, S.A. Fratantoni, M.J. Smit, I.J. de Esch, R. Leurs, Synthesis and pharmacological characterization of novel inverse agonists acting on the viral-encoded chemokine receptor US28, Bioorg. Med. Chem. 14 (2006) 7213–7230.
[14] Y. Sekine-Aizawa, R.L. Huganir, Imaging of receptor trafficking by using alpha-bungarotoxin-binding-site-tagged receptors, Proc. Natl. Acad. Sci. USA 101 (2004) 17114–17119.
[15] D. Maurel, J. Kniazeff, G. Mathis, E. Trinquet, J.P. Pin, H. Ansanay, Cell surface detection of membrane protein interaction with homogeneous time-resolved fluorescence resonance energy transfer technology, Anal. Biochem. 329 (2004) 253–262.
[16] J.F. Mercier, A. Salahpour, S. Angers, A. Breit, M. Bouvier, Quantitative assessment of beta 1- and beta 2-adrenergic receptor homo- and heterodimerization by bioluminescence resonance energy transfer, J. Biol. Chem. 277 (2002) 44925–44931.
[17] R. Case, E. Sharp, T. Benned-Jensen, M.M. Rosenkilde, N. Davis-Poynter, H.E. Farrell, Functional analysis of the murine cytomegalovirus chemokine receptor homologue M33: ablation of constitutive signaling is associated with an attenuated phenotype in vivo, J. Virol. 82 (2008) 1884–1898.
[18] L. El-Asmar, J.Y. Springael, S. Ballet, E.U. Andrieu, G. Vassart, M. Parmentier, Evidence for negative binding cooperativity within CCR5-CCR2b heterodimers, Mol. Pharmacol. 67 (2005) 460–469.
[19] D. Sohy, M. Parmentier, J.Y. Springael, Allosteric transinhibition by specific antagonists in CCR2/CXCR4 heterodimers, J. Biol. Chem. 282 (2007) 30062–30069.
[20] J.Y. Springael, P.N. Le Minh, E. Urizar, S. Costagliola, G. Vassart, M. Parmentier, Allosteric modulation of binding properties between units of chemokine receptor homo- and hetero-oligomers, Mol. Pharmacol. 69 (2006) 1652–1661.