CHAPTER THIRTEEN

EBV, the Human Host, and the 7TM Receptors: Defense or Offense?

Kristine Niss Arfelt, Suzan Fares, Mette M. Rosenkilde[1]
Faculty of Health and Medical Sciences, Department of Neuroscience and Pharmacology, Laboratory for Molecular Pharmacology, University of Copenhagen, Copenhagen, Denmark
[1]Corresponding author: e-mail address: rosenkilde@sund.ku.dk

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Abstract

Being present in around 90% of the worldwide population, Epstein–Barr virus (EBV) is an exceptionally prevalent virus. This highly successful virus establishes a latent infection in resting memory B cells and is maintained in a balance between viral homeostasis on one side and antiviral defense of the immune system on the other side. The life cycle of EBV is dependent on many viral proteins, but EBV also regulates a number of endogenous proteins. 7TM receptors and ligands of viral and host origin are examples of such

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proteins. 7TM receptors are highly druggable and they are among the most popular class of investigational drug targets. The 7TM receptor encoded by EBV-BILF1, is known to downregulate cell surface MHC class I expression as part of the immune evasion strategy of EBV. However, the functional impact of the relationship between EBV and the regulated endogenous 7TM receptors and ligands is still unclear. This is for instance the case for the most upregulated 7TM receptor EBI2 (EBV-induced gene 2 or GPR183). Whereas some regulated genes have been suggested to be involved in the EBV life cycle, others could also be important for the antiviral immune defense. As many of these 7TM receptors and ligands have been shown to be modulated in EBV-associated diseases, targeting these could provide an efficient and specific way to inhibit EBV-associated disease progression. Here, we will review current knowledge on EBV infection, the immune defense against EBV and 7TM receptors and ligands being either encoded or manipulated by EBV.

1. EBV INFECTION

Epstein–Barr virus (EBV, human herpesvirus 4) was discovered 50 years ago, when Epstein, Achong, and Barr used electron microscopy to identify viral particles in Burkitt's lymphoma cells.[1] It belongs to the lymphocryptovirus (LCV) genus of the gammaherpesvirus subfamily[2] (Fig. 1A). The EBV genome, which consists of a linear, double-stranded DNA molecule that encodes close to 100 viral genes, is enclosed in a nuclear capsid surrounded by a protein tegument, which in turn, is surrounded by a glycoprotein-coated viral envelope.[6] The glycoproteins are important for virus tropism, host selectivity, and infection.

1.1. Viral infection, entry, and tropism

It is generally believed that primary infection with EBV occurs virus in saliva from already infected persons, which infects B cells and epithelial cells in the Waldeyers tonsillar ring in the oropharynx. From Waldeyers ring, the virus replicates and further infects resting naïve B cells trafficking through the oropharynx or resting naïve B cells resident in the tonsillar crypts.[6] The mechanisms of virus entry into epithelial cells and B cells differ, but both depend on the attachment and membrane fusion mediated by the envelope glycoproteins. For B cell entry, the virion attaches to the B cell surface by binding of the viral gp350/220 to the complement component receptor CD21 (CR2); binding is associated with triggering of signaling and endocytosis.[7] After attachment and endocytosis, the glycoprotein gp42, which forms a tripartite complex with gH/gL, interacts with the major histocompatibility

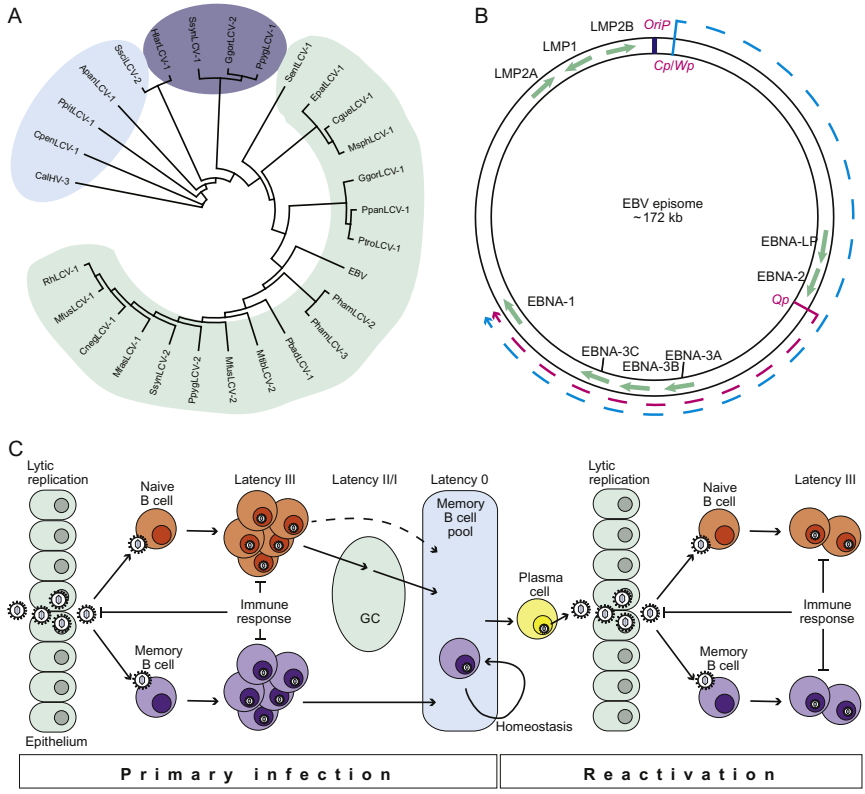

Figure 1 Overview of lymphocryptovirus, genome organization, and life cycle of EBV. (A) Dendrogram of the lymphocryptovirus (LCV) genus of the gammaherpes virus subfamily. The phylogenetic analysis was based on the amino acid sequence for the DPOL gene sequences from amino acid no 1–693 (for EBV). Amino acid sequence alignments were made using MAFFT.[3] The dendrogram was built using the maximum likelihood method in the PhyML 2.2.0 plug-in.[4] The LG nucleotide substitution model was applied. The major clades A (LCV hosted by New World monkeys), B and C (LCV hosted by Old World monkeys) are shown in blue, green, and purple, respectively. (B) Schematic overview of the EBV episome with indication of latent protein-coding genes (black) and promoters (pink). The latent genes are marked by green arrows. The latent origin of replication, OriP, is shown in dark blue. The dotted, long, and outer blue arrow shows the genes transcribed from the Cp and Wp promoter (all the EBNAs), whereas the dotted, inner, and pink arrow shows the transcription promoted from Qp (EBNA-1 only). (C) Proposed life cycle of EBV: the virus establishes a lytic infection in epithelial cells and/or B cells. The B cells express the growth-promoting latency III program and enter a state of highly proliferating lymphoblasts. Many of these are detected and removed by the immune system, but some express the latency II and I programs and secure entry into the memory B cell pool, where the virus can reside without immune detection. The

(*Continued*)

complex (MHC) class II and triggers membrane fusion mediated by the glycoproteins gH/gL and gB.[8] Attachment of virions to epithelial cells may occur through binding of gp350/220 to CD21, which is expressed at low levels on tonsillar epithelial cells, but also through an interaction of the viral BMRF-2 protein with integrins on the epithelial surface.[9] Unlike B cells, epithelial cells do not express MHC class II. The fusion with these cells is triggered by the interaction between cellular integrins and the gH/gL glycoproteins. The gB glycoprotein mediates the fusion, which does not require endocytosis.[10] Following fusion with both epithelial cells and B cells, virion capsids are transported to deliver linear viral DNA to the nucleus.[6]

The expression of gp42 on the viral envelope determines the viral tropism for either B cells or epithelial cells. The interaction of gH with an epithelial receptor is inhibited by gp42, meaning that gp42 expressing virions preferentially infect B cells.[11] Interestingly, virions produced by B cells do not express gp42 as gp42 molecules bind to MHC class II in the endoplasmic reticulum (ER) and are degraded. Thus, virions from B cells show tropism for epithelial cells. Conversely, virions produced in epithelial cells express gp42 and show tropism for B cells. The virions shed in saliva have been shown to be B cell tropic, suggesting that in the persisting infection the epithelial cells are used for B cell tropic virion amplification.[12] This observation also suggests that B cells, rather than epithelial cells, may be the initial target of virus infection when transmitted via saliva.

1.2. Lytic replication

EBV is well known for establishing an immediate latent infection in B cells, which provide the reservoir for the virus in the persisting infection. The lytic replication phase thus only occurs in B cells upon reactivation and in

Figure 1—Cont'd persisting virus infection is continuously maintained via homeostasis of the infected memory B cells. Infected memory B cells differentiating into plasmacells may activate lytic replication and virus shedding via epithelial cell infection. The reactivation is efficiently repressed by the memory immune response. Establishment of the latency in memory B cells has been proposed to occur via (1) direct infection and growth promotion of memory B cells. (2) Infection of naïve B cells and virus-driven transformation into memory B cells in a process, which mimics the antigen-driven memory cell differentiation of noninfected naïve B cells. This virus-driven transformation can either occur through the GCs, which represents a hostile environment or through GC-like differentiation outside the actual GCs (dotted arrow). *Adapted from Ref. 5.*

epithelial cells upon virus entry. The lytic replication is initiated by expression of the immediate-early genes, which act as transactivators for the early and late lytic genes. The most important immediate-early gene BZLF1 acts as a transactivator of late lytic genes and is critical to the initiation of lytic replication.[13] The early gene products have functions such as replication, metabolism, and blockade of antigen processing, whereas late gene products have more structural functions such as viral capsid antigens and immune evasion.[14]

1.3. Latent infection

When the linear viral DNA reaches the nucleus, it forms a circular DNA and initiates a short-prelatent phase, which is characterized by coexpression of both latent and some lytic genes. The latent genes activate the resting B cells to become highly proliferating lymphoblasts. No virions are produced in this phase and the lytic genes expressed interact with miRNAs to ensure cell cycle progression, protection from apoptosis and immune evasion.[6,15,16] The transformation efficiency of resting naïve B cells into highly proliferating lymphoblasts is only 1–10%.[17,18] This can be partly explained by a cellular DNA damage response initiated by the increased expression of the latent genes and resulting in apoptosis of virus-infected cells.[19]

An intriguing aspect of EBV infection is that infection of B cells differs dramatically between *in vivo* and *in vitro* settings. *In vivo*, EBV has the ability to establish a persisting latent infection in memory B cells, whereas infection of B cells *in vitro* results in an immortalized lymphoblastoid cell line (LCLs).[6] So, how can a growth-promoting virus end up in a quiescent state in resting memory B cells? The difference between these two outcomes of infection is the expression pattern of latent viral genes. Although the viral genome encodes nearly 100 protein-coding genes, only a small subset is expressed during latency. LCLs have been used as a platform to study these genes as all of them are expressed in the lymphoblastoid cells. Full latent gene expression is also present in newly infected B cells *in vivo*,[6] but in the *in vivo* settings EBV establishes several different patterns of gene expression, which are recognized in the various cancers associated with the virus. The latent protein-coding genes, along with their expression pattern and functions, are listed in Table 1 and shown in the EBV episome in Fig. 1B. In addition, abundant noncoding RNAs (EBERs) and viral miRNAs are also expressed during latency, but will not be discussed further.[26]

Table 1 EBV latency genes with latency program and function

Latent gene	Latency program	Function
EBNA-1	I, II, and III	Gene regulation, replication, and maintenance of the viral genome through regulation of viral promoters[20]
EBNA-2	III	Transactivator of viral and cellular genes, virus-induced growth transformation[21]
EBNA-LP	III	Coactivation with EBNA-2 and virus-induced growth transformation[21]
EBNA-3A	III	Virus-induced growth transformation and survival[6]
EBNA-3B	III	Viral tumor suppressor[22]
EBNA-3C	III	Virus-induced growth transformation and survival[6]
LMP1	II and III	CD40 mimic promoting B cell growth and survival[23]
LMP2A	II and III	B cell receptor mimic promoting B cell development and survival[24]
LMP2B	II and III	Modulator of LMP2A activation[25]

The fact that EBV is known to infect resting naïve B cells, but is maintained in the differentiated memory B cell pool, has led Thorley-Lawson and colleagues to postulate the germinal center (GC) model for EBV infection (Fig. 1C). In this model, the EBV infection is suggested to mimic the antigen-driven differentiation from naïve B cells to memory B cells via class switch recombination and somatic hypermutations in the GCs.[27] The GC model suggests that expression of latency program III induces B cell proliferation and formation of GCs. In GCs, the gene expression switches to latency program II with expression of LMP1 and LMP2A. LMP1 constitutively mimics the CD40 signal,[28] which induces survival and proliferation of infected B cells *in vitro*.[29] LMP2A, on the other hand, mimics the B cell receptor signaling properties and thereby bypasses induction of anergy and induces survival of the infected cells.[24] *In vitro* studies and studies with transgenic animals suggest that these two genes provide the necessary signals for the infected B cells to survive the critical selection in the GCs.[23] In addition, LMP1 induces class switching and somatic hypermutations by upregulation of activation-induced cytidine deaminase,[30] which is the most important regulator of secondary antibody maturation. After exit from the GCs, the EBV-infected cells differentiate into memory B cells and express

latency program 0 (expression of noncoding RNAs but no viral proteins) or latency program I (expression of noncoding RNAs and EBNA-1 only). Expression of EBNA-1 ensures the viral homeostasis by replication and hence maintenance of the viral genome when infected memory B cells enter cell division.[31]

There is some controversy concerning the GC model. An argument in support of the model is the fact that the persisting virus infection is restricted to memory B cells. Also, the existence of the latency III program, which turns resting B cells into highly proliferating lymphoblastoids and thereby puts both the host and virus at risk for cancer development, suggests that successful latent infection cannot be achieved by simply infecting resting memory B cells.[27] Opposing the GC model, studies have shown that EBV infects both naïve and memory B cell populations.[32] The lack of somatic hypermutations in memory B cells sampled during infectious mononucleosis also indicates that the GC reaction is not required for the differentiation into memory B cells.[33] Also, very few EBV-infected cells are present in the GCs of both infectious mononucleosis and normal EBV-infected lymph tissue.[34,35] In summary, studies suggest that the GC model plays a role in the persisting EBV infection and that it is complemented by either direct infection of memory B cells or by differentiation of memory B cells located outside the GCs. It is important to state that in the absence of a good *in vivo* model for EBV, all studies on EBV-encoded or -regulated genes are made on samples from patients with EBV-associated diseases, *in vitro* experiments, or transgenic animals expressing one or two of the encoded or regulated genes. Thus, these studies cannot encompass the complex *in vivo* interactions between viral genes, or between virus and host. To fully understand these, there is a requirement for further studies in a reliable EBV *in vivo* model.

1.4. Regulation of latency, replication, and virus reactivation

As shown in Fig. 1B, the viral genome encodes three different promoter regions: Wp, Cp, and Qp. The virus uses Wp to ensure expression of EBNA-1–3C after initial infection. The use of Wp is transient and EBNA-1 expression induces promoter switch to Cp, which is used for full latent gene expression (program III). In latency I, the genome switches promoter to Qp resulting in the expression of EBNA-1 only. EBNA-1 also binds and activates the latent origin, OriP, and thereby ensures viral homeostasis during latent infection.[6] The regulatory mechanism behind the

promoter switches determining virus life cycle through prelatent and latent phases followed by reactivation and lytic virus replication has long been unknown. In particular, the switch from a quiescent latent state to lytic replication has been a subject of attention for EBV researchers. Recent studies suggest that the virus takes advantage of the host epigenetic mechanisms in control of its promoter usage and different phases.[36] The viral genome in newly EBV-infected cells is, as is also the case for other herpesviruses and retroviruses, epigenetically naïve (has no epigenetic modifications).[37–39] Using the host cell epigenetic machinery, the virus genome acquires methylations and histone modifications during latency.[36] These modifications vary among the different latency programs. Thus, the Cp promoter has been found to be hypermethylated and silent in tumors expressing the latency I (Burkitt's lymphoma) or II (Hodgkin lymphoma), whereas it is unmethylated in LCLs and posttransplant lymphoproliferative disorders (PTLDs) expressing latency III.[40,41]

A solution to the question of how the virus switch from latency to lytic mode has also emerged within recent years: the immediate-early lytic transactivator BZLF1 has the capacity to bind to highly methylated DNA only. Thus, although this gene is expressed in the prelatent phase, it cannot bind to its binding motif in the unmethylated DNA. During latency, the DNA becomes increasingly methylated forming the preferred binding motif for BZLF1 and can then overcome epigenetic repression.[42]

2. IMMUNE RESPONSE AND IMMUNE EVASION

Up to 10% of the host B cells are infected with EBV during acute infectious mononucleosis.[43] Most of these cells are effectively cleared by the immune system, but some downregulate viral gene expression and differentiate into safety in the resting memory B cell pool. In the persisting infection, the virus and host coexist, so that homeostasis of the infected memory B cell pool is maintained by continuous low-level virus shedding while the immune system ensures that no full blown lytic replication is initiated. The number of infected B cells in the persisting infection is around 1–50 per 10^6 B cell.[43]

The host uses both the innate and adaptive immune response to eliminate infected B cells. The innate immune response relies heavily on virus recognition by Toll-like receptors (TLR) followed by interferon (IFN)-γ secretion and activation of natural killer (NK) cells. In particular, TLR3 expressed in classical dendritic cells (DCs) recognizes double-stranded

RNA such as EBV EBERs and induces secretion of IFN-γ, antiviral cytokines, and NK cell activation.[44] TLR9 expressed in plasmacytoid DCs recognizes unmethylated CpG motifs in the epigenetically naïve viral DNA[45] and induces IFN-γ secretion and NK cell activation.[46] The importance of NK cells is suggested by the finding that the levels of NK cells are significantly increased in patients with infectious mononucleosis.[47] In addition, recent *in vivo* studies of infectious mononucleosis in mice with reconstituted human immune components showed that depletion of NK cells led to loss of immune control and enhanced symptoms.[48]

The innate immune system ensures virus inhibition until the adaptive immune system is ready to launch a much more comprehensive antiviral response, which mainly consists of cytotoxic CD8 T cells and antibody production induced by CD4+ T cells to a minor degree.[43] Initially, cytotoxic T cells (CTL) are directed against lytic epitopes such as the immediate-early gene BZLF1. Later in the infection, the CTL are directed against latent epitopes, in particular.[43] In contrast to the CD8+ T cell response, the CD4+ T cell response is very modest and seems to be directed against a different set of early and late lytic epitopes as compared to the CD8+ T cell response.[49] The important role of the immune response and especially the adaptive immune response in the inhibition of EBV infection can be illustrated by the fact that a large number of patients with EBV-associated diseases have immunodeficiencies related to either genetic, iatrogenic (PTLD), or other diseases (like HIV).[6]

Like other herpesviruses, EBV has adapted to the immune system for instance by expressing many genes with immune evasive properties. Among these, LMP1 and BGL5 downregulate TLR9 in the latent and lytic phases, respectively.[50,51] Also, the late lytic function as a deubiquitinase in the TLR signaling pathway and thereby inhibits TLR signaling.[52] The CD8+ T cell cytotoxic response is inhibited by the downregulation of viral epitope presentation on MHC class I molecule on the cell surface by LMP1, BILF1, BGL5, viral IL-10 homolog BCRF1, and BNLF2a.[15,53–56] BCRF1 has also been shown to have broad inhibitory effects on the immune system by impairing NK cell-mediated killing of infected B cells, interference with CD4+ T cell activity and modulation of cytokine responses.[15] Finally, the CD4+ T cell response is inhibited by binding of the viral glycoprotein gp42 to MHC class II and the ensuing lowered recognition of CD4+ cells.[57]

The host-virus coexistence is completely dependent on the balance between a strong immune response inhibiting virus replication and immune evasion and survival of the virus with periodic shedding to enable

transmission to new hosts. In the process of ensuring this balance, the virus manipulates 7TM receptors and their ligands.[58–60] Thus, like many other herpesviruses, EBV encodes a 7TM receptor and in addition regulates the expression of both 7TM receptors and ligands in the host. In the following parts of the review, we will give an overview of the 7TM receptor exploitation and regulation of EBV along with the potential druggability of these receptors.

3. EBV-BILF1—A VIRUS-ENCODED 7TM RECEPTOR WITH IMMUNE EVASIVE FUNCTIONS

The EBV-encoded BILF1 receptor (EBV-BILF1) is thought to be implicated in the immune evasion strategy of EBV.[56,61,62] This orphan 7TM receptor is expressed at significant levels during the early lytic phase of the virus infection, intermediate levels in LCLs and nasopharyngeal carcinoma derived C666-1 line and at low levels during the viral latent phase.[63,64] Several studies have shown that the pivotal role in immune evasion played by EBV-BILF1 occurs during the lytic replication rather than the latent phase.[56,61–64] In addition to EBV, the BILF1 gene is present in the two so far characterized nonhuman primate LCVs, Old World rhesus LCV-1 (RhLCV-1), and New World callitrichine herpesvirus-3 (CalHV-3).[62,65] Furthermore, ungulate gammaherpesviruses belonging to the genera *Macavirus* (Porcine lymphotropic herpesviruses 1–3, Alcelaphine herpesvirus 1, and Ovine herpesvirus 2) and *Percavirus* (Equine herpesvirus 2) encode putative 7TM receptors at the homologous genomic position. Although these BILF1 receptor homologs remain uncharacterized, the strong conservation reflects a significance of this gene in the viral pathogenesis.

3.1. Immune evasion strategy of EBV-BILF1

Unlike most of the virus-encoded 7TM receptors[59] BILF1 is not a chemokine receptor and therefore does not sequester chemokines in order to circumvent the host immune system. BILF1, however, uses another strategy, which is the downregulation of MHC class I cell surface expression (Fig. 2A) and inhibition of the cytotoxic $CD8^+$ T cell recognition of the virus-infected cells.[56,61,62] The exact mechanism by which BILF1 targets the MHC class I molecules is far from being understood; however, a study by Zuo *et al.*[61] reported that BILF1 is involved in the MHC class I downregulation by targeting the molecules on both the endocytic and the exocytic pathways. The mechanism of the MHC class I targeting on

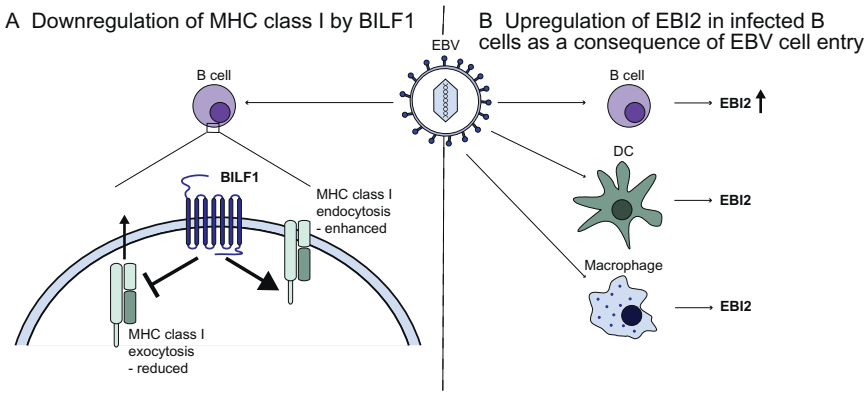

Figure 2 Suggested roles of the viral EBV-BILF1 and the human EBI2 receptor. (A) The EBV-encoded 7TM receptor BILF1 has immune evasive properties by reducing the cell surface levels of MHC class I molecule. This is achieved by BILF1-mediated induction of endocytosis of MHC class I molecules from the cell surface or by inhibition of the normal exocytic pathway transferring newly synthesized MHC class I molecules to the cell surface. (B) EBV infection induces expression of EBI2 on the B cell surface. Whether EBI2 plays a role in the virus life cycle or in the antiviral immune defense is yet unknown. Under normal physiological conditions, EBI2 is also expressed in DCs and macrophages. These cells could also express EBI2 upon EBV infection and thereby assist the antiviral immune response.

the endocytic pathway may involve a putative physical association of BILF1 with the MHC class I molecule and further enhancement of the MHC class I lysosomal degradation.[56,61] In contrast, the exocytic pathway targeting of the MHC class I molecules by BILF1 is not fully understood, albeit a physical association of BILF1 with the MHC class I molecules in the ER has been reported.[56] It is noteworthy that this physical association in the ER does not seem to hinder MHC class I trafficking to Golgi suggesting that the EBV association with MHC class I in the ER may instead interfere with the transporter associated with antigen processing (TAP)-associated glycoprotein or the TAP-complex binding.[56,65]

The wild-type EBV-BILF1 receptor was shown to potentiate internalization[61,62] and degradation of the MHC class I molecules resulting in a reduction of MHC class I on the cell surface.[61] Conversely, mutational studies of the DRY-like EKT motif in the bottom of TM3 of EBV-BILF1 showed that the K122A mutation impaired the ability of BILF1 to induce MHC class I endocytosis[61] and lead to abolished signaling properties of the receptor.[61,66] This motif is partially conserved among virus-encoded 7TM receptors[67] and has also been shown to impact the signaling of other

virus-encoded receptors like the KSHV-ORF74,[68,69] HCMV-US28,[70,71] and MCMV-M33.[72] Interestingly, it was shown to be dispensable for proper G-protein coupling in the CXC-chemokine receptor ORF74 encoded by Equine herpesvirus 2, as this receptor lacks the most important positive charge in the middle of this motif.[73,74]

Another region with impact on MHC class I surface expression has been identified in the C-terminus of EBV-BILF1, as truncation of this (ΔC) impairs the ability of the receptor to target the MHC class I molecule for lysosomal degradation and downregulation.[61,62]

Nevertheless, as mentioned above, BILF1 is not the only player and it acts in accord with three other (BGLF5, BNLF2a, and vIL-10) to downregulate the MHC class I molecule cell surface expression.[15,53,54,56] BILF1 can be detected as soon as 4 h postinfection.[62] The initial expression of BILF1 occurs during the host cell protein synthesis suppression, which usually happens under the effect of BGLF5 as well as the TAP inhibition mediated by BNLF2a.[62] The transient expression of BNLF2a as well as possible detection of BILF1 16 h postinfection makes it compelling to suggest that BILF1 also is capable of targeting the MHC class I molecules in the late lytic cycle.[62] Furthermore, a marked impairment of CD8+ T cell recognition of EBV-infected cells was reported by Zuo *et al.*[61] and this clearly reflects that the effect of BILF1 on MHC class I molecule is significant. It is noteworthy that BILF1 targets multiple forms of MHC class I molecules including HLA-A, B, and E with a minimal effect on HLA-C. This broad inhibition of multiple subsets of the MHC class I molecule may help the virus escape the immune system and persist in the host suggesting that BILF1 is important for the immune evasion strategy of EBV.[62,65] Downregulation of MHC class I is also exhibited by the RhLCV-1 BILF1 homolog, suggesting evolutionary conservation of function at least for Old World primates.[62]

3.2. Signaling and tumorigenesis of EBV BILF1

In addition to its immune evasive effects, EBV-BILF1 is highly tumorigenic both *in vitro* and *in vivo*.[66] It signals in a constitutive ligand-independent manner through Gαi as it inhibits forskolin-induced adenylate cyclase activity in a gene–dose-dependent manner.[63,66] This constitutive signaling is shared with other virus-encoded receptor families such as US28 from HCMV[70,75–77] and the ORF74 receptors from human herpesvirus 8 (KSHV-ORF74),[69,78–80] Herpesvirus Saimirii,[81] Equine herpesvirus

2,[73] and murine gammaherpesvirus 68 (MHV68).[82] Most of these viral receptors also bind multiple chemokines in a broad spectrum manner, and scavenge these from the surroundings of virus-infected cells as part of an immune evasion strategy.[78,79,81,83–85] Lyngaa *et al.*[66] reported that BILF1 induces NIH 3T3 cell transformation and vascular endothelial growth factor secretion and *in vivo* tumor formation in nude mice. Importantly, these properties were linked to the constitutive activity of EBV-BILF1, as they were ameliorated by an activity-silencing point-mutation in the conserved DRY-like EKT motif in the bottom of TM3. Similar tumorigenesis, also with linkage to constitutive activity, has been shown for US28 and KSHV-ORF74.[78,80,86] This suggests that the constitutive activity of EBV-BILF1 could be implicated in EBV-associated cancer pathogenesis.[63–66]

4. EBI2: AN ENDOGENOUS 7TM RECEPTOR MANIPULATED BY EBV

EBI2 was identified in 1993, when Kieff and colleagues used subtractive hybridization of DNA from Burkitt's lymphoma cells to screen for upregulated genes in EBV-infected cells.[87] They found, being more than 200-fold upregulated (hence the name: EBV-induced gene 2). Since 1993, this finding has been confirmed in four different studies describing EBI2 upregulation in both lytic and latent settings[29,88–90] (see Table 2).

The name of EBI2 suggests that EBV directly induces expression of the receptor, but the fact is that we, after more than two decades, still do not know if EBI2 is *induced by EBV* or if it *is induced as part of the immune response* to the virus infection. As described in detail below, EBI2 has moved from being an orphan receptor with no known biological function to an oxysterol-induced receptor with important functions in the immune system within a few years; in addition, the molecular basis for activation and inhibition of EBI2 has been studied extensively.

4.1. A family of oxysterols acts as ligands for the EBI2 receptor

When first identified, EBI2 was proposed to have a polypeptide cytokine ligand.[87] It was thus surprising, when two groups simultaneously identified oxysterols as the endogenous ligands for EBI2.[91,92] Importantly, although 7α25-dihydroxycholesterol (7α,25-OHC) is the most potent ligand, the two groups discovered a family of oxysterols, all able to activate the EBI2

Table 2 Expression pattern of EBI2 in various cell types in both lytic replication and latency

Phase of infection/ gene		EBI2 expression	Assay system	References
Lytic		Upregulated	Akata and B95.8 cell RT-qPCR	90
Latency	0			
	I	>200-fold	Subtractive hybridization of mRNA in BL cells	87
		Upregulated	Akata cell RT-qPCR	90
	II			
	III	>4-fold	LCLs	89
		>2-fold	Gene expression profiling of PTLD samples	88
		Upregulated	B95.8 cell RT-qPCR	90
LMP1-induced		Rapid upregulation	Run-on RNA assays with conditional LMP1 expressing cells	29

receptor. Further studies on the EBI2–oxysterol interaction has revealed that EBI2 is strongly active through Gαi and that it signals through both G-protein-dependent and -independent pathways, although the receptor seems to be biased toward the former.[90–93] A defined set of anchor residues in EBI2 consisting of an arginine in the extracellular end of TM helix 2 and tyrosine residues in TM-3 and -6 are highly important as alanine substitutions of these residues significantly decreases oxysterol binding and EBI2 activation.[93,94] In addition, *ex vivo* proliferation studies on mouse B cells expressing human EBI2 showed that EBI2 induces proliferation, which can be inhibited by treatment with a small-molecule EBI2 antagonist.[95] This proliferation was also observed when comparing the growth of B cells from wt mice to that of B cells from mice with targeted deletion of EBI2.[95]

Oxysterols are oxidized derivatives of cholesterol, which regulate many cellular functions and have also been recognized for having functions within the innate and adaptive immune response. Hence, the oxysterol activated liver X receptors (LXRs) can rescue macrophages from pathogen-induced apoptosis, induce pathogen clearance and inhibit an aberrant inflammatory response.[96–98] Also, LXRs have an important checkpoint function on B and T cell proliferation as activated B and T cells induce oxysterol metabolism

and inhibition of LXR leading to proliferation.[99] Accordingly, antigen-challenged mice lacking LXRs develop lymphoid hyperplasia.[99] Finally, LXRs are negative regulators of Th17 helper cells, which are implicated in autoimmune diseases and expression of LXRs in an *in vivo* model of multiple sclerosis thus improved disease.[100]

4.2. Roles of the EBI2–oxysterol axis in the immune response

EBI2 was initially shown to be expressed primarily in B cells and later studies confirmed this and added expression in DCs, macrophages, and to a lesser degree T cells and NK cells.[87,90–92,101] Interestingly, abundant expression of EBI2 in activated B cells is opposed by downregulation in GCs, leading researchers to the biological role of EBI2.[89,102] In 2009, two groups simultaneously published EBI2 as an important regulator of B cell localization in the lymphoid follicle.[103,104] Together with chemokine receptors CXCR4, CXCR5, and CCR7 and a highly controlled expression of these and their ligands (CXCL12, CXCL13, and CCL21, respectively), EBI2 mediates the segregation of B cells between the inner and outer follicle during antibody responses (for a recent review, see Ref. 105). Shortly, during the primary early antibody response activated B cells express high levels of EBI2 and migrate toward the inner- and outer follicular areas where oxysterol accumulation is generated by stromal cells.[103,104,106,107] Here, the B cells commit to one of two paths: plasmablast differentiation and antibody production by continuous expression of EBI2 or GC formation by downregulation of EBI2 by BCL-6.[102–104] The importance of EBI2 in the immune response is illustrated by a greatly reduced early antibody response in EBI2-deficient mice.[104]

Recent studies have expanded the role of EBI2 in the immune response to include directed localization of DCs in the marginal zone (MZ) bridging channels.[108,109] CD4 expressing DCs reside in the MZ bridging channels and survey the blood for antigens. Upon detection of antigen, DCs migrate toward the T cell zone and thereby initiate T helper cell activation and immune response. Two subsequent studies found that EBI2 expression and accumulation of 7α,25-OHC in the MZ bridging channel were required for generation of CD4+ DCs and localization of these to the MZ bridging channel.[108,109] EBI2 deficiency in DCs resulted in reduced activation of CD4+ T cells as well as reduced antibody response.[109] Also, EBI2 functions as a negative regulator of interferon responses in plasmacytoid and myeloid DCs, which could possibly balance aberrant TLR-mediated interferon responses to foreign and self-nucleic acids.[110]

In addition to its role in the adaptive immune response, EBI2 may also play a role in the innate immune response through actions on macrophages. Recently, macrophages were shown to express both EBI2 and the enzymes required for 7α,25-OHC production. Lipopolysaccharide (LPS) stimulation of a macrophage cell line leads to activation of EBI2, calcium mobilization and directed cell migration, suggesting that EBI2 indeed does play a role in macrophage-mediated immune responses.[101]

4.3. A potential role for the EBI2–oxysterol axis in EBV infection

Although very little is known of the role of EBI2 in EBV infections, knowledge on the role of EBI2 and in particular oxysterols in the general antiviral immune defense is steadily emerging. Production of oxysterols by macrophages might thus represent an antiviral host strategy as treatment of macrophages with TLR ligand LPS or type I interferons leads to an increase of 25-OHC oxysterol and its synthesizing enzyme cholesterol-25-hydroxylase (CH25H) both *in vitro* and *in vivo*.[111–113] *In vitro* studies with expression of CH25H or treatment with 25-OHC broadly inhibits enveloped viruses, among them herpes simplex, HIV, and MHV68.[114] *In vivo* studies with CH25H-deficient mice showed an increased susceptibility to MHV68 and also, 25-OHC treatment of humanized mice reduced HIV replication.[114] These effects of CH25H and 25-OHC are probably due to antiviral effects of the molecules themselves and not mediated by altered EBI2 activity.[114] On the contrary, studies on time course-dependent changes in the macrophage transcriptome after stimulation with *Salmonella* strains revealed that EBI2 messenger RNA (mRNA) levels increased up to 20-fold[115] directly linking EBI2 to the anti-*Salmonella* immune response.

The high expression of EBI2 during EBV infection suggests that it is required for either a successful primary and persisting EBV infection or in the immune response toward EBV infection. It has previously been suggested that EBV-mediated upregulation of EBI2 could ensure positioning of EBV-infected cells in a survival-promoting niche.[103] Thus, upregulation of EBI2 would keep infected B cells from participating in GC formation and risk GC-induced apoptosis. This is supported by the fact that EBI2-mediated migration is promoted by CD40 engagement[116] and that the CD40-mimic LMP1 has been shown to directly induce EBI2 in *in vitro* settings.[29] However, the high expression of EBI2 during latency program I,[87,90] when most of the viral genome is shutdown and only EBNA-1 is expressed, hints toward a role of EBI2 in the immune surveillance instead.

This is supported by the previously described finding that also *Salmonella* strains induce EBI2 upregulation in macrophages.[115]

Although the biological function of EBI2 has primarily been described for the antibody production in the adaptive immune response, antibodies (as described above) play a minor role in the immune anti-EBV response. The expression of EBI2 during EBV infection has for now focused on expression levels in B cells, but considering that EBI2 is also expressed and has biologic functions in macrophages and DCs, it would be interesting to measure expression of EBI2 in these cell compartments during EBV infection. Thus, it could be hypothesized that EBV infection also results in EBI2 upregulation in macrophages and DCs and that this could be part of an antiviral response mediated by these cells (Fig. 2B). As such, it has been shown that virus-infected macrophages can directly activate NK cells and thereby kill EBV-infected cells.[117] Also, DCs in the MZ bridging zone, which are activated by virus, activate CD4+ T cells, which in turn mediate antibody response as well as CD8+ CTL activation.[109] In addition, CD4+ T cells can acquire cytotoxic activity themselves and *in vivo* cytotoxicity assays in mice persistently infected with MHV68 show CD4 T cell-dependent killing of MHV68-infected cells.[118]

5. MANIPULATION OF THE HOST IMMUNE SYSTEM 7TM RECEPTORS AND LIGANDS BY EBV—THE CHEMOKINE SYSTEM

Chemokines induce chemotaxis of leukocytes through interactions with 7TM chemokine receptors. Approximately, 40 chemokines and 18 functional chemokine receptors have been identified in humans. Based on the pattern of conserved cysteine residues in the N-terminal region they are classified into four subfamilies: CC, CXC, C, and CX3C.[119] By controlling leukocyte migration, the chemokine system has important functions in coordinating the immune system, leukocyte homeostasis, lymphocyte activation, and the host immune response to infectious pathogens.[119] It is therefore not surprising that many viruses have been shown to modulate the chemokine system, several of them by encoding chemokine receptors or ligands.[60,120] EBV has not yet been shown to encode either chemokine receptors or ligands, but the system is being markedly manipulated during EBV infection, as some chemokines and chemokine receptors are upregulated, while others are downregulated (Fig. 3). Hence, the chemokine system may play an important role in the tissue localization

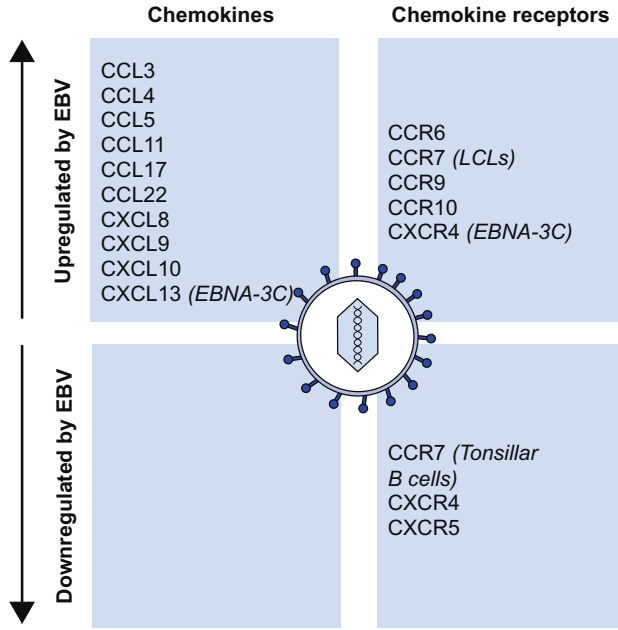

Figure 3 Regulation of the human chemokine system during EBV infections. EBV upregulates and downregulates both chemokine receptors and chemokine ligands. Notably, CCR7 is upregulated in Burkitt's lymphoma cells and LCLs, but time-dependent studies in tonsillary B cells showed that CCR7 was downregulated 7 days after infection suggesting that CCR7 is differentially expressed by EBV depending on B cell origin.[121] Also, CXCR4 expression is low in LCL with full latency III program expression, whereas LCLs expressing only EBNA-3C showed high expression of CXCR4 as well as its ligand CXCL13.

of EBV-infected B cells and thereby contribute to the pathogenesis of EBV-associated diseases.[121]

A study profiling chemokine expression in LCLs revealed high expression levels of CCR6, CCR7, and CCR10 and low expression of CXCR4 and CXCR5 in EBV immortalized cells.[122] Accordingly, LCLs showed a markedly increase in migration in response to the ligands of CCR6 (CCL20), CC7 (CCL21), and CCR10 (CCL28), but showed only weak migration in response to the ligands of CXCR4 (CXCL12) and CXCR5 (CXCL13).[122] Selective expression of EBNA-2, LMP1 or both in an EBV negative cell line showed that EBNA-2 directly induces expression of CCR6, while both EBNA-2 and LMP1 downregulate CXCR4. In contrast neither CCR10 or CXCR5 was directly induced by EBNA-2 or LMP1 and the regulation of these two chemokine receptors was more likely a consequence of the plasmablast state of the

LCLs.[122] CXCR4 has also been found to be upregulated in EBNA-3B-deficient LCLs suggesting that this viral protein is a negative regulator of CXCR4.[123] In addition, BILF1 has been found to heterodimerize with CXCR4 and thereby inhibit CXCR4-mediated signaling.[124] The importance of the chemokine pattern described above can be deduced from the fact that also human herpesviruses 6A, 6B, and 7 has been shown to induce CCR7 expression and reduce CXCR4 expression.[125,126] Interestingly, a study examining the lymphoproliferation in EBV-infected huSCID mice showed that the axis was important for lymphoma development as CXCR4 was highly expressed on the tumors and inhibition of the CXCR4/CXCL12 axis reduced tumor development.[127] Consistently, the gene, which is expressed in latency III program and is important for growth transformation of infected B cells, upregulated the levels of both CXCL12 and CXCR4.[128] The diverging expression of CXCR4 by the viral tumor suppressor EBNA-3B (downregulation)[123] and the growth transforming EBNA-3C (upregulation)[128] taken together with increased expression of CXCR4 in EBV-mediated huSCID lymphoma development[127] clearly suggests that CXCR4 is important for the growth transformation of infected B cells. Another study on the effect of EBV on CXCR5 and CCR7 expression in tonsillary B cells showed that 2 days after EBV infection there were minor changes in the expression levels of CXCR5. By day 7, however, the expression levels of both CXCR5 and CCR7 go down and both of the aforementioned receptors were no longer expressed at the cell surface by day 14. Also, the chemotactic response to CXCL13 and CCL21 was reduced by day 2, when CXCR5 and CCR7 was still expressed, suggesting that the virus impairs chemokine-directed migration even in the presence of the receptors.[121]

The histologic finding that EBV-infected B cells in infectious mononucleosis tend to avoid the GCs and instead accumulate under the epithelium of certain mucosal tissues[34,129] could be explained by the above-mentioned expression patterns of chemokine receptors on EBV-infected B cells. CXCR4 and CXCR5 respond to their ligands, which are highly expressed in the center of lymphoid follicles and thereby direct B cells to GC formation.[105] CCL20 and CCL28, on the other hand, are normally expressed by the epithelial cells of mucosal tissues such as salivary glands and the tonsils.[130–132] As such, downregulation of CXCR4 and CXCR5 and upregulation of CCR6 and CCR10 could ensure migration toward the tonsillary epithelial cells.[122]

EBV also induces the expression of the mRNA levels of CCR9, which is responsible for B cell homing to mucosal tissues, as well as C5AR1, the receptor for the complement factor C5a. Alterations of the expression level

of the aforementioned molecules may cause the EBV-infected cells to be retained in the interfollicular region of the tonsils.[121]

The manipulation of the chemokine system by EBV seems to be in favor of the virus infection, but it is likely that some of the regulation also reflects cell protection against the intruding virus and antiviral immune responses. As such, it was found that EBV-infected cells as well as EBV negative cells expressing LMP1 upregulate the expression of CCL17 and CCL22, known to preferentially attract Th2 cells and regulatory T cells via CCR4. This expression pattern would benefit the virus and skew the immune response away from a Th1 and CTL response. In contrast, EBV-infected cells were found to upregulate CCL3, CCL4, and CCL5, which are known to attract Th1 cells and activated CTL via CCR5.[133] Expression of CCL5 was also found to be elevated in LMP1 expressing cells as well as Burkitt's lymphoma cell lines.[134] Thus, the chemokine system may work in favor of both the virus and the immune system.

Regardless of its effect on EBV infection, the chemokine system has been found to be regulated in both nasopharyngeal cancer and Hodgkin lymphoma. As such, Hodgkin and Reed–Sternberg (HRS) cells from Hodgkin lymphoma and nasopharyngeal carcinoma cells have been shown to express CXCL8 and CCL17.[135] Furthermore, Hodgkin lymphoma cells express high levels of CXCL9, CXCL10, CCL3, CCL5, and CCL11.[136]

6. EBV-ASSOCIATED DISEASES

Primary EBV infection often occurs in young immunocompetent children and is asymptomatic or present with nonspecific mild symptoms.[6] However, given that EBV was originally identified in Burkitt's lymphoma cells and has the unique ability to transform resting B cells into highly proliferating lymphoblasts, it is not surprising that this virus is associated with a number of cancers in both immunocompromised and immunocompetent patients. In addition, EBV has been associated with autoimmune diseases involving infected B cells (see Table 3 for an overview of confirmed EBV-associated diseases).

6.1. Infectious mononucleosis

In adolescent and young people, primary infection with EBV may lead to infectious mononucleosis, which is usually a self-limiting disease with symptoms ranging from mild to severe. More than 50% of patients with

Table 3 Confirmed EBV-associated malignant and nonmalignant diseases

Disease	EBV association	Cell of origin	Latency program
Nonmalignant			
Infectious mononucleosis			
Malignant			
Immunocompetent			
Burkitt's lymphoma			
– Endemic	95–100%	GC or post-GC B cell	I
– Sporadic	15–85%	GC or post-GC B cell	I
Hodgkin lymphoma (classical)			
– Nodular sclerosing	20–40%	Post-GC B cell	II
– Lymphocyte-depleted	80–90%	Post-GC B cell	II
– Lymphocyte-rich	<10%	Post-GC B cell	II
– Mixed cellularity	60–75%	Post-GC B cell	II
Extranodal NK/T cell lymphoma	100%	$CD3^-$, $CD56^+$ T cell (derived from NK cell)	II
Nasopharyngeal carcinoma			
– Nonkeratinizing	100%	Undifferentiated epithelial cell	I/II
– Keratinizing	30–100%	Undifferentiated epithelial cell	I/II
Gastric carcinoma			
– Lymphoepithelioma like	90–100%	Undifferentiated gastric epithelial cell	I
– Conventional	5–15%	Undifferentiated gastric epithelial cell	I
Immunocompromised			
Lymphoproliferative disease in congenital immune deficiency	100%	GC or post-GC B cell	III
Posttransplant lymphoproliferative diseases (PTLDs)			

Continued

Table 3 Confirmed EBV-associated malignant and nonmalignant diseases—cont'd

Disease	EBV association	Cell of origin	Latency program
– Early lesions and polymorphic <1 year after transplant	90–100%	GC or post-GC B cell	II/III
– Monomorphic >1 year after transplant	50%	GC or post-GC B cell	II/III
Leiomyosarcomas	100%	Smooth muscle cell	III
HIV malignancies			
– Burkitt's lymphoma	55%	GC B cell	I
– Hodgkin lymphoma	100%	Post-GC B cell	II
– Diffuse large B cell lymphoma (DLBCL)—centroblast	30%	GC or post-GC B cell	I
– DLBCL—immunoblast	90%	GC or post-GC B cell	III
– Primary CNS lymphoma	100%	GC or post-GC B cell	III
– Primary effusion lymphoma	90–100%	GC or post-GC B cell	I

The diseases are shown along with association, cell of origin, and latency program expressed in the implicated cells.

infectious mononucleosis present with the triad of fever, lymphadenopathy, and pharyngitis.[137] The peripheral blood shows leukocytosis with an increase in mononuclear cells, CD8+ T cells, NK cells, and a decrease in CD4+ T cells.[6] The symptoms observed in infectious mononucleosis are due to the increased level of CD8+ cells and the cytokines they secrete.[138] Infected B cells in the throat of infectious mononucleosis patients vary in morphology as well as expression of viral genes. Thus, the infected cells express variations of latency programs III, II, and I.[33,139] In the periphery, more than 50% of the memory B cells are infected and these memory cells are resting and express the latency 0 program.[140] Interestingly, even in the early massively infectious phase of infectious mononucleosis, the restriction of virus infection to the memory B cell compartment is intact.[140] The risk of EBV positive Hodgkin lymphoma is increased after infectious mononucleosis,[141] which has been proposed to be due to MHC class I-restricted EBV-specific CTL responses in the early anti-EBV immune response.[142]

6.2. Diseases in immunocompetent patients

In the immunocompetent host, EBV is primarily associated with Burkitt's lymphoma and Hodgkin lymphoma originating in B cells; and nasopharyngeal carcinoma and gastric carcinoma originating in epithelial cells. Burkitt's lymphoma is a malignancy most commonly located in the abdomen and jaw. Three distinct clinical forms of Burkitt's lymphoma are recognized: endemic (African), sporadic (nonendemic), and immunodeficiency-associated. In central parts of Africa and New Guinea, Burkitt's lymphoma occurs holoendemic with malaria. The strong association of EBV with Burkitt's lymphoma is based on the high frequency of tumors that carry the virus in endemic areas (97%)[143] and the presence of clonal EBV in all tumor cells.[144] How EBV participates in the pathogenesis of Burkitt's lymphoma is still unclear. The tumor originates from GC B cells and expresses the latency program I.[145] All Burkitt's lymphoma cells carry one of three characteristic chromosomal translocations (from chromosome 8 to chromosome 14) that place the cellular oncogene c-myc under the control of the Ig heavy chain or one of the light-chain loci.[145] Classical Hodgkin lymphoma is characterized by the presence of large multinucleated B-lineage cells called HRS cells. There has also been found in some cases of infectious mononucleosis, thereby supporting the increased risk of Hodgkin lymphoma following a severe infectious mononucleosis.[129] However, EBV is only present in 40–60% of cases of Hodgkin lymphoma.[146] EBV-positive Hodgkin lymphoma cells origin in post-GC B cells and express latency program II.[6]

EBV infection of epithelial cells is associated with several carcinomas, among them nasopharyngeal carcinoma and gastric carcinoma. Nonkeratinizing nasopharyngeal carcinoma is extremely common in certain high-risk groups such as the Southern Chinese and Inuits and is almost always EBV positive.[147,148] The cancers originate in epithelial cells and express latency program 0.[149] Other cofactors are involved in the pathogenesis and both environmental and genetic susceptibilities have been implicated.[23] Almost 10% of gastric carcinomas worldwide origin in EBV-infected cells[150] making it the most common EBV-associated cancer.[6] All EBV-associated gastric carcinoma cells express latency program I and EBNA-1, which has been associated with promoted cancer cell survival.[151]

6.3. Diseases in immunocompromised patients

Impaired T cell immune surveillance of EBV-infected B cells caused by HIV-associated genetic or iatrogenic (transplantations) immunosuppression

is associated with lymphomas and to a lesser extent leiomyosarcomas. PTLDs- and HIV-associated lymphomas constitute the most abundant lymphomas in immunocompromised patients. PTLDs include a heterogeneous group of lymphomas, of which almost all carry the virus and express the latency program III.[144] The incidence varies from 1% to 20%, depending upon the transplantation type and level of the immunosuppression.[139] The lymphomas mostly originate in GC B cells or post-GC B cells. PTLDs arising early after transplantation are almost always associated with EBV, whereas PTLDs arising more than 1 year after transplantation are monomorphic displaying mutations in genes such as BCL-6, p53, and c-myc and are less often associated with EBV.[152]

Similar to PTLDs, HIV-associated lymphomas are a highly heterogeneous group, with a varying degree of association to EBV. EBV positive HIV-cancers mostly originate in GC or post-GC B cells and express different latency programs.[153]

Besides cancers, EBV has been associated with several autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and systemic lupus.[6] It is very difficult to demonstrate the causative role of EBV in these diseases as almost all (90%) are infected with EBV. The linkage between EBV and multiple sclerosis is, however, growing stronger as 99% of multiple sclerosis patients are EBV positive[154] and infectious mononucleosis increases the risk of multiple sclerosis by 2.3-fold.[155] In addition, EBV-infected B cells have been discovered in the CNS of multiple sclerosis patients.[156]

7. DRUG-TARGET POTENTIAL

7TM receptors and in particular class A receptors to which the 7TM receptors mentioned in this review belong, are highly druggable molecules. Approximately, 35% of all currently marketed drugs target class A 7TM receptors.[157] There is no specific antiviral treatment for EBV. Current treatment strategies for EBV-associated lymphomas include B cell antibodies (rituximab, chimeric, and monoclonal antibody against CD20), reducing immunosuppression, EBV-specific CTL infusion, and chemotherapy for PTLDs; and anti-HIV treatment for HIV-associated lymphomas.[158,159] The use of rituximab in particular has dramatically changed the overall survival of PTLD patients, but a strategy directed specifically against EBV-infected cells and genes, which play a role in the EBV-mediated pathogenesis, could likely reduce the incidence of malignancies. As such, BILF1 constitutes an obvious target as it plays a role for EBV immune evasion[56,61,62] and has oncogenic

properties both *in vitro* and *in vivo*.[66] EBI2 has been shown to have proliferative effects *ex vivo*[95] and in addition, the receptor is upregulated in PTLDs.[88] Though the role of EBI2 in EBV life cycle is still uncertain, the aforementioned knowledge suggests that EBI2 could play a role in EBV-mediated lymphoma development and therefore EBI2 constitutes another potential drug target. Finally, the chemokine expression pattern is skewed in both Hodgkin lymphoma and nasopharyngeal carcinoma[135] meaning that inhibition of some chemokine receptors could prove beneficial in the antiviral treatment strategy.

8. CONCLUSIONS

In summary, EBV encodes one 7TM receptor and manipulates endogenous 7TM receptors, some of which are involved in the virus life cycle and some of which may be involved in the antiviral host defense. In the case of EBI2, we are still unclear on whether the upregulation is virus mediated or immune system mediated. Nevertheless, EBI2 is upregulated in EBV-associated PTLD and, together with BILF1 and the chemokine receptors constitute potential drug targets in EBV-mediated diseases. Further studies exploring the relation between these receptors and EBV are highly warranted.

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