EBV vaccine and antibodies

I. FIELD OF THE INVENTION

[0001] The present invention relates, inter alia, to a composition comprising the BILF2 glycoprotein of Epstein-Barr-Virus (EBV), a nucleic acid encoding the BILF2 glycoprotein of EBV, or an extracellular vesicle (EV) or EBV-virus-like particle (VLP) displaying at its surface the BILF2 glycoprotein of EBV, for use in a method of eliciting antibodies against said BILF2 glycoprotein which reduce infectivity of EBV for human epithelial cells. Preferably, said composition elicit antibodies that prevent infection of human epithelial cells by EBV.

II. BACKGROUND

[0002] Epstein-Barr-Virus (EBV) is a ubiquitous human herpes viruses that infects over 90% of the population world-wide with a life-long persistence in its host. In most cases, primary infection occurs during early childhood and is usually asymptomatic. In contrast, if infection is retarded and takes place during adolescence or adulthood, it is regularly symptomatic, causing a benign lymphoproliferative syndrome termed Infectious Mononucleosis (IM) in up to 50% of cases. Although the disease is normally self-limiting, prolonged forms of IM, a frequent, complex disease called Myalgic Encephalitis/Chronic Fatigue Syndrome (ME/CFS) or rare Chronic Active EBV infection (CAEBV) with fatal outcome have been reported. Clinically apparent IM has also been found to significantly increase the risk to develop Hodgkin disease and other type of lymphoma later in life. Today, it is also generally accepted that IM is an independent risk factor for multiple sclerosis. In addition, EBV is causally associated with a heterogeneous group of malignant diseases such as nasopharyngeal carcinoma, gastric carcinoma, and various types of lymphoma, so that the WHO classifies EBV as a class I carcinogen.

[0003] Beside the above described medical condition caused by EBV, patients with primary or secondary immune defects such as transplant recipients are at high risk for EBV-associated diseases as a consequence of the detrimental effect of immunosuppressive agents on the immune-control of EBV-infected B-cells. EBV-associated posttransplant lymphoproliferative disease (PTLD) is an important form of posttransplant complications, occurring in up to 20% of organ recipients. Importantly, immunocompromised transplant recipients who are immunologically naive for EBV at the onset of immunosuppression are at a particular high risk of developing life-threatening EBV+ PTLD due to a primary EBV infection, e.g. often caused

after transplantation via transmission of the virus through a donor organ due to the high prevalence of EBV. Due to impaired T-cell immunity that results from exposure to immunosuppressive drugs, these patients are unable to effectively prime EBV-specific T-cells that play a critical role in controlling proliferation of EBV-infected B-cells. In contrast, patients who are EBV-seropositive at transplant have a much lower risk for developing PTLD, demonstrating the essential role of EBV-specific T-cells poised to eliminate virally infected cells and control EBV. In general, patients who are EBV-seronegative before transplantation are at a much higher risk to develop EBV-associated diseases, since transmission of donor EBV in transplanted organs or natural infection with the virus causes lymphoproliferative disease in EBV-seronegative recipients after transplantation. As with many virus-associated diseases a promising approach for preventing and/or treating virus infection and its consequences in the host is vaccination, which is also true in the case of reducing the high risk of PTLD in seronegative patients by immunizing them to EBV prior to the transplantation.

[0004] First trials in humans with candidate vaccines were performed already in the 1990s using a recombinant vaccinia virus expressing the major EBV membrane antigen gp350/220 yielding increased titers of EBV-neutralizing antibodies. The gp350/220 glycoprotein is encoded by the viral BLLF1 gene. More recently, different peptide-based prophylactic vaccines aimed at seroconversion and prevention of IM in healthy volunteers and in children awaiting kidney transplantation have been described. In these children, neutralizing antibodies were detected in four recipients. However, immune responses declined rapidly and were unlikely to affect post-transplant events.

[0005] To this end, EBV is a ubiquitous human herpes viruses that infects over 90% of the population worldwide with a life-long persistence in its host. Despite typically causing a self-limiting mild illness or no symptoms at all during initial infection in early childhood, EBV infection is implicated in the pathogenesis of multiple forms of cancer and multiple sclerosis and can trigger more serious illness in patients with primary or secondary immune defects. These EBV-associated diseases provide strong arguments for the development and improvement of methods to reduce the infectivity of EBV that are both safe and efficient in coping with subsequent virus infections and virus-associated diseases.

[0006] However, persistent EBV infection and the limited evidence of immune selection of viral antigenic variants indicate that *in vivo* neutralization of EBV infection is suboptimal. Thus, the technical problem underlying the present invention is the provision of an EBV vaccine that allows controlling infectivity of EBV and thus EBV infection.

[0007] The technical problem is solved by the subject-matter as defined in the claims, described in the description, and illustrated in the Figures.

III. SUMMARY

[0008] In summary, the inventors of the present invention provide data showing that Epstein-Barr virus-like particles (EB-VLPs) do not only contain the majority of viral glycoproteins but also induce a multitude of glycoprotein-specific antibodies in immunized rodents. Among them the inventors discovered antibodies with novel specificities including BILF2 and identified some that interfere with viral infection of typical target cells of EBV, i.e., B cells and epithelial cells. Despite the EBV membrane glycoprotein BILF2 being largely uncharacterized and seemingly comprising only a small fraction of total EBV membrane antigens, the inventors discovered that immunization of rodents with EB-VLPs generated antibodies with previously uncharacterized specificities, including BILF2. Such antibodies were capable of interfering with viral infection and further characterization of the immune response to EBV in both humans and rodents revealed that BILF2-targeting antibodies surprisingly constitute a core element of the adaptive immune response to EBV infection and following immunization with EB-VLPs or other immunization strategies.

[0009] Furthermore, it was demonstrated that the antibodies provided by the present invention compete with antibodies of human sera directed against BILF2 for the same epitope which attests to the fact that the antibodies of the present invention will have prophylactic and/or therapeutic value as is evidenced by Liu et al. (2019) who showed that 94% of healthy EBV-positive humans had IgA antibodies against BILF2. Such antibodies were obtained by the inventors of the present invention by immunizing rodents with a composition of the present invention as described herein.

[0010] Namely, the inventors immunized rodents with compositions comprising BILF2, e.g. EB-VLPs or extracellular vesicles (EVs), which carry BILF2 in their membranes and which elicited antibodies which reduce infectivity of EBV for human epithelial cells or can even prevent infection. This finding is surprising, because BILF2 is a more or less uncharacterized EBV protein and antibodies obtained thus far had no potential to prevent infection of non-B cells (see page 2549, left column, last paragraph and page 2549, right column, first paragraph of Mackett et al., 1990). As said, the antibodies provided by the present invention compete with antibodies of human sera directed against BILF2 for the same epitope which attests to the fact that the antibodies of the present invention will have a prophylactic and/or therapeutic value. In fact, EBV-infected cancers of epithelial origin are well known in the field. All tumor cells of nasopharyngeal carcinoma (NPC) and about 10% of cases of gastric carcinoma are infected with EBV, which has been WHO classified a class 1 human carcinogen capable of inducing various tumors in humans. Thus, antibodies provided by the present invention can interfere with EBV infection of epithelial cells thus preventing human cancers.

[0011] In sum, the present invention provides an EBV vaccine which can induce a broad humoral immune response that can protect from various EBV associated human diseases. Accordingly, antibodies obtained by immunizing rodents with an EBV vaccine of the present invention can also protect from various EBV associated human diseases, since they reduce the infectivity of EBV for human epithelial cells. An additional conclusion is that a vaccine based on BILF2 alone can induce an immune response, which can interfere with EBV infection of epithelial cells and thus prevent EBV-associated disease in human. Also, a vaccine based on BILF2 in combination with other EBV (glyco-)proteins can induce an immune response which can interfere with EBV infection of both epithelial cells and B-cells.

[0012] Accordingly, the present inventions relates to a composition comprising

a) the BILF2 glycoprotein of Epstein-Barr-Virus (EBV),

b) a nucleic acid encoding the BILF2 glycoprotein of EBV, or

c) an extracellular vesicle (EV) or EB-virus-like particle (VLP) displaying at its surface the BILF2 glycoprotein of EBV,

for use in a method of eliciting antibodies against said BILF2 glycoprotein which reduce infectivity of EBV for human epithelial cells.

[0013] Preferably, in said composition said antibodies prevent infection of human epithelial cells by EBV.

[0014] Preferably, in said composition said BILF2 glycoprotein is produced by mammalian cells and isolated therefrom.

[0015] Preferably, in said composition said nucleic acid is DNA or RNA, preferably wherein said DNA is comprised by a vector.

[0016] Preferably, said composition comprises

a) RNA encoding the BILF2 glycoprotein of EBV, or

b) an extracellular vesicle (EV) or EB-virus-like particle (VLP) displaying at its surface the BILF2 glycoprotein of EBV,

for use in a method of eliciting antibodies against said BILF2 glycoprotein which reduce infectivity of EBV for human epithelial cells.

[0017] Preferably in said composition, said EV or VLP expresses BILF2, either alone or in combination with one or more other EBV glycoproteins.

[0018] Preferably in said composition, said other EBV glycoproteins may comprise gp350, gB, gp42, gH, gL, gM, gN, BMRF2, BDLF2, BDLF3, BILF1, and BARF1.

[0019] Preferably in said composition the VLP further comprises one or more EBV structural proteins.

[0020] Preferably in said composition the one or more EBV structural proteins include fusion (F), matrix (M) (also called tegument), or nucleocapsid (N).

[0021] Preferably said composition further comprises one or more pharmaceutically acceptable carriers.

[0022] Preferably, said composition further comprises one or more adjuvants.

[0023] Preferably, said composition shall be used in a method of treatment or prevention of a disease.

[0024] Preferably, said composition shall be used in a method of treatment or prevention of a disease, wherein the disease is cancer, preferably a cancer of epithelial origin, or an infection, preferably an EBV infection, most preferably an EBV infection of epithelial cells.

[0025] Preferably, said composition shall be used in a method of treatment or prevention of a disease, wherein the disease is cancer and the cancer is selected from the group consisting of meduloblastoma, retinoblastoma, Burkitt's lymphoma, Hodgkin´s lymphoma, oral cancer, skin cancer, basaliom, acute myeloid leukemia, pancreatic cancer, colorectal cancer, endometrial cancer, biliary tract cancer, liver cancer, myeloma, multiple myeloma, prostate cancer, stomach cancer, kidney cancer, bone cancer, soft tissue cancer, head and neck cancer, glioblastoma multiforme, astrocytoma, melanoma, lung cancer, esophageal cancer, gastric cancer, breast cancer, ovarian cancer, mesothelioma cancer, bladder cancer, anal cancer, chondrosarcoma cancer, osteosarcoma cancer, sarcoma cancer, primitive neuroectodermal cancer (primitive neuroectodermal tumor (PNET)), and combinations thereof.

[0026] The present invention further provides an antibody directed against the BILF2 glycoprotein of EBV, wherein said antibody binds to an epitope within amino acids 41 to 160 of SEQ ID NO: 25 and is capable of reducing the infectivity of EBV for human epithelial cells. Such an antibody is obtainable (or is obtained) by immunizing, e.g. rodents with a composition of the present invention. The present invention further provides said antibody for use in a method for passive immunization against EBV, and/or for use in a method for reducing the infectivity of EBV for human epithelial cells, and/or for use in a method for preventing infection of human epithelial cells by EBV.

[0027] Preferably, an antibody of the present invention binds to an epitope within amino acids 80 to 160 or 129 to 143 of SEQ ID NO: 25 and is capable of reducing the infectivity of EBV for human epithelial cells.

[0028] Preferably, said antibody is

(a) an antibody comprising a heavy chain variable region having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence shown in SEQ ID NO: 7 and a light chain variable region having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence shown in SEQ ID NO: 8;

(b) an antibody comprising a heavy chain variable region having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence shown in SEQ ID NO: 15 and a light chain variable region having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence shown in SEQ ID NO: 16;

(c) an antibody comprising a heavy chain variable region having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence shown in SEQ ID NO: 23 and a light chain variable region having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence shown in SEQ ID NO: 24; or

(d) an antibody binding to the same epitope as that in the EBV BILF2 protein shown in SEQ ID NO: 25 to which any one of the antibodies (a) to (c) bind.

[0029] It is further preferred that an antibody of the present invention is

(a) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 1, heavy chain CDR2 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 2, and heavy chain CDR3 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 3, and

a light chain variable region comprising light chain CDR1 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 4, light chain CDR2 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 5, and light chain CDR3 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 6;

(b) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 9, heavy chain CDR2 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence

as set forth in SEQ ID NO: 10, and heavy chain CDR3 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 11, and

a light chain variable region comprising light chain CDR1 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 12, light chain CDR2 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 13, and light chain CDR3 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 14;

(c) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 17, heavy chain CDR2 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 18, and heavy chain CDR3 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 19, and

a light chain variable region comprising light chain CDR1 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 20, light chain CDR2 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 21, and light chain CDR3 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 22; or

(d) an antibody binding to the same epitope as that in the EBV BILF2 protein shown in SEQ ID NO: 25 to which any one of the antibodies (a) to (c) bind.

[0030] It is further preferred that an antibody of the present invention is

(a) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 1, heavy chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 2, and heavy chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 3, and

a light chain variable region comprising light chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 4, light chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 5, and light chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 6;

(b) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 9, heavy chain CDR2

having the amino acid sequence as set forth in SEQ ID NO: 10, and heavy chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 11, and

a light chain variable region comprising light chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 12, light chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 13, and light chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 14;

(c) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 17, heavy chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 18, and heavy chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 19, and

a light chain variable region comprising light chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 20, light chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 21, and light chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 22; or

(d) an antibody binding to the same epitope as that in the EBV BILF2 protein shown in SEQ ID NO: 25 to which any one of the antibodies (a) to (c) bind.

[0031] Preferably, for an antibody of the present invention said epitope is determined by pepspot analysis, amino acid replacement analysis and/or deletion analysis of the ectodomain of BILF2 as shown in SEQ ID NO: 25. The ectodomain of BILF2 encompasses amino acids 18 to 212 of SEQ ID NO: 25.

[0032] Preferably, said antibody is for use in a method for passive immunization against EBV.

[0033] Preferably, said antibody is for use in a method for reducing the infectivity of EBV for human epithelial cells.

[0034] Preferably, said antibody is for use in a method for preventing infection of human epithelial cells by EBV.

[0035] The present invention further comprises a method for diagnosing whether a human subject has antibodies which reduce infectivity of EBV for human epithelial cells, comprising determining in a serum sample obtained from said subject whether antibodies compete with any of the antibodies described above for epitope binding.

IV. DETAILED DESCRIPTION

[0036] The composition of the invention comprises the BILF2 glycoprotein of Epstein-Barr-Virus (EBV), a nucleic acid encoding the BILF2 glycoprotein of EBV, or an extracellular vesicle (EV) or EB-virus-like particle (VLP) displaying at its surface the BILF2 glycoprotein of EBV, for

use in a method of eliciting antibodies against said BILF2 glycoprotein which reduce infectivity of EBV for human epithelial cells.

[0037] Preferably, such antibodies prevent infection of human epithelial cells by EBV.

[0038] Preferably, antibodies which are elicited by a composition of the present invention are antibodies as defined herein, e.g. they are neutralizing, they bind an epitope within amino acids 40 to 160, preferably 81 to 160 or 129 to 143 of BILF2.

[0039] For the purpose of eliciting such antibodies, a composition of the present invention is administered to a human (subject) by means and methods known in the art. Administration of a composition may be effected by different routes including, but not limited to, parenteral administration (e.g., intramuscular, subcutaneous, intravenous, or intradermal injection), oral administration, transdermal administration, and nasal administration. Methods of delivery are not limited to the above described ones, and any means for intracellular delivery can be used. Other methods of intracellular delivery contemplated by the methods of the present invention include, but are not limited to, liposome encapsulation, nanoparticles, etc.. Likewise, an antibody of the present invention can also be administered to a human (subject) by the afore-described routes or methods.

[0040] A human (subject) as referred to herein may be “in need” of being administered a composition and/or an antibody of the present invention. A subject “in need” of being administered a composition or antibody of the present invention is in need of a reduction of infectivity of EBV for human epithelial cells or for prevention of infection of human epithelial cells. Such a subject includes a subject that exhibits and/or is at risk of exhibiting one or more symptoms of the diseases described herein.

[0041] Thus, administering a composition or antibody of the present invention to a subject in need includes prophylactic administration of the composition (i.e., before the disease and/or one or more symptoms of the disease are detectable) and/or therapeutic administration of the composition (i.e., after the disease and/or one or more symptoms of the disease are detectable). The invention's compositions and antibodies are also useful for a subject “at risk” for disease. The term “at risk” refers to a subject that is predisposed to contracting and/or expressing one or more symptoms of the disease. This predisposition may be genetic (e.g., a particular genetic tendency to expressing one or more symptoms of the disease, such as heritable disorders, etc.), or due to other factors (e.g., environmental conditions, exposures to detrimental compounds, including carcinogens, present in the environment, etc.). The term subject “at risk” includes subjects “suffering from disease”, i.e., a subject that is experiencing one or more symptoms of the disease. It is not intended that the present invention be limited to any particular signs or symptoms. Thus, it is intended that the present invention encompasses subjects that are experiencing any range of disease, from sub-clinical symptoms

to full-blown disease, wherein the subject exhibits at least one of the indicia (e.g., signs and symptoms) associated with the disease.

[0042] Compositions of the present invention which elicit antibodies as described herein are preferably for use as a vaccine, e.g. a prophylactic or therapeutic vaccine.

[0043] Both a prophylactic or therapeutic vaccine as described herein elicits antibodies against EBV BILF2 which reduce infectivity of EBV for human epithelial cells.

[0044] A vaccine usually provides for active immunization. In the context of the present invention, compositions as described herein elicit antibodies against EBV BILF2 which reduce infectivity of EBV for human epithelial cells.

[0045] A vaccine increases the immunity of a subject against EBV infection. Therefore, such compositions are for use as "EBV vaccine" and can refer to a treatment that increases the immunity of a subject against EBV. Therefore, in particular embodiments, a vaccine may be administered prophylactically, for example to a subject that is immunologically naive (e.g., no prior exposure or contact with EBV). In particular embodiments, a vaccine may be administered therapeutically to a subject who has been exposed to EBV. Thus, a vaccine can be used to ameliorate a symptom associated with EBV infection such as a lymphoproliferative disorder. A vaccine can also reduce the risk of a carcinoma (e.g., nasopharyngeal and/or gastric) or a smooth muscle tumor.

[0046] In particular embodiments a prophylactic vaccine can reduce, control, or eliminate a primary infection with EBV.

[0047] In particular embodiments, a therapeutic vaccine can reduce, control, or eliminate EBV reactivation. In particular embodiments, a reduction in EBV reactivation can be determined by measuring expression of EBV lytic genes. Namely, detection of fewer cells with latent or lytic EBV genes or detection of lower expression levels of EBV genes can indicate a reduction in EBV load (fewer latently EBV infected cells) and reactivation (fewer cells that support de novo virus production).

[0048] The skilled artisan will appreciate that the immune system generally is capable of producing an innate immune response and an adaptive immune response. An innate immune response generally can be characterized as not being substantially antigen specific and/or not generating immune memory. An adaptive immune response can be characterized as being substantially antigen specific, maturing over time (e.g., increasing affinity and/or avidity for antigen), and in general can produce immunologic memory. Even though these and other functional distinctions between innate and adaptive immunity can be discerned, the skilled artisan will appreciate that the innate and adaptive immune systems can be integrated and therefore can act in concert.

[0049] EBV infection of epithelial target cells is a multistep process. In the first step, EBV binds to target cells using various host cell surface receptors and multiple viral envelope glycoproteins. In some cases, binding of EBV virions can induce signaling pathway activation. After binding to the cell surface receptors, the viral and host membranes merge (either by direct membrane fusion or fusion with the endosomal membrane). The viral capsid is then transported in the cytosol to the nuclear periphery. Once at a nuclear pore, the viral genome is released into the nucleus through a nuclear pore.

[0050] "Infectivity" of EBV in the context of the present invention is EBV's capacity to invade cells, in particular epithelial cells of a human. Thus, infectivity results in infection and subsequent diseases in humans. Hence, for the purpose of the present invention, the term "infectivity" encompasses infection. Namely, without being bound by theory, infection being the result of infectivity is a two-step process. After infection (i) EBV enters an initial phase after which it becomes latent in the infected cells for days, probably weeks or months. Upon reactivation (ii) the latently infected cells turn into a virus factory, whereby cells are biochemically exhausted and succumb. In other words, EBV has a biphasic life cycle: latent infection - break - lytic reactivation and virus release, whereby EBV produces progeny. This is a speciality of EBV unknown in any other virus species.

[0051] As used herein, the term "infection" refers to the invasion of a host by EBV. EBV is considered to be "infectious" when it is capable of invading a human, and replicating or propagating within the human. "EBV infection" specifically refers to invasion of a human, such as cells and tissues of the human by EBV. As described herein, EBV invades a human through epithelial cells and compositions of the present invention are capable of eliciting antibodies against BILF2 of EBV which reduce infectivity of EBV for human epithelial cells. Also, antibodies as described herein reduce infectivity of EBV for human epithelial cells.

[0052] A "reduction in infectivity of human epithelial cells" as used herein may be used equally as the term "neutralization in infectivity of human epithelial cells". Said term means/is the property of antibodies which are elicited by the compositions as described herein. Accordingly, compositions as described herein elicit neutralizing antibodies. As used herein, the term "eliciting antibodies" is defined as inducing antibodies against EBV BILF2.

[0053] The term "reduction or neutralization in infectivity of human epithelial cells" refers to a percent decrease in EBV infectivity of human epithelial cells in the presence of the antibody, as compared to EBV infectivity in the absence of the antibody. For example, if half as many cells in a sample become infected by EBV in the presence of an antibody, as compared to in the absence of the antibody, this can be calculated as 50% neutralization. In particular embodiments, "neutralization or reduction in infectivity of human epithelial cells" can refer to at least 40% neutralization (or reduction), at least 50% neutralization (or reduction), at least 60%

neutralization (or reduction), at least 70% neutralization (or reduction), at least 80% neutralization (or reduction), 90% neutralization (or reduction), 95% neutralization (or reduction) or even 100% neutralization (or reduction).

[0054] That means that if there is an at least 40% neutralization (or reduction), only 60% of human epithelial cells are infected by EBV. Likewise, if there is an at least 50% neutralization (or reduction), only 50% of human epithelial cells are infected by EBV. Similarly, if there is an at least 60% neutralization (or reduction), only 40% of human epithelial cells are infected by EBV. Also, if there is an at least 70% neutralization (or reduction), only 30% of human epithelial cells are infected by EBV. Further, if there is an at least 80% neutralization (or reduction), only 20% of human epithelial cells are infected by EBV; if there is an at least 90% neutralization (or reduction), only 10% of human epithelial cells are infected by EBV; if there is an at least 95% neutralization (or reduction), only 5% of human epithelial cells are infected by EBV, or if there is 100% neutralization (or reduction), no human epithelial cells are infected by EBV. Thus, the higher the neutralization (or reduction), the fewer human epithelial cells are infected by EBV.

[0055] Ideally, however, the neutralization (or reduction) is such that antibodies as described herein prevent infection of human epithelial cells by EBV.

[0056] As epithelial cells any human epithelial cells may be used including human epithelial cell lines. Also HEK293 cells may be used, since HEK293 are known to be a proxy of human epithelial cells.

[0057] The neutralization assay which is preferably applied in the context of the present invention is described in Example 6. Briefly, HEK293 target cells are used as EBV's epithelial cell targets. Raw hybridoma cell culture supernatant or purified monoclonal antibodies are incubated with EBV stock. The GFP-encoding EBV stock contains $1x10^6$ green Raji units (GRUs) per mL. The mixture was added to HEK293 cells supplemented with fetal calf serum, mixed and plated in single wells of a multiwell cluster plate. Cells were trypsinized, resuspended and analyzed by flow cytometry and a high throughput sample unit. GFP positive cells were gated and the mean percentage of GFP positive cells in controls (about 15%) free of hybridoma cell culture supernatant or purified monoclonal antibodies was set to 100% for normalization.

[0058] "Epstein-Barr Virus", "EBV", "human herpesvirus 4" and "HHV-4" interchangeably refer to an oncogenic human herpesvirus. EBV is the cause of, inter alia, acute infectious mononucleosis (AIM or IM, also known as glandular fever). It is also associated with particular forms of cancer, such as Hodgkin's lymphoma. Burkitt's lymphoma, nasopharyngeal carcinoma, and conditions associated with human immunodeficiency virus (HIV), such as hairy leukoplakia and central nervous system lymphomas. EBV infects B cells of the immune system and epithelial cells. Once the virus's initial lytic infection is brought under control, EBV latently

persists in the individual's B cells for the rest of the individual's life due to a complex life cycle that includes alternate latent and lytic phases.

[0059] Beside the above described medical condition caused by EBV, patients with primary or secondary immune defects like transplant recipients are at particular risk for EBV-associated diseases as a consequence of the detrimental effect of immunosuppressive agents on the immune-control of EBV-infected B-cells. EBV-associated PTLD is an important form of post-transplant complications, occurring in up to 20% of organ recipients (Everly et al., 2007; Taylor et al., 2005). Importantly, immunocompromised transplant recipients who are immunologically naïve for EBV at the onset of immunosuppression are at a particular high risk of developing life-threatening EBV+ post-transplant lymphoproliferative disease (PTLD) due to a primary EBV infection, e.g. often caused after transplantation via transmission of the virus through a donor organ due to the high prevalence of EBV. Due to impaired T-cell immunity that results from exposure to immunosuppressive drugs, these patients are unable to effectively prime EBV-specific T-cells that play a critical role in controlling proliferation of EBV-infected B-cells. In contrast, patients who are EBV-seropositive at transplant have a much lower risk for developing PTLD, demonstrating the essential role of EBV-specific T-cells poised to eliminate virally infected cells. In general, patients who are EBV-seronegative before transplantation are at a much higher risk to develop EBV-associated diseases, since transmission of donor EBV in transplanted organs or natural infection with the virus causes lymphoproliferative disease in EBV-seronegative recipients after transplantation (e.g. Mendoza et al., 2006; Swerdlow et al., 2000). As with many virus-associated diseases a promising approach for preventing and/or treating virus infection and its consequences in the host is vaccination, which is also true in the case of reducing the high risk of PTLD in seronegative patients by immunizing them to EBV prior to the transplantation.

[0060] Despite causing acute disease and being implicated in the pathogenesis of life threatening illnesses, effective methods to reduce the infectivity of EBV have still not been brought to clinical application after decades of intense research. The present inventors have previously disclosed an Epstein-Barr-Virus Vaccine (WO 2012/025603) that comprised a virus-like particle obtainable by transfecting a human cell with a modified EBV genome that was packaging-deficient, replication deficient, and unable to transform B-cells. This was produced for use in a method for eliciting a therapeutic or prophylactic EBV-specific CD8+ cellular immune response and a humoral immune response in a human individual. The present invention brings further improvement and refinement to this discovery by defining the role of a specific EBV glycoprotein, BILF2, in eliciting and mediating adaptive, antibody-mediated immune responses, and confirming that immunization with BILF2 is sufficient to induce a long-lasting adaptive immune response that can limit the infectivity of BILF2 in epithelial cells.

[0061] The composition of the present invention comprises the BILF2 glycoprotein of Epstein-Barr-Virus (EBV), a nucleic acid encoding the BILF2 glycoprotein (gp75/55) of EBV, or an extracellular vesicle (EV) or EBV-virus-like particle (VLP) displaying at its surface the BILF2 glycoprotein of EBV.

[0062] "BILF2" when used herein refers to the BILF2 protein from EBV. Said term may, however, also refer to the nucleic acid (comprising a nucleotide sequence) encoding the BILF2 protein.

[0063] Commonly, proteins or polypeptides (including fragments thereof, preferably biologically active fragments, and peptides, usually having less than 30 amino acids) comprise one or more amino acids coupled to each other via a covalent peptide bond (resulting in a chain of amino acids). The term "polypeptide" as used herein describes a group of molecules, which consist of more than 30 amino acids. Polypeptides may further form multimers such as dimers, trimers and higher oligomers, i.e., consisting of more than one polypeptide molecule. Polypeptide molecules forming such dimers, trimers etc. may be identical or non-identical. The corresponding higher order structures of such multimers are, consequently, termed homo- or heterodimers, homo- or heterotrimers etc.. An example for a heteromultimer is an antibody molecule, which, in its naturally occurring form in humans, consists of two identical light polypeptide chains and two identical heavy polypeptide chains. The terms "polypeptide" and "protein" also refer to naturally modified polypeptides/proteins, wherein the modification is effected e.g. by post-translational modifications like glycosylation, acetylation, phosphorylation and the like. A "polypeptide" when referred to herein may also be chemically modified such as pegylated. Such modifications are well known in the art.

[0064] Commonly, the term "nucleic acid" is well known to the skilled person and encompasses DNA (such as cDNA) and RNA (such as mRNA). The nucleic acid can be double stranded and single stranded, linear and circular. Said nucleic acid molecule is preferably comprised in a vector which may preferably be comprised in a host cell, e.g. for its propagation.

[0065] BILF2 is a single pass type 1 membrane glycoprotein and, thus, sometimes the term "BILF2 glycoprotein" may be used. Nothing has been published about BILF2 since its original description as 'gp78/55' found in the virion, which has a protein backbone with a mass of around 28 kDa and a large component of N- or O-linked glycans (Mackett et al., 1990). BILF2 was first characterized in 1990 (Mackett et al., 1990), whose role in EBV remains poorly understood. BILF2 is an envelope glycoprotein encoded by a late lytic gene termed BILF2 (hence the name of the glycoprotein). It has been proven that BILF2 derived small peptides can be identified on the surface of EBV-infected cells by CD8+ T cells from patients with infectious mononucleosis (IM) (Taylor et al., 2015, Pudney et al., 2005). IgG antibodies against

BILF2 were detected in 73% of children with endemic Burkitt's lymphoma (BL), a B-cell non-Hodgkin's lymphoma that is almost 100% EBV positive (Coghill et al., 2020).

[0066] A preferred BILF2 protein which is encompassed by the term “BILF2” has the amino acid sequence shown in SEQ ID NO: 25. “BILF2”, however, does not only encompass the preferred amino acid sequence shown in SEQ ID NO: 25. It may also encompass variants thereof which have an identity of at least 60%, 70%, 80%, 90% or more with the amino acid sequence shown in SEQ ID NO: 25. It may also encompass fragments thereof. Such fragments have a length of at least 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230 or 240 amino acids of the amino acid sequence shown in SEQ ID NO: 25.

[0067] A preferred BILF2 nucleotide sequence is shown in SEQ ID NO: 26 or 27, respectively. A BILF2 nucleotide sequence, however, does not only encompass the preferred nucleotide sequence shown in SEQ ID NO: 26 or 27. It may also encompass variants thereof which have an identity of at least 60%, 70%, 80%, 90% or more with the nucleotide sequence shown in SEQ ID NO: 26 or 27. It may also encompass fragments thereof. Such fragments have a length of at least 150, 180, 210, 240, 270, 300, 330, 360, 390, 420, 450, 480, 510, 540, 570, 600, 630, 660, 690 or 720 nucleotides of the nucleotide sequence shown in SEQ ID NO: 26 or 27.

[0068] The term “identity” refers to a relationship between the sequences of two or more polypeptides (e.g. antibodies or antigens, such as BILF2) or polynucleotides (nucleotide sequences or nucleic acids), as determined by comparing the sequences. Identity also refers to the degree of sequence relatedness between or among sequences as determined by the number of matches between strings of two or more amino acid residues or nucleic acid residues. Identity measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (e.g., “algorithms”). Identity of related antigens or nucleic acids can be readily calculated by known methods. “Percent (%) identity” as it applies to polypeptide or polynucleotide sequences is defined as the percentage of residues (amino acid residues or nucleic acid residues) in the candidate amino acid or nucleic acid sequence that are identical with the residues in the amino acid sequence or nucleic acid sequence of a second sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent identity. Methods and computer programs for the alignment are well known in the art. It is understood that identity depends on a calculation of percent identity but may differ in value due to gaps and penalties introduced in the calculation. Generally, variants of a particular polynucleotide or polypeptide (e.g., antigen) have at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% but less than 100% sequence identity to that particular reference polynucleotide or polypeptide as determined by sequence alignment programs and parameters described herein and known to those skilled in the art. Such tools for alignment include those of the BLAST suite (Stephen F.

Altschul, et al. (1997), "Gapped BLAST and PSTBLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402). Another popular local alignment technique is based on the Smith-Waterman algorithm (Smith, T.F. & Waterman, M.S. (1981).

[0069] For example, an appropriate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, (1981), Advances in Applied Mathematics 2:482-489. This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff, Atlas of Protein Sequences and Structure, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov (1986), NucL Acids Res. 14(6):6745-6763. An exemplary implementation of this algorithm to determine percent identity of a sequence is provided by the Genetics Computer Group (Madison, Wis.) in the "BestFit" utility application. The default parameters for this method are described in the Wisconsin Sequence Analysis Package Program Manual, Version 8 (1995) (available from Genetics Computer Group, Madison, Wis.). A preferred method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, Calif). From this suite of packages the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated the "Match" value reflects "sequence identity". Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, for example, another alignment program is BLAST, used with default parameters. For example, BLASTN and BLASTP can be used using the following default parameters: genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+ GenBank CDS translations+Swiss protein+Spupdate+PIR. Details of these programs can be found at the following internet address: https://blast.ncbi.nlm.nih.gov/.

[0070] In the present invention, BILF2 may be presented to the immune system on the surface of an extracellular vesicle (EV) or virus-like particle (VLP). The term “extracellular vesicle”, as used in the present invention, refers to lipid bilayer-delimited particles that range from between 20 to 10000 nanometres and are formed by secretion from cells into the extracellular space. The term “extracellular vesicles” as used herein may also mean “nanoparticles”. Thus, it is also encompassed by the present invention that the EVs are nanoparticles and *vice versa*. Therefore, in the context of the present invention, extracellular vesicle (EV) or EB-virus-like particle (VLP) displaying at its surface the BILF2 glycoprotein of EBV may also be nanoparticles, such as EVs, carrying the BILF2 glycoprotein. The term “virus-like particle” (or “particle”) as used in the present invention relates to a particulate conglomerate of EBV

polypeptides and membrane lipids while being devoid of EBV DNA, and is thereby replication deficient. VLPs in the context of the present invention may be subsumed under the term “nanoparticles”.

[0071] Preferably, an EV or VLP expresses BILF2 alone or in combination with one or more other EBV glycoproteins. Said other EBV glycoproteins may comprise gp350, gB, gp42, gH, gL, gM, gN, BMRF2, BDLF2, BDLF3, BILF1, and BARF1.

[0072] Said VLP may further comprise one or more EBV structural proteins. Said one or more EBV structural proteins include fusion (F), matrix (M), or nucleocapsid (N).

[0073] The composition of the present invention, particularly if BILF2 is presented via EVs or VLPs, may in addition also elicit a cellular immune response including a CD4+ and/or CD8+ T cell response. The term “CD4+ or CD8+ T cell response” refers to a T cell immune response that is characterized by observing a high proportion of immunogen specific CD4+ T cells or CD8+ T cells within the population of total responding T cells following vaccination. The total immunogen-specific T cell response can be determined by, e.g., an IFN-gamma ELISPOT assay. The immunogen-specific CD4+ or CD8+ T cell immune response can be determined, e.g., by an intracellular cytokine staining (ICS) assay (Horton et al. (2007), J Immuol Methods 323(1): 39-54).

[0074] The particle is "devoid of EBV DNA", which means the particle does not comprise EBV DNA, i.e., no EBV DNA can be detected. Specifically, the term "DNA", in accordance with the present invention, includes any DNA, such as cDNA or genomic DNA. Further included by the term "DNA" as used in this context are DNA mimicking molecules known in the art such as synthetic or semisynthetic derivatives of DNA and mixed polymers, both sense and antisense strands. Such DNA mimicking molecules or DNA derivatives according to the invention include phosphorothioate nucleic acid. Accordingly, the particle must not comprise DNA sequences that are identical to EBV DNA sequences, wherein said sequences preferably relate to EBV gene sequences. Furthermore, the particle must not comprise nucleic acid sequences that share at least a (for each value) 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80% and at least 75% sequence identity to a wildtype EBV nucleic acid sequence. The degree of sequence identity of nucleic acid sequences can be calculated by well-known methods by the person skilled in the art and may comprise the automatic execution of algorithms effecting the alignment of sequence data and calculation of sequence homologies. Also, the particle may not comprise a DNA sequence that upon expression generates a polypeptide that functionally resembles an EBV polypeptide, wherein the functional resemblance preferably concerns B-cell transformation capacity. Methods to test functional similarity of polypeptides include in silico as well as in vitro, ex vivo and in vivo tests that are well-known to the skilled person in the art. For example, to determine functional resemblance one can generate deletion mutants of EBV

and perform complementation analysis. Specifically, a deletion mutant of EBV is characterised in that the sequence of a gene that encodes, e.g. a polypeptide that is essential in B-cell transformation, is deleted. As a result, said deletion mutant is not capable of transforming B-cells. To test the functional resemblance of a DNA sequence to the deleted EBV sequence, said DNA to be tested is supplied to the deletion mutant, e.g., by incorporation of said DNA to be tested into the genomic DNA of said deletion mutant. Subsequently, it is determined whether the thus modified deletion mutant is capable of transforming B-cells, i.e., complements the deletion and results in an EBV that functionally resembles a wildtype EBV. Variations of said complementation analysis are known in the art and can further be adapted to the specific needs of the skilled person on the basis of his technical knowledge in the field.

[0075] EVs and VLPs, in the present invention, may be used in order to expose the immune system to BILF2 and thereby are intended to be used in a method of eliciting antibodies. In the context of the present invention, “a method of eliciting antibodies” refers to a protocol or procedure intended to stimulate the immune system to produce specific antibodies against an antigen of interest in a living organism. By eliciting the production of antibodies, and subsequent retention of the capacity to produce said antibodies during re-exposure to the relevant antigen via persistent memory B cell generation, such a method is anticipated to result in adaptive immunity that diminishes the infectivity and/or virulence of a relevant pathogen – here EBV.

[0076] In the present invention, BILF2 may also be presented to the immune system via a composition that comprises a nucleic acid encoding the BILF2 glycoprotein of EBV. Said nucleic acid may be DNA or RNA. Said DNA or RNA has an open reading frame encoding BILF2. In some embodiments, the RNA (e.g., mRNA) further comprises a (at least one) 5' UTR, 3' UTR, a poly-A tail and/or a 5' cap.

[0077] Nucleic acids comprise a polymer of nucleotides (nucleotide monomers), also referred to as polynucleotides. Nucleic acids may be or may include, for example, deoxyribonucleic acids (DNAs), ribonucleic acids (RNAs), threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs, including LNA having a b-D-ribo-configuration, a-LNA having an a-L-ribo-configuration (a diastereomer of LNA), 2'-amino-LNA having a 2'-amino-functionalization, and 2'-amino-a-LNA having a 2'-amino-functionalization), ethylene nucleic acids (ENA), cyclohexenyl nucleic acids (CeNA) and/or chimeras and/or combinations thereof.

[0078] Messenger RNA (mRNA) is any ribonucleic acid that encodes a (at least one) protein (a naturally-occurring, non-naturally-occurring, or modified polymer of amino acids) and can be translated to produce the encoded protein in vitro, in vivo, in situ or ex vivo. In the context of the present invention such a protein is BILF2. The skilled artisan will appreciate that, except

where otherwise noted, nucleic acid sequences set forth in the instant application may recite "T"s in a representative DNA sequence, but where the sequence represents RNA (e.g., mRNA), the "T"s would be substituted for "U"s. Thus, any of the DNAs disclosed and identified by a particular sequence identification number herein also disclose the corresponding RNA (e.g., mRNA) sequence complementary to the DNA, where each "T" of the DNA sequence is substituted with "U."

[0079] An open reading frame (ORF) is a continuous stretch of DNA or RNA beginning with a start codon (e.g., methionine (ATG or AUG)) and ending with a stop codon (e.g., TAA, TAG or TGA, or UAA, UAG or UGA). An ORF typically encodes a protein. It will be understood that the sequences disclosed herein may further comprise additional elements, e.g., 5' and 3' UTRs, but that those elements, unlike the ORF, need not necessarily be present in a vaccine of the present disclosure.

[0080] In some embodiments, a nucleic acid encoding BILF2 is codon optimized, e.g. the nucleotide sequence shown in SEQ ID NO: 27. Codon optimization methods are known in the art. Codon optimization, in some embodiments, may be used to match codon frequencies in target and host organisms to ensure proper folding; bias GC content to increase mRNA stability or reduce secondary structures; minimize tandem repeat codons or base runs that may impair gene construction or expression; customize transcriptional and translational control regions; insert or remove protein trafficking sequences; remove/add post translation modification sites in encoded protein (e.g., glycosylation sites); add, remove or shuffle protein domains; insert or delete restriction sites; modify ribosome binding sites and mRNA degradation sites; adjust translational rates to allow the various domains of the protein to fold properly; or reduce or eliminate problematic secondary structures within the polynucleotide. Codon optimization tools, algorithms and services are known in the art - non-limiting examples include services from GeneArt (Life Technologies), DNA2.0 (Menlo Park CA) and/or proprietary methods. In some embodiments, the open reading frame (ORF) sequence is optimized using optimization algorithms.

[0081] In some embodiments, a codon optimized sequence shares less than 95% sequence identity to a naturally-occurring or wild-type sequence ORF (e.g., a naturally-occurring or wild-type mRNA sequence encoding BILF2). In some embodiments, a codon optimized sequence shares less than 90% sequence identity to a naturally-occurring or wild-type sequence (e.g., a naturally-occurring or wild-type mRNA sequence encoding BILF2). In some embodiments, a codon optimized sequence shares less than 85% sequence identity to a naturally-occurring or wild-type sequence (e.g., a naturally-occurring or wild-type mRNA sequence encoding BILF2). In some embodiments, a codon optimized sequence shares less than 80% sequence identity to a naturally-occurring or wild-type sequence (e.g., a naturally-occurring or wild-type mRNA sequence encoding BILF2). In some embodiments, a codon optimized sequence shares less

than 75% sequence identity to a naturally-occurring or wild-type sequence (e.g., a naturally-occurring or wild-type mRNA sequence encoding BILF2).

[0082] In some embodiments, a codon optimized sequence shares between 65% and 85% (e.g., between about 67% and about 85% or between about 67% and about 80%) sequence identity to a naturally-occurring or wild-type sequence (e.g., a naturally-occurring or wild-type mRNA sequence encoding BILF2). In some embodiments, a codon optimized sequence shares between 65% and 75% or about 80% sequence identity to a naturally-occurring or wild-type sequence (e.g., a naturally-occurring or wild-type mRNA sequence encoding BILF2).

[0083] In some embodiments, a codon-optimized sequence encodes BILF2 that is as immunogenic as, or more immunogenic than (e.g., at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 100%, or at least 200%, or more), than BILF2 encoded by a non-codon-optimized sequence.

[0084] When transfected into mammalian host cells, the modified mRNAs have a stability of between 12-18 hours, or greater than 18 hours, e.g., 24, 36, 48, 60, 72, or greater than 72 hours and are capable of being expressed by the mammalian host cells.

[0085] In some embodiments, a codon optimized RNA may be one in which the levels of G/C are enhanced. The G/C-content of nucleic acid molecules (e.g., mRNA) may influence the stability of the RNA. RNA having an increased amount of guanine (G) and/or cytosine (C) residues may be functionally more stable than RNA containing a large amount of adenine (A) and thymine (T) or uracil (U) nucleotides.

[0086] As described herein, a nucleic acid encoding BILF2 is a DNA which may be comprised by a vector. A vector can be a circular plasmid or a linear nucleic acid. The circular plasmid and linear nucleic acid are capable of directing expression of a particular nucleotide sequence – in the context of the present invention the nucleic acid comprised by a vector is DNA - in an appropriate cell. The vector comprising the nucleic acid construct may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components.

[0087] In the present invention, the method of eliciting antibodies against BILF2 reduces the infectivity of EBV for human epithelial cells. This is highly relevant to transmission and initial infection of EBV in humans, which commonly occurs via salivary transmission and the resultant infection of epithelial cells of the oropharynx from where infection of B cells can then occur (Houene et al., 2021).

[0088] Besides comprising the composition described above, a composition in accordance with the invention may further comprise pharmaceutically acceptable carriers which include

any carrier that does not itself elicit an immune response or any other adverse reaction harmful to the individual receiving the composition.

[0089] The term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" means solvents, dispersion media, coatings, antibacterial agents and antifungal agents, isotonic agents, and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. In certain embodiments, the pharmaceutically acceptable carrier or excipient is not naturally occurring.

[0090] Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and lipid aggregates such as, e.g. oil droplets or liposomes. Further suitable pharmaceutically acceptable carriers are well-known in the art. Additionally, said carriers may function as further immunostimulating agents which will be described in more detail below. Further, the composition may comprise diluents such as, e.g. water, saline, glycerol, ethanol etc.. Furthermore, substances necessary for formulation purposes may be comprised in a vaccine such as emulsifying agents and/or pH buffering substances. Any combination of the above-mentioned substances may be part of a composition in accordance with the invention as needed.

[0091] In some embodiments of the present invention, the described composition shall be administered along with one or more adjuvants. As used herein, “adjuvant” means a substance that increases or modulates the immune response of a subject as such. Adjuvants are often used to modify or augment the effects of a vaccine by stimulating the immune system to respond to the vaccine more vigorously, and thus providing increased immunity to a particular disease. Some adjuvants accomplish this task by mimicking specific sets of evolutionarily conserved molecules, so called pathogen-associated molecular patterns, which include liposomes, lipopolysaccharide, molecular cages for antigens, components of bacterial cell walls, and endocytosed nucleic acids such as RNA, double-stranded RNA, single-stranded DNA, and unmethylated CpG dinucleotide-containing DNA. In this context, an adjuvant is used to enhance an immune response to the plasmid and/or EBV vectors as described herein and in the context of the present invention.

[0092] In some embodiments of the present invention, the composition as described herein is for use in a method of treatment or prevention of a disease, preferably wherein the disease is cancer, preferably a cancer of epithelial origin, or an infection, preferably an EBV infection, most preferably an EBV infection of epithelial cells.

[0093] Preferably, the composition as described herein shall be used in a method of treatment or prevention of a disease, wherein the disease is cancer and the cancer is selected from the

group consisting of meduloblastoma, retinoblastoma, Burkitt's lymphoma, Hodgkin´s lymphoma, oral cancer, skin cancer, basaliom, acute myeloid leukemia, pancreatic cancer, colorectal cancer, endometrial cancer, biliary tract cancer, liver cancer, myeloma, multiple myeloma, prostate cancer, stomach cancer, kidney cancer, bone cancer, soft tissue cancer, head and neck cancer, glioblastoma multiforme, astrocytoma, melanoma, lung cancer, esophageal cancer, gastric cancer, breast cancer, ovarian cancer, mesothelioma cancer, bladder cancer, anal cancer, chondrosarcoma cancer, osteosarcoma cancer, sarcoma cancer, primitive neuroectodermal cancer (primitive neuroectodermal tumor (PNET)), and combinations thereof.

[0094] The term "preventing" when used in the context of a disease or disease condition means prophylactic administration of a composition that stops or otherwise delays the onset of a pathological hallmark or symptom of a disease or disorder.

[0095] The term "treating" when used in the context of a disease or disease condition means ameliorating, improving or remedying a disease, disorder, or symptom of a disease or condition associated with the disease, or may mean completely or partially stopping, on a molecular level, the biochemical basis of the disease, such as halting replication of a virus, etc..

[0096] The present invention further provides an antibody directed against the BILF2 glycoprotein of EBV, wherein said antibody binds to an epitope within amino acids 41 to 160 of SEQ ID NO: 25 and is capable of reducing the infectivity of EBV for human epithelial cells. Such an antibody may be obtainable (or may be obtained) by immunizing, e.g. rodents with a composition of the present invention; see also Examples 4 to 6. The present invention further provides said antibody for use in a method for passive immunization against EBV, and/or for use in a method for reducing the infectivity of EBV for human epithelial cells, and/or for use in a method for preventing infection of human epithelial cells by EBV.

[0097] Preferably, said antibody binds to an epitope within amino acids 80 to 160 or 129 to 143 of SEQ ID NO: 25 and is capable of reducing the infectivity of EBV for human epithelial cells.

[0098] Preferably, for an antibody of the present invention said epitope is determined by pepspot analysis and/or deletion analysis of the ectodomain of BILF2 as shown in SEQ ID NO: 25. The ectodomain of BILF2 encompasses amino acids 18 to 212 of SEQ ID NO: 25.

[0099] An "epitope" includes any determinant capable of being bound by an antibody. An epitope is a region of a polypeptide that is bound by an antibody that targets that region of a polypeptide. Such a region of a polypeptide includes specific residues that directly contact the antibody. Among the specific residues are amino acids, carbohydrate chains hooked on amino acids, or both. In particular embodiments, an "epitope" denotes the binding site of an antibody on a polypeptide. The antibody either binds to a linear epitope, (e.g., an epitope including a

stretch of 5 to 15 consecutive amino acids), or the antibody binds to a three-dimensional structure formed by the spatial arrangement of several short stretches of the polypeptide target. Three-dimensional epitopes recognized by an antibody, e.g., by the epitope recognition site or paratope of an antibody, can be thought of as three-dimensional surface features of an epitope molecule. These features fit precisely (in)to the corresponding binding site of the antibody and thereby binding between the antibody and its target polypeptide is facilitated. In particular embodiments, an epitope can be considered to have two levels: (i) the "covered patch" which can be thought of as the shadow an antibody would cast; and (ii) the individual participating side chains and backbone residues. Binding is then due to the aggregate of ionic interactions, hydrogen bonds, and hydrophobic interactions.

[0100] In order to define the epitope targeted by an antibody, the epitope must be determined. Appropriate methods to define said epitope includes pepspot analysis and/or deletion analysis.

[0101] The peptide scan (pepspot assay) is routinely employed to map linear epitopes in a polypeptide antigen. The primary sequence of the polypeptide is synthesized successively on activated cellulose with peptides overlapping one another. The recognition of certain peptides by the antibody to be tested for its ability to detect or recognize a specific antigen/epitope is scored by routine colour development (secondary antibody with, e.g. horseradish peroxidase and 4-chloronaphthol and hydrogenperoxide), by a chemoluminescence reaction or similar means known in the art. In the case of, inter alia, chemoluminescence reactions, the reaction can be quantified. If the antibody reacts with a certain set of overlapping peptides one can deduce the minimum sequence of amino acids that are necessary for reaction; see illustrative Example 5 and Table 2.

[0102] The same assay can reveal two distant clusters of reactive peptides, which indicate the recognition of a discontinuous, i.e. conformational epitope in the antigenic polypeptide.

[0103] Deletion analysis is done by individual deletions of parts of a polypeptide. The resulting deletion variants are subsequently tested whether or not antibodies bind to said deletion variants. In case of non-binding the deleted amino acids may be part of the epitope.

[0104] Preferably, an antibody of the present invention is

(a) an antibody comprising a heavy chain variable region having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence shown in SEQ ID NO: 7 and a light chain variable region having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence shown in SEQ ID NO: 8;

(b) an antibody comprising a heavy chain variable region having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence shown in SEQ ID

NO: 15 and a light chain variable region having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence shown in SEQ ID NO: 16;

(c) an antibody comprising a heavy chain variable region having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence shown in SEQ ID NO: 23 and a light chain variable region having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence shown in SEQ ID NO: 24; or

(d) an antibody binding to the same epitope as that in the EBV BILF2 protein shown in SEQ ID NO: 25 to which any one of the antibodies (a) to (c) bind.

[0105] Preferably, an antibody of the present invention is

(a) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 1, heavy chain CDR2 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 2, and heavy chain CDR3 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 3, and

a light chain variable region comprising light chain CDR1 having an amino acid sequence with at least 80%, 90% or 95%% identity to the amino acid sequence as set forth in SEQ ID NO: 4, light chain CDR2 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 5, and light chain CDR3 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 6;

(b) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 9, heavy chain CDR2 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 10, and heavy chain CDR3 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 11, and

a light chain variable region comprising light chain CDR1 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 12, light chain CDR2 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 13, and light chain CDR3 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 14;

(c) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 17, heavy chain CDR2 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 18, and heavy chain CDR3 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 19, and

a light chain variable region comprising light chain CDR1 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 20, light chain CDR2 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 21, and light chain CDR3 having an amino acid sequence with at least 80%, 90% or 95% identity to the amino acid sequence as set forth in SEQ ID NO: 22; or

(d) an antibody binding to the same epitope as that in the EBV BILF2 protein shown in SEQ ID NO: 25 to which any one of the antibodies (a) to (c) bind.

[0106] Preferably, an antibody of the present invention is

(a) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 1, heavy chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 2, and heavy chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 3, and

a light chain variable region comprising light chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 4, light chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 5, and light chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 6;

(b) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 9, heavy chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 10, and heavy chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 11, and

a light chain variable region comprising light chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 12, light chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 13, and light chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 14;

(c) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 17, heavy chain CDR2

having the amino acid sequence as set forth in SEQ ID NO: 18, and heavy chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 19, and

a light chain variable region comprising light chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 20, light chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 21, and light chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 22; or

(d) an antibody binding to the same epitope as that in the EBV BILF2 protein shown in SEQ ID NO: 25 to which any one of the antibodies (a) to (c) bind.

[0107] An antibody binds "the same epitope" as a reference antibody, e.g. an antibody as defined herein in items (a) to (c), when the two antibodies recognize identical or sterically overlapping epitopes. The most widely used and rapid methods for determining whether two epitopes bind to identical or sterically overlapping epitopes are competition assays, which can be configured in all number of different formats, using either labeled antigen or labeled antibody.

[0108] Amino acid sequence modifications of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibodies of the present invention are prepared by introducing appropriate nucleotide changes into the nucleic acid encoding such antibodies, or by peptide synthesis.

[0109] Such modifications include, for example, deletions from, and/or insertions into, and/or substitutions of, residues within the amino acid sequences of the antibodies. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antibodies, such as changing the number or position of glycosylation sites. Preferably, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids may be substituted in a CDR, while 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 25 amino acids may be substituted in the framework regions (FRs). The substitutions are preferably conservative substitutions. Additionally or alternatively, 1, 2, 3, 4, 5, or 6 amino acids may be inserted or deleted in each of the CDRs (of course, dependent on their length), while 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 25 amino acids may be inserted or deleted in each of the FRs.

[0110] Another type of variant is an amino acid substitution variant. These variants have preferably at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid residues in the antibody replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the CDRs of the heavy and/or light chain, in particular the hypervariable regions, but FR alterations in the heavy and/or light chain are also contemplated.

[0111] For example, if a CDR sequence encompasses 6 amino acids, it is envisaged that one, two or three of these amino acids are substituted. Similarly, if a CDR sequence encompasses 15 amino acids it is envisaged that one, two, three, four, five or six of these amino acids are substituted.

[0112] Generally, if amino acids are substituted in one or more or all of the CDRs of the heavy and/or light chain, it is preferred that the then-obtained "substituted" sequence is at least 80%, 90% or 95%identical to the "original" CDR sequence. This means that it is dependent of the length of the CDR to which degree it is identical to the "substituted" sequence. For example, a CDR having 5 amino acids is preferably 80% identical to its substituted sequence in order to have at least one amino acid substituted. Accordingly, the CDRs of the antibody may have different degrees of identity to their substituted sequences, e.g., CDRL1 may have 80%, while CDRL3 may have 90%.

[0113] Preferred substitutions (or replacements) are conservative substitutions. However, any substitution is envisaged as long as the antibody retains its capability to bind to BILF2 and/or its CDRs have an identity to the then substituted sequence (at least 80%, 85% or 90% to the "original" CDR sequence).

[0114] Substantial modifications in the biological properties of the antibodies of the present invention are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties: (1) hydrophobic: norleucine, met, ala, val, leu, ile; (2) neutral hydrophilic: cys, ser, thr; (3) acidic: asp, glu; (4) basic: asn, gln, his, lys, arg; (5) residues that influence chain orientation: gly, pro; and (6) aromatic: trp, tyr, phe.

[0115] Non-conservative substitutions may entail exchanging a member of one of these classes for another class. Any cysteine residue not involved in maintaining the proper conformation of the antibody may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability.

[0116] A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further development may have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e.g. 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibody variants thus

generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g. binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and BILF2. Such contact residues and neighbouring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

[0117] Other modifications of the antibodies are contemplated herein. For example, the antibody may be linked to one of a variety of non-proteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The antibody may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatine-microcapsules and poly(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules), or in macroemulsions.

[0118] The term “antibody” in its various grammatical forms is used herein in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, monomers, dimers, multimers, multispecific antibodies (e.g., bispecific antibodies), heavy chain-only antibodies, three chain antibodies, TCAs, single chain Fv (scFv), nanobodies, etc., and also includes antibody fragments, such as Fab or $F(ab)_2$ so long as they exhibit the desired biological activity. Antibodies may be murine, human, humanized, chimeric, or derived from other species. Antibodies of the present invention are preferably monoclonal antibodies.

[0119] The term antibody may reference a full-length heavy chain, a full length light chain, an intact immunoglobulin molecule; or an immunologically active portion of any of these polypeptides, i.e., a polypeptide that comprises an antigen binding site that immunospecifically binds an antigen of a target of interest or part thereof, such targets including, but not limited to, cancer cell or cells that produce autoimmune antibodies associated with an autoimmune disease. The immunoglobulin disclosed herein can be of any type (e.g., IgG, IgE, IgM, IgD, and IgA), class (e.g., IgGl, IgG2, IgG3, IgG4, IgAl and IgA2) or subclass of immunoglobulin molecule, including engineered subclasses with altered Fc portions that provide for reduced or enhanced effector cell activity. Light chains of the subject antibodies can be kappa light chains

(V kappa) or lambda light chains (V lambda). The immunoglobulins can be derived from any species.

[0120] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. Monoclonal antibodies in accordance with the present invention can be made by the hybridoma method and can also be made via recombinant protein production methods.

[0121] The term “variable”, as used in connection with antibodies, refers to the fact that certain portions of the antibody variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FRs). The variable domains of native heavy and light chains each comprise four FRs, largely adopting a [3-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the P-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

[0122] The term “hypervariable region” when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a “complementarity determining region” or “CDR” (e.g., residues 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a “hypervariable loop” residues 26-32 (Hl), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). In some embodiments, “CDR” means a complementary determining region of an antibody. “Framework Region” or “FR” residues are

those variable domain residues other than the hypervariable region/CDR residues as herein defined.

[0123] Antibody residues herein are numbered according to the Kabat numbering system and the EU numbering system. The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-113 of the heavy chain) (e.g., Kabat et al., Sequences of Immunological Interest. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The "EU numbering system" or "EU index" is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat et al., supra). The "EU index as in Kabat" refers to the residue numbering of the human IgG EU antibody. Unless stated otherwise herein, references to residue numbers in the variable domain of antibodies mean residue numbering by the Kabat numbering system. Unless stated otherwise herein, references to residue numbers in the constant domain of antibodies mean residue numbering by the EU numbering system.

[0124] Preferably, an antibody of the present invention or an antibody elicited by a composition of the present invention is a "neutralizing antibody". A neutralizing antibody is an antibody that, upon binding to its epitope within BILF2, e.g., an epitope as described herein, can reduce infectivity of EBV for human epithelial cells, preferably these antibodies prevent infection of human epithelial cells by EBV. Neutralizing antibodies are also useful as protein therapeutics to prevent or treat viral infection. Neutralizing antibodies against EBV typically function by blocking a virus from entering a cell. To enter a cell, EBV first attaches to the cell surface through an interaction between a protein on the surface of the virus and a receptor binding site of a cell surface protein. Following this attachment, the virus membrane can fuse with the cell membrane, allowing the contents of the virus to be inserted into the cell. Viral fusion also occurs through interaction of a viral protein with an epitope on an antigen of a cell protein. The interactions resulting in viral attachment to a cell and the interactions resulting in viral fusion to a cell are distinct, each involving different viral proteins and different cellular proteins. Thus, neutralizing antibodies could block EBV entry into cells by preventing virus/cell protein interactions leading to attachment and/or fusion.

[0125] An antibody of the present invention which is directed against BILF2 is preferably specifically interacting with (or specifically binding to) BILF2. As used herein, the terms "specifically interacting", "specifically binding" or "specifically bind(s)" mean that a binding domain exhibits appreciable affinity for BILF2 and, generally, does not exhibit significant reactivity with proteins or antigens other than BILF2. "Appreciable affinity" includes binding with an affinity of about 10^{-6} M (KD) or stronger. Preferably, binding is considered specific when binding affinity is about 10^{-9} to 10^{-6} M, preferably 10^{-9} to 10^{-7} M or 10^{-9} to 10^{-8} M; see also Table 2. Accordingly, an antibody of the present invention has preferably a KD of about 10^{-11} to 10^{-10} M, of about 10^{-10} to 10^{-9} M, of about 10^{-9} to 10^{-8} M or 10^{-8} to 10^{-7} M. Whether an antibody

specifically reacts with or binds to BILF2 can be tested readily by, inter alia, comparing the reaction of said antibody with a target protein or antigen with the reaction of said antibody with proteins or antigens other than BILF2. Preferably, an antibody of the present invention does not essentially bind or is not capable of binding to proteins or antigens other than BILF2. The term “does not essentially bind”, or “is not capable of binding” means that an antibody of the present invention does not bind another protein or antigen other than BILF2, i.e., does not show reactivity of more than 30%, preferably not more than 20%, more preferably not more than 10%, particularly preferably not more than 9%, 8%, 7%, 6% or 5% with proteins or antigens other than BILF2, whereby binding to BILF2, respectively, is set to be 100%.

[0126] Antibodies as described herein can passively transfer immunity and thus protect against EBV infection of human epithelial cells.

[0127] Said antibody may be further used in a method for passive immunization against EBV.

[0128] Preferably, said antibody is for use in a method for passive immunization against EBV.

[0129] Preferably, said antibody is for use in a method for reducing the infectivity of EBV for human epithelial cells.

[0130] Preferably, said antibody is for use in a method for preventing infection of human epithelial cells by EBV.

[0131] The present invention further provides for a method for diagnosing whether a human subject has antibodies which reduce infectivity of EBV for human epithelial cells, comprising determining in a serum sample obtained from said subject whether antibodies compete with any of the antibodies as described herein, in particular antibodies binding to an epitope within amino acids 41 to 160 of BILF2, preferably within amino acids 80 to 160 or 129 to 143 of BILF2, for epitope binding.

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[0132] The present invention may also be summarized in the following items:

(1) A composition comprising
   a) the BILF2 glycoprotein of Epstein-Barr-Virus (EBV),
   b) a nucleic acid encoding the BILF2 glycoprotein of EBV, or
   c) an extracellular vesicle (EV) or EB-virus-like particle (VLP) displaying at its surface the BILF2 glycoprotein of EBV

   for use in a method of eliciting antibodies against said BILF2 glycoprotein which reduce infectivity of EBV for human epithelial cells.

(2) The composition for the use of item 1, wherein said antibodies prevent infection of human epithelial cells by EBV.

(3) The composition of any one of the preceding items, wherein said BILF2 glycoprotein is produced by mammalian cells and isolated therefrom.

(4) The composition of item 1 or 2, wherein said nucleic acid is DNA or RNA.

(5) The composition for the use of item 4, wherein said DNA is comprised by a vector.

(6) The composition of item 1, wherein said EV or VLP expresses BILF2, either alone or in combination with one or more other EBV glycoproteins.

(7) The composition of item 6, wherein said other EBV glycoproteins may comprise gp350, gB, gp42, gH, gL, gM, gN, BMRF2, BDLF2, BDLF3, BILF1, and BARF1.

(8) The composition of item 6 or 7, wherein the VLP further comprises one or more EBV structural proteins.

(9) The composition of any one of items 6 to 8, wherein the one or more EBV structural proteins include fusion (F), matrix (M), or nucleocapsid (N).

(10) The composition of any one of the preceding items, further comprising one or more pharmaceutically acceptable carriers.

(11) The composition for the use of any one of the preceding items, further comprising one or more adjuvants.

(12) The composition of any one of items 1 to 11 for use in a method of treatment or prevention of a disease.

(13) The composition of item 12, wherein the disease is cancer, preferably a cancer of epithelial origin, or an infection, preferably an EBV infection, most preferably an EBV infection of epithelial cells.

(14) The composition of item 12 or 13, wherein the disease is cancer and the cancer is selected from the group consisting of meduloblastoma, retinoblastoma, Burkitt's

lymphoma, Hodgkin´s lymphoma, oral cancer, skin cancer, basaliom, acute myeloid leukemia, pancreatic cancer, colorectal cancer, endometrial cancer, biliary tract cancer, liver cancer, myeloma, multiple myeloma, prostate cancer, stomach cancer, kidney cancer, bone cancer, soft tissue cancer, head and neck cancer, glioblastoma multiforme, astrocytoma, melanoma, lung cancer, esophageal cancer, gastric cancer, breast cancer, ovarian cancer, mesothelioma cancer, bladder cancer, anal cancer, chondrosarcoma cancer, osteosarcoma cancer, sarcoma cancer, primitive neuroectodermal cancer (primitive neuroectodermal tumor (PNET)), and combinations thereof.

(15) An antibody directed against the BILF2 glycoprotein of EBV, wherein said antibody binds to an epitope within amino acids 41 to 160 of SEQ ID NO: 25 and is capable of reducing the infectivity of EBV for human epithelial cells.

(16) The antibody of item 15, wherein said antibody binds to an epitope within amino acids 80 to 160 or 129 to 143 of SEQ ID NO: 25 and is capable of reducing the infectivity of EBV for human epithelial cells.

(17) The antibody of item 15 or 16, wherein said antibody is

(a) an antibody comprising a heavy chain variable region having an amino acid sequence with at least 80% identity to the amino acid sequence shown in SEQ ID NO: 7 and a light chain variable region having an amino acid sequence with at least 80% identity to the amino acid sequence shown in SEQ ID NO: 8;

(b) an antibody comprising a heavy chain variable region having an amino acid sequence with at least 80% identity to the amino acid sequence shown in SEQ ID NO: 15 and a light chain variable region having an amino acid sequence with at least 80% identity to the amino acid sequence shown in SEQ ID NO: 16;

(c) an antibody comprising a heavy chain variable region having an amino acid sequence with at least 80% identity to the amino acid sequence shown in SEQ ID NO: 23 and a light chain variable region having an amino acid sequence with at least 80% identity to the amino acid sequence shown in SEQ ID NO: 24; or

(d) an antibody binding to the same epitope as that in the EBV BILF2 protein shown in SEQ ID NO: 25 to which any one of the antibodies (a) to (c) bind.

(18) The antibody of any one of the items 15 to 17, wherein said antibody is

(a) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 1, heavy chain CDR2 having an amino acid

sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 2, and heavy chain CDR3 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 3, and
a light chain variable region comprising light chain CDR1 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 4, light chain CDR2 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 5, and light chain CDR3 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 6;

(b) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 9, heavy chain CDR2 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 10, and heavy chain CDR3 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 11, and
a light chain variable region comprising light chain CDR1 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 12, light chain CDR2 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 13, and light chain CDR3 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 14;

(c) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 17, heavy chain CDR2 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 18, and heavy chain CDR3 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 19, and
a light chain variable region comprising light chain CDR1 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 20, light chain CDR2 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 21, and light chain CDR3 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 22; or

(d) an antibody binding to the same epitope as that in the EBV BILF2 protein shown in SEQ ID NO: 25 to which any one of the antibodies (a) to (c) bind.

(19) The antibody of any one of the items 15 to 18, wherein said antibody is

(a) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 1, heavy chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 2, and heavy chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 3, and
a light chain variable region comprising light chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 4, light chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 5, and light chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 6;

(b) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 9, heavy chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 10, and heavy chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 11, and
a light chain variable region comprising light chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 12, light chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 13, and light chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 14;

(c) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 17, heavy chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 18, and heavy chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 19, and
a light chain variable region comprising light chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 20, light chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 21, and light chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 22; or

(d) an antibody binding to the same epitope as that in the EBV BILF2 protein shown in SEQ ID NO: 25 to which any one of the antibodies (a) to (c) bind.

(20) The antibody of any one of items 15 to 19, wherein said epitope is determined by pepspot analysis and/or deletion analysis of the ectodomain encompassing amino acids 18 to 212 of BILF2 as shown in SEQ ID NO: 25.

(21) The antibody of any one of items 15 to 20 for use in a method for passive immunization against EBV.

(22) The antibody of any one of items 15 to 21 for use in a method for reducing the infectivity of EBV for human epithelial cells.

(23) The antibody of any one of items 15 to 22 for use in a method for preventing infection of human epithelial cells by EBV.

(24) A method for diagnosing whether a human subject has antibodies which reduce infectivity of EBV for human epithelial cells, comprising determining in a serum sample obtained from said subject whether antibodies compete with any of the antibodies of items 15 to 20 for epitope binding.

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[0117] Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to the specific embodiments of the present invention described herein. Such equivalents are intended to be encompassed by the present invention.

[0118] Unless otherwise stated, the following terms used in this document, including the description and items, have the definitions given below.

[0119] It is to be noted that as used herein, the singular forms "a", "an", and "the", include plural references unless the context clearly indicates otherwise. Thus, for example, reference to "a reagent" includes one or more of such different reagents and reference to "the method" includes reference to equivalent steps and methods known to those of ordinary skill in the art that could be modified or substituted for the methods described herein.

[0120] Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the present invention described herein. Such equivalents are intended to be encompassed by the present invention.

[0121] The term "and/or" wherever used herein includes the meaning of "and", "or" and "all or any other combination of the elements connected by said term".

[0122] The term "about" or "approximately" as used herein means within 20%, preferably within 10%, and more preferably within 5% of a given value or range. It includes, however, also the concrete number, e.g., about 20 includes 20.

[0123] Throughout this specification and the items which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. When used herein the term “comprising” can be substituted with the term “containing” or “including” or sometimes when used herein with the term “having”.

[0124] When used herein “consisting of" excludes any element, step, or ingredient not specified in the item element. When used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the item.

[0125] In each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms.

[0126] It should be understood that this invention is not limited to the particular methodology, protocols, material, reagents, and substances, etc., described herein and as such can vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the items.

[0127] Other embodiments are within the following items. In addition, where features or aspects of the present invention are described in terms of Markush groups, those skilled in the art will recognize that the present invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0128] Of course, whenever herein a composition or antibody is disclosed for use in a method for treatment or the like, this means that such a composition or antibody can also be applied in a method for treatment. For example, such a disclosure is equally suitable for a method of treatment comprising administering such a composition or antibody.

[0129] All publications cited throughout the text of this specification (including all patents, patent applications, scientific publications, manufacturer’s specifications, instructions, etc.) are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such disclosure by virtue of prior invention. To the extent the material incorporated by reference contradicts or is inconsistent with this specification, the specification will supersede any such material.

V. BRIEF DESCRIPTION OF THE FIGURES

[0130] Figure 1 shows BILF2 monoclonal antibodies and control antibodies tested for neutralizing characteristics with EB-VLPs and a human B cell line, Daudi cells. Note that RLU = relative light units; normalized to 100%.

[0131] Figure 2 shows BILF2 monoclonal antibodies and control antibodies tested with 2089 EBV stock on HEK293 cells, a proxy of human epithelial cells, for neutralizing characteristics. Note that % infected HEK293 cells are normalized to 100%.

[0132] Figure 3 shows human sera of EBV-positive individuals with serum antibodies that compete with neutralizing monoclonal antibodies and interfere with binding to their discrete epitopes on the BILF2 glycoprotein.

[0133] Figure 4 shows serum antibodies directed against BILF2 after immunization of BALB/c mice with four different classes of BILF2 antigen (BILF2 protein, BILF2 EVs) or BILF2 encoding nucleic acids (BILF2 DNA, BILF2 mRNA) detected in a sandwich ELISA.

[0134] Figure 5 shows titers of EBV neutralizing antibodies in sera of mice immunized with four different classes of BILF2 antigens (BILF2 protein, BILF2 EVs) or BILF2 encoding nucleic acids (BILF2 DNA, BILF2 mRNA) tested with EBV stocks and HEK293 target cells.

[0135] Figure 6 shows the correlation of individual sera from 28 mice after three immunizations with four different types of BILF2 antigens in two assays. Titers as measured in an BILF2 specific sandwich ELISA (x-axis) versus titers obtained in VNT (y-axis) are plotted. The statistical analysis revealed a Spearman r value of 0.7949 with a 95% confidence interval between 0.5921 to 0.9030, and a two-tailed P value of <0.0001 indicative of a strong correlation of data obtained in both assays.

VI. SEQUENCES

The present invention provides and refers herein to the following sequences:

SEQ ID NO:	Sequence	
1	NYWMH	VH-CDR1 antibody 28H3
2	YINPITGYTEYNQKFKD	VH-CDR2 antibody 28H3
3	DVRPFYYAMDY	VH-CDR3 antibody 28H3
4	RSSQSLVHSNGNTYLH	VL-CDR1 antibody 28H3
5	KVSNRFS	VL-CDR2 antibody 28H3
6	SQSTHVPPT	VL-CDR3 antibody 28H3
7	QVQLQQSGTELAKPGASVRMSCKASGYTFT**NYWMH**WVKQRP GQGLEWIG**YINPITGYTEYNQKFKD**KATLTADRSSSTAYIQLNSL TSDDSAVYYCAR**DVRPFYYAMDY**WGQGTSVTVSS (CDRs are underlined and in bold, Framework regions (FR) are between said CDRs)	VH region antibody 28H3
8	DVAMTQTPLSLPVSLGDQASISC**RSSQSLVHSNGNTYLH**WYLQ KPGQSPKLLIY**KVSNRFS**GVPDRFSGSGSGTDFTLKITRVEAED LGVYFC**SQSTHVPPT**FGGGTKLEIKRA (CDRs are underlined and in bold, Framework regions (FR) are between said CDRs)	VL region antibody 28H3

9	SDYTWN	VH-CDR1 antibody 27F5
10	SITYSGITSYNLSLKS	VH-CDR2 antibody 27F5
11	GTALFTH	VH-CDR3 antibody 27F5
12	RSSQNLVHSNGNTYLH	VL-CDR1 antibody 27F5
13	KVSNRFS	VL-CDR2 antibody 27F5
14	SQSTHVPHT	VL-CDR3 antibody 27F5
15	DVQLQESGPGLVKPSQSLSLTCTVSGYSIT**SDYTWN**WIRQFPG NKLEWMG**SITYSGITSYNLSLKS**RISVTRDTSKNQFFLQLNSVT AEDTATYFCAR**GTALFTH**WGQGTLVTVSA (CDRs are underlined and in bold, Framework regions (FR) are between said CDRs)	VH region antibody 27F5
16	DVVMTQTPLSLPVSLGDQASISC**RSSQNLVHSNGNTYLH**WYLQ RPGQSPKFLIF**KVSNRFS**GAPDRFSGSGSGTDFTLKISRVEAED LGVYFC**SQSTHVP HT**FGRGTKLEIKRA (CDRs are underlined and in bold, Framework regions (FR) are between said CDRs)	VL region antibody 27F5
17	TYGMCVG	VH-CDR1 antibody 9B8
18	SIWWNGDKDYNPSLEN	VH-CDR2 antibody 9B8
19	IGYYFDY	VH-CDR3 antibody 9B8
20	QASQDIGNWLA	VL-CDR1 antibody 9B8
21	SAISLAD	VL-CDR2 antibody 9B8
22	LQRYSNPWT	VL-CDR3 antibody 9B8
23	QVTLKESGPGILQPSQTLSLACSFSGFSLS**TYGMCVG**WIRQSS GKGLEWLA**SIWWNGDKDYNPSLEN**RLTISKDTSNNQVFLKITN METAEAGTYYCTR**IGYYFDY**WGQGVLVSVSS	VH region antibody 9B8
24	DIQMTQSPASLSASLEEVVTITC**QASQDIGNWLA**WYQQKPGKS PQLLIH**SAISLAD**GIPSRFSGSRSGTQYSLKIKRVQVEDTGTYYC **LQRYSNPWT**FGG GTKLELKRA	VL region antibody 9B8
25	MTHLVLLLCCCVGSVCAFFSDLVKFENVTAHAGARVNLTCSVPS NESVSRIELGRGYTPGDGQLPLAVATSNNGTHITNGGYNYSLTL EWVNDSNTSVSLIIPNVTLAHAGYYTCNVTLRNCSVASGVHCN YSAGEEDDQYHANRTLTQRMHLTVIPATTIAPTTLVSHTTSTSHR PHRRPVSKRPTHKPVTLGPFPIDPWRPKTTWVHWALLLITCAVV APVLLIIIISCLGWLAGWGRRRKGWIPL	BILF2 amino acid sequence in the EBV virus strain B95-8 and other EBV strains such as Akata and M81

26	ATGACTCATTTAGTGCTTCTATTGTGCTGCTGTGTGGGGTCTG TTTGTGCATTTTTCTCAGATCTGGTTAAATTTGAAAATGTCACC GCCCATGCAGGTGCTAGAGTGAATCTCACCTGCAGCGTTCC TAGCAATGAGTCGGTCTCTCGGATCGAGTTGGGCCGGGGAT ACACTCCCGGGGATGGACAACTGCCCTTGGCCGTAGCTACT TCCAACAACGGAACTCATATAACTAACGGGGGTTACAATTATA GCCTTACCCTAGAATGGGTAAATGACTCCAACACCTCCGTCT CTCTGATAATCCCCAATGTGACCCTTGCGCATGCAGGTTACTA CACGTGCAATGTGACACTGAGAAATTGCAGTGTGGCCTCCG GGGTCCACTGCAATTACTCTGCAGGGGAAGAAGACGACCAA TACCATGCCAACCGGACCCTCACTCAGCGTATGCATCTTACT GTTATCCCAGCCACTACCATAGCCCCCACCACATTAGTCTCC CACACCACAAGCACCAGTCACAGGCCCCATAGGCGGCCTGT TTCTAAACGTCCCACGCATAAGCCAGTGACCCTGGGCCCTTT TCCAATCGATCCCTGGCGCCCTAAGACCACCTGGGTGCACT GGGCCCTGCTCCTAATCACTTGTGCTGTGGTGGCTCCGGTG CTCCTCATCATTATCATCTCCTGTCTGGGGTGGCTGGCGGGC TGGGGGAGGCGGCGCAAGGGCTGGATACCCCTGTGA	BILF2 DNA sequence of the EBV virus strain B95-8
27	ATGACCCATCTTGTGCTGCTTCTGTGCTGCTGTGTGGGCTCC GTGTGTGCCTTCTTCAGTGACCTGGTGAAGTTTGAAAATGTC ACAGCCCACGCCGGGGCCCGGGTGAACCTGACCTGTTCAG TGCCCAGCAATGAATCTGTCAGCCGCATCGAGCTGGGGAGA GGATACACACCTGGAGATGGCCAGCTGCCCCTGGCCGTGGC CACATCCAACAACGGCACACACATCACCAATGGAGGCTACAA CTACTCCCTGACTCTGGAGTGGGTCAATGACAGCAACACCTC TGTGTCTCTGATCATTCCAAATGTGACCCTGGCTCATGCTGG GTACTACACCTGCAACGTGACACTGAGGAACTGCAGCGTGG CTTCTGGAGTCCACTGCAATTACAGCGCTGGAGAGGAGGAT GACCAGTACCATGCCAACAGGACCCTCACCCAGAGGATGCA CCTGACAGTCATCCCAGCCACCACAATTGCCCCCACAACTTT GGTGAGCCACACCACCAGCACCTCCCACCGCCCCCACAGG AGGCCAGTGTCCAAGCGGCCCACCCACAAGCCTGTCACCTT GGGCCCCTTCCCCATCGACCCCTGGAGGCCCAAGACAACCT GGGTGCACTGGGCCCTGCTGCTCATCACATGTGCTGTGGTG GCACCTGTGCTCCTGATTATTATCATCAGCTGCCTGGGCTGG CTGGCAGGCTGGGGCCGCCGCAGGAAAGGATGGATCCCTC TGTGA	codon-optimized BILF2 DNA sequence of the EBV virus strain B95-8
28	PLAVATSNNGTHITN	amino acids 65-79 of BILF2
29	HCNYSAGEEDDQYHA	amino acids 129-143 of BILF2
30	ATGACCCATCTTGTGCTGCTTCTGTGCTGCTGTGTGGGCTCC GTGTGTGCCTTCTTCAGTGACCTGGTGAAGTTTGAAAATGTC ACAGCCCACGCCGGGGCCCGGGTGAACCTGACCTGTTCAG TGCCCAGCAATGAATCTGTCAGCCGCATCGAGCTGGGGAGA GGATACACACCTGGAGATGGCCAGCTGCCCCTGGCCGTGGC CACATCCAACAACGGCACACACATCACCAATGGAGGCTACAA CTACTCCCTGACTCTGGAGTGGGTCAATGACAGCAACACCTC TGTGTCTCTGATCATTCCAAATGTGACCCTGGCTCATGCTGG GTACTACACCTGCAACGTGACACTGAGGAACTGCAGCGTGG CTTCTGGAGTCCACTGCAATTACAGCGCTGGAGAGGAGGAT GACCAGTACCATGCCAACAGGACCCTCACCCAGAGGATGCA CCTGACAGTCATCCCAGCCACCACAATTGCCCCCACAACTTT	BILF2 encoding open reading frame in the pVAX1 expression plasmid 7617.SA1

	GGTGAGCCACACCACCAGCACCTCCCACCGCCCCCACAGG AGGCCAGTGTCCAAGCGGCCCACCCACAAGCCTGTCACCTT GGGCCCCTTCCCCATCGACCCCTGGAGGCCCAAGACAACCT GGGTGCACTGGGCCCTGCTGCTCATCACATGTGCTGTGGTG GCACCTGTGCTCCTGATTATTATCATCAGCTGCCTGGGCTGG CTGGCAGGCTGGGGCCGCCGCAGGAAAGGATGGATCCCTC TGTGA	
31	GAAUAAACUAGUAUUCUUCUGGUCCCCACAGACUCAGAGA GAACCCGCCACC AUGACCCAUCUUGUGCUGCUUCUGUGCUGCUGUGUGGGC UCCGUGUGUGCCUUCUUCAGUGACCUGGUGAAGUUUGAAA AUGUCACAGCCCACGCCGGGGCCCGGGUGAACCUGACCU GUUCAGUGCCCAGCAAUGAAUCUGUCAGCCGCAUCGAGCU GGGGAGAGGAUACACACCUGGAGAUGGCCAGCUGCCCCU GGCCGUGGCCACAUCCAACAACGGCACACACAUCACCAAU GGAGGCUACAACUACUCCCUGACUCUGGAGUGGGUCAAUG ACAGCAACACCUCUGUGUCUCUGAUCAUUCCAAAUGUGAC CCUGGCUCAUGCUGGGUACUACACCUGCAACGUGACACUG AGGAACUGCAGCGUGGCUUCUGGAGUCCACUGCAAUUACA GCGCUGGAGAGGAGGAUGACCAGUACCAUGCCAACAGGAC CCUCACCCAGAGGAUGCACCUGACAGUCAUCCCAGCCACC ACAAUUGCCCCCACAACUUUGGUGAGCCACACCACCAGCA CCUCCCACCGCCCCCACAGGAGGCCAGUGUCCAAGCGGCC CACCCACAAGCCUGUCACCUUGGGCCCCUUCCCCCAUCGAC CCCUGGAGGCCCAAGACAACCUGGGUGCACUGGGCCCUG CUGCUCAUCACAUGUGCUGUGGUGGCACCUGUGCUCCUG AUUAUUAUCAUCAGCUGCCUGGGCUGGCUGGCAGGCUGG GGCCGCCGCAGGAAAGGAUGGAUCCCUCUGUGAUGA CUCGAGCUGGUACUGCAUGCACGCAAUGCUAGCUGCCCCU UUCCCGUCCUGGGUACCCCGAGUCUCCCCCGACCUCGGG UCCCAGGUAUGCUCCCACCUCCACCUGCCCCACUCACCAC CUCUGCUAGUUCCAGACACCUCCCAAGCACGCAGCAAUGC AGCUCAAAACGCUUAGCCUAGCCACACCCCCACGGGAAAC AGCAGUGAUUAACCUUUAGCAAUAAACGAAAGUUUAACUAA GCUAUACUAACCCCAGGGUUGGUCAAUUUCGUGCCAGCCA CACCCUGGAGCUAGCAAAAAAAAAAAAAAAAAAAAAAAAAA AAAGCAUAUGACUAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAA-3‘	BILF2 encoding mRNA of a synthetic T7 RNA polymerase generated transcript The central part of the sequence is underlined and indicates the part of the mRNA, which codes for BILF2, the preceding sequence is the 5‘ untranslated region (UTR), the succeeding part is the 3’ UTR of the mRNA including its terminal poly A region.
32	ATGACCCATCTTGTGCTGCTTCTGTGCTGCTGTGTGGGCTCC GTGTGTGCCTTCTTCAGTGACCTGGTGAAGTTTGAAAATGTC ACAGCCCACGCCGGGGCCCGGGTGAACCTGACCTGTTCAG TGCCCAGCAATGAATCTGTCAGCCGCATCGAGCTGGGGAGA GGATACACACCTGGAGATGGCCAGCTGCCCCTGGCCGTGGC CACATCCAACAACGGCACACACATCACCAATGGAGGCTACAA CTACTCCCTGACTCTGGAGTGGGTCAATGACAGCAACACCTC TGTGTCTCTGATCATTCCAAATGTGACCCTGGCTCATGCTGG GTACTACACCTGCAACGTGACACTGAGGAACTGCAGCGTGG CTTCTGGAGTCCACTGCAATTACAGCGCTGGAGAGGAGGAT GACCAGTACCATGCCAACAGGACCCTCACCCAGAGGATGCA CCTGACAGTCATCCCAGCCACCACAATTGCCCCCACAACTTT GGTGAGCCACACCACCAGCACCTCCCACCGCCCCCACAGG AGGCCAGTGTCCAAGCGGCCCACCCACAAGCCTGTCACCTT GGGCCCCTTCCCCATCGACCCCTGGAGGCCCAAGACAACCT GGGTGCACTGGGCCCTGGCAGGCTGGGGCCGCCGCAGGA AAGGATGGATCCCTCTGGGCGAGAACCTGTACTTCCAGGGC	BILF2 encoding open reading frame lacking BILF2‘s transmembrane domain (ΔTM) in the expression plasmid 7703.TA1

	GGCGAGCAGGGCCCCGCAGATGACCCAGGAGAAGGGCACC ATCATCATCACCATTAA	
33	EQGPADDPGEG	EBNA1 tag

VII. EXAMPLES

[0136] Example 1: Production of EB-VLPs

[0137] The inventors of the present invention have engineered a vaccine candidate, termed Epstein-Barr virus-like particles (EB-VLPs). EB-VLPs are enveloped vesicles that mimic the composition of EBV particles and contain many different viral proteins such as glycoproteins exposed on the vesicles' membrane, various tegument proteins and the many components that form the viral capsid contained in the lumen of the EB-VLPs. EB-VLPs are released from producer cells that carry the genetic viral information in a stable form. To induce synthesis of EB-VLPs in the producer cell line and their release into the cell culture supernatant, the viral lytic phase in the cells is induced by adding a drug to the producer cells, which are engineered to enter EBV's lytic phase and to release EB-VLPs. They are harvested from the cell culture supernatant and purified to be subsequently used as the active pharmaceutical ingredient in the EBV vaccine candidate (see WO 2012/025603).

[0138] Example 2: Analysis of the protein composition of EB-VLPs

[0139] The inventors of the present invention analyzed the protein composition of highly purified EB-VLPs by mass-spectrometry. EB-VLPs were found to contain more than 50 viral proteins from all three classes of virion proteins (envelope, i.e., glycoproteins, tegument and capsid) as expected.

[0140] Example 3: Analysis of the immune response to EB-VLPs

[0141] Mice and rats were immunized with EB-VLPs with the aim to obtain monoclonal antibodies recognizing the many different viral proteins and to identify antibodies with neutralizing characteristics directed against viral (glyco-)proteins present on the surface of EB-VLPs.

[0142] Specifically, mice and rats were immunized in a prime-boost scheme with EB-VLPs, which were purified according to a standard process. Briefly, EB-VLPs were harvested from the cell culture supernatant of producer cells that carry a genetically modified EBV genome with deletions in several EBV genes, one of which is indispensable for packaging genomic viral DNA into infectious virus particles. In these cells, EB-VLP production can be induced by a conditional molecular switch, which, upon activation, induces the so-called lytic phase of EBV that turns the producer cells into EB-VLP factories. The producer cell line releases EB-VLPs into the supernatant for four days, when the supernatant is collected, cells and cell debris are

removed by low speed centrifugation and EB-VLPs are pelleted by ultracentrifugation. EB-VLPs are further purified by flotation and equilibrium density gradient ultracentrifugation in discontinuous iodixanol (Optiprep, Sigma-Aldrich) density gradients. EB-VLPs are collected by fractionation as described (Roessler et al., 2023). Animals received between 10^9 to 10^{10} EB-VLP particles by subcutaneous and intraperitoneal injections with CpG as adjuvants in a prime-boost scheme with two to three-month intervals.

[0143] The individual rodent sera were also used to probe protein dot-blots encompassing the complete library of viral proteins (plus appropriate controls) purified from HEK293 cells transiently transduced with 86 individual expression plasmids.

[0144] In addition, the rodent sera were used to detect single EBV glycoproteins by quantitative flow cytometry analysis on HEK293 or HEK293T cells, which express single, individual EBV glycoproteins on their cell surface.

[0145] Besides other antigens in these proteome and glycoprotein screens, the present inventors found two viral glycoproteins, gp350/220 and BILF2, to be two dominant targets of the murine humoral immune response.

[0146] gp350/220 is a transmembrane protein embedded in the membrane of infectious EBV particles and equally present in the membrane of EB-VLPs (own mass spec and nanoflow analyses).

[0147] In contrast, BILF2, which is also one of the 13 known or predicted glycoproteins of EBV was first described in 1990 (Mackett et al., 1990), but has not raised much interest in the EBV community.

[0148] As a consequence, very little is known about BILF2 and no function has been assigned. Occasionally, bits and pieces regarding BILF2 can be found scattered in the prior art, also suggesting that BILF2 could be a target of human immune responses in principle. Of note, the BILF2 protein sequence is 100% conserved in all prototypic EBV strains such as B95.8, AG876, and M81 suggesting that the glycoprotein has an important role in EBV's biology and, probably, pathogenesis and immunity.

[0149] The present inventors immunized several BALB/c mice and Wistar rats with EB-VLPs with the primary aim to generate monoclonal antibodies directed against antigens contained in the active pharmaceutical ingredient in the EBV vaccine candidate. In primary screens after

somatic fusion of murine spleen cells, the inventors observed a very high frequency of hybridoma supernatants that contained antibodies directed against EBV (see Table 1). The inventors found that approximately 58% of these hybridoma supernatants contained antibodies directed against gp350/220 whereas 24% contained BILF2 specific antibodies. The remaining supernatants contained antibodies directed against other EBV glycoproteins or viral proteins that could not be immediately identified.

[0150] Table 1: Frequencies of EBV positive candidates (IgG only) in primary screens for monoclonal antibodies after prime boost vaccination.

	# EBV positive / # all candidates	% EBV positive candidates
Mouse 1	145/361	40%
Mouse 2	103/378	27%
Mouse 3	120/264	45%
Mouse 4	119/378	31%
Rat 1	89/188	47%
Rat 2	326/600	54%
Rat 3	338/668	51%

Mice and rats were immunized with 10^9 to 10^{10} EB-VLPs per dose with CpG

[0151] Example 4: Analysis of the immune response to extracellular vesicles (EVs) solely expressing BILF2.

[0152] Similarly, the inventors immunized BALB/c mice with extracellular vesicles (EVs) that express solely the BILF2 protein (embedded in their membrane) and which were termed BILF2-EVs. To generate BILF2-EVs, HEK293 cells were transduced with a codon-optimized BILF2 expression vector to express BILF2 ectopically. The manipulated cells released EVs in high quantities decorated with BILF2 protein on their surface into the cell culture supernatant as expected. Similarly to EB-VLPs, BILF2-EVs were harvested, purified and concentrated to be used as antigen in immunization experiments with the aim to generate BILF2 specific monoclonal antibodies.

Animals received between 10^9 to 10^{10} BILF2-EVs via subcutaneous and intraperitoneal injections with CpG as adjuvants in a prime-boost scheme with two to three-month intervals.

[0153] Example 5: Sequencing of antibodies directed against BILF2 and their characterization

[0154] From these two principal approaches using EB-VLPs or BILF2-EVs for immunization, the inventors cloned rat and mouse monoclonal antibodies with BILF2 specificity and different IgG isotypes.

[0155] Specifically, spleen cells of the immunized animals were used for somatic fusions according to conventional hybridoma technology. Supernatants from hybridoma cell cultures were used to screen for antibodies with specificity towards EBV glycoproteins. For this the inventors used flow cytometry and cells of the induced EB-VLP producer cell line mixed with an equal number of control HEK293 cells. To distinguish between both cell populations, one of which was stained with celltrace violet (CTV, ThermoFisher Scientific) prior to incubating the cell mixture with hybridoma supernatant and, after washing, with an appropriately fluorochrome coupled secondary antibody. Appropriate gating strategies allowed the identification of hybridomas with specificities towards viral glycoproteins.

[0156] To identify antigen specificity, HEK293 cells that express single or combinations of two viral glycoproteins on their surface were generated by transient or stable transfection of expression vectors encoding the individual EBV glycoproteins or combinations thereof. These cells were used similarly as in the primary screens described above. With interesting candidates multiple rounds of limited dilution subcloning of hybridoma cells were performed to reach monoclonality and identify antibody specificities of selected candidates. In the primary screen, the majority of hybridomas released antibodies directed against gp350/220 or BILF2, among many other specificities, a selections of which were subcloned to monoclonality.

Sequencing

[0157] Specifically, total RNA from selected monoclonal hybridoma cell lines was isolated using standard protocols. The 5' RACE System for rapid amplification of cDNA ends (ThermoFisher Scientific) was used to generate cDNA, which was subsequently PCR amplified using isotype-specific primer pairs. The PCR products were sequenced (Sanger sequencing, MWG Eurofins) and the obtained sequences were analyzed and annotated using the algorithms provided by Abysis (http://www.abysis.org/abysis, UCL).

[0158] DNA sequences of RT-PCR amplification products were analyzed using the Abysis software to identify and annotate the predicted CDRs pursuant to Kabat (http://www.abysis.org/abysis/sequence_input/key_annotation/key_annotation.cgi).

Determination of KD values of antibodies directed against BILF2

[0159] HEK293 cells, which express BILF2 at moderate stable level were transiently transduced with an expression plasmid encoding BILF2. These cells were mixed with an equal number of control HEK293 cells, which were stained with celltrace violet (CTV, ThermoFisher Scientific) prior to incubating the mixed cells with serial dilutions starting at 40 µg/mL of purified monoclonal antibodies directed against BILF2, which are listed in Table 2. After incubation at 37°C for 60 min, the cell mixture was washed and incubated with appropriately fluorochrome coupled secondary antibodies directed against either rat or mouse IgG. After flow cytometry using a Fortessa Instrument (Becton Dickinson) HEK293 control cells and cells expressing BILF2 were gated according to CTV staining and mean fluorescence intensities (MFI) were obtained from both cell types. MFI from control HEK293 cells were subtracted from MFI values obtained cells expression BILF2. After baseline correction the results across all diluted samples were plotted and analyzed using the non-linear fit (sigmoidal, 4PL) model provided Prism (version 10.2, Graphpad). Based on initial antibody concentration, mean KD_{50} values were obtained from three to four independent experiments, as shown in Table 2.

Epitope mapping of monoclonal antibodies specific to a BILF2 protein

[0160] Two different methods were used.
(i) A peptide library covering the amino acid sequence of BILF2 consisting of 60 peptides with 15 amino acids each was spotted and arrayed on nitrocellulose membranes together with appropriate controls and incubated with monoclonal antibodies shown in Table 2. Their binding to individual peptides was detected with secondary, fluorescence-labeled antibodies by infrared fluorescence, which was recorded with a CCD camera. Four monoclonal antibodies resulted in clear signals.
(ii) BILF2 full-length protein or BILF2 mutants with individual deletions of 40 amino acids each in the ectodomain of BILF2 (encompassing amino acids 18 to 212 of BILF2 as shown in SEQ ID NO: 25) were transiently expressed in HEK293 cells. The cells were incubated with appropriate dilutions of the monoclonal antibodies and their binding to the cell surface of BILF2 expressing cells was monitored by flow cytometry using appropriately fluorochrome coupled secondary antibodies directed against either rat or mouse IgG.

[0161] Table 2: Overview of seven BILF2 specific monoclonal antibodies and their characteristics

Monoclonal antibody	Species	Immunized with	Ig class	KD_{50} [nM]†	BILF2 epitope	LC, HC sequence	Comments
25A2	mouse	EB-VLP	IgG2a	4.4	aa 161-200	-	--
28H9	mouse	BILF2-EV	IgG2a	-	aa 65-79*	-	--
27H11	mouse	BILF2-EV	IgG2a	-	aa 65-79*	-	--
26G5	mouse	BILF2-EV	IgG2b	15.5	aa 65-79*	-	--
27F5	mouse	BILF2-EV	IgG1	1.4	aa 129-143**	Yes	neutralizing
28H3	mouse	BILF2-EV	IgG3	5.5	aa 41-160	Yes	neutralizing
9B8	Rat	EB-VLP	IgG2b	12.1	aa 41-160	Yes	neutralizing

* peptide #17 of BILF2: PLAVATSNNGTHITN (SEQ ID NO: 28)

** peptide #33 of BILF2: HCNYSAGEEDDQYHA (SEQ ID NO: 29)

† KD_{50} values were determined by flow cytometry

[0162] It is of note that antibodies 27F5 and 28H3 partially compete for epitope binding in an antibody competition assay which may be indicative that their epitope may be partially overlapping, while antibody 9B8 does not compete for epitope binding with either antibody 27F5 or 28H3.

[0163] Thus, when antibodies of the present invention bind to an epitope within amino acids 40-160 of BILF2 shown in SEQ ID NO: 25 they are capable of neutralizing EBV infection of epithelial cells. Preferably, the epitope is within amino acids 80-160 of BILF2 shown in SEQ ID NO: 25 or within amino acids 129-143 of BILF2 shown in SEQ ID NO: 25.

[0164] Example 6: Testing of antibodies directed against BILF2 for reducing or blocking EBV infection of B-cells or epithelial cell

[0165] Two different VNTs were used to assess monoclonal antibodies for their neutralizing characteristics. To test them with regard to EBV's target cells, B cells and epithelial cells, i.e., Daudi and HEK293 cells, respectively, were used.

[0166] Daudi cells were used as human B cells in combination with EB-VLPs as described in Roessler et al. (Roessler et al., 2023). For this, Daudi cells were stably transduced with a retroviral vector encoding the larger part of a split nanoluciferase termed LgBiT as a C-terminal fusion protein with human CD63. Similarly, EB-VLPs were equipped with the smaller part of

the split nanoluciferase, termed HiBiT. The materials and the setup of the assay are described in detail (Roessler et al., 2023). Briefly, 1 to 2 µg of purified monoclonal antibodies in 40 µL PBS or 40 µL of raw hybridoma cell culture supernatant were incubated with 30 µL EB-VLPs at 37°C for 60 min. In a volume of 50 µL $4x10^4$ Daudi cells were added and incubated at 37°C for 4 hours to allow adherence, fusion and uptake of EB-VLPs by the cells. Nanoluciferase activities in Daudi cells incubated with EB-VLPs were quantitated as described (Roessler et al., 2023).

[0167] HEK293 target cells were used as EBV's epithelial cell targets. 40 µL of raw hybridoma cell culture supernatant or 1 to 2 µg of purified monoclonal antibodies in 40 µL PBS were incubated with 50 µL of EBV stock for 60 min. The GFP-encoding 2089 EBV stock contained $1x10^6$ green Raji units (GRUs) per mL (Steinbrück et al., 2015). The mixture was added to $2x10^4$ HEK293 cells in RPMI1640 supplemented with 10% fetal calf serum and usual additives contained in 500 µL, mixed and plated in single wells of a 48 well cluster plate for 72 hours. Cells were trypsinized, resuspended and analyzed by flow cytometry using a Fortessa instrument (Beckton Dickinson) and a high throughput sample unit. GFP positive cells were gated and the mean percentage of GFP positive cells in controls (about 15%) free of hybridoma cell culture supernatant or purified monoclonal antibodies was set to 100% for normalization.

B-cell infection

[0168] The inventors tested the monoclonal antibodies whether they interfered with or neutralized viral infection of human B cells, the prime target of EBV in vivo and in vitro.

[0169] None of the purified antibodies (or hybridoma supernatants prior to purification) reduced or blocked infection in virus neutralization tests using EB-VLPs as virus stock and Daudi cells as susceptible human B cell targets (Fig. 1). Generation and purification of EB-VLPs and the validated Daudi cell-based EBV neutralization test are described in detail (Roessler et al., 2023).

Epithelial cell infection

[0170] EBV also infects certain human epithelial cells in vitro and is consistently associated with two EBV positive tumor entities in patients with epithelial cancer. All cases of Nasopharyngeal Cancer (NPC) and about 10% of cases of gastric cancer (GC) are EBV positive documenting that EBV can infect human epithelial cells in vivo. In immunocompromised individuals such as late stage AIDS patients, EBV can also be found in epithelial cells of the tongue where EBV is associated with a syndrome called oral hairy leucoplakia, which is characterized by EBV replicating in squamous epithelial cells of the

organ. In EBV positive individuals who control the virus, EBV has not been detected in normal epithelial cells of the nasopharynx or the tongue suggesting that EBV controllers mount an immune response that prevents infection of epithelial cells or eliminates EBV-infected epithelial cells.

[0171] Several epithelial models for EBV infection are in use such as AGS cells and the HEK293 cell line, which both have been extensively employed as a model of epithelial cell entry of infectious EBV or cell based fusion assays to study the interaction of viral ligands and cellular receptors. The inventors used viral stocks of the 2089 strain of EBV (Delecluse et al., 1998) with a recombinant viral genome that encodes the GFP gene to aid in monitoring infection of cells quantitatively. As epithelial cells the inventors used HEK293 cells as described previously (Janz et al., 2000).

[0172] The inventors infected HEK293 cells with calibrated virus stocks of the 2089 EBV strain such that about 12 to 17% of the target cells expressed GFP three days later. The inventors found this rate of infection to be in a range reflecting a linear dose effect in this model. The virus dose corresponds to about $5x10^4$ green Raji units (GRU) as defined by Steinbrück et al., when the virus stock is used to infect Raji cells (Steinbrück et al., 2015). In virus neutralization tests using individual monoclonal antibodies directed against BILF2 as well as control antibodies, we used this dose contained in 50 microL of the EBV stock. The virus stock was incubated with different amounts of antibodies diluted in PBS in a total volume of 90 microL at 37°C for 60 to 90 minutes. The virus-antibody mixture was transferred to $2x10^4$ HEK293 cells contained in 500 microL cell culture medium in wells of a 48 well cluster plate and incubated for three days prior to analysis by flow cytometry for the percentage of GFP-positive cells.

[0173] In this infection model, the inventors found that one of the established BILF2 specific monoclonal antibodies, 28H3, neutralized EBV to an extent comparable to two neutralizing antibodies directed against the viral gH/gL glycoprotein complex (25G11 and 26B5 in Fig. 2). Two other BILF2 antibodies interfered with epithelia cell infection to some extent (27F5 and 9B8 in Fig. 2) whereas the remaining BILF2 specific antibodies did not reveal a phenotype.

[0174] The gH/gL complex is known to be essential for EBV infection of both B cells and epithelial cells suggesting that antibodies against BILF2 and gH/gL can have additive effects in abrogating infection of human epithelial cells.

Control antibodies

[0175] The inventors also tested control antibodies in this infection model. The three monoclonal antibodies directed against gp42 of EBV, which neutralize B cell infection (Fig. 1), have no appreciable function in preventing infection of epithelial cells (Fig. 2). This is because gp42 makes physical contact with HLA class II molecules only present on B cells but not on epithelial cells. Thus, gp42 has no functionality in the prefusion complex that targets different receptors (but not HLA molecules) on epithelial cells. As a consequence, gp42 specific antibodies are not expected to block infection of epithelial cells (Chesnokova et al., 2009).

[0176] Another control antibody (1D2) binds the cellular surface marker CD47, which is very strongly expressed on HEK293 cells. 1D2 does not interfere with EBV infection of epithelial cells either (Fig. 2) indicating that a protein corona of antibodies covering the surface of HEK293 cells is no hindrance to EBV in infecting these cells.

[0177] Given these observations, it seems that the viral BILF2 glycoprotein has a unique function in the infection process of epithelial cells. Antibodies raised against BILF2 can neutralize EBV infection of epithelial but not of B cells, which highlights the antibodies' special role in fighting EBV.

[0178] Example 7: Sequencing of BILF2 antibodies

[0179] The variable regions of the light and heavy chains of the three monoclonal antibodies with neutralizing functions were determined, i.e., monoclonal antibody 27F5, 28H3 and 9B8; see Table 3.

[0180] Table 3:

	28H3	27F5	9B8
VH CDRs	SEQ ID NOs: 1-3	SEQ ID NOs: 9-11	SEQ ID NOs: 17-19
VL CDRs	SEQ ID NOs: 4-6	SEQ ID NOs: 12-14	SEQ ID NOs: 20-22
VH region	SEQ ID NO: 7	SEQ ID NO: 15	SEQ ID NO: 23
VL region	SEQ ID NO: 8	SEQ ID NO: 16	SEQ ID NO: 24

[0181] Example 8: Testing sera from EBV carrying humans for BILF2 antibodies

[0182] The inventors were intrigued by another related finding analyzing human sera. They tested sera from about 100 healthy human volunteers using their protein dot-blot assay that encompasses the entire proteome library of 86 EBV proteins. They found that serum antibodies directed against BILF2 are most prevalent and more frequent than serum antibodies directed

against gp350/220 in sera of this group of donors (Table 4). This information was obtained with sera from EBV-positive adults who apparently control viral infection.

[0183] The inventors also found that only small fractions of human sera detected other viral glycoproteins such as gp42 (BZLF2), gH, gL, and gB (BALF4).

[0184] This was a surprising finding because their relative protein abundance in EB-VLPs did not correlate with the prevalence of EBV glycoprotein-specific human serum antibodies. It thus appears as if BILF2 is the least abundant viral glycoprotein found in EB-VLPs as listed in Table 4, but BILF2 is top with respect to its immunogenicity in humans surpassing even gp350/220, which is the most abundant viral glycoprotein in this rank order.

[0185] As already pointed out, in mice and rats immunized with EB-VLPs the inventors found that among the animals´ humoral immune response, antibodies directed against gp350 and BILF2 prevailed reflecting the situation in human EBV controllers.

[0186] Table 4: BILF2, the least abundant EBV glycoprotein has the highest serum antibody prevalence and titer in EBV infected, healthy individuals

EBV glycoproteins (n = 13)	**Mass spec rank order***	**Serum antibody prevalence**	**Serum titer (RFU) †**	**Origin¶**	**Function**
BILF2	38‡	>95%	0.8	EBV	?
gp350/220 (BLLF1)	5	70%	0.5	EBV	CD21 ligand
gp42 (BZLF2)	20	30%	0.2	EBV	MHCII ligand
gB (BALF4)	18	30%	0.3	Common	Fusogenic
BDLF3	10	10-20%	0.1	EBV	?
gH (BXLF2)	8	<10%	<0.05	Common	Prefusion complex
gL (BKRF2)	23	<5%	<0.05	Common	
BMRF2	22	<5%	<0.05	common (?)	Integrin attachment?
BDLF2	11	<5%	<0.05	EBV	?

gM (BBRF3)	6	<5%	<0.05	Common	Virion assembly and release?
BARF1	36	<5%	<0.05	EBV	Decoy CSF1 receptor immune evasion
gN (BLRF1)	not detected	<5%	<0.05	Common	Virion assembly and release?
BILF1	not detected	<5%	<0.05	EBV	constitutively active G-protein coupled receptor

* rank order #1 indicates the most abundant EBV protein out of 53 detected according to the iBAQ algorithm

‡ compared to gp350/220 as reference, BILF2 abundance is reduced by a factor of 84

† Relative fluorescent units (max = 2.0; background corrected)

EBV: found exclusively in EBV strains or in related non-human primate EBVs;
common: homologues in other herpesviruses

[0187] Example 9: Competition assay between BILF2 antibodies of the invention and antibodies from human sera

[0188] The inventors also asked whether the BILF2 specific monoclonal antibodies of the invention bind to epitopes against which EBV positive individuals also mount individual antibodies.

[0189] If human sera contain antibodies that bind to epitopes which are also recognized by the three neutralizing monoclonal antibodies 27F5, 28H3 or 9B8, and a non-neutralizing antibody a competition assay might help to identify such human antibodies, which are likely important to prevent infection of epithelial cells in humans.

[0190] The inventors designed a competition assay using an HEK293 cell population, which consists of BILF2 positive (about 25 to 30%) and BILF2 negative cells. For this purpose, HEK293 cells were transiently transfected with an expression plasmid encoding BILF2. Only a fraction of cells (about 30%) express BILF2 protein on their surface whereas the majority of the cells does not.

[0191] The cells were incubated at room temperature for 60 min with serial dilutions of sera from three EBV-positive (top row panels) and three EBV-negative individuals (bottom panels) shown in Figure 3 to allow binding of human BILF2 specific serum antibodies to all epitopes on the EBV glycoprotein.

[0192] The cells were washed with 1% BSA and subsequently incubated with four individual monoclonal antibodies as shown in Figure 3 labeled with Alexa 488. The concentrations of the four fluorochrome-labeled antibodies were carefully adjusted to provide a robust signal but to not compete with human antibodies bound to BILF2 epitopes.

[0193] After incubation at 4°C for 20 min, the cells were washed and analyzed by flow cytometry using a BD Fortessa instrument. Serial dilutions of the six sera are plotted on the x-axis, the percentages of Alexa 488 positive cells are shown on the y-axis.

[0194] High concentrations of sera from EBV-positive individuals prevent the binding of BILF2 specific monoclonal antibodies indicating that their epitopes are blocked by human serum antibodies and thus are not accessible for the BILF2 specific monoclonal antibodies.

[0195] Analyzing the serum of three EBV-positive individuals revealed a reduced binding of all four monoclonal antibodies to BILF2 positive cells as a function of the concentration of the human serum antibodies. Upon serial dilution of the sera, the BILF2 epitopes become gradually accessible which result in an increase of Alexa 488 positive cells as the monoclonal antibodies can bind to their cognate epitopes in the absence of human antibodies.

[0196] In Figure 3 with sera from EBV-positive individuals, at low serum dilutions (1:8, 1:16 etc.) all four monoclonals showed a reduced binding which was not observed at high serum dilutions (1: 256).

[0197] This effect was not seen with sera from three EBV-negative individuals suggesting that the competitive effect at low serum dilutions (i.e., high antibody concentrations) is specific and indicative of the binding of human antibodies to BILF2 epitopes. Accordingly, sera of EBV-negative individuals do not contain antibodies that competed with BILF2 specific monoclonal antibodies in binding to the fraction of BILF2 positive cells indicated by almost flat horizontal lines in the lower panels.

[0198] Three of the four monoclonal antibodies, i.e., 27F5, 28H3 and 9B8 used in the competition assay have neutralizing functions (Fig. 2) suggesting that human serum antibodies might also neutralize EBV to prevent and interfere with its infecting human epithelial cells.

[0199] Antibody 26G5 does not interfere with EBV infecting epithelial cells as demonstrated in the virus neutralization tests.

[0200] Example 10: Immunization of BALB/c mice with four different BILF2 antigens

[0201] Four different types of BILF2 antigens were prepared to immunize four groups of mice employing a prime-boost-boost scheme. Two BILF2 antigen encoding nucleic acids and two different types of BILF2 protein encompass (i) an expression vector plasmid DNA; (ii) a formulation of a synthetic mRNA; (iii) a purified adjuvanted BILF2 protein; and (iv) a preparation of extracellular vesicles (EVs) that carry BILF2 protein (in addition of proteins of cellular origin) on their surface.

[0202] Ad (i)

[0203] A codon-optimized BILF2 encoding synthetic DNA was designed to express the viral protein BILF2 in its native form. The synthetic DNA fragment was cloned into a conventional vector plasmid and its sequence was confirmed. Using two appropriate PCR primers, the coding sequence of BILF2 was amplified and inserted into a version of the expression vector plasmid pVAX1. After transformation of the E. coli strain DH5alpha and selection with kanamycin, the final pVAX1-based expression plasmid of BILF2 was established, termed 7617.SA1 and sequenced to confirm its identity. Plasmid DNA was isolated from large cultures of the E. coli clone using the Endofree Plasmid Maxi Kit by Qiagen. Endotoxin concentration was determined to be 0.03 EU/100 microL at a plasmid DNA concentration of 1 microgram/microL. 100 microgram of plasmid DNA per mouse was injected intramuscularly as a single dose. The sequence of the BILF2 encoding part of the plasmid 7617.SA1 is provided in SEQ ID NO: 30.

[0204] Ad (ii)

[0205] A custom-made mRNA was ordered from PackGene Biotech Inc. encoding the codon optimized BILF2 open reading frame according to our specification. The mRNA was transcribed from an appropriately designed plasmid DNA as template (after its appropriate restriction enzyme cleavage) using T7 RNA polymerase and N1-methylpseudouridine

(N1meψ) triphosphate in combination with three unmodified triphosphate ribonucleotides for RNA synthesis to bypass innate immune response and to increase translation efficiency in vivo. At the 5' end, a Cap-1 structure was added and the RNA was encapsidated within Lipid Nanoparticles (LNPs) at a concentration of 0.2 mg RNA/mL. The formulation contained <1 EU/mL endotoxin and was frozen in small aliquots and stored at -80°C until use. The formulation was diluted and mice were immunized with 10 microgram RNA per dose contained in 100 microL, which was applied intramuscularly. The sequence of the BILF2 encoding synthetic mRNA is provided in SEQ ID NO: 31.

[0206] Ad (iii)

[0207] BILF2 protein was prepared from cell culture supernatants of HEK293T cells transiently transfected with an expression plasmid encoding a modified version of BILF2. The expression plasmid 7703.TA1 encodes a codon-optimized open reading frame of BILF2 with a carboxy-terminal TEV cleavage site followed by an EBNA1 epitope and a tag composed of six histidine residues. The single pass transmembrane domain of BILF2 was deleted (ΔTM) to foster the release and secretion of the modified BILF2 protein into the supernatants of transfected cells. The backbone of the expression plasmid 7703.TA1 is a pVAX1 vector modified to carry an SV40 promoter/enhancer element that also encompasses the SV40 origin of DNA replication to allow plasmid DNA amplification and to enhance expression of BILF2 in transfected HEK293T cells. The sequence of the modified BILF2 encoding part of the plasmid 7703.TA1 is provided in SEQ ID NO: 32.

[0208] HEK293T cells were transiently transfected with 7703.TA1 plasmid DNA and the cell culture supernatant was harvested three days post transfection. After low speed centrifugation Ni-beads (Pierce Inc) were added in the presence of 10 mM imidazole. After overnight binding, the Ni-beads were washed intensively and the bound protein was released by adding 0.5 M imidazole. The solution was concentrated using Amicon Ultra 15 centrifugal filters (cut-off of 30 kDal), and the buffer was exchanged to obtain a final concentration of 250 microgram/mL BILF2 protein in PBS/10% glycerol as determined by a sandwich ELISA using highly purified BILF2 protein as reference and standard. 60 nmol/mL ODN 1688 was added as adjuvant. The mice were injected with 100 microL per single dose which was applied intramuscularly.

[0209] Ad (iv)

[0210] Extracellular vesicles equipped with BILF2 protein on their surface were isolated from cell culture supernatants of HEK293T cells transiently transfected with the expression plasmid

7689.PA1 for three days. The 7689.PA1 DNA is identical to the sequence in 7617.SA1, which encodes the codon-optimized BILF2 protein (SEQ ID NO: 30) but, in addition, 7689.PA1 carries the SV40 origin of DNA replication to boost BILF2 protein expression in HEK293T cells upon transient DNA transfection. After two low speed centrifugation steps to remove cells and cellular debris, EVs were concentrated by ultracentrifugation, the pellet was resuspended overnight in the residual volume and EVs were further purified by density equilibrium centrifugation on a discontinuous iodixanol (Optiprep, Sigma-Aldrich) equilibrium density gradient as described in detail (Roessler et al., 2023; Roessler et al., 2022). Fraction 2 and 3 were collected, diluted in PBS and concentrated by sedimentation in an ultracentrifuge. After removal of most of the supernatant, the EVs were resuspended in the remaining volume. The content of BILF2 protein was determined using a sandwich ELISA with highly purified BILF2 protein as reference protein. The preparation was further diluted with PBS to obtain a final concentration of 22 microgram/mL BILF2 protein corresponding to about $5x10^{11}$ EVs/mL as determined by nanoparticle tracking analysis (NTA) using a ZetaView PMX110 instrument (Particle Metrix). BILF2 EVs were stored in small aliquots at -80°C prior to use. Mice were injected with 100 microL per single dose which was applied intramuscularly.

[0211] Immunization scheme

[0212] BALB/c mice approximately 7 weeks of age received three intramuscular administrations of the four antigens on the study days 1, 31 and 61. Table 5 provides an overview of the antigens and the immunization scheme. The dosing was administered following a prime-boost-boost regimen. Blood was collected from all animals on study day 1 before immunization, then on study day 61 prior to the last immunization and terminal blood sampling was collected on study day 75. Serum was prepared and stored at -20°C. Animal housing and caretaking, immunizations, and blood collections were performed by a commercial CRO (Aurigon Labs Ltd, Pálya utca 2, 2120 Dunakeszi Hungary).

[0213] Table 5: Experimental groups and doses

Group number	Route of administration	Antigen	Dose [/animal]	Dose concentration [/mL]	Administered volume [µL/animal]	No. of animals	Animal ID	
							male	female
1	intramuscular	BILF2 DNA	100 µg	1 mg	100	8	11-14	15-18

2		BILF2 mRNA	10 µg	0.1 mg	100	6	21-23	24-26
3		BILF2 protein (ODN 1688)	25 µg (6 nmol)	250 µg (60 nmol)	100	6	31-33	34-36
4		BILF2 EVs	$5x10^{10}$ EVs (2.2 µg BILF2 protein)	$5x10^{11}$ EVs (22 µg BILF2 protein)	100	8	41-44	45-48

[0214] **Detection and quantitation of BILF2 specific serum antibodies**

[0215] A BILF2 specific sandwich ELISA was developed using BILF2 protein ectopically expressed in HEK293T cells and released into the cells´ supernatant. To do so, the cells were transiently transfected with the expression plasmid 7741.IA1 encoding BILF2 with its transmembrane deleted and a carboxy-terminal EBNA1 tag (EQGPADDPGEG) (SEQ ID NO: 33). After three days of cultivation in medium with 2% fetal calf serum, only, the cell culture supernatant was harvested, purified by low speed centrifugation and analyzed for the concentration of BILF2. This analysis was performed in a separate ELISA together with a highly purified BILF2 protein preparation as standard and reference and a BILF2 specific monoclonal antibody coupled to HRP for detection of BILF2. The cell culture supernatant was found to contain 2.2 µg/mL BILF2 protein. For control purposes, a comparable cell culture supernatant was generated from non-transfected HEK293T cells. Both preparations were aliquoted and stored at -80°C until use.

[0216] To set up the BILF2 specific ELISA, individual wells of a 96-well cluster plate (F96 Maxisorp Nunc Immuno plate; Thermo Scientific #439454) were incubated with the capture antibody, an EBNA1 tag-specific monoclonal rat antibody termed E1BS 1H4 (Grässer et al., 1994) at 4°C overnight. After washing and blocking steps, 6 ng EBNA1-tagged BILF2 protein prepared as described in the previous paragraph and diluted in 100 microL PBS was added per well to capture and immobilize BILF2 protein. As a control and to assess non-specific binding of mouse serum immunoglobulin to the capture antibody or plastic surface in later steps, supernatant from non-transfected HEK293T cells was used accordingly. To allow protein binding the 96-well cluster plate was incubated at room temperature for 4 hours. Thereafter the plate was washed to remove unbound proteins.

[0217] Mouse sera were diluted in cell culture supernatant from non-transfected HEK293T cells and incubated for 60 min at room temperature. Serial dilutions of the absorbed sera were prepared in a standard U-bottom 96-well cluster plate in PBS and transferred to the plate containing the immobilized BILF2 protein and controls. After overnight incubation at 4°C, the plate was washed and mouse serum immunoglobulin was detected with an HRP coupled anti-

mouse antibody. Its binding was revealed using TMB (3,3',5,5'-Tetramethylbenzidine), a substrate of HRP, which was quantitated by stopping the reaction after 10 min and using a Clariostar plate reader instrument. OD measurements of serial serum dilutions obtained with and without BILF2 antigens were recorded, the values subtracted and analyzed using the Prism software (Graphpad Inc.) and its 4-Parameter Logistic (4PL) model to describe and calculate the midpoint of the sigmoidal dose-response curve and its corresponding serum dilution as the antibody titer.

[0218] **Detection and quantitation of EBV-neutralizing serum antibodies in a virus neutralizing test (VNT)**

[0219] The inventors learnt that certain monoclonal antibodies directed against BILF2 can inhibit EBV infection of HEK293 cells (Graham et al., 1977) but not of Daudi cells, a human B cell line (Klein et al., 1968).

[0220] In line with "Epithelial cell infection" as described herein in Example 6, the concentration of neutralizing serum antibodies was determined by determining the VNT50 titer in virus neutralization test with HEK293 cells. VNT50 refers to the dilution of a serum sample that prevents infection in 50% of replicate inoculations. This measurement quantifies the level of neutralizing antibodies that can effectively counteract EBV in sera of BILF2 immunized mice. Serial dilutions of mouse sera starting with an initial 1:8 dilution were tested as described herein. The inventors determined the percentage of GFP-positive cells by flow cytometry and analyzed the data using the Prism software (version 10.4, Graphpad Inc.) to calculate the VNT50 titer.

[0221] **Results**

[0222] Mouse sera collected before the first immunization on day 1, prior to the second immunization on day 61 and at the end of the experiment on day 75 were analyzed for serum antibodies directed against BILF2 in an ELISA. The titers are shown in Figure 4. No BILF2 specific antibodies were detected in non-immunized mice but already after prime-boost immunization on day 61 all but a single animal (mouse m31 immunized with BILF2 protein) produced substantial antibody levels. Two weeks after the second boost immunization on day 75, BILF2 specific antibody levels had increased even further. The two groups of mice immunized with BILF2 encoding mRNA or BILF2 EVs had much higher levels of BILF2 antibodies with mean values of 1:6,112 or 1:4,219 on day 75 compared to mice immunized with BILF2 DNA or BILF2 protein with mean values of 1:704 or 1:809, respectively.

[0223] All sera collected at the end of the experiments on day 75 were analyzed for antibodies with neutralizing functions using EBV stocks and HEK293 cells as targets. The results, i.e., VNT50 titers are shown in Figure 5. Titers higher than 1:10 were considered to have

neutralizing antibodies consistent with titers of mouse sera collected on day 1 or other BALB/c control sera. At the end of the animal experiment all mice of the two groups immunized with BILF2 encoding mRNA or BILF2 EVs had antibodies which neutralized EBV infection of HEK293 cells. With the exception of a single mouse (m18) mice immunized with BILF2 DNA or BILF2 proteins did not mount detectable levels of EBV neutralizing antibodies.

[0224] Thus, these results demonstrate that RNA encoding BILF2 and EVs displaying at their surface the BILF2 glycoprotein of EBV provide for antibodies neutralizing EBV infection of HEK293 cells which serve as surrogate for human epithelial cells. Accordingly, RNA encoding BILF2 and EVs displaying at their surface the BILF2 glycoprotein provide for antibodies against said BILF2 glycoprotein which reduce infectivity of EBV for human epithelial cells, preferably which prevent infection of human epithelial cells by EBV.

[0225] Accordingly, in view of these results, it is apparent that prior art attempts which used a DNA expression plasmid for BILF2 (WO 2019/161163) or suggested BILF2 protein as immunogen (Mackett et al.) fail to provide for antibodies neutralizing EBV infection.

[0226] The inventors asked if the titers of the two different read-outs correlate or not. Figure 6 shows the plot of the two data sets and a statistical analysis revealed a Spearman r value of 0.7949 with a 95% confidence interval between 0.5921 and 0.9030, and a two-tailed P value of <0.0001. The analysis suggested that high levels of BILF2 serum antibodies correlate strongly with neutralizing activity.

[0227] The results indicate that 10 microgram mRNA or non-adjuvanted EVs that carry 2.2 microgram BILF2 protein applied three times in a prime-boost-boost regimen in BALB/c mice can induce antibodies, which can inhibit EBV infection of HEK293 cells. On the contrary, the concentration or the quality of serum antibodies induced in mice immunized three times with 100 microgram DNA encoding BILF2 or immunized with 25 microgram BILF2 protein adjuvanted with ODN 1688 failed to inhibit EBV infection of HEK293 cells.

[0228] Of note, lipid nanoparticles (LNPs) in mRNA-based vaccines act as adjuvant because LNPs enhance immunity through several mechanisms. LNPs boost B-cell responses, induce key cytokines, particularly IL-6, which is crucial for the differentiation of follicular T helper cells, trigger innate immunity, and enhance antigen presentation among other stimulating effects. In contrast, EVs carrying a foreign viral glycoprotein, here BILF2 of EBV, come with their intrinsic potential to act as highly immunogenic particulate antigen structure. Without being bound by theory, it is likely that EVs, when combined with appropriate adjuvants, could trigger a much more potent immune response against, e.g., BILF2 compared to using LNP-encapsulated mRNA as a vaccine candidate.

Cited literature

Coghill, AE, Proietti, C, Liu, Y, Krause, L, Bethony, J, Prokunina-Olsson, L, Obajemu, A, Nkrumah, F, Biggar, RJ, Bhatia, K, Hildesheim, A, Doolan, DL, Mbulaiteye (2020), 'The Association between the Comprehensive Epstein-Barr Virus Serologic Profile and Endemic Burkitt Lymphoma', *Cancer Epidemiol Biomarkers Prev.* 29(1):57-62, https://pubmed.ncbi.nlm.nih.gov/31619404/

Delecluse, HJ, T Hilsendegen, D Pich, R Zeidler, and W Hammerschmidt (1998), 'Propagation and recovery of intact, infectious Epstein-Barr virus from prokaryotic to human cells.', *Proc. Natl. Acad. Sci. U S A*, 95 (14), 8245-50; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9653172.

Everly, MJ, Bloom, RD, Tsai, DE, Trofe, J (2007), Transplantation: Posttransplant Lymphoproliferative Disorder', *Annals of Pharmacotherapy,* 41(11), 1850-1858; https://pubmed.ncbi.nlm.nih.gov/17940127/

Hutt-Fletcher, LM (2015), 'EBV glycoproteins: where are we now?', *Future Virol.*, 10(10): 1155-1162; https://pubmed.ncbi.nlm.nih.gov/26843889/

Graham, Frank L, J Smiley, WC Russell, and R Nairn (1977), 'Characteristics of a human cell line transformed by DNA from human adenovirus type 5.', *J Gen Virol*, 36 (1), 59-74; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=886304.

Grässer, F A, P G Murray, E Kremmer, K Klein, K Remberger, W Feiden, G Reynolds, G Niedobitek, L S Young, and N Mueller-Lantzsch (1994), 'Monoclonal antibodies directed against the Epstein-Barr virus-encoded nuclear antigen 1 (EBNA1): immunohistologic detection of EBNA1 in the malignant cells of Hodgkin's disease.', *Blood*, 84 (11), 3792-98; https://pubmed.ncbi.nlm.nih.gov/7949135.

Gu, AD, Xie, YB, Mo, HY, Jia, WH, Li, MY, Li, M, Chen, LZ, Feng, QS, Liu, Q, Qian, CH, Zeng, YX (2008),'Antibodies against Epstein–Barr virus gp78 antigen: a novel marker for serological diagnosis of nasopharyngeal carcinoma detected by xMAP technology', *Journal of General Virology*, 89(5) https://pubmed.ncbi.nlm.nih.gov/18420792/

Gu, AD, Lu, LX, Xie, YB, Chen, LZ, Feng, QS, Kang, T, Jia, WH, Zeng, YX (2009), 'Clinical values of multiple Epstein-Barr virus (EBV) serological biomarkers detected by xMAP technology', *Journal of Translational Medicine*, 7(73) https://pubmed.ncbi.nlm.nih.gov/19698162/

Houene, G, Trier, NH (2021), 'Epstein-Barr Virus and Systemic Autoimmune Diseases', *Front Immunol.*, 11, https://pubmed.ncbi.nlm.nih.gov/33488588/

Janz, A, M Oezel, C Kurzeder, J Mautner, D Pich, M Kost, W Hammerschmidt, and HJ Delecluse (2000), 'Infectious Epstein-Barr virus lacking major glycoprotein BLLF1 (gp350/220) demonstrates the existence of additional viral ligands.', *J Virol*, 74 (21), 10142-52; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11024143.

Klein, E, G Klein, JS Nadkarni, JJ Nadkarni, H Wigzell, and P Clifford (1968), 'Surface IgM-kappa specificity on a Burkitt lymphoma cell in vivo and in derived culture lines.', *Cancer Res*, 28 (7), 1300-10; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=4174339

Liu, Z.; Yu, K. J.; Coghill, A. E.; Brenner, N.; Cao, S.-M.; Chen, C.-J.; Chen, Y.; Doolan, D. L.; Hsu, W.-L.; Labo, N.; Middeldorp, J. M.; Miley, W.; Simon, J.; Wang, C.-P.; Waterboer, T.; Whitby, D.; Xie, S.-H.; Ye, W.; Hildesheim, A. (2019): Multilaboratory Assessment of Epstein Barr Virus Serologic Assays: the Case for Standardization. In: J Clin Microbiol 57 (11), e01107-19. DOI: 10.1128/JCM.01107-19.

Mackett, M, MJ Conway, JR Arrand, RS Haddad, and LM Hutt-Fletcher (1990), 'Characterization and expression of a glycoprotein encoded by the Epstein-Barr virus BamHI I fragment.', *J Virol*, 64 (6), 2545-52; https://pubmed.ncbi.nlm.nih.gov/2159529.

Mendoza F, Kunitake, H, Laks, H, Odim, J (2006), ' Post-transplant lymphoproliferative disorder following pediatric heart transplantation', *Pediatric Transplantation*, 10(1), 60-66; https://pubmed.ncbi.nlm.nih.gov/16499589/

Pudney, VA, Leese, AM, Rickinson, AB, Hislop, AD (2005), 'CD8+ immunodominance among Epstein-Barr virus lytic cycle antigens directly reflects the efficiency of antigen presentation in lytically infected cells', *J Exp Med*, 201(3):349-60; https://pubmed.ncbi.nlm.nih.gov/15684323/

Roessler, Johannes, Dagmar Pich, Verena Krähling, Stephan Becker, Oliver T. Keppler, Reinhard Zeidler, and Wolfgang Hammerschmidt (2023), 'SARS-CoV-2 and Epstein–Barr virus-like particles associate and fuse with Extracellular Vesicles in virus neutralization tests', *Biomedicines*, 11 (11), 2892; http://dx.doi.org/10.3390/biomedicines11112892.

Roessler, J, D Pich, M Albanese, PR Wratil, V Krähling, JC Hellmuth, C Scherer, M von Bergwelt-Baildon, S Becker, OT Keppler, A Brisson, R Zeidler, and W Hammerschmidt (2022), 'Quantitation of SARS-CoV-2 neutralizing antibodies with a virus-free, authentic test.', *PNAS Nexus*, 1 (2), pgac045; https://pubmed.ncbi.nlm.nih.gov/36382127.

Steinbrück, L, M Gustems, S Medele, TF Schulz, D Lutter, and W Hammerschmidt (2015), 'K1 and K15 of Kaposi's sarcoma-associated herpesvirus are partial functional homologues of latent membrane protein 2A of Epstein-Barr virus.', *J Virol*, 89 (14), 7248-61; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=25948739.

Swerdlow, AJ, Higgins, CD, Hunt, BJ, Thomas, JA, Burke, MM, Crawford, DH, Yacoub, MH (2000), ' RISK OF LYMPHOID NEOPLASIA AFTER CARDIOTHORACIC TRANSPLANTATION: A Cohort Study of the Relation to Epstein-Barr Virus', *Transplantation*, 69(5), 897-904; https://pubmed.ncbi.nlm.nih.gov/10755547/

Taylor, AL, Marcus, R, Bradley, JA (2005), 'Post-transplant lymphoproliferative disorders (PTLD) after solid organ transplantation', *Crit Rev Oncol Hematol.*, 56(1), 155-67, https://pubmed.ncbi.nlm.nih.gov/15979320/

Taylor, GS, Long, HM, Brooks, JM, Rickinson, AB, Hislop, AD (2015), 'The Immunology of Epstein-Barr Virus–Induced Disease', *ANNUAL REVIEW OF IMMUNOLOGY,* 33:787-821 https://pubmed.ncbi.nlm.nih.gov/25706097/

CLAIMS

1. A composition comprising

a) RNA encoding the BILF2 glycoprotein of EBV, or

b) an extracellular vesicle (EV) or EB-virus-like particle (VLP) displaying at its surface the BILF2 glycoprotein of EBV,

for use in a method of eliciting antibodies against said BILF2 glycoprotein which reduce infectivity of EBV for human epithelial cells.

2. The composition for use of claim 1, wherein said antibodies prevent infection of human epithelial cells by EBV.

3. The composition of claim 1 or 2, wherein said EV or VLP expresses BILF2, either alone or in combination with one or more other EBV glycoproteins.

4. The composition of claim 3, wherein said other EBV glycoproteins may comprise gp350, gB, gp42, gH, gL, gM, gN, BMRF2, BDLF2, BDLF3, BILF1, and BARF1.

5. The composition of any one of the preceding claims, wherein the VLP further comprises one or more EBV structural proteins.

6. The composition of claim 5, wherein the one or more EBV structural proteins include fusion (F), matrix (M), or nucleocapsid (N).

7. The composition of any one of the preceding claims, further comprising one or more pharmaceutically acceptable carriers.

8. The composition for any one of the preceding claims, further comprising one or more adjuvants.

9. The composition of any one of the preceding claims for use in a method of treatment or prevention of a disease.

10. The composition of claim 9, wherein the disease is cancer, preferably a cancer of epithelial origin, or an infection, preferably an EBV infection, most preferably an EBV infection of epithelial cells.

11. The composition of claim 9 or 10, wherein the disease is cancer and the cancer is selected from the group consisting of meduloblastoma, retinoblastoma, Burkitt's lymphoma, Hodgkin´s lymphoma, oral cancer, skin cancer, basaliom, acute myeloid leukemia, pancreatic cancer, colorectal cancer, endometrial cancer, biliary tract cancer, liver cancer, myeloma, multiple myeloma, prostate cancer, stomach cancer, kidney cancer, bone cancer, soft tissue cancer, head and neck cancer, glioblastoma multiforme, astrocytoma, melanoma, lung cancer, esophageal cancer, gastric cancer, breast cancer, ovarian cancer, mesothelioma cancer, bladder cancer, anal cancer, chondrosarcoma cancer, osteosarcoma cancer, sarcoma cancer, primitive neuroectodermal cancer (primitive neuroectodermal tumor (PNET)), and combinations thereof.

12. An antibody for use in a method for passive immunization against EBV, wherein the antibody is directed against the BILF2 glycoprotein of EBV, wherein said antibody binds to an epitope within amino acids 41 to 160 of SEQ ID NO: 25 and is capable of reducing the infectivity of EBV for human epithelial cells.

13. An antibody for use in a method for reducing the infectivity of EBV for human epithelial cells, wherein the antibody is directed against the BILF2 glycoprotein of EBV, wherein said antibody binds to an epitope within amino acids 41 to 160 of SEQ ID NO: 25 and is capable of reducing the infectivity of EBV for human epithelial cells.

14. An antibody for use in a method for preventing infection of human epithelial cells by EBV, wherein the antibody is directed against the BILF2 glycoprotein of EBV, wherein said antibody binds to an epitope within amino acids 41 to 160 of SEQ ID NO: 25 and is capable of reducing the infectivity of EBV for human epithelial cells.

15. The antibody of any one of claims 12 to 14, wherein said antibody binds to an epitope within amino acids 80 to 160 or 129 to 143 of SEQ ID NO: 25 and is capable of reducing the infectivity of EBV for human epithelial cells.

16. The antibody of any one of claims 12 to 15, wherein said antibody is
    (a) an antibody comprising a heavy chain variable region having an amino acid sequence with at least 80% identity to the amino acid sequence shown in SEQ ID NO: 7 and a light chain variable region having an amino acid sequence with at least 80% identity to the amino acid sequence shown in SEQ ID NO: 8;
    (b) an antibody comprising a heavy chain variable region having an amino acid sequence with at least 80% identity to the amino acid sequence shown in SEQ ID

NO: 15 and a light chain variable region having an amino acid sequence with at least 80% identity to the amino acid sequence shown in SEQ ID NO: 16;

(c) an antibody comprising a heavy chain variable region having an amino acid sequence with at least 80% identity to the amino acid sequence shown in SEQ ID NO: 23 and a light chain variable region having an amino acid sequence with at least 80% identity to the amino acid sequence shown in SEQ ID NO: 24; or

(d) an antibody binding to the same epitope as that in the EBV BILF2 protein shown in SEQ ID NO: 25 to which any one of the antibodies (a) to (c) bind.

17. The antibody of any one of the claims 12 to 16, wherein said antibody is

(a) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 1, heavy chain CDR2 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 2, and heavy chain CDR3 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 3, and
a light chain variable region comprising light chain CDR1 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 4, light chain CDR2 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 5, and light chain CDR3 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 6;

(b) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 9, heavy chain CDR2 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 10, and heavy chain CDR3 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 11, and
a light chain variable region comprising light chain CDR1 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 12, light chain CDR2 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 13, and light chain CDR3 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 14;

(c) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 17, heavy chain CDR2 having an amino acid

sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 18, and heavy chain CDR3 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 19, and

a light chain variable region comprising light chain CDR1 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 20, light chain CDR2 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 21, and light chain CDR3 having an amino acid sequence with at least 80% identity to the amino acid sequence as set forth in SEQ ID NO: 22; or

(d) an antibody binding to the same epitope as that in the EBV BILF2 protein shown in SEQ ID NO: 25 to which any one of the antibodies (a) to (c) bind.

18. The antibody of any one of the claims 12 to 17, wherein said antibody is

(a) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 1, heavy chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 2, and heavy chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 3, and

a light chain variable region comprising light chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 4, light chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 5, and light chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 6;

(b) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 9, heavy chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 10, and heavy chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 11, and

a light chain variable region comprising light chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 12, light chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 13, and light chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 14;

(c) an antibody comprising a heavy chain variable region comprising heavy chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 17, heavy chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 18, and heavy chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 19, and

a light chain variable region comprising light chain CDR1 having the amino acid sequence as set forth in SEQ ID NO: 20, light chain CDR2 having the amino acid sequence as set forth in SEQ ID NO: 21, and light chain CDR3 having the amino acid sequence as set forth in SEQ ID NO: 22; or

(d) an antibody binding to the same epitope as that in the EBV BILF2 protein shown in SEQ ID NO: 25 to which any one of the antibodies (a) to (c) bind.

19. The antibody of any one of claims 12 to 18, wherein said epitope is determined by pepspot analysis, amino acid replacement analysis and/or deletion analysis of the ectodomain encompassing amino acids 18 to 212 of BILF2 as shown in SEQ ID NO: 25.