New Strategies and Patent Therapeutics in EBV-Associated Diseases

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Abstract: Epstein-Barr virus (EBV) is a virus present all throughout the world that causes infectious mononucleosis (IM) and is highly associated with certain malignancies. This study is a review of current knowledge concerning the pathogenic mechanisms of EBV in tumor and auto-immune diseases and the different new strategies to treat EBV associated pathologies.

Phenomena surrounding the proliferation and immortalization of B lymphocytes, the mechanisms of immune escape and the role of CD8+ and CD4+ T cells in the infection by EBV are explained.

An analysis is made of the role of EBV proteins during the biological events that take place in primary infection, persistent chronic infection together with an update of the approaches of novel patented therapeutics.

Currently there is no vaccine protecting against EBV-associated disorders and no treatment that may inhibit or eliminate their progression. Thus, it is crucial to obtain additional information on the function and importance of genes that play a role on the development of those diseases with which it is associated, as well as on the humoral and cellular immune processes involved in them.

Keywords: Epstein-Barr virus, multiple sclerosis, prevention, treatment.

1. INTRODUCTION

Epstein-Barr virus (EBV) is a gammaherpesvirus that ubiquitously infects humans–over 90% of the world's adult population is infected– and persists for the whole lifetime of the person [1]. At present, it is thought that primary EBV-infection occurs in the oropharynx *via* exchange of cell-free virus or productively infected cells in saliva but the finding of individuals who do not have EBV specific memory cytotoxic T lymphocyte (CTL) response, indicates that B lymphocytes, and not oropharyngeal epithelial cells, are required for primary EBV infection [2]. The virus mainly binds to B cells, through interaction of the gp350/220 present in the envelope of the virus with cell receptor CD21, this interaction fosters infection. Later the EBV is able to produce a latent infection, mainly in B cells, although latent forms of the virus may also be hosted by the nasopharyngeal epithelial cells and some T type cells [3].

In vitro, latent infected cells may express three types of latency depending on the genes that are expressed and on the differentiation status of the infected B cell [4]. Thus, naïve B cells express latent EBV nuclear antigens (EBNA) EBNA1, 2, 3A, 3B, 3C, -LP, latent membrane protein 1 (LMP1), LMP2A and LMP2B, known as growth program (latency III). Germinal centre cells and memory B cells express EBNA1, LMP1 and LMP2A, known as default program (latency II) and, lastly, EBNA1 is the only protein expressed in peripheral blood B cells, known as latency program (latency I) [5]. Only EBNA1 is expressed in all EBV-positive proliferating cells in healthy EBV carriers [4] and in all EBV-associated malignancies [6].

EBV infection of B cells is mainly controlled by CD8+ CTL, in addition to natural killer (NK) cells. CD4+ T cell response is probably also important at this time as a source either of effector cells or of cytokine help for the massive CD8+ T cell [7]. However, immune response is not always enough to control the infection, thus resulting in an active chronic infection by EBV [8]. In a smaller number of patients, the proliferation of cells infected by the virus is associated with the development of lymphoproliferative processes, epithelial neoplasias and autoimmune diseases [9-11].

2. REPLICATIVE CYCLE

The virus penetrates the cell wall (Fig. **1**) and migrates to the nucleus, where replication occurs. The genome is transcribed in three different phases, each of which depends upon reading the genes in the previous phase.

The initial, or immediate phase begins after the entry of the virus and leads to the synthesis of primary or regulatory proteins, which are necessary for the later synthesis of nucleic acids and structural proteins. They are located in the nucleus of the cell, are antigenic and are present throughout the viral cycle. These proteins are encoded by the EBV-BZLF-1 open reading frame.

In the second, or early phase, early and late-early genes are transcribed, giving rise to specific proteins such as phosphorylases and polymerase DNA for the synthesis of viral DNA. Antigen proteins are also synthesised. These regulate

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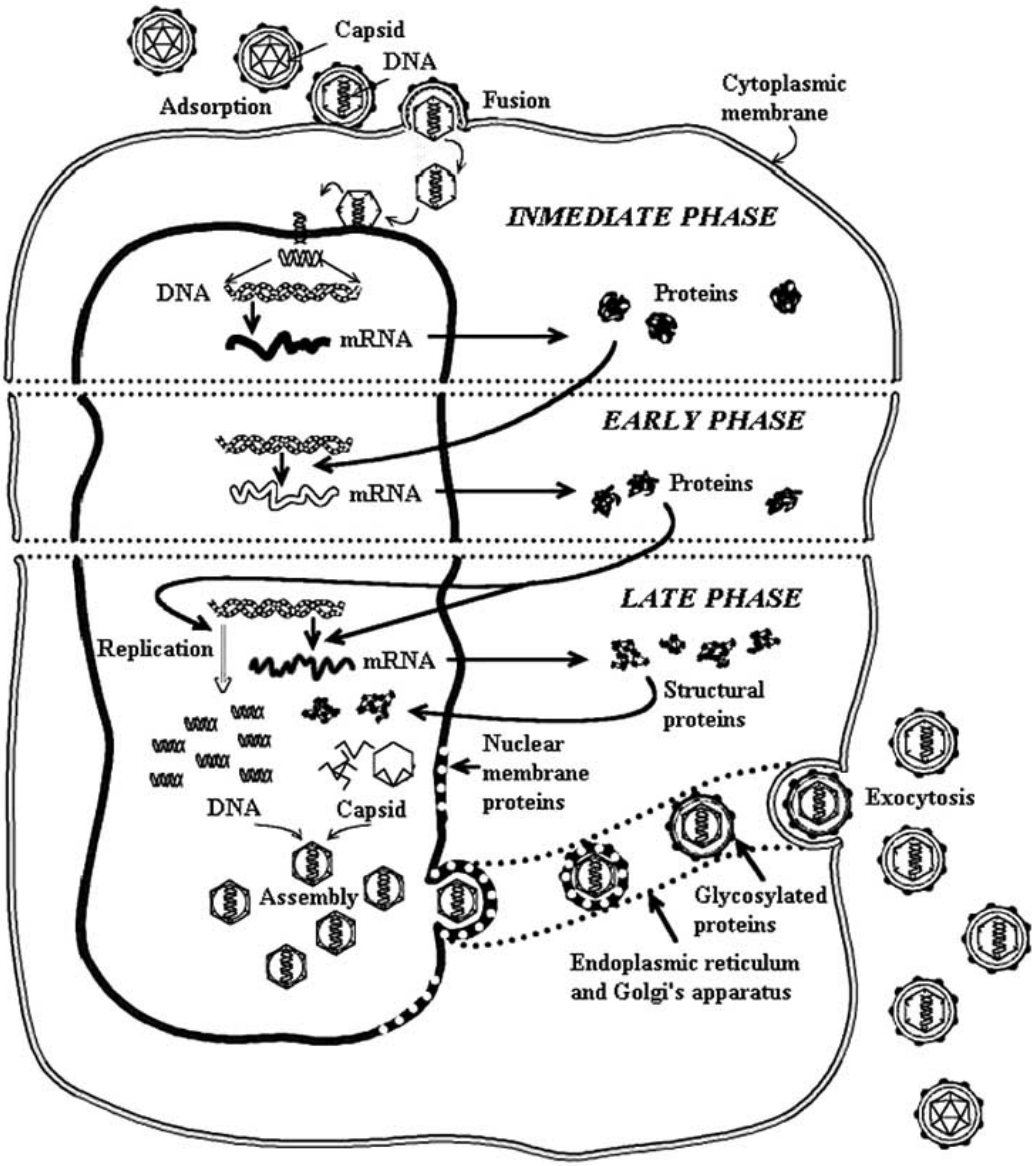

Fig. (1). Replicative EBV phases.

the subsequent phases and are located initially in the cell membrane and diffused throughout the cytoplasm, later to become restricted to the nucleus.

During the third, late phase, most of the late genes of the virus are transcribed and structural proteins are synthesised such as those of the capsid [viral capsid antigens (VCAs), a very specific one of 18 kDa and others of 40 and 135 kDa], and those of the envelope [late membrane antigens (LMAs)]. Capsids, which then incorporate the DNA, and glycoproteins, located initially in the nuclear membrane and Golgi's apparatus, are generated. The capsids approach the glycoproteins to create new complete particles.

Once formed, the virus leaves the cell, first emerging slowly through the Golgi apparatus and then lysing the cell due to its massive exit. Empty extracellular capsids and protein macromolecules, all of them antigenic, are frequently present during this process. These phases are successive and may coexist in the same cell.

3. EBV-ASSOCIATED DISEASES

EBV primary infection normally occurs during childhood and it is generally asymptomatic, although in half of the cases, it appears as infectious mononucleosis (IM) during adolescence. IM is a self-limited lymphoproliferative disease which may be considered an immune pathology with general symptoms caused by pro-inflammatory cytokines produced by T cells infiltrated in tissues throughout the whole body [12]. When the immune system is unable to control the infection, the patient suffers active chronic EBV infection [8].

3.1. EBV-Associated Tumors

EBV has been linked to various lymphoproliferative processes and epithelial neoplasias in immunodepressed and immunocompetent persons. Amongst them we may note posttransplantation lymphoproliferative disorder (PTLD) patients, which suffer a complication occurring in receptors of organ transplants that have been treated with immunosup-

pressors [13]; HIV-associated lymphomas, where HIV-induced immunodeficiency may increase the traffic of EBV-infected B cells that would lead to a wide variety AIDS-related lymphomas [14]; Burkitt's lymphoma (BL), a pathological entity initially described in Africa, is the most common childhood lymphoma in western countries and represents approximately 5% of all adults lymphomas [15,16]; Hodgkin's lymphoma (HL) in which a malignant population of Reed-Sternberg mononuclear and multinuclear cells is produced, in addition to reactive inflammatory cells [17], and nasopharyngeal carcinoma (NPC), a nasopharynx lymphoepithelioma that is extremely common in Southeast Asia and in Africa [18].

EBV-associated tumors are characterized by mRNA transcription of a restricted number of EBV genome regions in all cell tumors. The pattern of gene transcription is different for different types of tumors (Fig. **2**).

There are different cell lines established from BL biopsies *in vitro*, at first they show the original biopsy cell phenotype (group I), on serial passage, some BL lines show appearance of lymphoblastoid cells (group II) or a group III cells with phenotype indistinguishable from that of *in vitro*-transformed LCLs. Those cellular changes are associated with a change in the form of viral latency. The BL line, group I cells, express only the viral genome maintenance protein EBNA1, together with EBERs and BARTs (latency I) [19,20]. *In vitro*-transformed LCLs cells, group III cells, express the full spectrum of viral antigens that are found in LCLs (latency III). This group III express all six EBNA species [20].

EBERs, EBNA1, BARTs and LMP1, LMP2A and LMP2B (latency II) have been detected in NPC [21] as well as in HL [22]. However, there are important quantitative differences between NPC and HL. NPC is best classified as displaying a latency I/II form of infection, immediate between those displayed by BL (latency I) and HL (latency II) [20]. In NPC, the latent viral gene EBNA1 and the EBER genes are expressed in all EBV-positive cases and LMP2A and LMP2B transcripts are amplifiable in most tumors. In HL, EBV is present in the multinuclear Reed-Sternberg cells, is characterized by expression of the latency II [23].

BL, HL, and NPC all develop over much longer latent periods, more typical of tumors with a complex multistage pathogenesis while the PTLD-like lymphomas have relatively short latency periods (Fig. **2**). The genetic pattern expressed in PTDL is EBERs, EBNA1, 2, 3A, 3B, 3C, -LP, BARTs, LMP1, LMP2A and LMP2B (latency III) [24,25] (Table **1**). PTLD may be initiated when a type of B cell be-

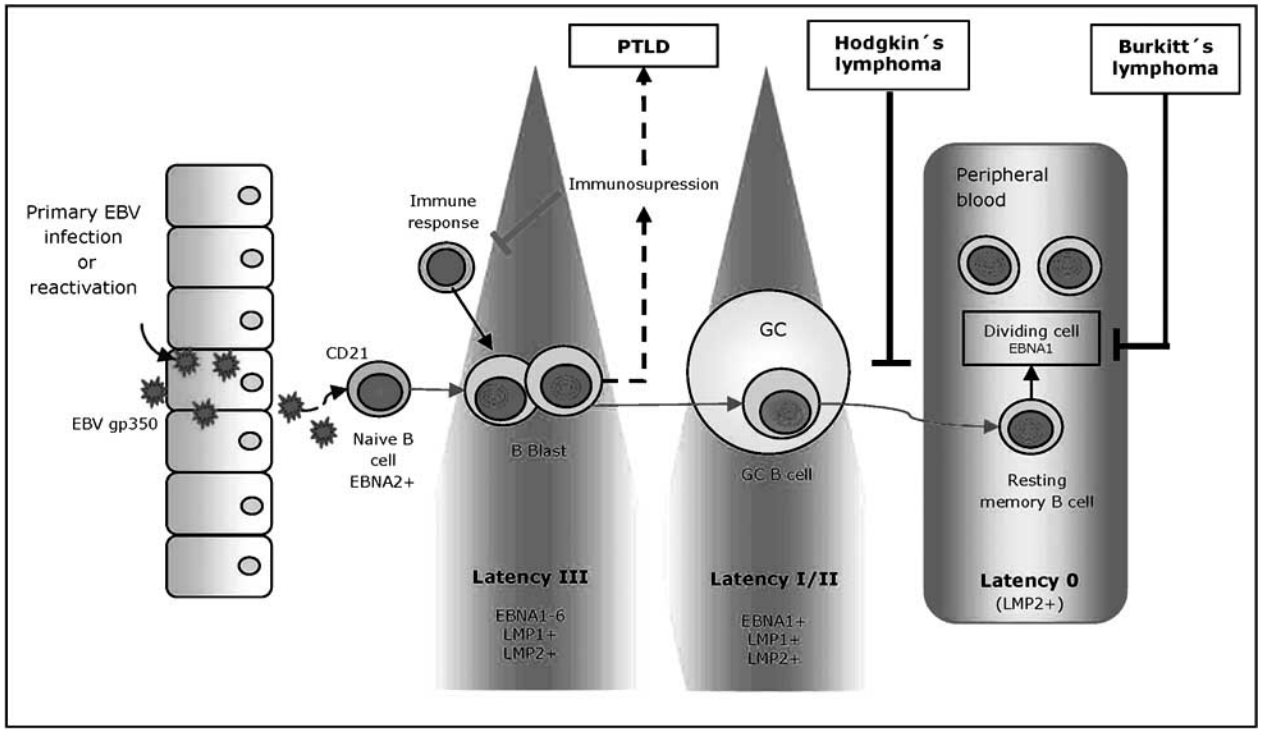

Fig. (2). Schematic representation of viral genes expression in the EBV life cycle and the EBV associated lymphomas.

Naive B cells are infected by EB-virions that enter the oropharyngeal lymph nodes by crossing epithelial barriers. Under influence of EBNA2-driven transcription program, naive B-cells differentiate into B blasts that express the full set of latent EBV genes and which are probably controlled by anti-EBV cytotoxic T-cell responses. The B blast further differentiates through the germinal center, this cell types express the latent membrane proteins LMP1 and LMP2, which provide them survival signals and growth in absence of antigen. Memory cells exit the cell cycle and enter the peripheral circulation. This memory cells are generally silent for all protein-encoding genes but may occasionally express LMP2. Memory B cells occasionally divide and express EBNA1 alone, which is essential for maintenance of the viral genome in the host cell. Hodgkin´s lymphoma arises from a virus-infected cell that is blocked at the GC stage, which results in constitutive expression of the latency II. Burkitt´s lymphoma arises from a GC that is entering the memory compartment. The cell expresses EBNA1 only. If the CTL response is suppressed, the blasts can lead to PTLD.

comes infected and expresses the latency III program, these cells cannot exit the latency III program, and they continue to proliferate owing to the absence of effective T-cell immunity [26]. The fact that most EBV-positive malignancies other than PTLD show highly restricted latent antigen expression, with loss in particular of the immunodominant EBNA3A, 3B, 3C proteins, emphasizes the difficulties inherent in trying to target these tumors by immunologic means [27,28].

3.2. EBV-Associated Autoimmune Diseases

EBV has recently been depicted as a possible triggering factor of some autoimmune diseases [11]. Amongst them we may highlight systemic lupus erythematosus (SLE), an autoimmune chronic inflammatory disease that generates a multisystemic rheumatic disorder which ultimately causes organ failure [29]; rheumatoid arthritis (RA), a widespread systemic autoimmune disease with infiltration of CD4+ T cells and NK cells into the synovia of the joints [30] and multiple sclerosis (MS), a neurology disease characterized by chronic inflammation and demyelination within the central nervous system (CNS) [31].

Epidemiology and serology studies show a clear relation between MS and EBV [32-34]. The role of EBV in MS is unknown, but there are some theories relating the immunopathology of the disease with the virus infection. One of these theories is based on the homology between the virus proteins and myelin proteins [35]; thus, MS would be the result of combining a hereditary predisposition and an unknown environmental factor that may maintain the autoreactivity of T cells through "molecular mimicry" [36,37].

During the study of auto-reactive CD4+ T cells in MS, Wucherpfenning and Strominger [38] characterized a DNA polymerase-derived epitope (BALF5) of EBV with a strong cross-reactivity with the myelin basic protein (MBP). Lünemman *et al.* [39] showed that the repertoire of specific anti-EBV T cells in MS patients is mainly composed of specific anti-EBNA1 CD4+ T cells and that the frequency was twice as much in cases than in controls. However, the frequency of EBNA1-specific CD8+ T cells was lower both in cases and in controls. These results show that EBNA1-mediated immunological control of latent EBV infection is deregulated at T cell level in patients with MS [39].

Another possible role of EBV in MS is based on EBV's capacity to induce α-B-crystallin protein on the surface of B cells through their infection, an event which would provoke a response of CD4+ T cells against it. This protein is identified as the major autoantigen abnormally expressed in the oligodendrocytes of the brains of MS patients [40]. We may also add that EBV infection of B cells produces retrovirus particles in active MS patients [41]; these endogenous retroviruses may transcribe a gene that codifies a protein of the retrovirus envelope with potential superantigen activity [42]. These superantigens may activate polyclonal T cells specific against myelinic antigens [43].

Cross-reaction of specific IgG against EBNA1 with autoantigen epitopes of Ro and Sm ribonucleoproteins [44] was found in systemic lupus erythematosus (SLE). EBNA1 is the only protein expressed in all latent infected cells, in all virus-associated tumors [10] and in autoimmune diseases associated with EBV (MS, RA and SLE) [11].

Both SLE and RA are characterized by high titres of anti-EBV antibodies and impaired T-cell responses to EBV antigens. These autoimmune diseases are both associated with an increased risk of lymphoma with a potential role for EBV [45].

Table 1. EBV Latent Gene Expression Patterns in EBV Associated Disorders

	BL	NHL	HL	NPC	PTLD	IM
EBNA1	+	+	+	+	+	+
EBNA2	-	-	-	-	+	+
EBNA3A	-	-	-	-	+	+
EBNA3B	-	-	-	-	+	+
EBNA3C	-	-	-	-	+	+
EBNALP	-	-	-	-	+	+
LMP1	-	+	+	+	+	+
LMP2A	-	+	+	+	+	+
LMP2B	-	+	+	+	+	+
BARTs	+	+	+	+	+	+
EBERs	+	+	+	+	+	+
OTHERS				BARF1		
LATENCY	I	II	II	II	III	III

BL: Burkitt's lymphoma; NHL: non-Hodgkin lymphomas; HL: Hodgkin's lymphoma; NPC: nasopharyngeal carcinoma; PTLD: posttransplant lymphoproliferative disorders; IM: infectious mononucleosis.

The EBV load in the peripheral blood of patients with RA has been investigated in two studies both of which showed that EBV load was about 10-fold higher in RA patients than in controls [46,47].

4. PROTEINS INVOLVED IN EBV PATHOGENESIS

The most plausible pathogenetic mechanism of EBV in autoimmune diseases and specifically in MS is the above-mentioned "molecular mimicry". The pathogenetic mechanism that may explain this theory implies both genes able to produce immortalization and proliferation of infected cells and genes involved in immune evasion, which are also essential to produce neoplasias.

4.1. Cell Proliferation and Immortalization

EBV infection gives infected cells the capacity to differentiate and to clonally expand through the LMP1 protein, which morphologically transforms B cells reducing the response to normal differentiation signals and promoting proliferation [48,49], as well as LMP2A [4]. LMP1, which is a lingand-independent cell-suface signalling molecule, provides a surrogate T-helper cell signal. LMP1 mimics the tumor necrosis factor receptor (TNFR) superfamily receptors by using their intracellular signaling factors TRAF and TRAD. CD40 (TNFR superfamily receptor), is a key receptor on germinal center B cells, which can deliver a survival signal. LMP1 is a functional homologue of CD40. *Via* their interaction with these factors and by mechanisms not yet fully understood LMP1 triggers two phosphorylation pathways that lead to the activation of transcription factors NF-κB and AP1. The result of signalling from both CD40 and LMP1 is to rescue B cells form apoptosis and drive their proliferation [4].

LMP2A can maintain these cells in absence of a competent B cell antigen receptor (BCR) that may translate signals. It has been observed in *in vitro* infected B cells that the LMP2A N-terminal cytoplasmic domain blocks the signal translating BCR, thus preventing the change from latent to lytic cycle, and maintaining latency [50]. This domain interacts *via* multiple phosphotyrosines arranged in ITAM- and SH2-protein binding motifs with the tyrosine kinases Lyn and Syk, as with BCR. This interaction diminishes Syk and Lyn from binding to the cytoplasmic B-cell, that would result in induction of the lytic cycle. LMP2A signalling does not cause B cells to grow, but delivers the tonic signal that is essential for the survival of all B cells [4,10]. LMP2A function is influenced and negatively regulated by LMP2B [51].

EBNA2 mainly acts as a transcription factor of cellular genes (*c-fgr*, *c-myc*), B cell activation markers such as CD23 and CD21, but also of other viral genes, including LMP1 and LMP2A [9,52]. EBNA2 interacts with transcription factor RBP-Jκ to promote conversion of resting B lymphocytes into lymphoblastoid B cells [53]. It has also been related to loss of somatic hypermutation of germinal centre B cells and, therefore, it is related to the differentiation and transformation of B cells [54].

Additionally, this proliferation, transformation and immortalization of B cells would have influence on another protein such as EBNA-LP, acts in the expression of cell genes essential for B cell outgrowth [55] in addition to increasing the ability of EBNA2 to transactivate the LMP1 gene [56].

EBNA 3A and 3C nuclear proteins are essential for EBV-mediated immortalization, but not EBNA 3B [10]. EBNA 3A-C regulate the expression of certain cell genes and bind to a variety of host proteins including different isoforms of the cellular transcription factor RBP Jκ [57]. The EBNA-3s compete for RBP-Jκ with EBNA-2 and Notch, and limit EBNA-2 strong up-regulation through RBP-Jκ [58,59]. Thus, EBNA2 and EBNA3 proteins work together to precisely control RBP-Jκ activity, thereby regulating the expression of cellular and viral promoters containing cognate Jκ sequence. EBNA3C acts as a regulating complex increasing the expression of LMP1, promoted by EBNA2 [60]. *In vitro*, EBNA3B has shown that it is not required for B cell immortalization, but that it is a highly maintained gene, such that it seems important for the infection of the host. It also mediates the regulation of CXCR4 chemokine, which may be an important strategy for the alteration of B cell homing in the infected host. The deletion of EBNA3B implies a reduction of the expression of EBNA3C and a lesser growth [61].

Lastly, EBNA1 protein has the ability to induce an increase in the expression of RAG1 and 2, which contribute to the reordering and recombination of immunoglobulin genes and of T cell receptor genes during the V(D)J recombination process, essential for the regeneration of mature T and B cells [62]. EBNA1 is a protein expressed both during the latent and the lytic phases, transcribed through various promoters (Wp, Cp, Qp or Fp) depending on the virus phase [10]. Amongst its functions, it is believed to play an important role in replication and mainly in the maintenance of the episome through interaction with chromosome proteins (HMG-1 and H1) [63]. This binding also guarantees the segregation of episomes in the progeny.

4.2. Immune Response Evasion

In IM there large numbers of "atypical mononuclear" cells in the blood, predominantly T lymphoblasts of the CD8+ subset but include some CD4+ T cells and also activated natural killer (NK)-like cells.

In vitro studies have demostrated that NK cells can inhibit the EBV-induced transformation of resting B cells if added within a few days of infection [64]. But the innate immune responses are of limited value, at least in the control of latent growth-transforming infections. EBV has not yet revealed any NK immune evasión strategy [65].

There are a highly expanded CD8+ T cell response and this issue has long been debated. CD8+ T cell response to latent cycle antigens, are markedly focused on immunodominant epitopes drawn from the EBNA3A, 3B, 3C family proteins, and less often from EBNA2, EBNA-LP, or LMP1 and apparently never from EBNA1 [28]. Although the CD8+ T lymphocyte response to latently infected cells is well characterized, very little is known about T cell controls over lytic infection. The primary CD8+ T cell response to EBV in IM patients contains multiple lytic antigen-specific reactivities, epitopes from immediate early (BZLF1, BRLF1) and several early (BMLF1, BMRF1, BALF2 and BALF5) proteins, at

levels at least as high as those seen against latent antigens [66,67].

CD4+ T cell responses are induced against a range of epitopes within latent cycle antigens. The responses to the immediate early protein BZLF1 were more common than responses to BMLF1 [68]. EBV infection therefore induces CD4+ T cell responses to multiple latent cycle epitopes, with the greatest concentration of epitopes being in the C-terminal half of EBNA1. The C-terminal half of the EBNA1 is a particularly rich source of CD4 epitopes for a range of different HLA class II alleles in Caucasian donors [69-71].

Additionally, it has been observed that the protein codified by the BGLF5 gene of the lytic cycle reduces the synthesis of host proteins in such a way that it may reduce the surface presentation of HLA molecules for recognition by T cells [72]. Lytic-phase BZLF1 or ZEBRA protein may also interfere in abrogation of IFNγ-induced MHC-II upregulation and contribute to the immune escape [73].

Besides all these mechanisms to avoid recognition by T-lymphocytes, the immune response during EBV infection may be suppressed by the virus through the expression of the lytic phase BCRF1 gene, the product of which is homologous with human IL-10 (vIL-10), which would act by suppressing Th1 cell response [74]. The expression of BARF1, a CSF-1 homologue, also interferes with cytokine production, something that may negatively alter the immune response [75].

B cells which, in normal conditions, are eliminated through apoptosis may increase their survival after EBV infection. BHRF1 is a prosurvival molecule from EBV that has a high degree of homology with bcl-2, a family of proteins that inhibits apoptosis [76,77].

The LMP1 protein through intracellular signalling also rescues B cells from signalling for apoptosis and drives them towards proliferation [78]. The LMP1 is a potent activator of NFκB, and the activation of NFκB is an important step for EBV-induced B cell immortalization.

Infected B cells may escape detection by CD8+ T cells through EBNA1. EBNA-1 is not presented properly to T cell since the glycine and alanine repeat domain (GAr) of EBNA-1 interferes with processing. There are evidences suggesting that the repetitive domain interferes with ubiquitin proteasome-dependent degradation and may thereby prevent the presentation of MHC-I-associated antigenic peptides [79]. While, the isolation of CD4+ or CD8+ EBNA1-specific CTL from healthy carriers implies the processing and presentation of EBNA1 epitopes *via* a proteasome-independent cross-priming pathway [80].

EBERs genes codify two small nuclear RNAs which seem to also participate in blocking apoptosis [81].

5. PROPHYLAXIS AND TREATMENT

5.1. Prophylaxis

Strategies based on simple or EBV-combined peptides and on peptides from T-cell immune response (Table **2**) have been employed in the development of vaccines.

5.1.1. Peptide Vaccines

Amongst the vaccines developed with viral proteins we may highlight that developed by Medimmune [82] and GlaxoSmithKline [83] with structural protein gp350. This vaccine was developed with the idea of preventing infection by EBV. Phase I/II clinical studies have shown that this recombinant glycoprotein [82,83] may elicit neutralizing antibodies [84]. This glycoprotein had proven effective in preventing the development of EBV-induced IM, but it had no efficacy in preventing asymptomatic EBV infection [85], although many cases of EBV-associated tumors and MS [86] may be prevented by a suitable vaccine that protects against EBV infection.

Two vaccines for EBNA1 have been developed within the possible vaccines that use EBNAs antigens, one patented by Akzo Nobel [87] and the other by Affinium Pharm [88]. There is another patent registered for EBNA2 [89], the aim of which is to apply it in PTDL prophylaxis. Two patents have been developed codifying peptide antigens of the BHRF1 region, such as p17 (EA-R) and p50 (EA-D) [90] for the prevention of IM and NPC [91]. Diatech PTY [92] developed vaccines based on mimotypes, molecules antigenically mimicking EBV determinants from VCA, EBNA and EA homologous sequences. This patent indicates that antibodies produced from these mimotypes may also recognize gp125 (BALF4) and p18 (VCA) [92].

Another group of patented polypeptides are EBNA1, LMP1 and LMP2 synthesized segments. The indications of this patent are mainly aimed against NPC, HL and PTLD [93]. There is another patent with very immunogenic peptides encoded within the EBV BFRF3 and BdRF1 open reading frames, expressing VCA-p18 or VCA-p40 capsid proteins, developed by Akzo Nobel [94]. Middeldorp [95] developed a vaccine using LMP1, LMP2 and BARF1 sequences, proteins which are expressed in the nuclear membranes of infected cells. The peptide that is present in all EBV-associated tumors and autoimmune diseases is EBNA1; thus, a possible vaccine would include EBNA1 added to another latent or lytic gene [96]. EBV infection is associated with autoimmune diseases and vaccine development has been suggested to try to prevent them. Harley *et al*. [97] tried to develop another form of immunization by using homologous peptides related with autoimmune diseases. The peptide used enclose homologues of an antigenic sequence identified in the EBV and it may reduce the amount of circulating antibodies with particular specificity in addition to re-inducing tolerance due to treatment with the antigen. Huber *et al*., [98] developed an immunization through peptides, specifically against the HERV-K18 superantigen induced by EBV when transactivating human endogenous retroviruses [42], since this superantigen may stimulate a great amount of T cells. This would protect against diseases such as IM, EBV-induced lymphomas, and EBV-associated autoimmune diseases such as SLE, RA, Sjogren's syndrome and MS.

5.1.2. CD8+ and CD4+ Lymphocyte Epitopes

Another type of vaccine is that based on CD8+ and CD4+ T cells specific response peptides. In this context, Elliot *et al*. [99] used a not yet patented vaccine with only

Table 2. Patented Vaccines Against EBV

PATENTED VACCINES	PUBLICATION NUMBER	APPLICANT PATENTS	PATENTS
gp350	U.S. 2007202122	Medimmune INC	[82]
gp350	W.O. 03094962	GlaxoSmithKline Biolog SA	[83]
EBNA1	W.O. 9406912	Akzo Nobel NV	[87]
EBNA1	W.O. 2004007536	Affinium Pharm INC	[88]
EBNA2	U.S. 2006257356	Foundation for Medical Educati	[89]
BHRF1	U.S. 2008175849	Ortho diagnostic systems INC	[90]
BHRF1	W.O. 9311267	Cancer Res Inst	[91]
VCA, EBNA, EA	W.O. 2007022557	Diatech PTY LTD	[92]
EBNA1, LMP1, LMP2	W.O. 2007065215	Savine Therapeutics PTY LTD	[93]
VCA-p18 VCA-p40	U.S. 6365717	Akzo Nobel NV	[94]
LMP1 + LMP2 + BARF1	AU. 2002235884	Cyto Barr BV	[95]
EBNA1 + lytic or latent gene	W.O. 2007097820	Univ. Ohio State Res Found	[96]
Peptide for autoantibodies	W.O. 0158481	Oklahoma Med Res Found	[97]
HERV-K18 superantigen	W.O. 0247720	Univ. Tufts	[98]
CD8+ T cell epitopes of EBV type A and B	W.O. 9745444	Queensland Inst Med Res; Commw Scient Ind Res Org; Univ. Melbourne; Inst Medical W & E Hall; Csl Ltd	[100]
CD8+ T cell epitopes of LMP1	W.O. 2004041849	Queensland Inst Med Res; Commw Scient Ind Res Org; Univ. Melbourne; Inst Medical W & E Hall; Csl Ltd	[101]
CD8+ T cell epitopes of gp85 or gp350 and LMP1 or LMP2	W.O. 9902550	Queensland Inst Med Res; Commw Scient Ind Res Org, Univ. Melbourne, Inst Medical W & E Hall; Csl Ltd	[1102]
CD4+ T cell epitopes of EBNA1, LMP1 and LMP2	U.S. 2009130134	Pancre, Auriault	[104]
Dendritic cells EBNA1	W.O. 0112215	Univ. Rockefeller	[107]
APC EBNA1 + LMP1	W.O. 2007049737	Medical and Biolog Lab Co LTD; Aichi Prefecture	[108]
Ad5F35-LMP2 recombinant adenovirus	CN. 1907493	Zeng YI	[111]
EBNA1 recombinant adenovirus	W.O. 03047634	Univ. Health Network	[113]

one CD8+ T cell epitope to generate immunity against IM. In phase I trial, they observed it produced a response specific T cells, but the number of subjects studied was too small for any conclusion to be reached. One vaccine that has recently been patented by Queensland Inst Med Res [100] is based on the development of CD8+ T cell epitopes against both EBV subtypes, which are distinguished through their differences in the genes codifying EBNA2, 3B and 3C. As indicated, the vaccination may be able to protect 90% of the Caucasian population. This same institution has also developed two vaccines based on LMP-1 or LMP-2 and gp85 or gp350 epitopes recognized by CD8+ T cells [101,102].

The study of latent antigen-specific CD4+ T cell responses has been motivated by the fact that most EBV-associated tumors are HLA class II–positive, even tumors such as Burkitt's lymphoma (BL), which is globally deficient in HLA class I antigen processing but retains class II processing function [103]. Pancre *et al.* [104] developed immunogenic peptides derived from EBV type I and II latency antigens comprising at least one CD4+ T cells epitope which can be recognized by the majority of individuals in the Caucasian population, the use of which may stimulate a strong response against CD4+ T cells in the immunotherapy of tumor pathologies associated with types I and II latency phases [105].

EBNA1, the only viral protein expressed in most BLs, shown that CD4+ T cell clones to at least some EBNA1 epitopes can recognize and kill appropriately HLA-matched LCLs and also EBNA1-positive BL lines [71,103]. Hence, CD4+ T cells could be useful therapeutically if they were able to recognize HLA class II–positive cells endogenously expressing the relevant latent protein [65].

It has been recently observed that the use of antigen presenting cells (APC) T-cell immunization strategies may obtain a more efficient immunization than the one obtained with lymphoblastoid cell lines (LCLs) [106]. Specifically, a vaccine based on this concept has been patented which elicits a strong CD4+ T cell response using dendritic cells (DCs) charged with EBNA1 [107].

It was found that an APC having mRNA of LMP1 and EBNA1 introduced therein can induce an EBV-specific cytotoxic T cell, which would eliminate an EBV-infected NK lymphoma [108]. These vaccines can confer a protective response to CD4+ and CD8+ T cells against EBV transformed B cells *in vitro* [109]. It has been observed and patented that lymphocyte production by DCs that are infected with recombinant adenovirus codifying LMP2 may be able to kill CNF cells [110,111]. CD4+ T cells specific for EBNA1 and CD8+ T cells specific for LMP2 may be generated [112] by infecting DCs with recombinant modified vaccinia Ankara virus (MVA). Following the same line of research, Liu *et al.* [113] tried to develop, *in vitro* and *in vivo*, a specific response of CD4+ and CD8+ T cells against EBNA1 for the treatment of cancer with an adenoviral SE1 vector, which would limit the cellular growth of the tumor and/or cause its death. This patent may also prove useful in MS in eliminating self-reactive B and T cells.

5.2. Treatment

Strategies based on chemical products, lymphocyte transfer and gene therapies have been developed for the treatment of EBV infection (Table **3**).

5.2.1. Chemical Treatments

Antiviral agents, such as acyclovir [114] and valacyclovir [115], DNA polymerase inhibitors, are the most used in the treatment of EBV infection, but the efficacy of both is limited because they only act if the virus uses its own DNA polymerase, therefore being effective only in lytic-phase but not in latent-phase cells. A study that assessed the efficacy of valganciclovir showed that in 47 liver transplantation children with a high viral load of EBV treated with valganciclovir, PTLD was suspected in only one child and a long valganciclovir treatment achieved undetectable EBV-DNA in 47% of patients [116]. Other antiviral agent, ganciclovir, showed that a treatment during 30 days decreased in 38% the risk of PTLD during the first year post-transplant in renal patients [117]. This study indicated that ganciclovir could be more potent than acyclovir. Anti-viral therapy appears to play a important role in reducing the risk of PTLD in post-transplant patients.

The use of an agent to induce virus-specific genes codifying kinases (thymidine kinase, BGLF4) that, when ex-

Table 3. Patented Treatments Against EBV

PATENTED TREATMENTS	PUBLICATION NUMBER	APPLICANT PATENTS	PATENTS
Geldanamycin (Hsp90-ATPase modulator)	W.O. 2009014759	Trustees of the Univ. of Columbia	[121]
Lipophilic phosphonoacid/ nucleoside + antiviral	W.O. 9838202	Univ. California (San Diego)	[122]
L-nucleoside (L-FMAU)	W.O. 9520595	Univ. Georgia; Univ. Yale	[123]
Simvastatin	W.O. 2005042710	US Government	[124]
1-(2-Fluoro-4-thio-ss-D-arabinofuranosyl)-5-methyluracil	W.O. 2009034945	Yamasa CORP; Univ. Kyoto	[125]
5-subtitued uracil-nucleoside	U.S. 2007197462	Univ. Emory	[126]
Inducing agents	E.P. 1886677	Perrine SP, Faller DV, White BF	[127]
EBNA1 specific lymphocytes transfer	W.O. 0112215	Univ Rockefeller	[107]
EBNA1 specific lymphocytes transfer	U.S. 2006188520	The Rockefeller University	[133]
Ad5F35-LMP2 recombinant adenovirus	CN. 1907493	Zeng YI	[111]
EBNA1 recombinant adenovirus	W.O. 03047634	Univ. Health Network	[113]
Proteasome inhibitors	W.O. 2004004749	Univ. Charite	[137]
ITAM inhibitors through LMP2A	U.S. 2007110760	Monroe *et al.*	[138]
RBP-J-EBNA2 mutant	W.O. 02098918	GSF Forschungszentrum Umwelt	[139]
RNAi (EBERs)	U.S. 2007202082	Jin *et al.*	[141]
gp42	U.S. 2009074719	Univ. Northwestern	[142]
EBNA3C-TAT peptide	W.O. 2008005506	Trustees of the Univ. of Pennsylvania	[143]
CtxB or EtxB/ LMP1 or LMP2 complex	W.O. 0247727	Univ. Bristol	[144]

pressed, phosphorylate the nucleoside analogue, ganciclovir, and transform it into its active form has been studied for some time [118]. Sodium butyrate and radiation, and arginine butyrate selectively activate the EBV thymidine kinase gene in latently infected EBV-positive tumor cells [119,120].

A proposed and patented chemical compound that may inhibit EBV replication in the host is a host chaperone protein activity modulator (Hsp90-ATPase), an ansamycin antibiotic, specifically, geldanamycin (17-dimethylaminoethylamino-17-demethoxy-geldanamycin). This Hsp90 activity modulator is an inhibitor of the expression of bcl2-associated anathogene 3 (BAG3) [121].

Another compound that has recently been proposed and patented is the combination of an antiviral and a lipophilic phosphonacid/nucleoside conjugate with high antiviral activity. The compound includes a phosphonacid/nucleoside conjugate in which the carboxyl group and phosphonyl groups of phosphonacid are sterified, such that the compound is comprised of at least a lipophilic group and a nucleoside group [122].

Another chemical compound indicated for the treatment of the hepatitis B virus and of the EBV is an L-nucleoside in which the R group is occupied by a purine or pyrimidine base. The active substance is 2'-fluoro-5-methyl-beta-L-arabinofuranosyluridine, also referred to as L-FMAU [123]. Simvastatin, which belongs to the family of drugs that inhibit the 3-hydroxy-3-methylglutaryl CoA reductase, binds specifically to a domain of lymphocyte function-associated antigen 1, (LFA-1) thus inhibiting its function [124]. This domain of cell adhesion is highly expressed in tumors with extranodal localization such as those that appear in immunocompromised patients with EBV-associated immunoblastic lymphoma in areas such as the brain, the lungs or the gastrointestinal tract. The administration of Simvastatin together with LCLs in immunodeficiency mice (SCID) has been observed to produce a delay in the development of EBV associated lymphomas and to prolong survival [124]. Other patented chemical compounds that may currently be used are 1-(2-Fluoro-4-thio-ss-D-arabinofuranosyl)-5-methyluracil [125], patented by Yamasa CORP and 5-substituted uracil-nucleoside, which acts as a thymidylate synthetase inhibitor. The latter is patented by Schinazi *et al.* [126] and may be used in cancer treatment. Inducing agents, such as the patent developed by Perrine [127], are also registered.

Anti-B lymphocyte chemical treatments, such as Rituximab, have also been observed to obtain a very good response, since B cells found in the meninges of MS patients may be acting as APC for T cells and therefore contributing to the propagation of local disease-relevant immune responses [128].

5.2.2. Lymphocyte Transfer

A proposed treatment approach is to try to increase the response of the immune system against the target organism. The predominant immunologic response in viral infections is carried out through CTL. This response, mediated by MHC-I, is directed against EBNA3A, 3B and 3C antigens and, with less frequency, against EBNA2, EBNA-LP, LMP1 and LMP2, in the case of EBV. Considering this, infusions of donor leukocytes containing CTL presensitized to EBV, were transferred to patients developing PTLD for the treatment of the disease, showing regression in some cases [129]. This method was refined through the transfusion of EBV-specific CTL lines and used at the moment of development of a tumor or as prophylaxis [130]. It has been suggested that to make the cell response more specific for solid organs transplant, all CTL that react with the host MHC may be selected from the donor for their transplant [131].

It has also been observed and patented that DCs may present EBNA1 to CD4+ T cells [132]. Thus, CTL have the ability to efficiently recognize and destroy tumor cells that express EBNA1 [107,133] although this statement is not in agreement with other authors [79].

Unfortunately, in NPC, HL and NHL, this therapy is difficult partly due to the fact that the stimulation of LCLs mainly occurs towards immunodominant T cells specific against EBNA3, which is not expressed in these tumors.

5.2.3. Gene Therapy

Gene therapy has been developed for EBV-related lymphomas expressing a suicide gene which was driven by EBNA2 dependent regulatory elements in cells latently infected with EBV [134].

Zeng Yi [111] and Liu *et al*. [113] carried out, and patented, *in vitro* studies focused on the treatment and prevention of EBV-positive cancers by administering a nucleic acid molecule comprising an LMP2 [111] or EBNA1-responsive promoter region [113] operatively linked to a gene necessary for viral replication, preferably in adenovirus replication, and optionally one or more heterologous genes [135, 136].

5.2.4. Other Therapies

Another possible therapy for EBV infection and for lymphoproliferative diseases is the use of proteosome inhibitors, such as PS-1 and PS-2 inhibitors, reversible inhibitors of chymotrypsin-like activity of the multicatalytic proteinase complex 20S proteasome, which has shown to prevent NF-κB factor transcription activation in response to TNF-α and to prevent NF-κB activation through the blockage of IκBβ decay and IκBβ protein [137]. A recently patented strategy has been the use of peptide homologues (synthetic ITAM analogues) that inhibit the interaction of proteins and immunoreceptor tyrosine-based activation motifs (ITAMs) of viral proteins. This invention showed that blocking association of the LMP2A ITAM with cellular molecules and blocking LMP2A ITAM-mediated signalling are effective strategies for treatment and prevention of metastases of EBV-induced malignancies. It is stated that this treatment may result in inhibition of BCR-induced proliferation [138].

Another form of treatment, patented by Kempkes [139] is a DNA-binding protein mutant, RBP-Jκ, able to bind to the Notch protein but unable to bind to EBNA2, since it presents an aminoacidic sequence with at least one mutation in the EBNA2 binding domain, thus preventing immortalization.

Such as stated by Mei *et al*. [140], a form of therapy that should be developed further is the use of drugs with latent proteins as targets, mainly proteins expressed in tumors, such

as LMP1, LMP2A or EBNA1, essential for immortalization, for cell proliferation and for evading immune responses. This may be manifested with the use of small interfering RNA targeting the LMP1 gene, thus reducing LMP1 expression and inducing apoptosis in EBV-infected LCLs. EBV codifies small RNAs, known as EBERs, which are abundant in infected cells. Using a vector system, based on the EBV promotor that codifies these EBERs, the interference RNA (RNAi) may be used as cytoplasmic or nuclear targets to silence genes and block the different RNAs levels. This new therapy is patented by Jin *et al*. [141].

As regards peptide-based treatments, Kirschner *et al*. [142] have recently created and patented peptide-based compounds (peptides, peptidomimetics, and other small molecules) to inhibit EBV-mediated membrane fusion with epithelial cells, B-cells, or both. The peptides are based on the EBV fusion protein gp42. These may have one or more functional or biological activities associated with gp42. This patent may be formulated for treating and preventing diseases associated with EBV infection, such as acute IM, B-cell lymphomas, other lymphoproliferative diseases associated with EBV.

Another patented treatment is the use of EBNA3C blended with a protein transduction domain (TAT) which may block the proliferation of the infected cell and the overgrowth of the transformed B cell, since this protein, which inhibits the action of EBNA3C induces the degradation of retinoblastoma proteins by inhibiting the translation of EBNA3C mRNA [143]. Morgan *et al*. [144] developed a fusion complex made up of a toxin and a viral antigen as treatment against EBV infection. Toxins used are a protein homologous to subunit B of *E. coli* heat-labile enterotoxin (EtxB) or a *Vibrio cholera toxin* (CtxB), which are able to bind to receptor GM1 of the cell plasmatic membrane and a second protein homologous to a viral antigen expressed in the surface as LMP1 or LMP2, which allow the internalization in the cell and the alteration of the antigen processing pattern of the viral presentation and/or tumor antigens. This patent was developed to be applied in neoplasias.

6. CONCLUSIONS

Up to now some important achievements have been made to inhibit IM progress, but the same does not hold true for asymptomatic EBV infection, which is the one involved in lymphoproliferative and autoimmune diseases.

The development of an effective prophylactic vaccine needs to bear in mind that neutralizing antibodies on their own or their combination with cell-mediated immune response against a single peptide component does not offer long-lasting sterile immunity against EBV, such as the case with gp350 envelope vaccine. However, the reduction of the viral load may be enough to prevent the development of diseases such as IM or PTLD during the primary infection, in addition to other diseases associated to the virus latent phase, such as autoimmune diseases and other malignant diseases. Thus, the development of vaccines based on stimulating T cell response, mainly CD8+ T cells, requires the aid of virus-specific CD4+ T cells *in vivo* and, therefore, CD4+ T cell stimulation needs to be taken into account when developing a vaccine.

CTL *in vitro* stimulation against proteins expressed in tumors may render this therapy very specific for this type of patients. Thus, this immunotherapy based on epitope-specific CTL clones reactivated and expanded *in vitro*, and the increase in the immune response through the vaccination with epitopes may be very useful against EBV-associated neoplasias. According to the patents developed up till now, the conclusion seems to be that EBNA1, as a promising CD4+ T cell antigen, should be combined with LMP1 and LMP2 for CD8+ T cell stimulation in such a vaccine for both prevention of symptomatic EBV infection as well as immunotherapy against EBV-associated malignancies.

Although the most effective targets to prevent or inhibit EBV infection are genes, we may prevent the development of tumors and other autoimmune diseases associated to B and T cell immortalization, such as MS, by developing gene therapies against genes that are important for cell immortalization and differentiation, such as EBNA1, EBNA2 or LMP1. The development of small interfering RNA targeting this type of genes may also be effective in reducing their expression. Thus, LMP1 or EBNA1 would be of great importance, since it may induce B-cell apoptosis.

For preventing and treating autoimmune diseases, it may also be useful to develop specific antibodies against autoantibodies that by molecular mimicry seems to play an important role in the development of such diseases.

Finally, the ideal strategy would be to develop a vaccine that obtain long-lasting protective immunity by studying the key proteins that contribute to the development of the diseases associated with the virus, contributing both to cellular and tumor immunity.

DECLARATION OF INTEREST

The authors have no conflict of interest to declare and no fee has been received for preparation of this manuscript.

REFERENCES

[1] Bornkamm, G.W.; Hammerschmidt, W. Molecular virology of Epstein-Barr virus. *Philos Trans. R. Soc. London B. Biol. Sci*., **2001**, *356*, 437-459.

[2] Faulkner, G.C.; Burrows, S.R.; Khanna, R., Moss, D.J.; Bird, A.G.; Crawford D.H. X-linked agammaglobulinemia patients are not infected with Epstein-Barr virus: implication for the biology of the virus. *J. Virol*., **1999**, *73*, 1555-1564.

[3] Young, K.A.; Chen, X.S.; Holers, V.M.; Hannan, J.P. Isolating the Epstein-Barr virus gp350/220 binding site on complement receptor type 2 (CR2/CD21). *J. Biol. Chem.*, **2007**, *282*, 36614-36625.

[4] Thorley-Lawson, D.A. Epstein-Barr virus: exploiting the immune system. *Nat. Rev. Immunol.*, **2001**, *1*, 75-82.

[5] Babcock, G.J.; Hochberg, D.; Thorley-Lawson, A.D. The expression pattern of Epstein-Barr virus latent genes *in vivo* is dependent upon the differentiation stage of the infected B cell. *Immunity*, **2000**, *13*, 497-506.

[6] Throley-Lawson, D.A.; Gross, A. Persistence of the Epstein-Barr virus and the origins of associated lymphomas. *N. Engl. J. Med*., **2004**, *350*, 1328-1337.

[7] Rickinson, A.B.; Moss, D.J. Human cytotoxic T lymphocytes response to Epstein-Barr virus infection. *Ann. Rev. Immunol*., **1997**, *15*, 405-431.

[8] Schwarzmann, F.; von Baehr, R.; Jager, M.; Prang, N.; Böhm, S.; Reischl, U.; Wolf, H.; Bieger, W.P. A case of severe chronic active

infection with Epstein-Barr virus: Immunologic deficiencies associated with a lytic virus strain. *Clin. Infect. Dis.*, **1999**, *29*, 626-631.
[9] Kutok, J.L.; Wang, F. Spectrum of Epstein-Barr virus-associated diseases. *Annu. Rev. Pathol. Mech. Dis.*, **2006**, *1*, 375-404.
[10] Middeldorp, J.M.; Brink, A.A.; van der Brule, A.J.; Meijer, C.J. Pathogenic roles for Epstein-Barr virus (EBV) gene products in EBV-associated proliferative disorders. *Crit. Rev. Oncol. Hematol.*, **2003**, *45*, 1-36.
[11] Niller, H.H.; Wolf, H.; Minarovits, J. Regulation and dysregulation of Epstein-Barr virus latency: implications for the development of autoimmune diseases. *Autoimmunity*, **2008**, *41*, 298-328.
[12] Vetsika, E.K.; Callan, M. Infectious mononucleosis and Epstein-Barr virus. *Expert Rev Mol. Med.*, **2004**, *6*, 1-16.
[13] Nalesnik, M.A. Clinical and pathological features of post-transplant lymphoproliferative disorders (PTLD). *Springer Semin. Immunopathol.*, **1998**, *20*, 325-342.
[14] Beral, V.; Peterman, T.; Berkelman, R.L.; Jaffe, H. AIDS-associated non-Hodgkin lymphoma. *Lancet*, **1991**, *337*, 805-809.
[15] Thorley-Lawson, D.A.; Allday, M.J. The curious case of the tumour virus: 50 years of Burkitt's lymphoma. *Nat. Rev. Microbiol.*, **2008**, *6*, 913-924.
[16] Bouffet, E.; Frappaz, D.; Pinkerton, R., Favrot, M.; Phillip, T. Burkitt's lymphoma: a model for clinical oncology. *Eur. J. Cancer*, **1991**, *27*, 504-509.
[17] Hjalgrim, H.; Smedby, K.E.; Rostgaard, K., Molin, D.; Hamilton-Dutoit, S.; Chang, E.T.; Ralfkiaer, E.; Sundström, C.; Adami, H.O.; Glimelius, B; Melbye, M. Infectious mononucleosis, childhood social environment, and risk of Hodgkin lymphoma. *Cancer Res.*, **2007**, *67*, 2382-2388.
[18] Zheng, H.; Li, L.L.; Hu, D.S.; Deng, X.Y.; Cao, Y. Role of Epstein-Barr virus encoded latent membrane protein 1 in the carcinogenesis of nasopharyngeal carcinoma. *Cell Mol. Immunol.*, **2007**, *4*, 185-196.
[19] Niedobitek, G.; Agathanggelou, A.; Rowe, M.; Jones, E.L.; Jones, D.B.; Turyaguma, P.; Oryema, J.; Wright, D.H.; Young, L.S. Heterogenous expression of Epstein-Barr virus latent protein in endemic Burkitt's lymphoma. *Blood*, **1995**, *86*, 659-665.
[20] Rickinson, A.; Kieff, E. In: *Fields Virology*, Fields, B.; Knipe, D.; Howley, P. Ed.; Lippincot-Raven: Philadelphia, **2001**; Vol. 1, pp. 2511–2573.
[21] Brooks, L.; Yao, Q.Y.; Rickinson, A.B.; Young, L.S. Epstein-Barr virus latent gene transcription in nasopharyngeal carcinoma cells: coexpression of EBNA1, LMP1, and LMP2 transcripts. *J. Virol.*, **1992**, *66*, 2689-2697.
[22] Pallesen, G.; Hamilton-Dutoit, S.J.; Rowe, M.; Young, L.S. Expression of Epstein-Barr virus latent gene products in tumor cells of Hodgkin's disease. *Lancet*, **1991**, *337*, 320-322.
[23] Deacon, E.M.; Pallesen, G.; Niedobitek, G.; Crocker, J.; Brooks, L.; Rickinson, A.B.; Young, L.S. Epsetin-Barr virus and Hodgkin´s: transcriptional analysis of virus latency in the malignant cells. *J. Exp. Med.*, **1993**, *177*, 339-349.
[24] Knowles, D.M. Immunodeficiency-associated lymphoproliferative disorders. *Mod. Pathol.*, **1999**, *12*, 200-217.
[25] Young, L.; Alfieri, C.; Hennessy, K.; Evans, H.; O´Hara, C.; Anderson, K.C.; Ritz, J.; Shapiro, R.S.; Rickinson, A.; Kieff, E. Expression of Epstein-Barr virus transformation-associated genes in tissues of patients with EBV lymphoproliferative disease. *N. Engl. J. Med.*, **1989**, *321*, 1080-1085.
[26] Thorley-Lawson, D.A.; Gross, A. Persistence of the Epstein-Barr virus and the origins of associated lymphomas. *N. Engl. J. Med.*, **2004**, *350*, 1328-1337.
[27] Steven, N.M.; Leese, A.M.; Annels, N.E.; Lee, S.P.; Rickinson, A.B. Epitope focusing in the primary cytotoxic T-cell response to Epstein-Barr virus and its relationship to T-cell memory. *J. Exp. Med.*, **1996**, *184*, 1801–1813.
[28] Murray, R.J.; Kurilla, M.G.; Brooks, J.M.; Thomas, W.A.; Rowe, M.; Kieff, E.; Rickinson, A.B. Identification of target antigens for the human cytotoxic T cell response to Epstein-Barr virus (EBV): implications for the immune control of EBV-positive malignancies. *J. Exp. Med.*, **1992**, *176*, 157–168.
[29] James, J.A.; Harley, J.B.; Scofield, R.H. Epstein-Barr virus and systemic lupus erythematosus. *Curr. Opin. Rheumatol.*, **2006**, *18*, 462-467.
[30] Sawada, S.; Takei, M. Epstein-Barr virus etiology in rheumatoid synovitis. *Autoimmun. Rev.*, **2005**, *4*, 106-110.
[31] Lassmann, H. Recent neuropathological findings in MS-implications for diagnosis and therapy. *J. Neurol.*, **2004**, *251*, IV2-IV5.
[32] Ascherio, A.; Munger, K.L. Environmental risk factors for multiple sclerosis. Part I: the role of infection. *Ann. Neurol.*, **2007**, *61*, 288-299.
[33] Phol, D.; Krone, B.; Rostasy, K.; Kahler, E.; Lehnert, M.; Wagner, H.J.; Gärtner, J.; Hanefeld, F. High seroprevalence of Epstein-Barr virus in children with multiple sclerosis. *Neurology*, **2006**, *67*, 2063-2065.
[34] Lünemann, J.D.; Huppke, P.; Roberts, S.; Brück, W.; Gärtner, J; Münz, C. Broadened and elevated humoral immune response to EBNA1 in pediatric multiple sclerosis. *Neurology*, **2008**, *71*, 1033-1035.
[35] Bray, P.F.; Luka, J.; Bray, P.F.; Culp, K.W.; Schlight, J.P. Antibodies against Epstein-Barr nuclear antigen (EBNA) in multiple sclerosis CSF, and two pentapeptide sequence identities between EBNA and myelin basic protein. *Neurology*, **1992**, *42*, 1798-1804.
[36] Gran, B.; Hemmer, B.; Vergelli, M.; McFarland, H.F.; Martin, R. Molecular mimicry and multiple sclerosis: degenerate T-cell recognition and the induction of autoimmunity. *Ann. Neurol.*, **1999**, *45*, 559-567.
[37] Lünemann, J.D.; Jelcić, I.; Roberts, S.; Lutterotti, A; Tackenberg, B.; Martin, R.; Münz, C. EBNA1-specific T cells from patients with multiple sclerosis cross react with myelin antigens and co-produce IFN-gamma and IL-2. *J. Exp. Med.*, **2008**, *205*, 1763-1773.
[38] Wucherpfennig, K.W.; Strominger, J.L.; Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. *Cell*, **1995**, *80*, 695-705.
[39] Lünemann, J.D.; Edwards, N.; Muraro, P.A.; Hayashi, S.; Cohen, J.I.; Münz, C.; Martin, R. Increased frequency and broadened specificity of latent EBV nuclear antigen-1-specific T cells in multiple sclerosis. *Brain*, **2006**, *129*, 1493-1506.
[40] Van Sechel, A.C.; Bajramovic, J.J.; van Noort, J.M. EBV-induced expression and HLA-DR-restricted presentation by human Bcells of alpha B-crystallin, a candidate autoantigen in multiple sclerosis. *J. Immunol.*, **1999**, *162*, 129-135.
[41] Haahr, S.; Munch, M. The association between multiple sclerosis and infection with Epstein-Barr virus and retrovirus. *J. Neurovirol.*, **2000**, *6*, S76-S79.
[42] Sutkowski, N.; Conrad, B.; Thorley-Lawson, D.A.; Huber, B.T. Epstein-Barr virus transactivates the human endogenous retrovirus HERV-K18 that encodes a superantigen. *Inmmunity*, **2001**, *15*, 579-589.
[43] Sutkowski, N.; Palkama, T.; Ciurli, C.; Sekaly, R.P.; Thorley-Lawson, D.A.; Huber, B.T. An Epstein-Barr virus-associated superantigen. *J. Exp. Med.*, **1996**, *184*, 971-980.
[44] McClain, M.T.; Heinlen, L.D.; Dennis, G.J.; Roebuck, J.; Harley, J.B.; James, J.A. Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry. *Nat. Med.*, **2005**, *11*, 85-89.
[45] Toussirot, E.; Roudier, J. Epstein-Barr virus in autoimmune diseases. *Best Pract. Res. Clin. Rheumatol.*, **2008**, *22*, 883-896.
[46] Saal, J.G.; Krimmel, M.; Steidle, M.; Gerneth, F., Wagner, S., Fritz, P.; Koch, S.; Zacher, J.; Sell, S.; Einsele, H.; Müller, C.A. Synovial Epstein-Barr virus infection increases the risk of rheumatoid arthritis in individuals with the shared HLA-DR4 epitope. *Arthritis Rheum.*, **1999**, *42*, 1485-1496.
[47] Balandraud, N.; Meynard, J.B.; Auger, I.; Sovran, H.; Mugnier, B.; Reviron, D.; Roudier, J.; Roudier, C. Epstein-Barr virus load in the peripheral blood of patients with rheumatoid arthritis. Accurate quantification using real-time polymerase chain reaction. *Arthritis Rheum.*, **2003**, *48*, 1223-1228.
[48] Kilger, E.; Kieser, A.; Baumann, M.; Hammerssschmidt, W. Epstein-Barr virus-mediated B-cell proliferation is dependent upon latent membrane protein 1, which simulates an activated CD40 receptor. *EMBO J.*, **1998**, *17*, 1700-1709.
[49] Chuang, H.C.; Lay, J.D.; Chuang, S.E.; Hsieh, W.C.; Chang, Y.; Su, I.J. Epstein-Barr virus (EBV) latent membrane protein-1 down-regulates tumor necrosis factor-alpha (TNF-alpha) receptor-1 and confers resistance to TNF-alpha-induced apoptosis in T cells: implication for the progression to T-cell lymphoma in EBV-associated hemophagocytic syndrome. *Am. J. Pathol.*, **2007**, *170*, 1607-1617.

[50] Caldwell, R.G.; Wilson, J.B.; Anderson, S.J.; Longnecker, R. Epstein-Barr virus LMP2a drives B-cell development and survival in the absence of normal B-cell receptor signals. *Immunity*, **1998**, *9*, 405-411.

[51] Rechsteiner, M.P.; Berger, C.; Zauner, L.; Sigrist, J.A.; Weber, M.; Longnecker, R.; Bernasconi, M.; Nadal, D. Latent membrane protein 2B regulates susceptibility to induction of lytic Epstein-Barr virus infection. *J. Virol.*, **2008**, *82*, 1739-1747.

[52] Cordier, M.; Calender, A.; Billaud, M.; Zimber, U.; Rousselet, G.; Pavlish, O.; Banchereau, J.; Tursz, T.; Bornkamm, G.; Lenoir, G.M. Stable transfection of Epstein-Barr virus (EBV) nuclear antigen 2 in lymphoma cells containing the EBV P3HR1 genome induces expression of B-cell activation molecules CD21 and CD23. *J. Virol.*, **1990**, *64*, 1002-1013.

[53] Yalamanchili, R.; Tong, X.; Grossman, S.; Johannsen, E.; Mosialos, G.; Kieff, E. Genetic and biochemical evidence that EBNA 2 interaction with a 63-kDa cellular GTG-binding protein is essential for B lymphocyte growth transformation by EBV. *Virology*, **1994**, *204*, 634–641.

[54] Boccellato, F.; Anastasiadou, E.; Rosato, P.; Kempkes, B.; Frati, L.; Faggioni, A.; Trivedi, P. EBNA2 interferes with the germinal center phenotype by downregulating BCL6 and TCL1 in non-Hodgkin's lymphoma cells. *J. Virol.*, **2007**, *81*, 2274-2282.

[55] McCann, E.M.; Kelly, G.L.; Rickinson, A.B.; Bell, A.I. Genetic analysis of the Epstein-Barr virus-coded leader protein EBNA-LP as a co-activator of EBNA2 funtion. *J. Gen. Virol.*, **2001**, *82*, 3067-3079.

[56] Peng, R.; Moses, S.C.; Tan, J.; Kremmer, E.; Ling, P.D. The Epstein-Barr virus EBNA-LP protein preferentially coactivates EBNA2-mediated stimulation of latent membrane proteins expressed from the viral divergent promoter. *J. Virol.*, **2005**, *79*, 4492-4505.

[57] Krauer, K.G.; Kienzle, N.; Young, D.B.; Sculley, T.B. Epstein-Barr virus nuclear antigen 3 and 4 interact with RBP-2N, a major isoform of RBP J kappa in B lymphocytes. *Virology*, **1996**, *226*, 346-353.

[58] Johannsen, E.; Miller, C.L.; Grossman, S.R.; Kieff, E. EBNA-2 and EBNA-3C extensively and mutually exclusively associate with RBPJkappa in Epstein-Barr virus-transformed B lymphocytes. *J. Virol.*, **1996**, *70*, 4179–4183.

[59] Waltzer, L.; Perricaudet, M.; Sergeant, A., Manet, E. Epstein-Barr virus EBNA3A and EBNA3C proteins both repress RBP-J kappa-EBNA2-activated transcription by inhibiting the binding of RBP-J kappa to DNA. *J. Virol.*, **1996**, *70*, 5909–5915.

[60] Marshall, D.; Sample, C. Epstein-Barr virus nuclear antigen 3C is a transcriptional regulator. *J. Virol.*, **1995**, *69*, 3624-3630.

[61] Chen, A.; Zhao, B.; Kieff, E.; Aster, J.C.; Wang, F. EBNA-3B- and EBNA-3C-regulated cellular genes in Epstein-Barr virus-immortalized lymphoblastoid cell lines. *J. Virol.*, **2006**, *80*, 10139-10150.

[62] Srinivas, S.; Sixbey, J.W. Esptein-Barr virus induction of recombinase-activating genes RAG1 y 2. *J. Virol.*, **1995**, *69*, 8155-8158.

[63] Humme, S.; Reisbach, G.; Feederle, R.; Delecluse, H.J.; Bousset, K.; Hammerschmidt, W; Schepers, A. The EBV nuclear antigen 1 (EBNA1) enhances B cell immortalization several thousandfold. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 10989-10994.

[64] Lotz, M.; Tsoukas, C.D.; Fong, S.; Carson, D.A.; Vaughan, J.H. Regulation of Epstein-Barr virus infection by recombinant interferons. Selected sensitivity to interferon-γ. *Eur. J. Immunol.*, **1985**, *15*, 520–525.

[65] Hislop, A.D.; Taylor, G.S.; Sauce, D.; Rickinson, A.B. Cellular responses to viral infection in humans: lessons from Epstein-Barr virus. *Annu. Rev. Immunol.*, **2007**, *25*, 587-617.

[66] Steven, N.M.; Annels, N.E.; Kumar, A.; Leese, A.M., Kurilla, M.G.; Rickinson, A.B. Immediate early and early lytic cycle proteins are frequent targets of the Epstein-Barr virus-induced cytotoxic T cell response. *J. Exp. Med.*, **1997**, *185*, 1605–1617.

[67] Pudney, V.A.; Leese, A.M.; Rickinson, A.B.; Hislop, A. D. CD8+ immunodominance among Epstein-Barr virus lytic cycle antigens directly reflects the efficiency of antigen presentation in lytically infected cells. *J. Exp. Med.*, **2005**, *201*, 349–360.

[68] Precopio, M.L.; Sullivan, J.L.; Willard, C.; Somasundaran, M.; Luzuriaga, K. Differential kinetics and specificity ofEBV-specificCD4+ andCD8+ Tcells during primary infection. *J. Immunol.*, **2003**, *170*, 2590–2598.

[69] Bickham, K.; Munz, C.; Tsang, M.L.; Larsson, M.; Fonteneau, J.F.; Bhardwaj, N.; Steinman, R. EBNA1-specific CD4+ T cells in healthy carriers of Epstein-Barr virus are primarily Th1 in function. *J. Clin. Invest.*, **2001**, *107*, 121–130.

[70] Leen, A.; Meij, P.; Redchenko, I.; Middeldorp, J.; Bloemena, E.; Rickinson, A.; Blake, N. Differential immunogenicity of Epstein-Barr virus latent-cycle proteins for human CD4+ T-helper 1 responses. *J. Virol.*, **2001**, *75*, 8649–8659.

[71] Voo, K.S.; Fu, T.; Heslop, H.E.; Brenner, M.K.; Rooney, C.M.; Wang, R.F. Identification of HLA-DP3-restricted peptides from EBNA1 recognized by CD4+ T cells. *Cancer Res.*, **2002**, *62*, 7195–7199.

[72] Rowe, M.; Glaunsinger, B.; van Leeuwen, D.; Zuo, J.; Sweetman, D.; Ganem, D.; Middeldorp, J.; Wiertz, E.J.; Ressing, M.E. Host shutoff during productive Epstein-Barr virus infection is mediated by BGLF5 and may contribute to immune evasion. *Proc. Natl. Acad. Sci. USA*, **2007**, *104*, 3366-3371.

[73] Morrison, T.E.; Mauser, A.; Wong, A.; Ting, J.P.; Kenney, S.C. Inhibition of IFN-gamma signalling by an Epstein-Barr virus immediate early protein. *Immunity*, **2001**, *15*, 787-789.

[74] Moore, K.W.; Vieira, P.; Fiorentino, D.F.; Trounstine, M.L.; Khan, T.A.; Mosmann T.R. Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein-Barr virus gene BCRF1. *Science*, **1990**, *248*, 1230-1234.

[75] Strockbine, L.D.; Cohen, J.I.; Farrah, T.; Lymanm, S.D.; Wagener, F.; DuBose, R.F.; Armitage, R.J.; Spriggs, M.K. The Epstein-Barr virus BARF1 gene encodes a novel, soluble colony-stimulating factor-1 receptor. *J. Virol.*, **1998**, *72*, 4015-4021.

[76] Desbien, A.L.; Kappler, J.W.; Marrack, P. The Epstein-Barr virus Bcl-2 homolog, BHRF1, blocks apoptosis by binding to a limited amount of Bim. *Proc. Natl. Acad. Sci. USA*, **2009**, *106*, 5663-5668.

[77] Henderson, S.; Huen, D.; Rowe, M.; Dawson, C.; Croom-Carter, D.; Rowe, M. Epstein-Barr virus-coded BHRF1 protein, a viral homologue of Bcl-2 protects human B-cells form programmed cell death. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 8479-8483.

[78] Uchida, J.; Yasui, T.; Takaoka-Shichijo, Y.; Muraoka, M.; Kulwichit, W.; Raab-Traub, N.; Kikutani, H. Mimicry of CD40 signals by Epstein-Barr virus LMP1 in B lymphocyte responses. *Science*, **1999**, *286*, 300-303.

[79] Levitskaya, J.; Sharipo, A.; Leonchiks, A.; Ciechanover, A.; Masucci, M.G. Inhibition of ubiquitin/proteasome-dependent protein degradation by the Gly-Ala repeat domain of the Epstein-Barr virus nuclear antigen 1. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 12616-12621.

[80] Ohga, S.; Nomura, A.; Takada, H.; Hara, T. Immunological aspects of Epstein-Barr virus infection. *Crit. Rev. Oncol. Hematol.*, **2002**, *44*, 203-215.

[81] Ruf, I.K.; Lackey, K.A.; Warudkar, S.; Sample, J.T. Protection from interferon-induced apoptosis by Epstein-Barr virus small RNAs is not mediated by inhibition of PKR. *J. Virol.*, **2005**, *79*, 14562-14569.

[82] Spaete, R.; Winthrop, J. Non-splicing variants of gp350/220. U.S. 2007202122, Aug 30, 2007.

[83] Denis MJ. Novel vaccination. W.O. 03094962, Nov 20, 2003.

[84] Moutschen, M.; Léonard, P.; Sokal, E.M.; Smets, F.; Haumont, M.; Mazzu, P.; Bollen, A.; Denamur, F.; Peeters, P.; Dubin, G.; Denis, M. Phase I/II studies to evaluate safety and immunogenicity of a recombinant gp350 Epstein–Barr virus vaccine in healthy adults *Vaccine*, **2007**, *25*, 4697-4705.

[85] Sokal, E.M.; Hoppenbrouwers, K.; Vandermeulen, C.; Moutschen, M.; Léonard, P.; Moreels, A.; Haumont, M.; Bollen, A.; Smets, F.; Denis, M. Recombinant gp350 vaccine for infectious mononucleosis: a phase 2, randomized, double-blind, placebo-controlled trial to evaluate the safety, immunogenicity, and efficacy of an Epstein-Barr virus vaccine in healthy young adults. *J. Infect. Dis.*, **2007**, *196*, 1749-1753.

[86] Thacker, E.L.; Mirzaei, F.; Ascherio, A. Infectious mononucleosis and risk for multiple sclerosis: a meta-analysis. *Ann. Neurol.*, **2006**, *59*, 499-503.

[87] Middelodorp, J.M. Epstein-Barr virus peptide and antibody against peptide thereof. W.O. 9406912, Mar 31, 1994.

[88] Frappier, L.; Holowaty, M.; Greenblatt, J.; Zeghouf, M. Novel interactions of the Epstein-Barr virus protein EBNA1, and compositions and methods related thereto. W.O. 2004007536, Jan 22, 2004.

[89] Celis, E. Epstein-Barr virus-specific immunization. U.S. 2006257356, Nov 16, 2006.
[90] Smith, R.S.; Pearson, G.R.; Parks, D.E.; Varghese, S.P. Immunoreactive peptides from Epstein-barr virus. U.S. 2008175849, Jul 24, 2008.
[91] Hickish, T.; Cunningham, D. Antiviral agent comprising antisense oligonucleotides complementary to the BHRF1 gene of Epstein-Barr virus. W.O. 9311267, Jun 10, 1993.
[92] Foley, M.; Casey, J.L.; Coley, A.M.; Devine, P.L. Mimotopes of Epstein-barr virus (EBV) epitopes. W.O. 2007022557, Mar 1, 2007.
[93] Thomson, S.A.; Duraiswamy, J.K.; Moss, D.J. Treatment of Epstein-Barr virus-associated diseases. W.O. 2007065215, Jun 14, 2007.
[94] Middeldorp, J.M.; Van Grunsven, W.M.J. Peptides and nucleic acid sequences related to the Epstein Barr virus. U.S. 6365717, Apr 2, 2002.
[95] Middeldorp, J.M. Method for the identification of extracellular domains of the epstein barr virus (EBV), tumor-associated latent membrane proteins and for the selection of antibody reagents reactive therewith. AU. 2002235884, Aug 12, 2002.
[96] Caligiuri, M.A.; Baiocchi, R.A. Viral gene products and methods for vaccination to prevent viral associated diseases. W.O. 2007097820, Aug 30, 2007.
[97] Harley, J.B.; James, J.A.; Kaufman, K.M. Diagnostics and therapy of Epstein-Barr virus in autoimmune disorders. W.O. 0158481, Aug 16, 2001.
[98] Huber, B.T.; Thorley-Lawson, D.A.; Sutkowski, N. Treatment and prevention of EBV infection and EBV-associated disorders. W.O. 0247720, Jun 20, 2002.
[99] Elliott, S.L.; Suhrbier, A.; Miles, J.J.; Lawrence, G.; Pye, S. J.; Le, T.T.; Rosenstengel, A.; Nguyen, T.; Allworth, A.; Burrows, S.R.; Cox, J.; Pye, D.; Moss, D.J.; Bharadwaj, M. Phase I trial of a CD8+ T-cell peptide epitope-based vaccine for infectious mononucleosis. *J. Virol.*, **2008**, *82*, 1448-1457.
[100] Khanna, R.; Kerr, B.M.; Misko, I.S.; Moss, D.J.; Burrows, S.R. EBV CTL epitopes. W.O. 9745444, Dec 4, 1997.
[101] Khanna, R.; Duraiswamy, J. Epstein Barr virus peptide epitopes, polyepitopes and delivery system therefore. W.O. 2004041849, May 21, 2004.
[102] Burrows, S.; Khanna, R. CTL epitopes from EBV. W.O. 9902550, Jan 21, 1999.
[103] Khanna, R.; Burrows, S.R.; Thomson, S.A.; Moss, D.J.; Cresswell, P.; Poulsen, L.M.; Cooper, L. Class I processing-defective Burkitt's lymphoma cells are recognised efficiently by CD4+ EBV specific CTLs. *J. Immunol.*, **1997**, *158*, 3619–3625.
[104] Pancre, V.; Auriault, C. T CD4+ Epitopes of Type I and II Latency Antigens of the Epstein-Barr Virus, Which Can Be Recognized by the majority of individuals in the caucasian populations and applications thereof. U.S. 2009130134, May 21, 2009.
[105] Nikiforow, S.; Bottomly, K.; Miller, G.; Münz, C. Cytolytic CD4+-T-cell clones reactive to EBNA1 inhibit Epstein–Barr virus-induced B-cell proliferation. *J. Virol.*, **2003**, *77*, 12088–12104.
[106] Subklewe, M.; Sebelin, K; Block, A.; Meier, A.; Roukens, A.; Paludan, C.; Fonteneau, J.F.; Steinman, R.M.; Münz, C. Dendritic cells expand Epstein Barr virus specific CD8+ T cell responses more efficiently than EBV transformed B cells. *Hum. Immunol.*, **2005**, *66*, 938-949.
[107] Steinman, R.M.; Muenz, C. Protective antigen of Epstein-Barr virus. W.O. 0112215, Feb 22, 2001.
[108] Kuzushima, K.; Ito, Y.; Okamura, A.; Akatsuma, Y.; Morishima, Y. Citotoxic T-cell epitope peptide capable of specifically attacking cell infected with Epstein-Barr virus and use thereof. W.O. 2007049737, May 3, 2007.
[109] Bickham, K.; Goodman, K.; Paludan, C.; Nikiforow, S.; Tsang, M.L.; Steinman, R.M.; Münz, C. Dendritic cells initiate immune control of Epstein–Barr virus transformation of B lymphocytes *in vitro*. *J. Exp. Med.*, **2003**, *198*, 1653-1663.
[110] Pan, S.H.; Tai, C.C.; Lin, C.S.; Hsu, W.B.; Chou, S.F.; Lai, C.C.; Chen, J.Y.; Tien, H.F.; Lee, F.Y.; Wang, W.B. Epstein-Barr virus nuclear antigen 2 disrupts mitotic checkpoint and causes chromosomal instability. *Carcinogenesis*, **2009**, *30*, 366-375.
[111] Zeng, Y.Z. Ad5F35-LMP2 recombinant adenovirus vaccine comprising Epstein-Barr virus latent membrane protein 2 gene. CN. 1907493, Feb 7, 2007.
[112] Leen, A.; Meij, P.; Redchenko, I.; Middeldorp, J.; Bloemena, E.; Rickinson, A.; Blake, N. Differential immunogenicity of Epstein–Barr virus latent-cycle proteins for human CD4+ T-helper 1 responses. *J. Virol.*, **2001**, *75*, 8649-8659.
[113] Liu, F.F.; Klamut, H.J.; Chia, M.; Li, J.H. Nucleic acids and methods for treating EBV-positive cancers. W.O. 03047634, Jun 12, 2003.
[114] Lycke, J.; Svennerholm, B.; Hjelmquist, E.; Frisén, L.; Badr, G.; Andersson, M.; Vahlne, A.; Andersen, O. Acyclovir treatment of relapsing-remitting multiple sclerosis. A randomized, placebo-controled, double-blind study. *J. Neurol.*, **1996**, *243*, 214-224.
[115] Bech, E.; Lycke, J.; Gadeberg, P.; Hansen, H.J.; Malmeström, C.; Anderson, O.; Christensen, T.; Ekholm, S.; Haahr, S.; Höllsberg, P.; Bergström, T.; Svennerholm, B.; Jakobsen, J. A randomized, double-blind placebo-controlled MRI study of anti-herpes virus therapy in MS. *Neurology*, **2002**, *58*, 31-36.
[116] Hierro, L.; Díez-Dorado, R.; Díaz, C.; De la Vega, A.; Frauca, E.; Camarena, C.; Muñoz-Bartolo, G.; González de Zárate, A.,; López Santamaría, M.; Jara, P. Efficacy and safety of valganciclovir in liver-transplanted children infected with Epstein-Barr virus. *Liver Transpl.*, **2008**, *14*, 1185-1193.
[117] Funch, D.P.; Walkeer, A.M.; Schneider, G.; Zivadeh, N.J.; Pescovitz, M.D. Ganciclovir and acyclovir reduce the risk of post-transplant lymphoproliferative disorder in renal transplant recipients. *Am. J. Transplant.*, **2005**, *5*, 2894-2900.
[118] Moore, S.M.; Cannon, J.S.; Tanhehco, Y.C.; Hamzeh, F.M.; Ambinder, R.F. Induction of Epstein-Barr virus kinases to sensitize tumor cells to nucleoside analogues. *Antimicrob. Agents. Chemother.*, **2001**, *45*, 2082–2091.
[119] Westphal, E.M.; Blackstock, W.; Feng, W.; Israel, B.; Kenney, S.C. Activation of lytic Epstein-Barr virus (EBV) infection by radiation and sodium butyrate *in vitro* and *in vivo*: a potential method for treating EBV-positive malignancies. *Cancer Res.*, **2000**, *60*, 5781-5788.
[120] Faller, D.V.; Mentzer, S.J,; Perrine, S.P. Induction of the Epstein-Barr virus thymidine kinase gene with concomitant nucleoside antivirals as a therapeutic strategy for Epstein-Barr virus-associated malignancies. *Curr. Opin. Oncol.*, **2001**, *13*, 360-367.
[121] Silverstein, S.J.; Kyratsous, C. Methods of inhibiting VZV replication and related compositions. W.O. 2009014759, Jan 29, 2009.
[122] Hostetler, K.; Kini, G.; Beadle, J.R. Phosphonate ester antiviral compounds. W.O. 9838202, Sep 3, 1998.
[123] Chu, C.K.; Cheng, Y.C.; Pai, B.S.; Yao, G. Q. L-Nucleosides for the treatment of hepatitis B-virus and Epstein-Barr virus. W.O. 9520595, Aug 3, 1995.
[124] Cohen, J.I.; Pesnicak, L.; Katano. H. Use of statin to kill EBV-transformed B cells. W.O. 2005042710, May 12, 2005.
[125] Kodama, E.; Matsuoka, M.; Ashida, N. Medicinal agent for disease associated with epstein-barr virus, and method for screening of the medicinal agent. W.O. 2009034945, Mar 19, 2009.
[126] Schinazi, R.F.; Shi, J.; Gustafson, E.; Fingeroth, J.D. Treatment of EBV and KHSV infection and associated abnormal cellular proliferation. U.S. 2007197462, Aug 23, 2007.
[127] Perrine, S.P.; Faller, D.V.; White, B.F. Use of an inducing agent for the treatment of blood, viral and cellular disorders. E.P. 1886677, Feb 13, 2008.
[128] Hauser, S.L.; Waubant, E.; Arnold, D.L.; Vollmer, T.; Antel, J.; Fox, R.J.; Bar-Or, A.; Panzara, M.; Sarkar, N.; Agarwal, S.; Smith, C.H. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N. Engl. J. Med.*, **2008**, *358*, 676-688.
[129] Papadopoulos, E.B.; Ladanyi, M.; Emanuel, D.; Mackinnon, S.; Boulad, F.; Carabasi, M.H.; Castro-Malaspina, H.; Childs, B.H.; Gillio, A.P.; Small, T.N. Infusions of donor leukocytes to treat Epstein–Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. *N. Engl. J. Med.*, **1994**, *330*, 1185–1191.
[130] Rooney, C.M.; Roskrow, M.A.; Smith, C.A.; Brenner, M.K.; Heslop, H.E. Immunotherapy for Epstein-Barr virus-associated cancers. *J. Natl. Cancer Inst. Monogr.*, **1998**, *23*, 89-93 .
[131] Hopwood, P.; Crawford, D.H. The role of EBV in post-transplant malignancies: a review. *J. Clin. Pathol.*, **2000**, *53*, 248-254.
[132] Münz, C.; Bickham, K.L.; Subklewe, M.; Tsang, M.L.; Chahroudi, A.; Kurilla, M.G.; Zhang, D.; O'Donnell, M.; Steinman, R.M. Human CD4(+) T lymphocytes consistently respond to the latent Ep-

stein-Barr virus nuclear antigen EBNA1. *J. Exp. Med.*, **2000**, *191*, 1649-1660.

[133] Steinman, R.M.; Muenz, C. Protective antigen of epstein barr virus. U.S. 2006188520, Aug 24, 2006.

[134] Franken, M.; Estabrooks, A.; Cavacini, L.; Sherburne, B.; Wang, F.; Scadden, D.T. Epstein-Barr virus-driven gene therapy for EBV-related lymphomas. *Nat. Med.*, **1996**, *2*, 1379-1382.

[135] Duraiswamy, J.; Bharadwaj, M.; Tellam, J.; Connolly, G.; Cooper, L.; Moss, D.; Thomson, S.; Yotnda, P. Khanna, R. Induction of therapeutic T-cell responses to subdominant tumor-associated viral oncogene after immunization with replication-incompetent polyepitope adenovirus vaccine. *Cancer Res.*, **2004**, *64*, 1483–1489.

[136] Duraiswamy, J.; Sherritt, M.; Thomson, S.; Tellam, J.; Cooper, L.; Connolly, G.; Bharadwaj, M.; Khanna, R. Therapeutic LMP1 polyepitope vaccine for EBV-associated Hodgkin disease and nasopharyngeal carcinoma. *Blood* **2003**, *101*, 3150-3156.

[137] Proesch, S.; Volk, H.D.; Krueger, D. Proteasome inhibitors for the treatment of herpesviridae infected individuals. W.O. 2004004749, Jan 15, 2004.

[138] Monroe, J.G.; Katz, E.; Murali, R. Methods and compositions targeting viral and cellular ITAM motifs, and use of same in identifying compounds with therapeutic activity. U.S. 2007110760, May 17, 2007.

[139] Kempkes, B. Novel targets for a therapeutic intervention in EBV related diseases. W.O. 02098918, Dec 12, 2002.

[140] Mei, Y.P.; Zhu, X.F.; Zhou, J.M.; Huang, H.; Deng, R.; Zeng, Y.X. siRNA targeting LMP1-induced apoptosis in EBV-positive lymphoma cells is associated with inhibition of telomerase activity and expression. *Cancer Lett.*, **2006**, *232*, 189-198.

[141] Jin, D.Y.; Choy, E.; Kok, K.H. Promoters for RNA interference. U.S. 2007202082, Aug 30, 2007.

[142] Kirschner, A.N.; Omerovic, J.; Longnecker, R.; Jardetzky, T.S. Peptides, peptidomimetics and small molecule inhibitors of the Epstein-Barr virus mediated fusion and entry process. U.S. 2009074719, Mar 19, 2009.

[143] Robertson, E.S. An EBV inhibitor and methods of making an using the same. W.O. 2008005506, Jan 10, 2008.

[144] Morgan, A.J.; Wilson, A.D.; Wong, K.W. Therapeutic agent comprising a B-subunit of a protein toxin in conjunction with EBV latent membrane protein useful for the treatment of diseases associated with the Epstein Barr Virus and neoplasias W.O. 0247727, Jun 20, 2002.

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