Vaccination against the Epstein Barr virus

Julia Rühl[1], Carol Sze Leung[2] and Christian Münz[1*]

[1]Viral Immunobiology, Institute of Experimental Immunology, University Hospital of Zürich, Zürich, Switzerland

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

[*]Correspondence: Christian Münz, Viral Immunobiology, Institute of Experimental Immunology, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Tel.: +41-44-635-3716, Fax: +41-44-635-6883, e-mail: christian.muenz@uzh.ch.

Running title: EBV vaccine candidates

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 heterologous prime-boost vaccinations with them, EBV derived virus like particles and viral envelope glycoprotein vaccine formulations are explored and are discussed in this review text. 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.

1. 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 lymphoma (2, 3) and is associated with epithelial, lymphocyte and smooth muscle derived tumors in humans (4). Around 2% of all malignancies in humans are associated with EBV with an annual incidence rate of 200'000 (5). In addition to these EBV associated malignancies, this virus is associated with immune pathologies that result from a hyperactivation of EBV induced T cell responses (6). These include syndromes that result from $CD8^+$ T cell lymphocytosis during symptomatic primary EBV infection called infectious mononucleosis (IM) (7), from virus induced cytokine production for the hyperactivation of myeloid cells resulting in hemophagocytic lymphohistiocytosis (HLH) (8) and possibly also from the autoimmune disease multiple sclerosis (MS) (9). 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 (10). 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) (11). Thus, both EBV associated malignancies and immune pathologies justify the 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 (12, 13). In B cells EBV expresses latent viral gene products from its circularized and increasingly chromatinized multi-copy extrachromosomal DNA (14). Initial expression of 6 nuclear antigens (EBNAs) and 2 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 (15). 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 (16), and then presumably amplifies virion production by lytic replication in epithelial cells for more efficient shedding into the saliva and further transmission (17). EBV associated pathologies originate from these different stages of the EBV life cycle. For example, post-transplant lymphoproliferative disease (PTLD) and some of the diffuse large B cell lymphomas (DLBCL) express latency III, Hodgkin and Burkitt lymphoma emerge from germinal centers with latency II or only EBNA 1 expression as the sole EBV protein (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.

2. 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 (18, 19). 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 (20-22). They need to carry the co-stimulatory and co-inhibitory molecules CD27, CTLA-4, SLAM protein family members like 2B4, 4-1BB and NKG2D in combination with the main activating receptors CD16 or the T cell receptor (23-33). Furthermore, they need to expand well after activation and depend on GATA2 and MCM4 for their differentiation (34-37). 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 (18). This profile fits well to $CD8^+$ T cells, natural killer (NK), NKT and $\gamma\delta$ T cells. Indeed, all of these cytotoxic lymphocyte populations have been shown to restrict EBV infection in the preclinical model of HIS mice (38-43). In addition the EBV specific $CD8^+$ T cells might have a particular PD-1^+Tim-3^+KLRG1^+CXCR5^+TCF-1^+ and BATF3^+ phenotype that allows them to control EBV infected B cells in germinal centers (11, 44, 45). These $CD8^+$ T cells recognize predominantly latent and early lytic EBV antigens (6). T cell lines have also been adoptively transferred to treat EBV associated malignancies, initially primarily PTLD (46). With respect to individual antigens EBNA1, LMP1 and LMP2 specific T cell lines have proven clinically efficacious in EBV associated lymphomas and nasopharyngeal carcinoma (47-49). Interestingly, T cells with these specificities have also been infused into MS patients with some clinical success (50, 51). Lytic EBV replication is in addition targeted by early differentiated $CD56_{dim}$NKG2A$^+$KIR$^-$ partially $CD16^+$ NK cells (39, 52, 53). Both $CD8^+$ T cells and NK cells significantly expand during IM (7, 53-56). In addition to early differentiated NK cells Vγ8Vδ2 T cells are elevated in a subset of children (57). They preferentially respond to Burkitt lymphoma cells with a latency I EBV gene expression that is also found in homeostatically proliferating EBV infected memory B cells (58). Finally, NKT cells preferentially respond to Hodgkin lymphoma and nasopharyngeal carcinoma cell lines (59). 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.

3. Recombinant viral vector vaccines

Recombinant viral vector vaccines are live viruses that are engineered to express extra proteins, which the vaccine is targeting to generate immunity. These vaccine platforms are relatively new and have several advantages over traditional vaccines. First, viral vectored vaccines can induce a broad range of immune responses, in particularly $CD8_+$ cytotoxic T lymphocyte (CTL) responses that are important in clearing virally infected cells. This contrasts with most of the existing vaccine formulas 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 they are 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 the vaccine. Third, viral vector vaccines have a high gene transduction efficiency (60) and can deliver the antigens to different cell types depending on the tropism of viral vectors used.

Many different viral vectors have been developed to use as vaccine candidates, including poxviruses, adenoviruses and Sendai virus. The choice of viral vectors for vaccine development mostly depends on the vector's specific properties such as immunogenicity, safety and infectivity. Also, the pre-existing immunity against the viral vectors in humans are often considered. Vaccinia virus and adenovirus are among the most widely used viral vectors, mainly due to their ability to induce antigen-specific $CD8_+$ T cell response. Currently there are many clinical trials testing diverse viral vector vaccines in different disease settings, mainly infectious diseases (61, 62).

The first EBV vaccine tested in humans used live recombinant vaccinia virus expressing the EBV membrane antigen BNLF-1 (LMP1) (63). While there were no significant EBV titer variations between vaccinated and unvaccinated adults, only 3 of 9 vaccinated infants were infected with EBV within 16 months after vaccination compared to 10 out of 10 in the unvaccinated control. However, this vaccine platform is no longer accepted due to the risk of adverse effects (64). A safer alternative is the multiplication incompetent attenuated pox viral vectors such as modified vaccinia virus Ankara (MVA) (65, 66). Indeed, a MVA vaccine encoding the EBV antigens EBNA1 and LMP2 (MVA-EL) has been developed as a therapeutic vaccine against EBV-positive cancer (67, 68).

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 study and 15 of 18 patients in the Hong Kong study. 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. Nevertheless, instead of using it as a direct vaccination, the adenovirus vectors encoding the LMP proteins or polyepitopes with or without EBNA1 were used to infect DCs or LCLs in vitro, so as to either expand EBV-specific T cells and infuse these back into patients or to adoptively transfer the infected DCs as cellular vaccination (49, 69-71). Considering the complexity of cellular vaccine approaches, adenovirus vectors encoding EBV antigens should be explored as a direct vaccination against EBV.

4. 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 the pre-existing neutralizing immunity (72, 73). Furthermore, the immunogenicity of these vectors can establish neutralizing responses that limit its capacity for secondary injection, requiring the use of different viral vectors in the boost vaccination. Indeed, heterologous prime-boost strategies using two antigen formulations has been regarded as a new and better way of immunization (74, 75).

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 (75-77). 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 (78). The same strategy has been applied to vaccine development against different diseases, including hepatitis C virus (HCV), Ebola virus and prostate cancer (79-81). Our group has also demonstrated that adenovirus prime and MVA boost vaccination against EBNA1 is efficient in eliciting comprehensive $CD4_+$ and $CD8_+$ T cell responses which can translate into protection against EBV antigen expressing lymphomas (82).

Though viral vectors generally elicit a higher magnitude of T cell responses, they are expensive to produce and usually take longer 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 (83-85). 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 (82, 84). As viral vector vaccines are known for their superiority in inducing $CD8_+$ T cell responses, we combined this approach with viral vector vaccines in order to stimulate strong $CD4_+$ and $CD8_+$ T cell responses (82). We have showed 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 the mice from T cell lymphoma challenge, with the later, however, having a better protection against B cell lymphomas. This is consistent with previous studies that also 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 (86). Thus, a heterologous prime-boost approach should be considered in the future development of a vaccine against EBV that aims to elicit T cell mediated immune control.

5. 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 (87, 88). 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 consisting of EBVgp350/220 ectodomain was shown to elicit strong, long-lasting neutralizing antibody responses in BALB/c mice, which were, however, not significantly higher than after vaccination with soluble gp350/220 (89). 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 (90). A different, but possibly even more promising approach, is to use VLPs based on the EBV particle. In order to reduce oncogenicity of EBV for vaccination, genetic elements and/or proteins involved in DNA packaging are deleted (91). Already 20 years ago, the first generation of cell lines that produce EBV VLPs were created by removing the terminal repeats (TRs), which prior had been identified as packaging signals of EBVs DNA (92-94). 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 (95). 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 (95).

Despite strong evidence of immune activation and good safety profile in mice, as a clinical vaccine the risk of remaining infectious oncogenic genomes of the early EBV VLPs remained high. Therefore, the development of EBV VLPs was further improved through the deletion of BFLF1/BFRF1A or BBRF1. In 2012, Pavlova et al. managed to create fully DNA free EBV VLPs. The BFLF1/BFRF1A mutant EBV strain elicited similar

strong CD4+ T cell responses as the EBV wildtype in vitro (96). 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 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 proficient than their counterparts without latent antigen. This partial inhibition of EBV infection in B cells could also be translated into experiments with 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 (97). Therefore, EBV derived VLPs might need to contain latent antigens in addition to the structural proteins to elicit protective immune responses.

6. Envelope protein formulations to elicit neutralizing antibodies

Gp350/220 is an EBV glycoprotein, which initiates the attachment of EBV to susceptible host cells expressing the complement receptor type 2 (CD21) and/or type 1 (CD35). 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 samples (98). 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 (99). 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 prevention of acute IM (100, 101). Therefore, improvements of the gp350 protein vaccination were conceived (102) and it was identified that dimers, trimers and tetramers of gp350 are leading to significantly higher neutralizing antibody titers in mice (103, 104). Multimerized gp350 seems, therefore, to elicit more potent B cell responses.

Improvement of gp350 protein vaccines was not only achieved by multimerization, but also by the addition of 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 stable emulsion (SE). Mice and rabbits that were vaccinated with GLA/SE-adjuvanted gp350 vaccines showed elevated EBV-neutralizing antibody titers. In vaccinated mice also high IgG titers and robust anti-gp350 $CD4_+$ T-cell responses could be detected (105). 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 (106) were generated by computer modeling (107). 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 stronger peptide vaccines which could be combined for the neutralizing epitopes of multiple EBV envelope glycoproteins.

When EBV is already attached to the host B cells through gp350 – CD21 interaction or for the infection of epithelial cells, proteins such as EBV gH/gL and EBV

gB become critical for the entry of EBV. Whereas gp350 is unique to EBV, gH/gL and gB are conserved among other herpesviruses. 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 (108). 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 (109). 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 a high amount of 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 (110). Vaccinated mice developed anti-gp350 titers that were about 1000-fold higher than in mice with soluble monomeric gp350 vaccine and were protective against a challenge with vaccinia virus expressing gp350. Cynomolgus macaques immunized with the gp350 nanoparticles also generated anti-gp350 titers that were 3- to 10-fold higher than with soluble monomeric gp350 protein (110). More recently, the same group investigated the immunization of non-human 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 (111). Because of the lack of a challenge of the vaccinated animals, it remains unclear whether these neutralizing antibody titers would inhibit EBV infection in vivo.

7. 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 (112, 113) 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 (7). Therefore, establishing or maintaining immune control of asymptomatic persistent EBV infection should probably the goal for EBV vaccination. In patients with already established EBV associated malignancies this might be an uphill battle. Furthermore, 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 are 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%) (7), followed by an increased risk for Hodgkin lymphoma and MS (114, 115). Even so natural immune control of EBV primarily relies on cytotoxic lymphocytes (18, 19), 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 (6). Therefore, all the above discussed EBV vaccine candidates could prevent IM and provide the proof of concept that immunization against EBV is possible.

Acknowledgements

Research in our laboratories is supported by Cancer Research Switzerland (KFS-4091-02-2017), KFSP-Precision$_{MS}$ of the University of Zurich, the Vontobel Foundation, the Baugarten Foundation, the Sobek Foundation, the Swiss Vaccine Research Institute, Roche, ReiThera 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.

References

1. Münz C. Latency and lytic replication in the oncogenesis of the Epstein Barr virus. Nat Rev Micobiol. 2019;17:691-700.
2. Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. Lancet. 1964;1:702-3.
3. Epstein MA, Henle G, Achong BG, Barr YM. Morphological and biological studies on a virus in cultured lymphoblasts from Burkitt's lymphoma. J Exp Med. 1964;121:761-70.
4. Shannon-Lowe C, Rickinson A. The Global Landscape of EBV-Associated Tumors. Front Oncol. 2019;9:713.
5. Cohen JI, Fauci AS, Varmus H, Nabel GJ. Epstein-Barr virus: an important vaccine target for cancer prevention. Sci Transl Med. 2011;3(107):107fs7.
6. Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. The immunology of Epstein-Barr virus-induced disease. Annu Rev Immunol. 2015;33:787-821.
7. Dunmire SK, Verghese PS, Balfour HH, Jr. Primary Epstein-Barr virus infection. J Clin Virol. 2018;102:84-92.
8. Al-Samkari H, Berliner N. Hemophagocytic Lymphohistiocytosis. Annu Rev Pathol. 2018;13:27-49.
9. Ascherio A, Munger KL, Lunemann JD. The initiation and prevention of multiple sclerosis. Nat Rev Neurol. 2012;8(11):602-12.
10. Johnson DB, McDonnell WJ, Gonzalez-Ericsson PI, Al-Rohil RN, Mobley BC, Salem JE, et al. A case report of clonal EBV-like memory CD4+ T cell activation in fatal checkpoint inhibitor-induced encephalitis. Nat Med. 2019;25(8):1243-50.
11. Chatterjee B, Deng Y, Holler A, Nunez N, Azzi T, Vanoaica LD, et al. CD8+ T cells retain protective functions despite sustained inhibitory receptor expression during Epstein-Barr virus infection in vivo. PLoS Pathog. 2019;15:e1007748.
12. Tugizov SM, Berline JW, Palefsky JM. Epstein-Barr virus infection of polarized tongue and nasopharyngeal epithelial cells. Nat Med. 2003;9(3):307-14.
13. Tugizov SM, Herrera R, Palefsky JM. Epstein-Barr virus transcytosis through polarized oral epithelial cells. J Virol. 2013;87(14):8179-94.
14. Frost TC, Gewurz BE. Epigenetic crossroads of the Epstein-Barr virus B-cell relationship. Curr Opin Virol. 2018;32:15-23.
15. Thorley-Lawson DA. EBV Persistence--Introducing the Virus. Curr Top Microbiol Immunol. 2015;390(Pt 1):151-209.

16. Laichalk LL, Thorley-Lawson DA. Terminal differentiation into plasma cells initiates the replicative cycle of Epstein-Barr virus in vivo. J Virol. 2005;79(2):1296-307.
17. Hutt-Fletcher LM. The Long and Complicated Relationship between Epstein-Barr Virus and Epithelial Cells. J Virol. 2017;91(1).
18. Latour S, Fischer A. Signaling pathways involved in the T-cell-mediated immunity against Epstein-Barr virus: Lessons from genetic diseases. Immunol Rev. 2019;291(1):174-89.
19. Damania B, Münz C. Immunodeficiencies that predispose to pathologies by human oncogenic gamma-herpesviruses. FEMS Microbiol Rev. 2019;43:181-92.
20. Katano H, Ali MA, Patera AC, Catalfamo M, Jaffe ES, Kimura H, et al. Chronic active Epstein-Barr virus infection associated with mutations in perforin that impair its maturation. Blood. 2004;103(4):1244-52.
21. Rohr J, Beutel K, Maul-Pavicic A, Vraetz T, Thiel J, Warnatz K, et al. Atypical familial hemophagocytic lymphohistiocytosis due to mutations in UNC13D and STXBP2 overlaps with primary immunodeficiency diseases. Haematologica. 2010;95(12):2080-7.
22. Cohen JI, Niemela JE, Stoddard JL, Pittaluga S, Heslop H, Jaffe ES, et al. Late-onset severe chronic active EBV in a patient for five years with mutations in STXBP2 (MUNC18-2) and PRF1 (perforin 1). J Clin Immunol. 2015;35(5):445-8.
23. Salzer E, Daschkey S, Choo S, Gombert M, Santos-Valente E, Ginzel S, et al. Combined immunodeficiency with life-threatening EBV-associatedlymphoproliferative disorder in patients lacking functional CD27. Haematologica. 2012.
24. Schwab C, Gabrysch A, Olbrich P, Patino V, Warnatz K, Wolff D, et al. Phenotype, penetrance, and treatment of 133 CTLA-4-insufficient individuals. J Allergy Clin Immunol. 2018.
25. van Montfrans JM, Hoepelman AI, Otto S, van Gijn M, van de Corput L, de Weger RA, et al. CD27 deficiency is associated with combined immunodeficiency and persistent symptomatic EBV viremia. J Allergy Clin Immunol. 2012;129(3):787-93 e6.
26. Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. Nat Genet. 1998;20(2):129-35.
27. Nichols KE, Harkin DP, Levitz S, Krainer M, Kolquist KA, Genovese C, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. Proc Natl Acad Sci U S A. 1998;95(23):13765-70.

28. Sayos J, Wu C, Morra M, Wang N, Zhang X, Allen D, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM [see comments]. Nature. 1998;395(6701):462-9.
29. Alosaimi MF, Hoenig M, Jaber F, Platt CD, Jones J, Wallace J, et al. Immunodeficiency and EBV-induced lymphoproliferation caused by 4-1BB deficiency. J Allergy Clin Immunol. 2019;144(2):574-83 e5.
30. Somekh I, Thian M, Medgyesi D, Gulez N, Magg T, Gallon Duque A, et al. CD137 deficiency causes immune dysregulation with predisposition to lymphomagenesis. Blood. 2019.
31. Chaigne-Delalande B, Li FY, O'Connor GM, Lukacs MJ, Jiang P, Zheng L, et al. Mg^{2+} regulates cytotoxic functions of NK and CD8 T cells in chronic EBV infection through NKG2D. Science. 2013;341(6142):186-91.
32. de Vries E, Koene HR, Vossen JM, Gratama JW, von dem Borne AE, Waaijer JL, et al. Identification of an unusual Fc gamma receptor IIIa (CD16) on natural killer cells in a patient with recurrent infections. Blood. 1996;88(8):3022-7.
33. Huck K, Feyen O, Niehues T, Ruschendorf F, Hubner N, Laws HJ, et al. Girls homozygous for an IL-2-inducible T cell kinase mutation that leads to protein deficiency develop fatal EBV-associated lymphoproliferation. J Clin Invest. 2009;119(5):1350-8.
34. Martin E, Palmic N, Sanquer S, Lenoir C, Hauck F, Mongellaz C, et al. CTP synthase 1 deficiency in humans reveals its central role in lymphocyte proliferation. Nature. 2014;510(7504):288-92.
35. Rigaud S, Fondaneche MC, Lambert N, Pasquier B, Mateo V, Soulas P, et al. XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. Nature. 2006;444(7115):110-4.
36. Biron CA, Byron KS, Sullivan JL. Severe herpesvirus infections in an adolescent without natural killer cells. N Engl J Med. 1989;320(26):1731-5.
37. Eidenschenk C, Dunne J, Jouanguy E, Fourlinnie C, Gineau L, Bacq D, et al. A novel primary immunodeficiency with specific natural-killer cell deficiency maps to the centromeric region of chromosome 8. Am J Hum Genet. 2006;78(4):721-7.
38. Strowig T, Gurer C, Ploss A, Liu YF, Arrey F, Sashihara J, et al. Priming of protective T cell responses against virus-induced tumors in mice with human immune system components. J Exp Med. 2009;206(6):1423-34.

39. Chijioke O, Muller A, Feederle R, Barros MH, Krieg C, Emmel V, et al. Human natural killer cells prevent infectious mononucleosis features by targeting lytic Epstein-Barr virus infection. Cell Rep. 2013;5(6):1489-98.
40. Yuling H, Ruijing X, Li L, Xiang J, Rui Z, Yujuan W, et al. EBV-induced human CD8+ NKT cells suppress tumorigenesis by EBV-associated malignancies. Cancer Res. 2009;69(20):7935-44.
41. Xiang Z, Liu Y, Zheng J, Liu M, Lv A, Gao Y, et al. Targeted activation of human Vgamma9Vdelta2-T cells controls epstein-barr virus-induced B cell lymphoproliferative disease. Cancer Cell. 2014;26(4):565-76.
42. Zumwalde NA, Sharma A, Xu X, Ma S, Schneider CL, Romero-Masters JC, et al. Adoptively transferred Vgamma9Vdelta2 T cells show potent antitumor effects in a preclinical B cell lymphomagenesis model. JCI Insight. 2017;2(13).
43. McHugh D, Caduff N, Murer A, Engelmann C, Deng Y, Zdimerova H, et al. Infection and immune control of human oncogenic gamma-herpesviruses in humanized mice. Philos Trans R Soc Lond B Biol Sci. 2019;374(1773):20180296.
44. He R, Hou S, Liu C, Zhang A, Bai Q, Han M, et al. Follicular CXCR5- expressing CD8+ T cells curtail chronic viral infection. Nature. 2016;537(7620):412-28.
45. Leong YA, Chen Y, Ong HS, Wu D, Man K, Deleage C, et al. CXCR5+ follicular cytotoxic T cells control viral infection in B cell follicles. Nat Immunol. 2016;17(10):1187-96.
46. Gottschalk S, Rooney CM. Adoptive T-Cell Immunotherapy. Curr Top Microbiol Immunol. 2015;391:427-54.
47. Icheva V, Kayser S, Wolff D, Tuve S, Kyzirakos C, Bethge W, et al. Adoptive transfer of epstein-barr virus (EBV) nuclear antigen 1-specific T cells as treatment for EBV reactivation and lymphoproliferative disorders after allogeneic stem-cell transplantation. J Clin Oncol. 2013;31(1):39-48.
48. Bollard CM, Tripic T, Cruz CR, Dotti G, Gottschalk S, Torrano V, et al. Tumor-Specific T-Cells Engineered to Overcome Tumor Immune Evasion Induce Clinical Responses in Patients With Relapsed Hodgkin Lymphoma. J Clin Oncol. 2018;36(11):1128-39.
49. Smith C, Tsang J, Beagley L, Chua D, Lee V, Li V, et al. Effective treatment of metastatic forms of Epstein-Barr virus-associated nasopharyngeal carcinoma with a novel adenovirus-based adoptive immunotherapy. Cancer Res. 2012;72(5):1116-25.

50. Pender MP, Csurhes PA, Smith C, Beagley L, Hooper KD, Raj M, et al. Epstein-Barr virus-specific adoptive immunotherapy for progressive multiple sclerosis. Mult Scler. 2014;20(11):1541-4.

51. Pender MP, Csurhes PA, Smith C, Douglas NL, Neller MA, Matthews KK, et al. Epstein-Barr virus-specific T cell therapy for progressive multiple sclerosis. JCI Insight. 2018;3(22).

52. Pappworth IY, Wang EC, Rowe M. The switch from latent to productive infection in epstein-barr virus-infected B cells is associated with sensitization to NK cell killing. J Virol. 2007;81(2):474-82.

53. Azzi T, Lunemann A, Murer A, Ueda S, Beziat V, Malmberg KJ, et al. Role for early-differentiated natural killer cells in infectious mononucleosis. Blood. 2014;124(16):2533-43.

54. Dunmire SK, Grimm JM, Schmeling DO, Balfour HH, Jr., Hogquist KA. The Incubation Period of Primary Epstein-Barr Virus Infection: Viral Dynamics and Immunologic Events. PLoS Pathog. 2015;11(12):e1005286.

55. Williams H, McAulay K, Macsween KF, Gallacher NJ, Higgins CD, Harrison N, et al. The immune response to primary EBV infection: a role for natural killer cells. Br J Haematol. 2005;129(2):266-74.

56. Hendricks DW, Balfour HH, Jr., Dunmire SK, Schmeling DO, Hogquist KA, Lanier LL. Cutting edge: NKG2C$_{hi}$CD57$_{+}$ NK cells respond specifically to acute infection with cytomegalovirus and not Epstein-Barr virus. J Immunol. 2014;192(10):4492-6.

57. Djaoud Z, Guethlein LA, Horowitz A, Azzi T, Nemat-Gorgani N, Olive D, et al. Two alternate strategies for innate immunity to Epstein-Barr virus: One using NK cells and the other NK cells and gammadelta T cells. J Exp Med. 2017;214(6):1827-41.

58. Hochberg D, Middeldorp JM, Catalina M, Sullivan JL, Luzuriaga K, Thorley-Lawson DA. Demonstration of the Burkitt's lymphoma Epstein-Barr virus phenotype in dividing latently infected memory cells in vivo. Proc Natl Acad Sci U S A. 2004;101(1):239-44.

59. Chung BK, Tsai K, Allan LL, Zheng DJ, Nie JC, Biggs CM, et al. Innate immune control of EBV-infected B cells by invariant natural killer T cells. Blood. 2013;122(15):2600-8.

60. Ura T, Okuda K, Shimada M. Developments in Viral Vector-Based Vaccines. Vaccines (Basel). 2014;2(3):624-41.

61. Kallel H, Kamen AA. Large-scale adenovirus and poxvirus-vectored vaccine manufacturing to enable clinical trials. Biotechnol J. 2015;10(5):741-7.
62. Nasar F, Matassov D, Seymour RL, Latham T, Gorchakov RV, Nowak RM, et al. Recombinant Isfahan Virus and Vesicular Stomatitis Virus Vaccine Vectors Provide Durable, Multivalent, Single-Dose Protection against Lethal Alphavirus Challenge. J Virol. 2017;91(8).
63. Gu SY, Huang TM, Ruan L, Miao YH, Lu H, Chu CM, et al. First EBV vaccine trial in humans using recombinant vaccinia virus expressing the major membrane antigen. Dev Biol Stand. 1995;84:171-7.
64. Casey CG, Iskander JK, Roper MH, Mast EE, Wen XJ, Torok TJ, et al. Adverse events associated with smallpox vaccination in the United States, January-October 2003. JAMA. 2005;294(21):2734-43.
65. Sanchez-Sampedro L, Perdiguero B, Mejias-Perez E, Garcia-Arriaza J, Di Pilato M, Esteban M. The evolution of poxvirus vaccines. Viruses. 2015;7(4):1726-803.
66. Parrino J, McCurdy LH, Larkin BD, Gordon IJ, Rucker SE, Enama ME, et al. Safety, immunogenicity and efficacy of modified vaccinia Ankara (MVA) against Dryvax challenge in vaccinia-naive and vaccinia-immune individuals. Vaccine. 2007;25(8):1513-25.
67. Taylor GS, Jia H, Harrington K, Lee LW, Turner J, Ladell K, et al. A recombinant modified vaccinia ankara vaccine encoding Epstein-Barr Virus (EBV) target antigens: a phase I trial in UK patients with EBV-positive cancer. Clin Cancer Res. 2014;20(19):5009-22.
68. Hui EP, Taylor GS, Jia H, Ma BB, Chan SL, Ho R, et al. Phase I trial of recombinant modified vaccinia ankara encoding Epstein-Barr viral tumor antigens in nasopharyngeal carcinoma patients. Cancer Res. 2013;73(6):1676-88.
69. Chia WK, Wang WW, Teo M, Tai WM, Lim WT, Tan EH, et al. A phase II study evaluating the safety and efficacy of an adenovirus-DeltaLMP1-LMP2 transduced dendritic cell vaccine in patients with advanced metastatic nasopharyngeal carcinoma. Ann Oncol. 2012;23(4):997-1005.
70. Bollard CM, Gottschalk S, Torrano V, Diouf O, Ku S, Hazrat Y, et al. Sustained complete responses in patients with lymphoma receiving autologous cytotoxic T lymphocytes targeting Epstein-Barr virus latent membrane proteins. J Clin Oncol. 2014;32(8):798-808.

71. Smith C, Cooper L, Burgess M, Rist M, Webb N, Lambley E, et al. Functional reversion of antigen-specific CD8+ T cells from patients with Hodgkin lymphoma following in vitro stimulation with recombinant polyepitope. J Immunol. 2006;177(7):4897-906.
72. Capone S, D'Alise AM, Ammendola V, Colloca S, Cortese R, Nicosia A, et al. Development of chimpanzee adenoviruses as vaccine vectors: challenges and successes emerging from clinical trials. Expert Rev Vaccines. 2013;12(4):379-93.
73. Dicks MD, Spencer AJ, Edwards NJ, Wadell G, Bojang K, Gilbert SC, et al. A novel chimpanzee adenovirus vector with low human seroprevalence: improved systems for vector derivation and comparative immunogenicity. PLoS One. 2012;7(7):e40385.
74. Lu S. Heterologous prime-boost vaccination. Curr Opin Immunol. 2009;21(3):346-51.
75. Kardani K, Bolhassani A, Shahbazi S. Prime-boost vaccine strategy against viral infections: Mechanisms and benefits. Vaccine. 2016;34(4):413-23.
76. Paris RM, Kim JH, Robb ML, Michael NL. Prime-boost immunization with poxvirus or adenovirus vectors as a strategy to develop a protective vaccine for HIV-1. Expert Rev Vaccines. 2010;9(9):1055-69.
77. Musich T, Robert-Guroff M. New developments in an old strategy: heterologous vector primes and envelope protein boosts in HIV vaccine design. Expert Rev Vaccines. 2016;15(8):1015-27.
78. Ewer KJ, O'Hara GA, Duncan CJ, Collins KA, Sheehy SH, Reyes-Sandoval A, et al. Protective CD8+ T-cell immunity to human malaria induced by chimpanzee adenovirus-MVA immunisation. Nat Commun. 2013;4:2836.
79. Swadling L, Capone S, Antrobus RD, Brown A, Richardson R, Newell EW, et al. A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory. Sci Transl Med. 2014;6(261):261ra153.
80. Venkatraman N, Ndiaye BP, Bowyer G, Wade D, Sridhar S, Wright D, et al. Safety and Immunogenicity of a Heterologous Prime-Boost Ebola Virus Vaccine Regimen in Healthy Adults in the United Kingdom and Senegal. J Infect Dis. 2019;219(8):1187-97.
81. Cappuccini F, Pollock E, Stribbling S, Hill AVS, Redchenko I. 5T4 oncofoetal glycoprotein: an old target for a novel prostate cancer immunotherapy. Oncotarget. 2017;8(29):47474-89.

82. Rühl J, Citterio C, Engelmann C, Haigh TA, Dzionek A, Dreyer JH, et al. Heterologous prime-boost vaccination protects from EBV antigen expressing lymphomas. J Clin Invest. 2019;129: 2071-2087.

83. Gurer C, Strowig T, Brilot F, Pack M, Trumpfheller C, Arrey F, et al. Targeting the nuclear antigen 1 of Epstein Barr virus to the human endocytic receptor DEC-205 stimulates protective T-cell responses. Blood. 2008;112:1231-9.

84. Leung CS, Maurer MA, Meixlsperger S, Lippmann A, Cheong C, Zuo J, et al. Robust T-cell stimulation by Epstein-Barr virus-transformed B cells after antigen targeting to DEC-205. Blood. 2013;121(9):1584-94.

85. Meixlsperger S, Leung CS, Ramer PC, Pack M, Vanoaica LD, Breton G, et al. CD141+ dendritic cells produce prominent amounts of IFN-alpha after dsRNA recognition and can be targeted via DEC-205 in humanized mice. Blood. 2013;121(25):5034-44.

86. Flynn BJ, Kastenmuller K, Wille-Reece U, Tomaras GD, Alam M, Lindsay RW, et al. Immunization with HIV Gag targeted to dendritic cells followed by recombinant New York vaccinia virus induces robust T-cell immunity in nonhuman primates. Proc Natl Acad Sci U S A. 2011;108(17):7131-6.

87. Paavonen J, Jenkins D, Bosch FX, Naud P, Salmeron J, Wheeler CM, et al. Efficacy of a prophylactic adjuvanted bivalent L1 virus-like-particle vaccine against infection with human papillomavirus types 16 and 18 in young women: an interim analysis of a phase III double-blind, randomised controlled trial. Lancet. 2007;369(9580):2161-70.

88. Chichester JA, Green BJ, Jones RM, Shoji Y, Miura K, Long CA, et al. Safety and immunogenicity of a plant-produced Pfs25 virus-like particle as a transmission blocking vaccine against malaria: A Phase 1 dose-escalation study in healthy adults. Vaccine. 2018;36(39):5865-71.

89. Ogembo JG, Muraswki MR, McGinnes LW, Parcharidou A, Sutiwisesak R, Tison T, et al. A chimeric EBV gp350/220-based VLP replicates the virion B-cell attachment mechanism and elicits long-lasting neutralizing antibodies in mice. J Transl Med. 2015;13:50.

90. Perez EM, Foley J, Tison T, Silva R, Ogembo JG. Novel Epstein-Barr virus-like particles incorporating gH/gL-EBNA1 or gB-LMP2 induce high neutralizing antibody titers and EBV-specific T-cell responses in immunized mice. Oncotarget. 2017;8(12):19255-73.

91. Adhikary D, Behrends U, Feederle R, Delecluse HJ, Mautner J. Standardized and highly efficient expansion of Epstein-Barr virus-specific CD4+ T cells by using virus-like particles. J Virol. 2008;82(8):3903-11.
92. Zimmermann J, Hammerschmidt W. Structure and role of the terminal repeats of Epstein-Barr virus in processing and packaging of virion DNA. J Virol. 1995;69(5):3147-55.
93. Delecluse HJ, Pich D, Hilsendegen T, Baum C, Hammerschmidt W. A first-generation packaging cell line for Epstein-Barr virus-derived vectors. Proc Natl Acad Sci U S A. 1999;96(9):5188-93.
94. Feederle R, Shannon-Lowe C, Baldwin G, Delecluse HJ. Defective infectious particles and rare packaged genomes produced by cells carrying terminal-repeat-negative Epstein-Barr virus. J Virol. 2005;79(12):7641-7.
95. Ruiss R, Jochum S, Wanner G, Reisbach G, Hammerschmidt W, Zeidler R. A virus-like particle-based Epstein-Barr virus vaccine. J Virol. 2011;85(24):13105-13.
96. Pavlova S, Feederle R, Gartner K, Fuchs W, Granzow H, Delecluse HJ. An Epstein-Barr virus mutant produces immunogenic defective particles devoid of viral DNA. J Virol. 2013;87(4):2011-22.
97. van Zyl DG, Tsai MH, Shumilov A, Schneidt V, Poirey R, Schlehe B, et al. Immunogenic particles with a broad antigenic spectrum stimulate cytolytic T cells and offer increased protection against EBV infection ex vivo and in mice. PLoS Pathog. 2018;14(12):e1007464.
98. Mutsvunguma LZ, Rodriguez E, Escalante GM, Muniraju M, Williams JC, Warden C, et al. Identification of multiple potent neutralizing and non-neutralizing antibodies against Epstein-Barr virus gp350 protein with potential for clinical application and as reagents for mapping immunodominant epitopes. Virology. 2019;536:1-15.
99. Tanner JE, Hu J, Alfieri C. Construction and Characterization of a Humanized Anti-Epstein-Barr Virus gp350 Antibody with Neutralizing Activity in Cell Culture. Cancers (Basel). 2018;10(4).
100. Sokal EM, Hoppenbrouwers K, Vandermeulen C, Moutschen M, Leonard P, Moreels A, et al. Recombinant gp350 vaccine for infectious mononucleosis: a phase 2, randomized, double-blind, placebo-controlled trial to evaluate the safety, immunogenicity, and efficacy of an Epstein-Barr virus vaccine in healthy young adults. J Infect Dis. 2007;196(12):1749-53.

101. Moutschen M, Leonard P, Sokal EM, Smets F, Haumont M, Mazzu P, et al. Phase I/II studies to evaluate safety and immunogenicity of a recombinant gp350 Epstein-Barr virus vaccine in healthy adults. Vaccine. 2007;25(24):4697-705.
102. Servat E, Ro BW, Cayatte C, Gemmell L, Barton C, Rao E, et al. Identification of the critical attribute(s) of EBV gp350 antigen required for elicitation of a neutralizing antibody response in vivo. Vaccine. 2015;33(48):6771-7.
103. Cui X, Cao Z, Sen G, Chattopadhyay G, Fuller DH, Fuller JT, et al. A novel tetrameric gp350 1-470 as a potential Epstein-Barr virus vaccine. Vaccine. 2013;31(30):3039-45.
104. Zhao B, Zhang X, Krummenacher C, Song S, Gao L, Zhang H, et al. Immunization With Fc-Based Recombinant Epstein-Barr Virus gp350 Elicits Potent Neutralizing Humoral Immune Response in a BALB/c Mice Model. Front Immunol. 2018;9:932.
105. Heeke DS, Lin R, Rao E, Woo JC, McCarthy MP, Marshall JD. Identification of GLA/SE as an effective adjuvant for the induction of robust humoral and cell-mediated immune responses to EBV-gp350 in mice and rabbits. Vaccine. 2016;34(23):2562-9.
106. Hoffman GJ, Lazarowitz SG, Hayward SD. Monoclonal antibody against a 250,000-dalton glycoprotein of Epstein-Barr virus identifies a membrane antigen and a neutralizing antigen. Proc Natl Acad Sci U S A. 1980;77(5):2979-83.
107. Tanner JE, Coincon M, Leblond V, Hu J, Fang JM, Sygusch J, et al. Peptides designed to spatially depict the Epstein-Barr virus major virion glycoprotein gp350 neutralization epitope elicit antibodies that block virus-neutralizing antibody 72A1 interaction with the native gp350 molecule. J Virol. 2015;89(9):4932-41.
108. Cui X, Cao Z, Chen Q, Arjunaraja S, Snow AL, Snapper CM. Rabbits immunized with Epstein-Barr virus gH/gL or gB recombinant proteins elicit higher serum virus neutralizing activity than gp350. Vaccine. 2016;34(34):4050-5.
109. Snijder J, Ortego MS, Weidle C, Stuart AB, Gray MD, McElrath MJ, et al. An Antibody Targeting the Fusion Machinery Neutralizes Dual-Tropic Infection and Defines a Site of Vulnerability on Epstein-Barr Virus. Immunity. 2018;48(4):799-811 e9.
110. Kanekiyo M, Bu W, Joyce MG, Meng G, Whittle JR, Baxa U, et al. Rational Design of an Epstein-Barr Virus Vaccine Targeting the Receptor-Binding Site. Cell. 2015;162(5):1090-100.

111. Bu W, Joyce MG, Nguyen H, Banh DV, Aguilar F, Tariq Z, et al. Immunization with Components of the Viral Fusion Apparatus Elicits Antibodies That Neutralize Epstein-Barr Virus in B Cells and Epithelial Cells. Immunity. 2019;50(5):1305-16 e6.
112. Sitki-Green D, Covington M, Raab-Traub N. Compartmentalization and transmission of multiple epstein-barr virus strains in asymptomatic carriers. J Virol. 2003;77(3):1840-7.
113. Walling DM, Brown AL, Etienne W, Keitel WA, Ling PD. Multiple Epstein-Barr virus infections in healthy individuals. J Virol. 2003;77(11):6546-50.
114. Hjalgrim H, Askling J, Rostgaard K, Hamilton-Dutoit S, Frisch M, Zhang JS, et al. Characteristics of Hodgkin's lymphoma after infectious mononucleosis. N Engl J Med. 2003;349(14):1324-32.
115. Thacker EL, Mirzaei F, Ascherio A. Infectious mononucleosis and risk for multiple sclerosis: a meta-analysis. Ann Neurol. 2006;59(3):499-503.

Figure legend

Figure 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.

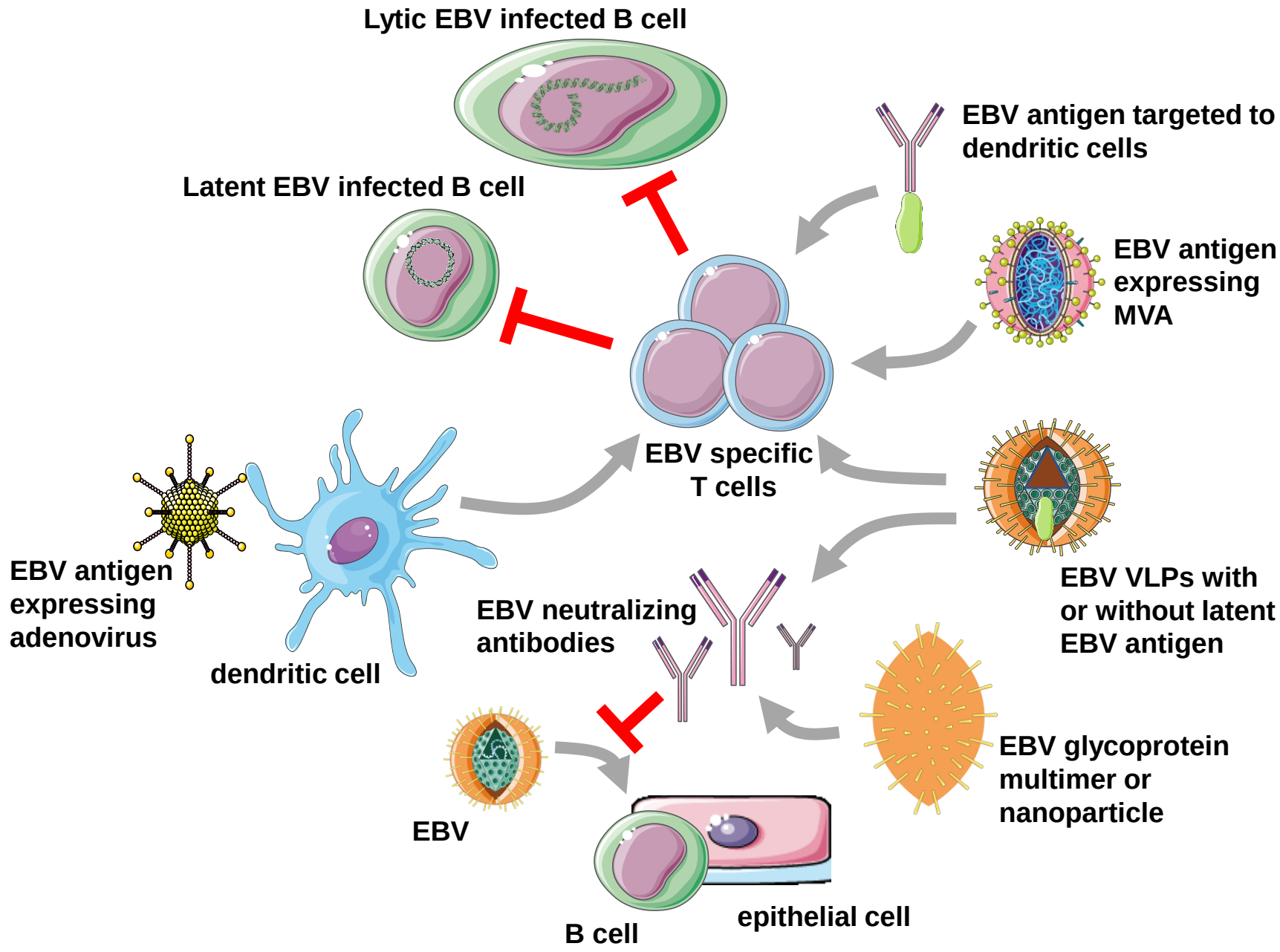
Lytic EBV infected B cell
Latent EBV infected B cell
EBV antigen targeted to dendritic cells
EBV antigen expressing MVA
EBV specific T cells
EBV VLPs with or without latent EBV antigen
EBV antigen expressing adenovirus
dendritic cell
EBV neutralizing antibodies
EBV glycoprotein multimer or nanoparticle
EBV
B cell
epithelial cell

Figure 1