A Frontier in EBV Vaccine Research: mRNA-Based Immunization with gp350, gp42, and gL Glycoproteins

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Author Bio

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Abstract

Epstein-Barr virus (EBV), an extremely common herpesvirus that affects 95% of the adult population, has been implicated in various medical conditions, including infectious mononucleosis, cancers, and autoimmune diseases, yet there is no working vaccine approved for it. Demonstrated by their success in combatting SARS-CoV-2, which became a pandemic-level health-threat in 2020, Messenger RNA (mRNA) vaccines offer a streamlined process that could expedite the development of an EBV vaccine. After providing an overview of mRNA vaccines and their applications, this paper discusses candidate EBV glycoproteins gp350, gp42, and gL for the development of a vaccine, selected based on the criteria of protein abundance, functional importance, stage of EBV infection, presence on the viral envelope, and role in immune evasion. The engineering process for these candidate mRNA vaccines involved the insertion of signal sequences, transmembrane domains, 5' and 3' untranslated regions, and poly (A) tails to ensure protein expression and localization. Potential next steps and their merits are discussed regarding mRNA synthesis. Immunofluorescence, Western blotting, and Enzyme-Linked Immunosorbent Assay ELISA) are suggested to visualize protein expression, confirm localization, and quantify protein levels, respectively. Subsequent steps and their obstacles are discussed, including in vitro testing on human cell lines, animal models, and human trials. This paper explores three candidate vaccines with the potential to reduce the prevalence of the elusive pathogen EBV and its associated diseases. Its findings lay the groundwork for developing mRNA vaccines targeting EBV-associated diseases, offering a promising avenue for research in the fields of preventive medicine and immunology.

Introduction

Currently, of all known human viruses, only approximately 10% have a working vaccine (Forni et al., 2022; Office of Infectious Disease and HIV/AIDS Policy, 2022). Messenger RNA (mRNA) vaccines have emerged as a groundbreaking innovation in the field of vaccinology. Most recently, the Nobel Prize was awarded to two scientists, Katalin Karikó and Drew Weissman, who made pivotal strides in mRNA synthesis. mRNA vaccines offer a promising approach to combat a wide range of infectious diseases, including those caused by complex pathogens like the Epstein-Barr virus (EBV). mRNA vaccines like the Moderna Spikevax vaccine and Pfizer BioNTech Comirnaty make up the majority of vaccines distributed in the wake of the SARS-CoV-2 pandemic from late 2020 to its declared end in 2023, with a campaign resulting in 13.51 billion doses administered globally as of August 5th, 2023 (Mathieu et al. 2023). The development of mRNA vaccines represents a paradigm shift in how immunization against infectious diseases is approached. Unlike traditional vaccines that rely on weakened or inactivated pathogens, mRNA vaccines utilize a revolutionary concept. In a much more streamlined and less costly development process, they provide the body with genetic instructions in the form of synthetic mRNA, enabling cells to produce harmless pieces of the target pathogen (Pardi et al., 2018).

The process of developing an RNA vaccine typically begins with the generation of the RNA encoding the antigen of interest. In vitro transcription, using a bacterial platform such as E. coli, is a common method used to synthesize the mRNA for the vaccine (Liang et al., 2000). This involves using a DNA template that encodes the target antigen. The DNA template contains a promoter region recognized by RNA polymerase, which initiates the transcription process. Promoters like the T7 promoter are commonly used for mass-producing mRNA, after which the RNA polymerase enzyme copies the DNA template, synthesizing the corresponding mRNA (Kang et al., 2023). This mRNA coding for antigenic proteins is encapsulated in lipid nanoparticles, facilitating its delivery into cells. Compared to viral vectors or DNA delivery, mRNA-based vaccines have a lower risk of integrating into the host genome. Additionally, mRNA acts more quickly since it only needs to reach the cytoplasm for translation, bypassing the need for nuclear transport. Upon translation of the mRNA, produced proteins prompt a robust and targeted immune response, harnessing the body's immunological memory by producing memory T and B lymphocytes. These memory T and B cells are effectively primed with a more rapid effector response and antibodies to efficiently recognize and fight the pathogen if encountered. Specifically, the proteins that mRNA vaccines code for primarily are extracellular proteins, particularly glycoproteins, proteins that have sugar molecules (glycans) attached to them. They are essential components of many biological processes, including cell signaling and immune recognition. Due to their accessibility on the cell surface, they are key markers that the immune system can readily recognize and respond to, ultimately conferring protection against the corresponding pathogen. mRNA vaccines have shown remarkable adaptability, making them suitable for addressing a broad spectrum of infectious diseases, from viral infections to bacterial pathogens (Pardi et al., 2018).

Epstein-Barr virus (EBV), a member of the herpesvirus family, is one of the most widespread human viruses—infecting 95% of adults worldwide—yet it has no clinically approved vaccine (Cohen, 2000). EBV has a double-stranded DNA (dsDNA) structure, with several main parts making up its 170 nanometers (nm): an outer lipid bilayer envelope, a central core, and an inner, irregularly shaped middle compartment (Cai et al., 2021). EBV infections are usually asymptomatic or present as mild illnesses, but this virus is also implicated in more complex and serious medical conditions. EBV's adaptability and complexity contribute to its diverse clinical manifestations, ranging from infectious mononucleosis (commonly known as mono) to its involvement in more severe disorders. One of the most notable aspects of EBV is its association with various types of cancer, including Burkitt lymphoma, nasopharyngeal carcinoma, and Hodgkin lymphoma; the World Health Organization (WHO) has categorized EBV as a class I oncogenic virus due to its role as a significant global health concern, attributed directly to roughly 200,000 newly diagnosed tumors each year across the world. This amounts to 1.5% of human cancer globally (Farrell, 2019). As recent evidence surfaces, EBV has also been implicated in autoimmune diseases, including multiple sclerosis (Bjornevik et al., 2022).

EBV has evolved sophisticated mechanisms to evade the immune system, persist in the host, and establish latent infections—where the virus lies dormant in host cells. EBV has a unique ability to promote uncontrolled cell growth by altering host-cell DNA, and with four latency phases, the virus can shut down certain immunogenic genes, allowing long-term persistence in host cells without detection. These distinctive characteristics make vaccine development tricky. Researchers are actively investigating the mechanisms underlying these connections, with the hope of uncovering potential therapeutic interventions. Though several attempts at clinical trials for EBV vaccines have been recorded, none have received approval and many face issues in efficacy or adjuvant selection (Cai et al., 2021). This is where newer mRNA vaccines come into play.

Methods

Table 1. Overview of selected candidate genes and their properties considered during selection.

Candidate Gene	Product	Genbank ID	Properties
BLLF1	gp350	QCG99665.1	The most abundantly expressed surface glycoprotein during both phases (most notably lytic) first viral attachment protein.
BKRF2	gL	UQK62613.1	Non-membrane bound part of the gH/gL glycoprotein complex. Essential for viral entry into the host cell via fusion of viral and plasma membranes.
BZLF2	gp42	QZL09798.1	Contributes to infection of B-cells (primary EBV reservoir) and acts as a tropism switch. Role in immune evasion by blocking T-cell receptors after fusion to the gH/gL complex ensures the virus is undetected. Extracellular protein.

Candidate Selection Considerations

Several criteria were used in selecting candidate EBV proteins, informed by the mechanisms that mRNA vaccines rely on to trigger an immune response as well as the primary goals for such a vaccine. These included protein abundance, functional importance, stage of EBV infection, presence of viral envelope, and role in immune evasion which are demonstrated in the product glycoproteins listed in Table 1. Each criterion deals with how a protein interacts with human host cells, including how often they are expressed extracellularly, which affects the likelihood of an immune response, and whether the virus can continue to proliferate without it. All of these factors must be considered in the creation of a vaccine prototype with high efficacy. A detailed explanation for each criterion follows below.

Abundance

The abundance of a candidate protein on the plasma membrane of EBV-infected cells can significantly impact its suitability as a vaccine target. Proteins that are heavily expressed during EBV infection are more likely to be exposed to the immune system and may elicit stronger immune responses (Salvatori, 2020). They are also more reliable targets that ensure, if successful, the vaccine can defend against most and not just some invading pathogens. As seen in Table 1. Gp350 was an attractive candidate for this reason; it is the most abundantly expressed glycoprotein during the lytic phase of the virus (Slabik, 2020). Examination of transcriptomic and proteomic data can provide insights into protein abundance.

Functional Importance

Consideration of the protein's role in EBV biology is crucial. Proteins that play essential roles in viral entry, immune evasion, or replication may be attractive candidates. This also prevents evolutionary vaccine resistance because the virus cannot adapt to function without said protein. For example, gp350 is involved in viral attachment to host cells, making it a key player in viral proliferation (Slabik, 2020). gL has a prominent role in viral entry and was therefore selected along with gp42 given its involvement in B-cell fusion and immune evasion mechanisms that make it a potential target to disrupt these strategies (Ressing, 2003).

Stages of EBV Infection

EBV undergoes both lytic and latent phases during infection. Proteins that are expressed during these phases may have different immunogenic properties. For example, proteins expressed during the lytic phase may be more readily recognized by the immune system, with a significantly higher frequency of CD8+ T-cells (Callan et al., 1998). It is less common to find reliably expressed proteins in the latent phase as the virus is not active, but gp350 holds promise due to its abundance in the lytic phase and its random expression in detectable amounts in the latent phase (Slabik et al., 2020).

Extracellular Expression

Proteins that are expressed extracellularly and exposed to the host immune system are what the mRNA vaccine mechanism relies on. This includes proteins like gp350, gL, and gp42 which interact with host cells during EBV attachment (Table 1).

Role in Immune Evasion

Proteins involved in immune evasion mechanisms and latency establishment, such as gp42 (Table 1), may be important for the virus's survival and can be attractive vaccine candidates, as without them miRNA production, the decreasing of immune recognition by CD8+ cells, and the decreasing of antiviral protein production would be disrupted.

Engineering the mRNA Vaccines

For each candidate protein, the amino acid sequences of EBV proteins gp350 (product of gene BLLF1), gp42 (product of gene BZLF2), and gL (product of gene BKRF2) were obtained from the NCBI Virus database in FASTA format. The same procedure was repeated for the SARS-CoV-2 surface glycoprotein.

Then, the protein structure prediction software InterProScan version 96.0 (referred to as InterPro in this paper) was utilized to identify signal sequences and transmembrane domains in the amino acid sequences. InterPro utilizes predictive models from databases including SignalP, employing bioinformatics algorithms such as TMHMM and Phobius, providing comprehensive information on protein sequences that can be used to inform the design process (Paysan-Lafosse et al., 2022).

The Moderna mRNA vaccine sequence was utilized as a model for designing the mRNA vaccines. To analyze the mRNA sequence of the Moderna vaccine model, SnapGene version 7.0.2 was employed to identify the locations of the 5' UTR, 3' UTR, and polyA tail in the model based on the sequence.

Next, the signal sequence and transmembrane domain were located within the SARS-CoV-2 Spike protein (e.g., obtained from InterProScan) and inserted into the respective N-terminus and C-terminus of the candidate protein's amino acid sequence where necessary. Lastly, the other elements of the mRNA vaccine model were inserted into the sequence. The design of the mRNA vaccine sequences for each candidate protein incorporated the following elements in order from 5' to 3':

1. 5' UTR sequence from the Moderna mRNA vaccine model
2. Open reading frame (ORF) of the candidate vaccine protein, which includes the engineered signal sequence and transmembrane domain, if applicable.
3. 3' UTR sequence from the Moderna mRNA vaccine model
4. Poly(A) tail

Results

Outlined are the design processes for each candidate protein based on Figure 1.

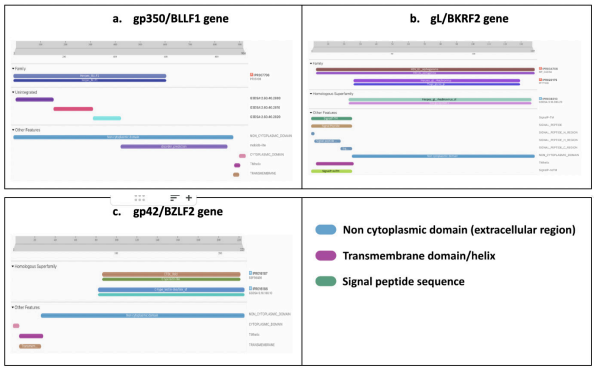

Figure 1. Interpro predicted glycoprotein expression, protein localization, and features. a) extracellular expression of amino acids 1-865, TM domain observed at the C-terminus region 870-892, N-terminus signal peptides absent. b) extracellular expression of amino acids 26-137, presence of N-terminus signal sequence 1-25, TM domain predicted for residues 4-26 (false positive attributed to a long stretch of hydrophobic amino acids), c) extracellular amino acids 28-223, TM domain predicted at region 7-29 (signal sequence present but not predicted in the figure due to lack of traditional signal sequence).

Further scrutiny and validation were warranted regarding Figure 1.c to confirm the accuracy of this prediction, particularly in the context of its potential role within the vaccine model. Kirschner et al. (2009) confirm that highly hydrophobic region 9-29 accounts for the transmembrane domain, as well as indicating gp42 is a type-II membrane protein. Therefore, it does not have a traditional signal sequence, as residues 9-29 serve to direct the insertion of the protein into the endoplasmic reticulum membrane.

Though in Figure 1.b Interpro appears to predict a transmembrane helix, upon further research Matsuura et al. (2010) indicate that gL has only a signal sequence and there is no transmembrane domain structure. The BKRF2 glycoprotein L was found to possess a signal sequence at the C-terminus with a hydrophobic stretch and no transmembrane helix. The software assessed the glycoprotein L for its propensity to form a transmembrane helix based on the presence of nonpolar/hydrophobic amino acids; however, such a helix formation was proved improbable. The system may not have been able to distinguish between a signal peptide and a transmembrane helix. Further analysis using a newer model of SignalP may be able to verify this (Peterson, 2011).

Engineering Signal Sequence and Modifying the ORF:

The gp350 protein was determined to lack a signal sequence. To ensure efficient protein synthesis and localization to the cell surface, a signal sequence from the Moderna mRNA vaccine model was incorporated. The first 18 amino acids from the Moderna vaccine model (known as the signal sequence) were inserted at the N-terminus of the BLLF1 mRNA. The original methionine (AUG) start codon of the glycoprotein was removed to maintain consistency with the signal sequence insertion.

Insertion of the Transmembrane Domain

To incorporate a transmembrane domain into the gL mRNA sequence, the transmembrane domain of the SARS-CoV-2 Spike protein was identified as amino acids 1214 to 1236. This stretch of 23 amino acids (from 1214 to 1236) was added into the ORF immediately before the stop codon. This addition involved inserting 69 bases corresponding to the amino acid sequence.

Incorporating the 5’ and 3’ UTRs and Poly(A) tail:

The 5’ untranslated region (UTR) from the Moderna mRNA vaccine model was added in front of the modified ORF for all candidate glycoprotein mRNA sequences, ensuring proper translation initiation. The 3’ UTR was appended at the end of the mRNA sequence to maintain the essential regulatory elements required for post-transcriptional control. The poly(A) tail, a chain of adenine nucleotides, was inserted after the 3’ UTR to improve stability and prevent RNA degradation.

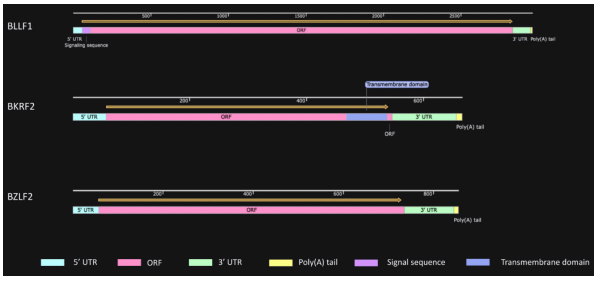

Figure 2. Graphical map of the engineered components of the vaccine from left to right. BLLF1 total 2957 bases, BKRF2 total 665 bases, BZLF2 total 854 bases

Discussion

As of now, a vaccine candidate for EBV has not made it past Phase III of the vaccine screening process or regulatory approval; however, with renewed interest in mRNA technology, a different approach harnessing mRNA vaccines may see success. As demonstrated by its use in streamlining an effective vaccine for SARS-CoV-2, mRNA technology can decrease the time and resources required to engineer new iterations for testing, a significant obstacle during the four decades since vaccine development began. To advance understanding and evaluate the efficacy of the proposed EBNA mRNA vaccine, it is essential to consider prospective methods being researched in the vaccine production and testing process. These methods are crucial for assessing whether the proposed mechanisms will effectively produce the desired immune response. Several testing approaches can be employed as next steps to examine various aspects of the vaccine development process.

RNA Generation

Optimizing mRNA synthesis is crucial for the successful development and production of RNA vaccines, which require the mass generation of mRNA, often in the form of in vitro transcription using bacteriophage RNA polymerase and PCR-amplified linearized templates or plasmids. One key consideration is the choice of promoter system used for in vitro transcription, which can significantly impact mRNA yield, purity, and stability. The T7 promoter system is commonly employed due to its high efficiency and specificity in driving transcription from a DNA template (Kang et al., 2023). However, alternative promoter systems such as SP6 and T3 should be considered, as each offers distinct transcriptional activity and regulatory control advantages.

Optimized RNA Modalities

Several alternative mRNA models are undergoing research for their lower-dose mRNA requirements, including self-amplifying mRNA (saRNA) and its structurally altered counterpart trans-amplifying mRNA (taRNA) (Rosa et al., 2021). saRNA involves inserting a viral replicase gene, typically, sequences from single-stranded RNA viruses like alphaviruses, and flaviviruses. When delivered to the cytoplasm, this type of mRNA produces high levels of the desired antigen without generating viral infectious particles, addressing safety concerns. In mouse models, a saRNA vaccine required a significantly lower dose compared to conventional mRNA vaccines to induce an immune response against H1N1/PR8 infection.

Trans-amplifying mRNA (taRNA) results from splitting self-amplifying mRNA into two templates: one containing the gene of interest and the other containing the replicase system. taRNA offers advantages over saRNA, including improved safety, versatility, and cost-effectiveness in manufacturing. Notably, taRNA has already demonstrated efficacy in protecting mice against influenza, inducing antibody production and providing protection.

mRNA Capping

Capping of in vitro transcribed (IVT) mRNA is essential for translation efficiency and intracellular stability (Ramanathan et al., 2016). This technique involves the addition of a cap structure to the mRNA's 5' end, promoting efficient protein synthesis. During the IVT reaction, substituting a portion of the guanosine triphosphate (GTP) substrate with a cap analog alongside ribonucleoside triphosphates (rNTPs) results in cap-1 mRNAs that trigger less immunogenicity in vivo (Rosa et al., 2021). While this method is faster and eliminates the need for a separate enzymatic reaction, it achieves an imperfect capping efficiency of approximately 60–80%. On the other hand, a separate enzymatic reaction using the vaccinia capping enzyme (VCC) and a methyl donor to cap the mRNA, achieves a higher capping efficiency of 100% (Rosa et al., 2021). However, this method requires an additional step and setup. Both methods may involve high production costs when considering large-scale manufacturing. To mitigate these costs, researchers may explore alternative expression systems such as yeast-based platforms, which offer lower production costs but may require additional optimization steps to achieve optimal mRNA yield and quality. A 2018 patent utilizes specific elements from the yeast Ty retrotransposon, enabling the production of RNAs, including mRNAs and long non-coding RNAs (lncRNAs), with features like a 3' polyA tail, a 5' methylguanosine cap, and potential methylation of adenine and cytosine residues (Pigeon et al., 2018). Although still requiring further research, this yeast-based platform allows cost-effective production of these RNAs in significant quantities, showing promise.

Uracil to Pseudouridine Replacement

It has been demonstrated that replacing uracil residues with pseudouridine (Ψ) can enhance the stability of in vitro transcribed RNA, reduce immunogenicity, and improve translation efficiency (Morais et al., 2021). Nucleotide modifications play a pivotal role in mRNA-LNP vaccine design. Unmodified mRNA molecules can trigger innate immune responses upon administration. To mitigate this, chemically modified nucleoside Ψ can be incorporated into the mRNA sequence. The choice of modifications should be guided by their impact on both the immune response and protein expression (Morais et al., 2021).

Testing for Protein Expression/ Localization

Immunofluorescence can be utilized to visualize the expression and localization of the EBNA protein within cells. Immunofluorescence involves using antibodies tagged with fluorescent molecules to bind to specific target proteins within the cell. When excited by a specific wavelength of light, the fluorophores' fluorescence allows visualization of the protein's location (Joshi & Yu, 2017). Cells are fixed, permeabilized to allow antibody penetration, and then incubated with primary antibodies against the target protein. After washing away unbound antibodies, cells are incubated with fluorescently labelled secondary antibodies that bind to the primary antibodies. Immunofluorescence allows tracking of the distribution of the target protein within the cellular context, providing insights into its subcellular localization and potential interactions with cellular components.

Western blotting is another technique that can be utilized, not for cellular localization but rather to confirm protein expression in cell lysates as it detects proteins based on their size and immunoreactivity. Protein lysates are separated by gel electrophoresis based on size and transferred onto a membrane (Yang et al., 2012). The membrane is then probed with antibodies against the protein of interest, followed by detection using chemiluminescent or chromogenic substrates. Post-imaging, the data can help confirm the expected expression of the intended proteins.

Enzyme-linked immunosorbent Assay (ELISA) uses antibodies linked with enzymes that react with a substrate to produce a detectable signal (usually colorimetric or chemiluminescent) proportional to the amount of target protein, as stated in StatPearls (Alhajj et al., 2023). ELISA is highly quantitative and can measure protein concentration accurately. Researchers can use it to assess the level of vaccine-encoded protein in samples, such as cell culture supernatants. Calibration curves and standardization are key considerations.

Further Testing and Limitations

Following verification of protein expression and localization, in vitro testing using human cell lines should be conducted to assess the stability, translational efficiency, and immunogenicity of the modified mRNA. These initial studies provide valuable insights into the performance of the vaccine candidate at the cellular level, helping to refine its design. In the vaccine development process what follows is usually animal model testing to evaluate the safety and immunogenicity of the mRNA vaccine candidate. It is important to monitor the immune responses of these animal models, including antibody production, T-cell activation, and cytokine profiles. These can verify the vaccine candidate's ability to induce strong immune responses against the virus and expose any adverse effects or inflammatory responses in the animal subjects. This would involve selecting appropriate animal models, such as mice or non-human primates to mimic human responses, preferably species that naturally are EBV hosts or are modified to interact with the virus as human cells do (Cai et al., 2021). Animal model selection is a key obstacle in mRNA vaccine development currently. The cotton-top tamarin was initially established as an experimental model for EBV infection and lymphoma development, first being used in 1985; however, the model's translatability to human EBV infection was limited (Escalante et al., 2022). Humanized mice, which develop lymphoma upon EBV infection, have also been employed, yet Escalante et al. note that their susceptibility to EBV infection and subsequent disease manifestation again differed from human EBV infection. Rabbits and Chinese tree shrews were found to be unreliable for the same reason as humanized mice; their epithelial cells were not infected by EBV, making it difficult to generalize for human testing. As of now, no ideal animal model has been designated for the testing of EBV vaccines.

If animal testing shows promising results, the candidate can then proceed onto ordered clinical trials on humans–from Phase I, administered to a handful of healthy volunteers, to Phase II with a larger group of participants including individuals at risk for EBV, and lastly a large scale Phase III diverse population trial (WHO, 2020). Despite efforts, the furthest a vaccine candidate for EBV has progressed is Phase II. Phase II of a prophylactic AS04 recombinant gp350 vaccine demonstrated a reduced incidence of infectious mononucleosis in the vaccinated group compared to the placebo group (Cai et al., 2021). However, the vaccine fell short of preventing initial EBV infection, its primary goal. mRNA vaccines hold promise in revitalizing these efforts to develop

an effective EBV vaccine by offering a versatile and potentially more efficient alternative to conventional vaccine strategies. As they can be rapidly designed and produced for numerous antigens, and their flexibility enables the exploration of different delivery methods and adjuvants, mRNA vaccines hold the potential to overcome challenges associated with traditional vaccine approaches.

Conclusion

With hundreds of researchers contributing to its development over several decades, mRNA vaccines are finally gaining recognition for their potential to solve various global health challenges where research with other methods has stagnated. With one such area being vaccination for EBV, an extensively studied virus for its role in various diseases, three protein targets show promise for immunization with new mRNA technology. Gp350, gL, and gp42 all have unique roles in EBV's complex biology and are expressed extracellularly, making them ideal candidates. By making several alterations to their genes to allow for uptake, protein synthesis, and stability, an mRNA vaccine can be created and tested with established methods such as immunofluorescence, Western Blotting, and ELISA. The versatility and speed of development associated with mRNA vaccines hold promise for revolutionizing the field of immunology and responding rapidly to emerging health threats, such as Epstein-Barr virus, fast-mutating pathogens like influenza, or even certain cancers.

References

Alhajj M, Zubair M, Farhana A. Enzyme-Linked Immunosorbent Assay. [Updated 2023 Apr 23]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK555922

Bjornevik, K., Cortese, M., Healy, B. C., Kuhle, J., Mina, M. J., Leng, Y., Elledge, S. J., Niebuhr, D. W., Scher, A. I., Munger, K. L., & Ascherio, A. (2022). Longitudinal analysis reveals high prevalence of epstein-barr virus associated with multiple sclerosis. Science, 375(6578), 296-301. https://doi.org/10.1126/science.abj8222

BKRF2 [Human gammaherpesvirus 4] [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004 – [cited 2023 Oct 19]. Available from: https://www.ncbi.nlm.nih.gov/protein/UQK62613.1

BLLF1 [Human gammaherpesvirus 4] [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004 – [cited 2023 Oct 19]. Available from: https://www.ncbi.nlm.nih.gov/protein/QCG99665.1

BLLF1 [Human gammaherpesvirus 4] [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004 – [cited 2023 Oct 19]. Available from: https://www.ncbi.nlm.nih.gov/protein/QCG99665.1

Cai, J., Zhang, B., Li, Y., Zhu, W., Akihisa, T., Li, W., Kikuchi, T., Liu, W., Feng, F., & Zhang, J. (2021). Prophylactic and therapeutic EBV vaccines: Major scientific obstacles, historical progress, and future direction. Vaccines, 9(11), 1290. https://doi.org/10.3390/vaccines9111290

Callan, M., Tan, L., Annels, N., Ogg, G., Wilson, J., O'Callaghan, C., Steven, N., McMichael, A., & Rickinson, A. (1998). Direct visualization of antigen-specific cd8+t cells during the primary immune response to epstein-barr virus in vivo. The Journal of Experimental Medicine, 187(9), 1395-1402. https://doi.org/10.1084/jem.187.9.1395

Cohen, J. I. (2015). Epstein–barr virus vaccines. Clinical & Translational Immunology, 4(1). https://doi.org/10.1038/cti.2014.27

Cohen, J. I., M.D. (2000). Epstein–Barr Virus Infection. New England Journal of Medicine, 342, 481-492. https:///doi.org/10.1056/nejm200008173430707

Dotmatics. (2023). SnapGene® software [Computer software]. https://snapgene.com
Used to modify nucleotide sequences and create figures.

Escalante, G. M., Mutsvunguma, L. Z., Muniraju, M., Rodriguez, E., & Ogembo, J.G. (2022). Four decades of prophylactic EBV vaccine research: A systematic review and historical perspective. Frontiers in Immunology, 13. https://doi.org/10.3389/fimmu.2022.867918

Farrell, P. J. (2019). Epstein–Barr virus and cancer. Annual Review of Pathology: Mechanisms of Disease, 14(1), 29-53. https://doi.org/10.1146/annurev-pathmechdis-012418-013023

Forni, D., Cagliani, R., Clerici, M., & Sironi, M. (2022). Disease-causing human viruses: Novelty and legacy. Trends in Microbiology, 30(12), 1232-1242. https://doi.org/10.1016/j.tim.2022.07.002

Joshi, S., & Yu, D. (2017). Immunofluorescence. Basic Science Methods for Clinical Researchers, 135-150. https://doi.org/10.1016/b978-0-12-803077-6.00008-4

Kang, D. D., Li, H., & Dong, Y. (2023). Advancements of in vitro transcribed mRNA (IVT mRNA) to enable translation into the clinics. Advanced Drug Delivery Reviews, 199, 114961. https://doi.org/10.1016/j.addr.2023.114961

Kirschner, A. N., Sorem, J., Longnecker, R., & Jardetzky, T. S. (2009). Structure of epstein-barr virus glycoprotein 42 suggests a mechanism for triggering receptor-activated virus entry. Structure, 17(2), 223-233. https://doi.org/10.1016/j.str.2008.12.010

Liang, S.-T., Xu, Y.-C., Dennis, P., & Bremer, H. (2000). MRNA composition and control of bacterial gene expression. Journal of Bacteriology, 182(11), 3037-3044. https://doi.org/10.1128/jb.182.11.3037-3044.2000

Lucie Pigeon, Rachid Rahmouni, Chantal Rabenadrasana-Pichon & Patrick Midoux (2018). Production of RNA by yeasts with recombinant pseudo-viral particles (World Patent No. WO2018/171946 A1). World Intellectual Property Organization https://patents.google.com/patent/WO2018171946A1/en

Mathieu, E., Ritchie, H., Rodés-Guirao, L., Appel, C., Giattino, C., Hasell, J., Macdonald, B., Dattani, S., Beltekian, D., Ortiz-Ospina, E., & Roser, M. (2020) Total COVID-19 vaccine doses administered. Our World in Data. https://ourworldindata.org/grapher/cumulative-covid-vaccinations

Matsuura, H., Kirschner, A. N., Longnecker, R., & Jardetzky, T. S. (2010). Crystal structure of the epstein-barr virus (EBV) glycoprotein h/glycoprotein L (gH/gL) complex. Proceedings of the National Academy of Sciences, 107(52), 22641-22646. https://doi.org/10.1073/pnas.1011806108

Morais, P., Adachi, H., & Yu, Y.-T. (2021). The critical contribution of pseudouridine to mRNA covid-19 vaccines. Frontiers in Cell and Developmental Biology, 9. https://doi.org/10.3389/fcell.2021.789427

Office of Infectious Disease and HIV/AIDS Policy (OIDP). (2022). Vaccines by disease. HHS.gov. https://www.hhs.gov/immunization/diseases/index.html

Pardi, N., Hogan, M. J., Porter, F. W., & Weissman, D. (2018). MRNA vaccines — a new era in vaccinology. Nature Reviews Drug Discovery, 17(4), 261-279. https://doi.org/10.1038/nrd.2017.243

Paysan-Lafosse T, Blum M, Chuguransky S, Grego T, Pinto BL, Salazar GA, Bileschi ML, Bork P, Bridge A, Colwell L, Gough J, Haft DH, Letunić I, Marchler-Bauer A, Mi H, Natale DA, Orengo CA, Pandurangan AP, Rivoire C, Sigrist CJA, Sillitoe I, Thanki N, Thomas PD, Tosatto SCE, Wu CH, Bateman A. InterPro in 2022. Nucleic Acids Research, Nov 2022. https://doi.org/10.1093/nar/gkac993

Petersen, T. N., Brunak, S., von Heijne, G., & Nielsen, H. (2011). SignalP 4.0: Discriminating signal peptides from transmembrane regions. Nature Methods, 8(10), 785-786. https://doi.org/10.1038/nmeth.1701

Ramanathan, A., Robb, G. B., & Chan, S.-H. (2016). MRNA capping: Biological functions and applications. Nucleic Acids Research, 44(16), 7511-7526. https://doi.org/10.1093/nar/gkw551

Ressing, M. E., van Leeuwen, D., Verreck, F. A. W., Keating, S., Gomez, R., Franken, K. L. M. C., Ottenhoff, T. H. M., Spriggs, M., Schumacher, T. N., Hutt-Fletcher, L. M., Rowe, M., & Wiertz, E. J. H. J. (2005). Epstein-Barr virus gp42 is posttranslationally modified to produce soluble gp42 that mediates HLA class II immune evasion. Journal of Virology, 79(2), 841-852. https://doi.org/10.1128/jvi.79.2.841-852.2005

Rosa SS, Prazeres DMF, Azevedo AM, Marques MPC. mRNA vaccines manufacturing: Challenges and bottlenecks. Vaccine. 2021 Apr 15;39(16):2190-2200. doi: 10.1016/j.vaccine.2021.03.038. Epub 2021 Mar 24. PMID: 33771389; PMCID: PMC7987532.

Salvatori, G., Luberto, L., Maffei, M., Aurisicchio, L., Roscilli, G., Palombo, F., & Marra, E. (2020). SARS-CoV-2 SPIKE protein: An optimal immunological target for vaccines. Journal of Translational Medicine, 18(1). https://doi.org/10.1186/s12967-020-02392-y

Slabik, C., Kalbarczyk, M., Danisch, S., Zeidler, R., Klawonn, F., Volk, V., Krönke, N., Feuerhake, F., Ferreira de Figueiredo, C., Blasczyk, R., Olbrich, H., Theobald, S. J., Schneider, A., Ganser, A., von Kaisenberg, C., Lienenklaus, S., Bleich, A., Hammerschmidt, W., & Stripecke, R. (2020). CAR-T cells targeting epstein-barr virus gp350 validated in a humanized mouse model of EBV infection and lymphoproliferative disease. Molecular Therapy - Oncolytics, 18, 504-524. https://doi.org/10.1016/j.omto.2020.08.005

Surface glycoprotein [Severe acute respiratory syndrome coronavirus 2] [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004 – [cited 2023 Oct 19]. Available from: https://www.ncbi.nlm.nih.gov/protein/YP_009724390.1

Synthetic construct HCV1146 Moderna (mRNA-1273) SARS-CoV-2 vaccine sequence [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004 – [cited 2023 Oct 19]. Available from: https://www.ncbi.nlm.nih.gov/nuccore/OK120841.1

World Health Organization: WHO. (2020). Clinical trials. www.who.int. https://www.who.int/health-topics/clinical-trials#tab=tab_1

Yang, P.-C., & Mahmood, T. (2012). Western blot: Technique, theory, and trouble shooting. North American Journal of Medical Sciences, 4(9), 429. https://doi.org/10.4103/1947-2714.100998