Cellular and Molecular Life Sciences (2020) 77:4315–4324
https://doi.org/10.1007/s00018-020-03538-3

Cellular and Molecular Life Sciences

REVIEW

Vaccination against the Epstein–Barr virus

Julia Rühl[1] · Carol S. Leung[2] · Christian Münz[1]

Received: 15 November 2019 / Revised: 8 January 2020 / Accepted: 21 April 2020 / Published online: 4 May 2020
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Abstract

Epstein–Barr virus (EBV) was the first human tumor virus being discovered and remains to date the only human pathogen that can transform cells in vitro. 55 years of EBV research have now brought us to the brink of an EBV vaccine. For this purpose, recombinant viral vectors and their heterologous prime-boost vaccinations, EBV-derived virus-like particles and viral envelope glycoprotein formulations are explored and are discussed in this review. Even so, cell-mediated immune control by cytotoxic lymphocytes protects healthy virus carriers from EBV-associated malignancies, antibodies might be able to prevent symptomatic primary infection, the most likely EBV-associated pathology against which EBV vaccines will be initially tested. Thus, the variety of EBV vaccines reflects the sophisticated life cycle of this human tumor virus and only vaccination in humans will finally be able to reveal the efficacy of these candidates. Nevertheless, the recently renewed efforts to develop an EBV vaccine and the long history of safe adoptive T cell transfer to treat EBV-associated malignancies suggest that this oncogenic γ-herpesvirus can be targeted by immunotherapies. Such vaccination should ideally implement the very same immune control that protects healthy EBV carriers.

Keywords Virus-like particles · Recombinant viral vectors · Glycoprotein multimers · Infectious mononucleosis · Cytotoxic lymphocytes · Neutralizing antibodies · Prophylactic · Therapeutic · Lymphoma · Carcinoma

Importance of EBV as a vaccination target

The Epstein–Barr virus (EBV) is a common human γ-herpesvirus with the most potent host cell transforming capacity of all infectious disease agents in vitro [1]. It was discovered 55 years ago in Burkitt's lymphoma [2, 3] and is associated with epithelial-, lymphocyte- and smooth muscle-derived tumors in humans [4]. The most prominent EBV-associated tumors are in addition to the still most common Sub-Saharan childhood tumor Burkitt's lymphoma, post-transplant lymphoproliferative disease (PTLD), diffuse large B cell lymphomas (DLBCL), Hodgkin's lymphoma, nasopharyngeal carcinoma and the 10% of gastric carcinoma that are positive for this virus [5]. Around 2% of all malignancies in humans are associated with EBV with an annual incidence rate of 200,000 [6]. In addition to these EBV-associated malignancies, this virus causes immune pathologies that result from a hyperactivation of EBV-induced T cell responses [7]. These include syndromes that result from $CD8^+$ T cell lymphocytosis during symptomatic primary EBV infection called infectious mononucleosis (IM) [8], from virus-induced cytokine production for the hyperactivation of myeloid cells resulting in hemophagocytic lymphohistiocytosis (HLH) [9] and possibly also the autoimmune disease multiple sclerosis (MS) [10]. Along the lines of EBV possibly setting up a pro-inflammatory environment in the brain of some MS patients, it was recently reported that encephalitis in at least one patient under immune checkpoint treatment blocking the inhibitory receptor PD-1 on T cells was associated with elevated EBV loads in blood and cerebrospinal fluid, as well as clonal expansion of T cells with EBV-specific T cell receptors in the brain [11]. Accordingly, loss of EBV-specific T cell-mediated immune control was observed upon PD-1 blockade in a preclinical model of EBV infection in mice with reconstituted human immune system components (HIS mice) [12]. Thus, both EBV-associated malignancies and immune pathologies justify the

✉ Christian Münz
christian.muenz@uzh.ch

[1] Viral Immunobiology, Institute of Experimental Immunology, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

[2] Ludwig Institute for Cancer Research, Nuffield Department of Medicine, University of Oxford, Oxford, UK

development of a vaccine against EBV, but which individual or combination of viral antigens should be targeted.

For the choice of vaccine antigen, the life cycle of EBV and its gene expression in the various virus-associated diseases needs to be considered. EBV is primarily transmitted via saliva exchange and most likely crosses the mucosal epithelial cell barrier by transcytosis to infect B cells in submucosal secondary lymphoid tissues like tonsils [13, 14]. In B cells EBV expresses latent viral gene products from its circularized and increasingly chromatinized multi-copy extra-chromosomal DNA [15]. Initial expression of six nuclear antigens (EBNAs) and two membrane proteins (LMPs) in the so called latency III program is curtailed with further B cell differentiation to just EBNA1, LMP1 and 2 (latency II) in germinal center B cells and to finally no viral protein expression in memory B cells (latency 0), the site of EBV persistence [16]. In homeostatically proliferating memory B cells, EBNA1 is transiently expressed as the only viral protein (latency I) [17]. The latent EBV proteins drive B cell proliferation allowing dissemination of the virus in the human body. From the reservoir in memory B cells EBV can reactivate upon plasma cell differentiation [18], and then presumably amplifies virion production by lytic replication in epithelial cells for more efficient shedding into the saliva and further transmission [19]. EBV-associated pathologies originate from these different stages of the EBV life cycle. For example, PTLD and some DLBCL express latency III, Hodgkin's and Burkitt's lymphoma emerge from germinal centers with latency II or latency I, respectively, and early lytic EBV antigen-specific $CD8^+$ T cells expand mainly during IM. Furthermore, early lytic EBV antigen expression has recently been recognized to enhance virus-associated tumor formation [1]. These considerations identify latent and early lytic EBV antigens as promising candidates for vaccines, but also envelope proteins are explored as targets of neutralizing antibody responses that could curb transmission.

Protective immune responses against EBV infection

With the classes of EBV antigens that could be targeted for vaccination against EBV- associated diseases in mind, the question arises which type of immune responses should be elicited. Information about protective immune responses against EBV can be gleaned from preclinical in vitro and in vivo models and clinical observations. Among the most informative clinical observations are primary immunodeficiencies that identify genetic lesions that predispose for EBV-associated diseases [20, 21]. These point towards cytotoxic lymphocytes as the main immune compartment that exerts immune control over EBV infection. The respective lymphocytes need to be positive for the cytotoxic granule machinery, including perforin, Munc13-4 and Munc18-2 [22–24]. They need to carry the co-stimulatory molecules CD27, SLAM protein family members like 2B4, 4-1BB and NKG2D, as well as the co-inhibitory CTLA-4 receptor in combination with the main activating receptors CD16 or the T cell receptor [25–35]. Furthermore, they need to expand well after activation and depend on GATA2 and MCM4 for their differentiation [36–39]. In contrast, EBV-specific immune control does not seem to depend on type I and II interferons, antibody production and MHC class II restricted T cell responses [20]. Particularly the absence of EBV-associated pathologies in patients with B^+ hypogammaglobunemia and Ig class-switch recombination deficiencies is surprising [40, 41]. Furthermore, since MHC class II deficiencies do not predispose for complications with EBV infection [42, 43], MHC class I-restricted helper T cell functions might compensate to maintain cytotoxic lymphocytes. These considerations point to $CD8^+$ T cells, natural killer (NK), NKT and γδ T cells as pillars of EBV-specific immune control. Indeed, all of these cytotoxic lymphocyte populations have been shown to restrict EBV infection in the preclinical model of HIS mice [44–49]. In addition the EBV- specific $CD8^+$ T cells might have a particular $PD\text{-}1^+Tim\text{-}3^+KLRG1^+CXCR5^+TCF\text{-}1^+$ and $BATF3^+$ phenotype that allows them to control EBV-infected B cells in germinal centers [12, 50, 51]. These $CD8^+$ T cells recognize predominantly latent and early lytic EBV antigens [7]. T cell lines have also been adoptively transferred to treat EBV-associated malignancies, initially primarily PTLD [52]. With respect to individual antigens EBNA1, LMP1- and LMP2-specific T cell lines have proven clinically efficacious in EBV- associated lymphomas and nasopharyngeal carcinoma [53–55]. Interestingly, T cells with these specificities have also been infused into MS patients with some clinical success [56, 57]. Lytic EBV replication is in addition targeted by early differentiated $CD56^{dim}NKG2A^+KIR^-$ partially $CD16^+$ NK cells [45, 58, 59]. Both $CD8^+$ T cells and NK cells significantly expand during IM [8, 59–62]. In addition to early differentiated NK cells, Vγ8Vδ2 T cells are elevated in a subset of children [63]. They preferentially respond to Burkitt lymphoma cells with a latency I EBV gene expression. Finally, NKT cells preferentially respond to Hodgkin's lymphoma and nasopharyngeal carcinoma cell lines [64]. Thus, while $CD8^+$ T cells target all EBV latencies and early lytic EBV replication, NK, NKT and γδ T cells seem to restrict lytic, latency II and latency I EBV infection, respectively. These might be the cytotoxic lymphocyte compartments on which immune control of EBV infection depends and that should be stimulated by EBV-specific vaccination.

Recombinant viral vector vaccines

Recombinant viral vector vaccines are live viruses that are engineered to express additional proteins, against which immune responses are desired [65, 66]. These vaccine platforms are relatively new and have several advantages over traditional vaccines. First, viral vector vaccines can induce a broad range of immune responses, particularly in $CD8^+$ cytotoxic T lymphocyte (CTL) responses that are important in clearing virally infected and tumor cells. This contrasts with most of the existing vaccine formulations that are designed to elicit primarily a humoral antibody response. The viral vector infects target cells and leads to antigen expression in the cytosol, where it can gain easy access to the classical MHC class I-processing pathway, and subsequent presentation of the resulting peptide epitopes on MHC class I molecules to stimulate an antigen-specific $CD8^+$ CTL response. Second, viruses are naturally immunogenic and therefore adjuvants themselves as they express a range of pathogen-associated molecular patterns (PAMPs) to initiate an inflammatory response. This adjuvant effect is crucial for enhancing the protective immune response elicited by vaccines. Third, viral vector vaccines have a high gene transduction efficiency [67] and can deliver the antigens to different cell types depending on the tropism of the used viral vectors.

Many different viral vectors have been developed to use as vaccine candidates, including poxviruses, adenoviruses and yellow fever virus [65]. The choice of viral vectors for vaccine development mostly depends on the vector's properties with respect to immunogenicity, safety and infectivity. Furthermore, the pre-existing immunity against the viral vectors in humans is often considered. Vaccinia virus and adenovirus are among the most widely used viral vectors, mainly due to their ability to induce antigen-specific T cell responses. Currently many clinical trials are ongoing to test diverse viral vector vaccines in different disease settings, mainly infectious diseases [68, 69].

The first EBV vaccine tested in humans used live recombinant vaccinia virus expressing the EBV membrane antigen BLLF1 (gp350) [70]. While there were no significant EBV titer variations between vaccinated and unvaccinated adults, only three of nine vaccinated infants were infected with EBV within 16 months after vaccination compared to ten out of ten in the unvaccinated control. However, this vaccine platform is no longer accepted due to the risk of adverse effects [71]. A safer alternative is the multiplication-incompetent-attenuated pox viral vector of modified vaccinia virus Ankara (MVA) [72, 73]. Indeed, a MVA vaccine encoding the EBV antigens EBNA1 and LMP2 (MVA-EL) has been developed as a therapeutic vaccine against EBV-positive cancer [74, 75]. This vaccine has been evaluated for safety and immunogenicity in phase I clinical trials in EBV-positive nasopharyngeal carcinoma (NPC) patients. MVA-EL was well tolerated and there was an increase in T cell responses against at least one antigen after vaccination in 8 of 14 patients in the UK and 15 of 18 patients in Hong Kong. However, the therapeutic efficacy of the MVA-EL has yet to be shown. A recombinant adenovirus vector has also been developed to induce EBV-specific T cell responses. However, instead of using it as a direct vaccination, facing pre-existing adenovirus immunity, the respective vectors encoding LMP polyepitopes with or without EBNA1 were used to infect DCs or EBV-transformed B lymphoblastoid cell lines in vitro, to either expand EBV-specific T cells and infuse these back into patients or to adoptively transfer the infected DCs as cellular vaccination [55, 76–78]. Considering the complexity of cellular vaccine approaches, adenovirus vectors that are shielded against pre-existing immunity and encoded EBV antigens should be explored for direct vaccination against EBV (Fig. 1).

Heterologous prime-boost vaccination

Early work on adenovirus vaccines used serotypes such as human adenovirus 5 (hAd5), but pre-existing immunity that can neutralize the viral vector is widespread in the human population, thus limiting its potency and hampering its clinical use. Chimpanzee adenovirus vectors were then developed to avoid this pre-existing neutralizing immunity [79, 80]. Unfortunately, the immunogenicity of these vectors can establish neutralizing responses that limit its capacity for secondary injections, requiring the use of different viral vectors during boost vaccination. Indeed, heterologous prime-boost strategies using two antigen formulations have been regarded as an improved way of immunization [81, 82].

Different combinations of heterologous prime-boost vaccines have been tested in animal models and some are undergoing efficacy testing in clinical trials, mainly against infectious diseases [82–84]. Among these, the combination of chimpanzee adenovirus and MVA has been shown to induce a strong $CD8^+$ T cell response that correlates with efficacy in humans against a liver-stage malaria antigen [85]. The same strategy has been applied to vaccine development against additional diseases, including hepatitis C virus (HCV), Ebola virus and prostate cancer [86–88]. Our group has also demonstrated that adenovirus prime and MVA boost vaccination against EBNA1 are efficient in eliciting comprehensive $CD4^+$ and $CD8^+$ T cell responses which can translate into protection against EBV antigen expressing lymphomas [89].

Though viral vectors generally elicit a higher magnitude of T cell responses, they are expensive to produce and

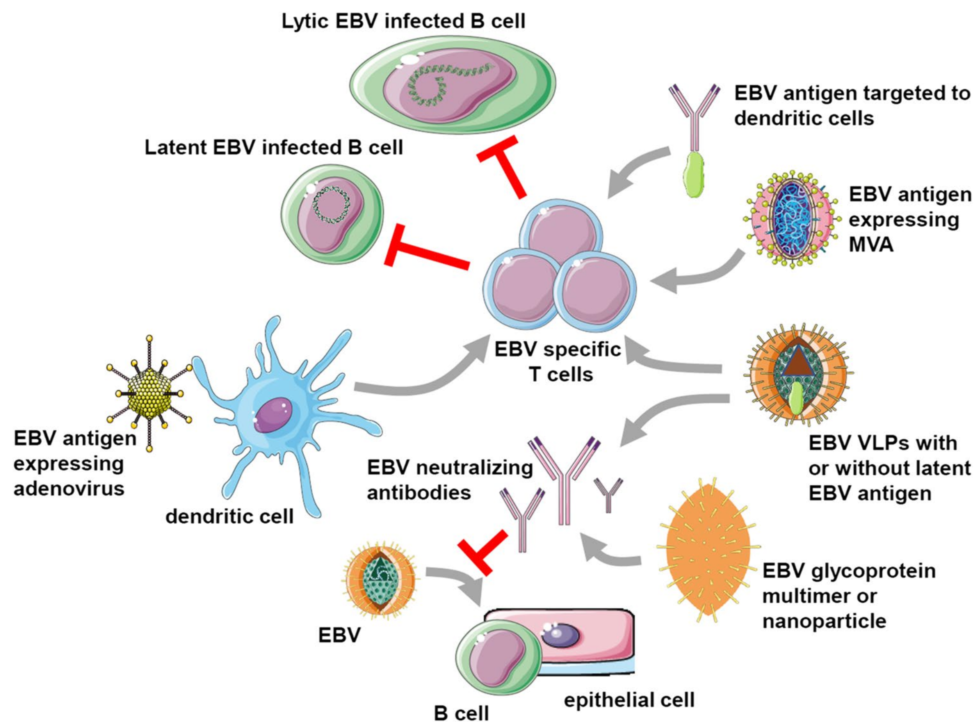

Fig. 1 EBV vaccine candidates. EBV-specific vaccination aims to either stimulate protective T cell responses (top half) or neutralizing antibodies (bottom half), that target latent and lytic EBV-infected B cells or prevent B and epithelial cell infection, respectively. For EBV-specific T cell stimulation, recombinant adenoviruses encoding latent EBV antigens are explored for dendritic cell infection, followed by T cell expansion in vitro for adoptive transfer or injection into patients with EBV-associated malignancies. Furthermore, latent EBV antigen targeting to dendritic cells with antibodies is investigated. Moreover, recombinant modified vaccinia virus Ankara (MVA) vectors expressing latent EBV antigens have been developed and tested in patients. Finally, EBV-derived virus-like particles (VLPs) have shown promising results in preclinical models, lowering EBV titers when a latent EBV antigen was transgenically expressed in the viral tegument. Neutralizing antibodies were also elicited with VLPs or EBV envelope proteins. These antibody responses were more potent after multimerization of the respective glycoproteins or their incorporation into nanoparticles. This figure was created in part with modified Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported License: https://smart.servier.com

usually take a long time to manufacture. In contrast to these, protein-based vaccines are generally safer and cheaper to produce. Our lab has developed a vaccine platform to deliver the EBV antigen EBNA1 to antigen presenting cells by fusing the antigen to a monoclonal antibody against the DC endocytic receptor DEC-205 [90–92]. This recombinant protein vaccine, adjuvanted with the double-stranded RNA polyI:C, has been shown to induce robust T cell responses, but mostly CD4$^+$ T cell responses and lacking CD8$^+$ T cell responses when tested in vivo [89, 91]. As viral vector vaccines are known for their superiority in inducing CD8$^+$ T cell responses, we combined this approach with viral vector vaccines to stimulate strong CD4$^+$ and CD8$^+$ T cell responses [89]. We have shown that this heterologous prime boost vaccination strategy is more efficient in inducing a protective T cell response than the homologous prime boost. The combination of the protein vaccine targeting DEC-205 and the adenovirus is only slightly less efficient than the adenovirus prime and MVA boost in protecting mice from T cell lymphoma challenge, with the later, however, being superior against B cell lymphomas. These findings are consistent with previous studies, indicating that human immunodeficiency virus (HIV) antigen targeting to DEC-205 had to be boosted with a recombinant poxviral vaccine to elicit protective responses in nonhuman primates [93]. Thus, heterologous prime-boost approaches should be considered in the future for the development of a vaccine against EBV that aims to elicit T cell mediated immune control.

Virus-like particles

Virus-like particles (VLPs) are defined as virus particles which do not contain any viral nucleic acids. The research efforts of the last decades led to the development of VLP vaccines, including human papillomavirus VLPs against cervical carcinoma and *Plasmodium falciparum* antigen displaying alfalfa mosaic virus VLPs against malaria [94, 95]. Because of their safety attributes and their ability to elicit virus-specific innate and adaptive immune responses

without harming the host, VLPs were also investigated as versatile tools for EBV vaccine development.

In 2015, a novel Newcastle disease virus (NDV) VLP platform displaying the EBVgp350/220 ectodomain was shown to elicit strong, long-lasting neutralizing antibody responses in BALB/c mice, which were, however, not significantly higher than responses induced by soluble gp350/220 [96]. The NDV VLP platform was subsequently used to incorporate additional EBV envelope and latent antigens. The combination of gH/gL-EBNA1 and gB/LMP2 into VLPs both led to the generation of high neutralizing titers and EBV-specific T cell responses in vaccinated BALB/c mice [97]. A different, but possibly even more promising approach, is to use VLPs based on the EBV particle. To reduce oncogenicity of EBV for vaccination, genetic elements and/or proteins involved in DNA packaging were deleted [98]. Already 20 years ago, the first generation of cell lines that produce EBV VLPs was created by removing the terminal repeats (TRs), which previously had been identified as packaging signals of EBVs DNA [99–101]. Those first EBV VLPs were able to bind human B and epithelial cells and did contain large amounts of viral particles, but no viral DNA. In 2011, Ruiss et al. developed EBV-derived VLPs in which the deletion of TRs was complemented with the deletion of potential EBV oncogenes namely EBNA2, 3A, 3B and 3C, LMP1 and BZLF1 for additional safety [102]. Those EBV VLPs were shown to be assembled and released via the endosomal sorting complex for transport (ESCRT). Infected B cells were capable of presenting multiple EBV antigens to $CD8^+$ and $CD4^+$ T cells, which led to significant T cell expansions in vitro. In immunized BALB/c mice, the EBV VLPs elicited EBV-specific humoral and cellular immune responses [102].

Despite strong evidence of immune activation and a good safety profile in mice, the risk of remaining infectious oncogenic genomes in the early EBV VLPs remained high. Therefore, the development of EBV VLPs was further improved through the deletion of the viral packaging and nuclear egress proteins BFLF1/BFRF1A or the portal protein BBRF1 for viral DNA insertion into the capsid. In 2012, Pavlova et al. managed to create fully DNA-free EBV VLPs. The BFLF1/BFRF1A mutant EBV strain elicited comparable $CD4^+$ T cell responses as the EBV wildtype in vitro [103]. Through these deletions, the pathogenic potential of the EBV VLPs was reduced, however the responses against structural and lytic components of EBV may not be sufficient for the creation of an effective EBV vaccine.

Therefore, more immunogenic EBV VLPs were created by fusing latent antigens such as EBNA1 and EBNA3C to the abundant major tegument protein BNRF1. Through this approach, the EBV VLPs were able to stimulate potent $CD4^+$ T cell responses against structural as well as latent EBV epitopes. In ex vivo cultures with human peripheral blood mononuclear cells, the EBV VLPs, which contained EBNA1 latent EBV antigen, could inhibit the outgrowth of EBV-infected B cells more proficiently than their counterparts without latent antigen. This partial inhibition of EBV infection in B cells could also be shown in vivo in HIS mice, while 100% of the PBS-treated mice got infected after EBV challenge, only 14% of the VLP-EBNA1-immunized mice had detectable viral loads in their peripheral blood [104]. Therefore, EBV-derived VLPs might need to contain latent antigens in addition to the structural proteins to elicit protective immune responses. Despite the improved safety profile of EBV-derived VLPs themselves, the low titers of these that can be produced by most cell lines and contaminants in the respective preparations that derive from the human producer cells remain concerns for this vaccination approach.

Envelope protein formulations to elicit neutralizing antibodies

Gp350/220 is an EBV glycoprotein, which initiates the attachment of EBV to susceptible host, primarily B cells expressing the complement receptor type 2 (CD21) and/or type 1 (CD35) [105]. Binding is further strengthened by the gp42 envelope protein interacting with MHC class II [106]. While these glycoproteins are specific for EBV, fusion of the viral envelope with cellular membranes is finally mediated by the gH/gL and gB proteins that are conserved among the herpesviruses [107]. Being crucial in the first step of EBV latent infection, gp350/220 is one of the antigenic candidates often in the focus of exploration for the development of a prophylactic EBV vaccine. In the past, multiple potent antibodies against the EBV gp350 protein were found in human blood [108]. The neutralizing antibody that has been mainly characterized is the monoclonal 72A1 antibody. The broad interest in the 72A1 antibody led to the development of a humanized anti-gp350 antibody which blocked EBV infection of B cells in vitro to equivalent levels as the mouse-human chimeric 72A1 antibody construct [109]. However, immunizing with the gp350 protein alone did not lead to a prevention of infection with EBV in a phase II clinical trial, but only to a partial reduction of acute IM [110, 111]. Therefore, improvements of the gp350 protein vaccination were conceived [112] and dimers, trimers and tetramers of gp350 elicited significantly higher neutralizing antibody titers in mice [113, 114]. Multimerized gp350 therefore seems to elicit more potent B cell responses.

Improvement of gp350 protein vaccines was not only achieved by multimerization, but also by the addition of immune-stimulating adjuvants. A study of Heeke et al. included the use of GLA/SE as an adjuvant in addition to the vaccination with gp350 in mice and rabbits. GLA/SE is composed of the synthetic TLR4 agonist glucopyranosyl

lipid A (GLA) integrated into a stable emulsion (SE). Mice and rabbits that were vaccinated with GLA/SE-adjuvanted gp350 vaccines showed elevated EBV-neutralizing antibody titers. Also, high IgG titers and robust anti-gp350 $CD4^{+}$ T cell responses could be detected in vaccinated mice [115]. Furthermore, by epitope mapping, it was found that the immune response against EBV's gp350 protein is mainly directed against one dominant neutralizing epitope of gp350. In an approach to focus the antibody response on this potent epitope, gp350 mimetic peptides with strong ionic, electrostatic or hydrogen bonds to the neutralizing region of the monoclonal antibody 72A1 [116] were generated by computer modeling [117]. In mice, those gp350 mimetic peptides elicited antibody responses that were able to block the interaction of 72A1 antibody and gp350. This technique may lead to more potent peptide vaccines which could contain the neutralizing epitopes of multiple EBV envelope glycoproteins.

In addition to improving EBV gp350-specific vaccination, antigen formulations have been extended to the other envelope proteins. Cui et al. compared the vaccination of rabbits with recombinant monomeric as well as multimeric EBV gH/gL and gB proteins to gp350 protein vaccines. The group found that vaccination with EBV gH/gL or gB protein vaccines elicited higher neutralization titers than gp350 protein vaccines [118]. These antibody titers were even increased when gH/gL and gB proteins were multimerized. Recently, Snijder et al. also used the proteins from the EBV fusion machinery as targets and the group isolated neutralizing human antibodies from memory B cells [119]. An anti-gH/gL antibody, AMMO1, showed potent inhibition of infection of B and epithelial cells in vitro. Therefore, vaccination for gp350 plus the herpesviral fusion complex might elicit the most comprehensive humoral immune responses to EBV,

Another promising approach for EBV vaccination, which also mainly focuses on the generation of neutralizing antibodies against viral glycoproteins, is the use of nanoparticles for the delivery of mutimerized and optimally spaced EBV antigens. In 2015, nanoparticles containing a portion of the ectodomain of gp350 including the complement receptor 2 binding site were used to vaccinate mice and monkeys [120]. Vaccinated mice developed anti-gp350 titers that were about 1000-fold higher than in mice that received a soluble monomeric gp350 vaccine and were protected against a challenge with vaccinia virus expressing gp350. Cynomolgus macaques immunized with the gp350 nanoparticles also generated anti-gp350 titers that were three- to ten-fold higher than with soluble monomeric gp350 protein [120]. More recently, the same group investigated the immunization of nonhuman primates with gH/gL- and gH/gL/gp42-based nanoparticles. Those highly immunogenic vaccines elicited virus-neutralizing antibody responses that were maintained for at least 3 months after vaccination. It could be shown that the vaccination-induced antibodies were able to inhibit the viral fusion with B and epithelial cells [121]. Because the vaccinated animals cannot be infected with EBV, it remains unclear whether these neutralizing antibody titers would inhibit EBV infection in vivo.

Conclusions and outlook

From the many approaches summarized above, it is clear that the time is ripe for vaccination against EBV-associated pathologies. From the frequent reinfections of healthy virus carriers with EBV [122, 123], it seems also clear that sterilizing immunity against EBV infection is probably utopic. Such immune protection would also have to be watertight, because if it would be transient and just delay primary EBV infection, the ensuing initial encounters with the virus would carry a higher risk for IM [8]. Therefore, establishing or maintaining immune control of asymptomatic persistent EBV infection should probably be the goal for EBV vaccination. In patients with already established EBV-associated malignancies, therapeutic vaccination might be an uphill battle due to established immunosuppressive mechanisms. Furthermore, prophylactic vaccination against these pathologies might be difficult to assess in initial clinical trials due to their low incidence rate, usually ranging below 50 per 100′000 individuals [4]. Therefore, the most likely scenario to test EBV-specific vaccine candidates is adolescents or young adults that are still EBV seronegative (around one third of this population) and who have a high risk to acquiring EBV with IM (30–50%) [8], followed by an increased risk for Hodgkin's lymphoma and MS [124, 125]. Even so natural immune control of EBV primarily relies on cytotoxic lymphocytes [20, 21], vaccine-induced EBV-neutralizing antibodies could convert IM into asymptomatic infection, because the elevated viral shedding into the saliva and $CD8^{+}$ T cell lymphocytosis driven by early lytic EBV antigens suggest that uncontrolled lytic replication contributes to IM [7]. Therefore, all the above discussed EBV vaccine candidates could prevent IM and provide the proof of concept that immunization against EBV is possible. In the end, however, vaccination or the endogenous immune response to asymptomatic EBV infection probably needs to establish long-lived immune control by cytotoxic $CD8^{+}$ T cells, which form the required cornerstone of natural immunity to this tumor virus. Thus, the development of an EBV-specific vaccine offers the possibility to design formulations that selectively elicit such cell-mediated immune control, which then also could be adapted to tumors that are not associated with viruses.

Acknowledgements Research in our laboratories is supported by Cancer Research Switzerland (KFS-4091-02-2017), KFSP-Precision^{MS}, HMZ ImmunoTargET and Cancer Research Center Zurich of

the University of Zurich, the Vontobel Foundation, the Baugarten Foundation, the Sobek Foundation, the Swiss Vaccine Research Institute, Roche, Novartis and the Swiss National Science Foundation (310030B_182827 and CRSII5_180323) to C.M., and Swiss National Science Foundation (P300P3_155374) to C.S.L.

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