Understanding the interplay between host immunity and Epstein-Barr virus in NPC patients

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Epstein-Barr virus (EBV) has been used as a paradigm for studying host–virus interactions, not only because of its importance as a human oncogenic virus associated with several malignancies including nasopharyngeal carcinoma (NPC) but also owing to its sophisticated strategies to subvert the host antiviral responses. An understanding of the interplay between EBV and NPC is critical for the development of EBV-targeted immunotherapy. Here, we summarize the current knowledge regarding the host immune responses and EBV immune evasion mechanisms in the context of NPC.

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INTRODUCTION

Epstein-Barr virus (EBV/HHV-4), which latently infects more than 90% of the world’s adult human population, is associated with nasopharyngeal carcinoma (NPC).

In NPC patients, EBV typically exists in a type II latency program (particularly the undifferentiated or poorly differentiated types). Type II latency is characterized by the expression of a subset of latent genes, including EBV-determined nuclear antigen 1 (EBNA1), latent membrane proteins (LMP1, LMP2A, and LMP2B), and several EBV non-coding RNAs (primarily EBER1 and EBER2). In addition, BamHI-A rightward transcripts (BARTs) and BamHI-A rightward frame 1 (BARE1) of EBV are expressed abundantly and detected consistently in NPC.

The detection of EBV in NPC and the prominent role of EBV in promoting tumor development support EBV as a potential therapeutic target for NPC. In fact, with the accumulation of knowledge regarding EBV oncogenicity and interactions between EBV and the host immune responses, immunological approaches, such as adoptive T-cell immunotherapy and vaccine-based strategies to induce EBV-specific T-cell responses, are emerging. In this review, we summarize the current understanding of how EBV stimulates the host immunity and the mechanisms exploited by EBV to circumvent immune responses in the context of NPC.

EVIDENCE FOR EBV CONTRIBUTING TO NPC

EBV factors detected in NPC patients

In the 1960s, antibodies against the EBV antigen were first identified in NPC patients, and subsequent studies reported higher levels of anti-EBV antibodies in NPC patients than in healthy controls. More direct and stronger evidence has been obtained regarding the detection of EBV DNA,10,11 protein antigens,2 and miRNA products1 in NPC patients. Viral DNA is considered a specific prognostic marker for both pre- and post-treatment NPC patients,11–17 regardless of prevalence in the region studied.18 Recent comprehensive profiles with methods that are more sensitive and specific (e.g., multiplexed stem-loop reverse transcription polymerase chain reaction19 and miRNA microarray20) identified panels of upregulated viral miRNAs in both NPC lesions and sera, some of which were shown to function as potential biomarkers for the diagnosis and prognosis of NPC.21

Mechanisms exploited by EBV products to promote NPC

A set of EBV latent genes have been identified that play an important role in NPC development, and multiple mechanisms including the restriction of cell homeostasis, the enhancement of cell mobility, and the induction of stem-like cancer cells were proposed.

EBNA1, which is expressed in all EBV-related tumors, is believed to be one of the most important viral proteins that promote NPC and is required for maintaining the viral latency in NPC.22,23 The introduction of EBNA1 enables EBV-negative NPC cells to grow more rapidly and to achieve increased metastasis in immunodeficiency mice.24 The potential mechanisms of EBNA1 function involve upregulation of tumor angiogenesis cytokines;25 degradation of promyelocytic leukemia (PML) protein, which is associated with p53 activation, DNA repair and cell apoptosis;26 inhibition of the anti-oncogenesis canonical p65 nuclear factor-κB (NF-κB) pathway;27 and induction of metastatic potential proteins28 as well as epithelial–mesenchymal transition (EMT).29

LMP1, another major viral oncoprotein, is closely associated with epithelial transformation30,31 and angiogenesis.32–34 LMP1 is detected primarily in preinvasive lesions, including dysplasia and carcinoma in
LMP2A and LMP2B are also expressed in NPC. These proteins negatively regulate LMP2A activity by binding to this protein, preventing its phosphorylation without altering its cellular localization. LMP2A possesses the ability to induce stem-like cancer cells, and it regulates the ability of infected NPC cells to grow faster and are more invasive. No direct evidence has been found for the role of LMP2B in NPC, although LMP2B itself may facilitate the spread and motility of epithelial cells.

Emerging evidence has revealed that EBERs, BARF1, and BARTs also contribute directly to NPC development. EBERs accelerate the growth of NPC cells and confer resistance against apoptotic stress. BARF1 is not expressed during EBV infection of the NPC-derived EBV-negative cell lines HONE-1 and CNE-1; however, when infected by a recombinant EBV carrying the BARF1 gene under the control of the SV40 promoter, the infected NPC cells grew faster and were more resistant to apoptosis compared with wild-type EBV-infected cells. BARTs are very abundant EBV transcripts in NPC, contain several open reading frames, and are precursors for 22 miRNAs. Their roles in NPC (for instance, miR-BART1 in cell transformation, miR-BART5 in anti-apoptotic activity, and miR-BART7 in EMT) were recently reviewed (Table 1).

### THE INTERPLAY BETWEEN HOST INNATE IMMUNITY AND EBV

**EBV mounts innate responses**

One major characteristic of NPC is the presence of abundant infiltrating leukocytes in tumor stroma where various cell types, including neutrophils, natural killer (NK) cells, monocytes/macrophages, and dendritic cells (DCs), are detected and represent the first defense line for EBV infection. Nevertheless, the interaction between EBV and the host innate immunity system is not fully understood.

Based on flow cytometry, EBV was shown to bind to the neutrophil surface with its major envelope glycoprotein gp350 and subsequently stimulate the production of antiviral cytokines, including interleukin 1α (IL-1α), IL-1β, chemokines IL-8, and macrophage inhibitory protein (MIP)-1. Conventional DCs (cDCs) and plasmacytoid DCs (pDCs), the two major human DC subsets, sense EBV products through Toll-like receptors. When challenged with either live EBV virions or unmethylated EBV DNA, pDCs were found to produce interferon-α (IFN-α). In addition, treatment of cDCs with EBERs induces the production of IFN-β, IFN-γ, and tumor necrosis factors (TNFs).

EBV-stimulated cDCs and pDCs can promote the cytotoxicity of NK cells through type I and II interferons, as well as chemokine receptors, acting on natural killer cells and other immune cells.

### Table 1 A brief summary of mechanisms exploited by EBV latent products to promote NPC formation and development

| General mechanisms | Viral products | Molecular mechanisms | References |
|--------------------|---------------|----------------------|------------|
| Promotion of transformation and angiogenesis | EBNA1, LMP1 | Mediates AP-1 to upregulate IL-8, VEGF, HIF-1α | 25 |
| Inhibition of apoptosis | EBERs | Upregulates IGF-1 | 54 |
| Induction of stem cell-like phenotype | LMP1 | Mediates NF-κB, ERK, and JNK pathways to induce endocan; mediates the degradation of polyhydroxylases 1 and 3 to upregulate HIF1-α | 32–34 |
| Enhancement of cell mobility | EBNA1, LMP1 | Activates hedgehog pathway to induce CD44V6, NGFR (p75NTR), and CCR4 | 46,47 |

**AMPK, AMP-activated protein kinase; AP-1, transcription activator-1; Bcl-2, B-cell lymphoma-2; CSC/CPC, cancer stem cells/cancer progenitor cells; CCR4, C-C chemokine receptor type 4; DICE1, deleted in cancer 1; EGFR, epidermal growth factor receptor; HIF-1α, hypoxia-inducible factor 1α; IGF-1, insulin-like growth factors; JNK, c-Jun N-terminal kinase; LMP1, AMPK-liver kinase B1-AMP-activated protein kinase; NGFR, nerve growth factor receptor; PARP, poly-ADP-ribose polymerase; PHGDH, phosphoglycerate dehydrogenase; PSAT1, phosphohydroxythreonine aminotransferase 1; PTEN, phosphatase and tensin homolog located on chromosome 10; PUMA, p53 upregulated modulator of apoptosis; STAT3, signal transducer and activator of transcription; TNFα, tumor necrosis factor-alpha inducible protein-2; VEGF, vascular endothelial growth factor; MEK-ERK, mitogen-activated protein kinase-extracellular signal-regulated kinase.**

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I IFNs. NK cells are potential targets for EBV infection because the gp85-gp25-gp42 complex of EBV can directly combine with human leukocyte antigen (HLA) class II molecules on NK cells. EBV can activate monocytes and macrophages. DUTPase of EBV induces macrophages to express and secrete TNF-α, IL-1β, and IL-6 via the MyD88-dependent activation of NF-κB. For monocytes, in addition to the inflammatory cytokines that are also produced by activated macrophages, EBV also stimulates production of several chemokines, including IFN-inducible protein-10 (IP-10), MIP-1, monocyte chemotactic protein-1 (MCP-1), and IL-8 at the mRNA level.

Evasion of innate immune responses

The establishment of life-long persistence in more than 90% of the worldwide adult human population clearly indicates that EBV has delicately evolved to evade the innate immune response. In addition to the above-mentioned latent genes, a portion of viral lytic antigens are frequently detected in NPC, probably due to EBV reactivation upon some poorly defined triggers. Recently, the mechanisms by which individual EBV products (including both lytic and latent genes) evade the innate immune response were reviewed. Here, we focus on summarizing two common and efficient strategies to circumvent the innate immune response in the NPC-induced modulation of phagocyte function and blockade of antiviral cytokines.

Modulation of phagocyte apoptosis and maturation. Subsequent to the finding that the binding of EBV to the surface of neutrophils induces inflammatory cytokine expression, Gosselin J et al. found that EBV penetrates neutrophils and localizes to their nuclei. After infecting neutrophils, EBV launches apoptosis by modulating the Fas/Fas ligand (L) pathway, as indicated by a significant increase in infecting neutrophils, EBV launches apoptosis by modulating the uptake of EBV and localizes to their nuclei. After infecting neutrophils, EBV launches apoptosis by modulating the Fas ligand (L) pathway, as indicated by a significant increase in both membrane-bound Fas/Fas-L and soluble Fas-L. This study was the first to explain why EBV cannot establish robust infection in neutrophils. EBV also impairs the phagocytic activity of primary monocytes by inhibiting protein kinase C (PKC) activity. Monocyte apoptosis caused by EBV contact during DC development results in a reduction in mature DCs. This reduction may provide EBV with a time window for productive replication by temporarily delaying the onset of immune responses. In addition to the decrease in the number of pDCs during EBV infection, the maturation of pDCs is also compromised, as indicated by reduced secretion of TNF-α, which can partly facilitate pDC development. pDCs have a dual role in defending viral infection, by secreting a high level of type I IFNs to inhibit viral replication directly and by initiating and tuning the specific adaptive immunity. EBV infection undermines the ability of pDCs to mature, thereby preventing these cells from mounting antiviral T-cell responses.

Blockade of antiviral cytokines. The apoptosis of innate effector cells results in a significant reduction in IFN production. In addition, certain EBV proteins and transcripts, such as EBERs and LMP2, can inhibit the type I IFN responses by disrupting IFN-stimulated transcription and by targeting IFN receptors for degradation.

Inducing the innate immune cells to produce antagonistic factors to block the function of those antiviral cytokines demonstrates another strategy by which EBV eludes the immune responses. For example, in addition to IL-1α and IL-1ß, EBV also initiates the production of their natural inhibitor IL-1 receptor antagonist (IL-1Ra). IL-1Ra competitively inhibits the binding of IL-1α and IL-1ß to their receptors. Moreover, IL-1Ra is secreted approximately 3200 and 610 times more than IL-1α and IL-1ß, respectively, from EBV-stimulated neutrophils, indicating another effective mechanism by which EBV counteracts the host innate immune response.

In addition, EBV prevents the production of prostaglandin E2 (PGE2) by monocytes by inhibiting the expression of inducible cyclooxygenase 2 (COX-2), a critical enzyme in the PGE2 biosynthesis pathway. This inhibition of COX-2 may be a result of EBV interfering with the activation of the NF-κB pathway, which plays an important role in COX-2 induction in monocytes. NF-κB is also critical for TNF-α induction, and consequently, EBV suppresses TNF-α secretion from lipopolysaccharide-treated monocytes by 70%–90%. Because simple contact between EBV and monocytes upregulates TNF-α, inhibition of the NF-κB pathway after EBV replication in monocytes may be a mechanism by which the virus shuts down further TNF-α production. Additional evidence of this mechanism may be needed. First, TNF-α suppression by EBV was not observed at a basal expression level, and second, the exact mechanism of this suppression may be largely attributable to monocyte apoptosis upon EBV penetration.

THE INTERPLAY BETWEEN HOST ADAPTIVE IMMUNITY AND EBV

Antibodies detected during EBV infection

EBV-specific antibodies, primarily immunoglobulin G (IgG) and IgA, are detected in the sera of NPC patients. These antibodies recognize various EBV targets, including EBV structural antigens (e.g., viral capsid antigen-proteins VCA-p18 and VCA-p40, glycoproteins gp350/220, and gp74), lytic antigens (e.g., Bam HI rightward reading frame 1 (BRLF1), Bam HI leftward reading frame 1 (BLZF1), and EBV-DNase), and latent antigens (e.g., EBNA1 and LMP1). One recent study that enrolled a relatively large number of samples studied the humoral immune response to EBV-encoded tumor-associated proteins in NPC patients. The results indicated that there exists a stronger IgG antibody response to EBNA1 than that of LMP1, LMP2, and BARF1. Except for EBNA1, only low IgA titers against LMP1, LMP2, and BARF1 were present. The marginal immunogenicity of LMPs and BARF1 to humoral immune responses may be due to their intrinsic properties (for example, rapid and complete secretion of BARF1 leaves little protein within or on the surfaces of cells for detection) and to their limited expression on the plasma membrane.

Cellular responses to EBV infection

Cellular immunity is essential for controlling EBV during both primary and persistent phases. The complete view of EBV-specific cellular immunity in NPC patients remains to be elucidated, despite the fact that many novel technical approaches have been introduced to assess CD8+ T and CD4+ T-cell responses to EBV. Circulating EBV-specific cytotoxic lymphocytes (CTLs) can be detected in NPC patients, and EBV-specific memory CTL responses can be reactivated in vitro after those cells were extracted from blood. Nevertheless, the antigen-specific CD8+ CTLs against several consistently expressed viral lytic genes, including BZLF1, BRLF1, BamHI-M leftward frame 1, BamHI-M rightward frame 1,
and BamHI-A leftward frame 2, are rarely found in NPC tumor lesions.\textsuperscript{113,114} In regard to latent antigens, Fogg MH \textit{et al.} found that CTLs targeting the EBNA1 significantly decrease in EBV-associated NPC patients.\textsuperscript{115} It is possible that presentation of EBNA1 by major histocompatibility complex (MHC) I molecules is diminished in tumors; however, this interesting finding requires further validation. For the subdominant latent antigens (LMP1, LMP2, and BARF1), CTLs specific to these proteins can be detected in most of NPC patients.\textsuperscript{111,116–118} CD4\textsuperscript{+} T cells play a pivotal role in supporting the production of high affinity antibodies, maintaining the number and biological function of CTLs, and possessing cytotoxic activities.\textsuperscript{119} However, the understanding of CD4\textsuperscript{+} T-cell responses to EBV is less clear due to the small size of the CD4\textsuperscript{+} compartment because of a lack of detectable CD4\textsuperscript{+} T-cell expansion during EBV infection.\textsuperscript{120} Most knowledge concerning CD4\textsuperscript{+} responses to EBV has been built on observations from either healthy EBV carriers or \textit{in vitro} experiments. For example, specific CD4\textsuperscript{+} T-cell clones or T-cell lines against EBV were evaluated by co-culture with autologous B-lymphoblastoid cell lines or DCs infected with recombinant vaccinia virus encoding individual lytic or latent proteins.\textsuperscript{109} Similar to the CD8\textsuperscript{+} T-cell response, a hierarchy of immunodominance of EBV antigens has been classified. EBNA1 and EBNA3 are the dominant targets, and LMPs and BARF1 are the subdominant targets.\textsuperscript{117,121,122} CD4\textsuperscript{+} T cells specific for EBNA1, LMPs, and BARF1 can be detected in NPC patients, albeit at low levels.\textsuperscript{111,117}

**Evasion of adaptive immune responses**

**Switching off immunodominant viral antigen expression.** EBV has developed multiple strategies to evade cellular immune responses during its long-term co-evolution with the host. Like all other herpesviruses, the major strategy EBV uses for establishing and maintaining latency in the face of the cellular immunity, particularly the CD8\textsuperscript{+} T-cell response, is to switch off the expression of most viral genes, particularly the viral genes with strong immunogenicities or that present a “non-immunogenic” phenotype that makes them invisible to the immune system. For example, several vital latent factors with high immunodominance, such as the EBNA3 family and EBNA2,\textsuperscript{123} are consistently absent in NPC patients. Nevertheless, when co-cultured \textit{in vitro} with autologous EBV-transformed lymphoblastoid cell lines, the virus-specific CTLs extracted from NPC patients sufficiently recognize antigens from the EBNA3 family.\textsuperscript{110}

**Impairment of the antigen-presenting HLA I or HLA II pathway.** NPC cells are positive for both HLA class I and II molecules; thus, these cells may present viral peptides to be recognized by both CD8\textsuperscript{+} and CD4\textsuperscript{+} T cells. However, EBV impairs both HLA I and HLA II antigen presentation pathways to circumvent T-cell surveillance. Notably, NPC cells retain their antigen presentation capacity when they are cultured \textit{in vitro}.\textsuperscript{110,124} EBNA1 is the primary target for the CD4\textsuperscript{+}, - but not the CD8\textsuperscript{+}, - T-cell response because ENBA1 is highly resistant to proteasomal digestion and thus is protected from being presented by MHC I molecules endogenously.\textsuperscript{125,126} This strategy is also utilized by latency-associated nuclear antigen 1, a homolog of EBNA1 in Kaposi sarcoma-associated herpes virus, to avoid being presented through the MHC class I pathway.\textsuperscript{127} Exogenously supplied EBNA1 can be presented by MHC class I molecules through a transporter associated with Ag processing (TAP)- independent pathway, whereas endogenously expressed EBNA1 can only be presented when the glycine-alanine repeat (GAr) domain of EBNA1 is deleted.\textsuperscript{128,129} Therefore, the GAr domain of EBNA1 is thought to control the presentation of endogenous EBNA1. However, further results have indicated that the GAr domain itself does not completely protect EBNA1 from presentation to CD8\textsuperscript{+} T cells.\textsuperscript{130–132}

The expression of LMP1 in human cells dramatically enhances HLA I processing,\textsuperscript{133,134} however, LMP1 is a poor CD8\textsuperscript{+} T-cell target \textit{in vivo}. Additionally, overall downregulation of HLA class I antigen presentation machinery (APM) was observed in NPC biopsies.\textsuperscript{135} This discrepancy may be explained by the finding that LMP1 induces c-myc-leucocytomatosis (c-Myc), which has been shown to downregulate HLA class I APM, subsequently counteracting the stimulatory effect of LMP1.\textsuperscript{136} In addition, the first transmembrane domain of LMP1 is able to mediate self-aggregation to severely impair the cis-presentation of an LMP1-derived epitope,\textsuperscript{136} demonstrating another novel mechanism of immune evasion.

Among the detectable EBV lytic antigens in NPC patients, BZLF1, BamHI-G leftward frame 5 (BGLF5), and BamHI-N leftward frame 2a (BNLF2a) are able to dysregulate the cellular immune response via various mechanisms. BGLF5, a DNase/alkaline exonuclease (AE) gene, exerts a host shutoff function to block the synthesis of host HLA I, thereby limiting CD8\textsuperscript{+} T-cell recognition.\textsuperscript{137} In addition, this shutoff function of BGLF5 is also involved in repressing DNA repair, inducing genomic instability in human epithelial cells.\textsuperscript{138} BZLF1 inhibits MHC class II expression by suppressing the transactivation of the transactivator class II, MHC, transactivator (CIITA),\textsuperscript{139} a critical transcriptional coactivator of MHC class II expression. BNLF2a specifically affects the presentation of immediate early and early proteins to HLA I molecules by inhibiting TAP and surface HLA I expression.\textsuperscript{140,141}

**Regulation of immuno-inhibitory biomolecules.** IL-10 is a well-known cytokine with immune-suppressive function. An association between increased IL-10 secretion and a significantly decreased number of cytotoxic T cells was observed in EBV-positive NPCs.\textsuperscript{142} Both EBV structural proteins and EBV-encoded miRNAs are involved in IL-10 induction. LMP1 was the first identified viral protein responsible for IL-10 induction via the activation of p38/stress-activated protein kinase 2 (SAPK2).\textsuperscript{143} In addition, EBER1 and EBER2 were shown to be associated with enhanced IL-10 expression at the transcription level through a novel signaling pathway independent of an IFN-inducible protein kinase R (PKR).\textsuperscript{144}

Decoy receptor 3 (DcR3), a recently identified molecule with immune inhibitory function, has the capacity to induce DC apoptosis via the formation of the death domain-containing receptor/death-inducing signaling complex.\textsuperscript{145} DcR3 also reduces MHC class II expression in tumor-associated macrophages.\textsuperscript{146} LMP1 was found to upregulate DcR3 expression via the NF-κB and phosphatidyl inositol 3-kinase (PI3K) signaling pathways.\textsuperscript{147} Because NPC-associated macrophages are positive for EBV,\textsuperscript{148} DcR3 may also be involved in immune evasion by EBV.

In addition, B7 homolog 1 (B7-H1), a T-cell inhibitory molecule, was upregulated during EBV infection of pDCs\textsuperscript{88} and NPC cell lines,\textsuperscript{149} and further studies are required to explore the role of B7-H1 in EBV immune evasion in NPCs.

**Induction of T regulatory cell activation and T-cell anergy.** T regulatory cells (Tregs), a subset of T cells with immune inhibitory functions, work in a cell-to-cell contact manner and secrete granzyme or cytokines such as IL-10 and transforming growth factor β (TGF-β).\textsuperscript{150–152} Tregs are consistently detected in the circulation and tumor
microenvironment in EBV-positive NPC, where approximately 12% of tumor-infiltrating leucocytes (TILs) in NPC harbor a Treg phenotype (CD4+CD25highforkhead box P3+). LMP1 dominantly induces Tregs to secrete IL-10, which suppresses the proliferation of mitogen or the withdrawal of Ag-stimulated T-effector cells and their release of IFN-γ. LALLFWL peptides of LMP1 show strong and direct inhibition of T-cell proliferation and NK cytotoxicity. This T-cell anergy is most likely attributable to the enhanced expression of IL-10 and TGF-β, resembling Treg responses. Tregs are also involved in the immune evasion of EBNA1 and LMP2 because Treg depletion restores EBNA1- and LMP2-specific CD8+ T-cell responses, as well as the immune control of EBV-infected cells in vitro (Table 2).

CONCLUDING REMARKS

NPC patients maintain efficient immune functions, including innate and adaptive immunities, to address EBV infection. However, this ancient virus has evolved multiple elaborate strategies to counteract and evade the host immunity, leading to its high prevalence among the human population. Seemingly, symbiosis is established between EBV and NPC that EBV facilitates NPC development by promoting the

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**Table 2** Strategies of cellular response evasion exploited by individual EBV antigens detected in NPC

| Strategies | Viral antigens | Mechanisms | References |
|------------|----------------|------------|------------|
| Switch off immunodominant viral antigens | e.g., EBNA2, the EBNA3 family | Not well-known, epigenetic modification? | 123 |
| Impair the HLA I or HLA II pathway | EBNA1, LMP1, LMP1, B2LF1 (Zta), BGLF5, BNLFL2a | Blocks proteasomal HLA II pathway degradation via the GAr domain; induces c-Myc via IL6 and the JAK3/STAT3 pathway; self-aggregation via its first transmembrane domain; suppresses the class II transactivator CIITA; directly shuts off host HLA I synthesis; inhibits TAP and surface HLA I expression | 128, 129, 135, 136, 139, 137, 138, 139 |
| Upregulate immune-inhibitory molecules | LMP1, EBERs, LMP1, EBV (specific antigen, not yet determined) | Induces IL-10 via p38/SAPK2; induce IL-10 via PKR-independent pathways; induces DcR3 via NF-κB and PI3K pathways; induces B7-H1 and ICOS-L | 143, 144, 147, 88 |
| Recruit Tregs and induce T-cell anergy | LMP1, LMP1 | Induces Tregs via chemokines (e.g., IL-10 and TGF-β); directly inhibits T-cell proliferation | 155, 156 |

ICOS-L, inducible costimulatory ligand; JAK3, Janus kinase 3.

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**Figure 1.** The interaction between EBV and the host immune system in NPC patients. NPC patients preserve efficient anti-EBV immunity while EBV has evolved multiple evasion strategies. A type of balance has been established for this interaction. The anti-EBV immune responses represent the “yang” or “positive” side of the Taiji diagram, and the EBV evasion mechanisms represent the “yin” or “negative” side.
growth of EBV-infected cells and by preventing apoptosis.\textsuperscript{157,158} EBV also counteracts the host immunity by modulating numerous cellular
signaling pathways,\textsuperscript{159} and an increased number of cancer cells provides
more potential neo-hosts for EBV (Figure 1). The limited knowledge regarding the virus–host interaction in the NPC environment and in systemic immune responses contributes to the failure or low efficacy of most EBV-targeted immunotherapies. More importantly, selection pressure-driven evolution constantly stimulate the emergence of EBV variants,\textsuperscript{160,161} which may be more oncogenic and less immunogenic than the parental strain. For instance, a recent study identified an EBV variant from NPC with unusually high tropism for epithelial cells but low tropism for B cells,\textsuperscript{162} suggesting the existence of EBV variants with increased NPC risk.

To date, the induction of an EBV antigen-specific T-cell response (primarily CD8\textsuperscript{T} T cells) in patients with vaccines and adoptive T-cell therapy are the two most common strategies for the immunological treatment of EBV-associated cancers. Because targeting only one specific antigen led to limited tumor regression in NPC patients,\textsuperscript{163–166} vaccines composed of multiple EBV antigens to activate T-cell responses that are more potent has emerged as a novel strategy. In this respect, two different teams constructed two recombinant viruses. The recombinant virus called Ad-SAVINE incorporates peptide sets from EBNA1, LMP1, and LMP2,\textsuperscript{167} and the other recombinant virus, called MVA-EL, contains an EBNA1/LMP2 fusion protein.\textsuperscript{168} Phase I trials in NPC patients showed that both of these vaccinia viruses activate CD4\textsuperscript{+} and CD8\textsuperscript{T} T-cell responses; encouraging clinical progress with full tolerance has been made.\textsuperscript{166–170}

However, many questions regarding host immunity and EBV remain to be addressed for the development of EBV-targeted therapy. For instance, the immunodominance hierarchy of individual viral antigens (particularly for EBV-encoding RNAs) and the crosstalk among multiple signaling pathways activated by EBV should be addressed. New technologies (for example, a molecular-based tag linkage method our lab developed that enables haplotype phasing greater accuracy and sensitivity for viral quasispecies determination\textsuperscript{171}) with higher sensitivity and precision to examine viral quasispecies in the NPC environment are required to monitor viral evolution. Exploring novel cellular factors or chemical substances that can reactivate EBV from latency will provide a promising strategy for treating EBV-related tumors by inducing cell lysis through viral reactivation. Greater attention should be given to the local suppression of EBV-specific immunity because immunosuppression contributes greatly to NPC development.

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