“EPSTEIN-BARR VIRUS: A CLOSER LOOK UNDER THE MICROSCOPE”

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INTRODUCTION

Even with countless discoveries made for the past few centuries since the invention of the microscope, microbiology—the study of life and living organisms at the microscopic level—continues to tantalize scientists with more and more questions and new corners to explore. When life was once thought to be smallest at the scale of an ant, observers were proven wrong with the first observation of cell samples from plant and animal tissues. Then, even smaller forms of cellular life were found in the form of bacteria, previously known collectively as germs, along with certain fungi and protists. By late 19th century, the scale was further refined with the observation of the virus, a biological enigma described as "contagium vivum fluidum", or ‘contagious living liquid’ by the Dutch scientist, Martinus Beijerinck (“Historical Highlights”, n.d.). It appears that each day, a new finding would surface in science—whether medical, agricultural, ecological, or just any other branch of science for that matter—and in this case, the remarkable understanding of the natural world that we are gradually gaining comes simply from looking more and more closely at life and the materials around us, literally at that.

With further advancements in microscopy, visualization and imaging technology; scientists are slowly coming closer and closer to unraveling the mysterious nature of viruses—chemical units resembling life but not exactly living, which as such, can not be termed as organisms—within the field of study called virology. Viruses have been identified as the culprit behind numerous diseases plaguing larger organisms such as plants, animals and the human population alike, alongside other microscopic pathogens like bacteria, fungi and protists. In humans, approximately 250 out of the estimated 1000 to 1500 types of viruses are identified to be pathogenic (“Viruses”, 2005). Among the diseases identified to be virus-related are Hepatitis, AIDS, common cold, and measles, in humans; and raspberry mosaic, tobacco mosaic, leaf curl, and tomato ringspot in plants (Behrendt, 2000 & “Viruses”, 2005).
However, instead of discussing the exhaustive list of pathogenic viruses and the syndromes they cause, we will instead focus on one type of virus, the Epstein-Barr virus, or EBV, in this paper. The essential topics of discussion include the history behind its discovery, its characteristics, morphological aspects, location, method of reproduction and finally its significance in human pathology.

**DISCOVERY**

The discovery of this virus was triggered by the observation of a surgeon working in Uganda, Dennis Burkitt, 1961, on the propagation of a certain tumor in African children that somehow had a geographic distribution corresponding to rainfall and temperature patterns. Based on this finding, he suggested that a biological factor was responsible for the spread of this disease, which affects about 8 in every 100,000 children in parts of Africa and Papua New Guinea and was also later known as Burkitt’s lymphoma. Work on identifying the agent then began with three researchers, M.A. Epstein, Y.M. Barr and B.G. Achong, who immediately began looking for possible cancer-causing viruses in samples of the tumour sent from Uganda to Britain. It was later identified in 1964, with the help of an electron microscope—a previously unknown member of the herpes family of viruses. The pathogen was then named after Epstein and Barr, in honor of their research ("Kissing EBV", 1997).

**CHARACTERISTICS & MORPHOLOGY**

The family of viruses to which Epstein-Barr virus—commonly known as EBV—belongs; Herpesviridae is found in all branches of vertebrates and comprises many human pathogens. The members belong to three subfamilies according to DNA content and structure (Alpha-, Beta-, and Gammaherpesviridae). Our virus of interest falls under the third subfamily and is thus identified as a Gammaherpesvirus—also denoted as $\gamma$-herpesvirus. Being a member of Herpesviridae, EBV naturally displays or possesses the
characteristics of this classification of viruses. These characteristics include the presence of a linear, double stranded DNA; enveloped particles within the range of 120-200nm in size; and a capsid which is cubic or icosahedral in structure (Ackerman, Berthiaume & Tremblay, 1998).

A virus is dependent on a living cell host for its reproductive cycle and genetic replication for they are otherwise biologically inert chemical structures and units. The process of EBV propagation follows this series of steps:

Viral capsids enter cells after fusion of their envelope with the plasma membrane; followed by viral replication and assembly, most of which takes place within the host cell’s nucleus. The infecting DNA from inside the invading capsid circularizes and undergoes replication by a rolling-circle mechanism, producing concatemers. Progeny DNA then enters preformed capsids which leave the nucleus by budding—through which
they acquire an envelope—and transit via the Golgi network of membranes. Once mature, the viruses depart the cell via exocytosis (Ackerman, Berthiaume & Tremblay, 1998).

According to an article by the National Center for Infectious Diseases, the herpesvirus EBV is one of the most common human viruses—occurring worldwide and infecting most people at one time in their life, or another (“Epstein-Barr”, n.d.). In the United States, up to 95% of adults between 35 to 40 years old have been infected by this virus. Infants grow susceptible to this virus as soon as maternal antibody protection disappears at birth. Infection with this virus is also prominent in many children, though not so in the United States and developed countries. When it strikes during adolescence or young adulthood, mononucleosis would ensue 35% to 50% of the time (“Epstein-Barr”, n.d.).
LOCATION

Inside the human body, the EBV exhibits the ability to infect only two major cell types which are the outer epithelial cells of the salivary gland, and the white blood cells known as B lymphocytes (B-cells) involved in the body’s immune responses to diseases and foreign threats. Infection with this virus initiates at the salivary gland, where a large quantity of the EBV is released into the saliva, enabling it to be propagated from one person to another. B-cells infected with EBV, on the other hand, will proliferate which, if not controlled by the immune system with a correct response, will result in the development of cancer in the particular individual who contracted this virus (“Kissing EBV”, 1997).

SIGNIFICANCE IN HUMAN PATHOLOGY

Individuals who are infected with the Epstein-Barr virus may retain it for life, but it does not necessarily lead to any diseases in them. As a matter of fact, it is known to infect nearly every person in developing countries, and more than 80% of the people in developed ones. Due to its transmission via intimate contact with the saliva of an infected person, it is dubbed the “kissing virus”. Glandular fever which is a symptom of infectious mononucleosis of the cells in the immune system, leading to swollen lymph glands, sore throat and fever, has since been called the “kissing disease” (“Epstein-Barr”, n.d. & “Kissing EBV”, 1997). In some cases, swelling of the spleen and liver involvement may also develop following the fever. Heart problems or involvement of the central nervous system are known to occur, but only very rarely. Infectious mononucleosis, to date, is almost never fatal and there are no known associations of this infection to pregnancy complications such as miscarriage or birth defects. Symptoms would generally resolve in 1 or 2 months, and the virus would then remain in dormant or latent state in a few cells in the infected person’s throat and blood for the rest of their life. It can also reactivate later on, but usually without causing any symptoms of illness (“Epstein-Barr”, n.d.).
Despite the generally harmless infection of this virus, it has actually been linked to the previously mentioned Burkitt’s lymphoma, and nasopharyngeal carcinoma, two rare cancers that are not normally diagnosed in the United States (“Epstein-Barr”, n.d.). The latter of the two cancers involves the growth of tumor in the nasal passages and throat which affects up to 2 per cent of people in southern China and also occurs in Southeast Asia, northern Africa and among Arctic peoples (“Kissing EBV”, 1997). Even though EBV seems to play an important role in these malignancies, other factors may also be involved; therefore it may just not be the sole cause of these diseases (“Epstein-Barr”, n.d.). In addition to that, EBV has also been proposed as a possible cause for Hodgkin’s disease—a type of cancer affecting cells of lymph nodes (“Kissing EBV”, 1997).

EBV infection is also prominent in individuals with reduced immunity, affecting—among others—organ transplant patients, as a tumor called ‘post-transplant lymphoproliferative disease’ due to their suppressed immunity to prevent organ rejection; AIDS sufferers, who commonly develop ‘oral hairy leukoplakia’, a condition involving considerable proliferation of the Epstein-Barr virus in cells along the edge of the tongue; and people in countries with high malaria incidence, by means of which Burkitt’s lymphoma prevalence in the very same countries is suggested to be linked. This suggestion is based on the fact that malaria leads to decreased immunity in sufferers, which might have played a crucial role in EBV infection towards Burkitt’s lymphoma (“Kissing EBV”, 1997).

In the body, the mechanism of EBV infection is by the production of 100 antigens during the active phase of the viral cycle, as opposed to only 10 antigens during the inactive phase—which include the Epstein-Barr virus nuclear antigens (EBNAs 1–6), and the latent membrane proteins (LMPs 1–3) (“Kissing EBV”, 1997).

The body’s immune response to the invasion of EBV comes in the form of cytotoxic T lymphocytes (T-cells) that combine with certain antigens carried by the virus and destroys cells that carry these specific antigens. However, when associated with Burkitt’s lymphoma and nasopharyngeal carcinoma, the virus appears to exhibit only one
antigen which is EBNA1. T-cells do not have the ability to combine with this particular antigen and as such, are unable to destroy cells carrying it as well. Consequently, EBV goes virtually ‘hidden’ from the immune system and its patrolling T-cells in these cases (“Kissing EBV”, 1997).

Clinical diagnosis of EBV infection can be done, in most cases, from the characteristic triad of fever, pharyngitis and lymphadenopathy lasting from 1 to 4 weeks in length. Results from serological tests include elevated white blood cell count and total number of lymphocytes, greater than 10% atypical lymphocyte presence, and positive response to a “mono” test. For patients with symptoms that suggest infectious mononucleosis, a positive Paul-Bunnell heterophile antibody test result is sufficiently diagnostic, and there is no need for further testing. Moderate-to-high levels of heterophile antibodies are observed during the first month of illness and decrease rapidly after the fourth week. False-positive results may be found in a small number of patients, and false-negative results may be obtained in 10% to 15% of patients, primarily in children younger than 10 years of age. It is extremely rare to find true outbreaks of genuine mononucleosis—there have also been a substantial number of pseudo-outbreaks which are due to laboratory error, as published in CDC's Morbidity and Mortality Weekly Report, vol. 40, no. 32, on August 16, 1991 (“Epstein-Barr”, n.d.).

Negative “mono” or heterophile test results may call for additional laboratory testing to differentiate EBV infections from a mononucleosis-like illness induced by other factors such as cytomegalovirus, adenovirus, or Toxoplasma gondii. Direct detection of EBV in blood or lymphoid tissues serves as a research tool and is not available for routine diagnosis. On the other hand, serologic testing remains the method of choice for diagnosing primary infection of this virus (“Epstein-Barr”, n.d.).

According to an article published by the National Center for Infectious Diseases, United States (n.d.), the process of diagnosing Epstein-Barr virus infection is summarized according to the following criteria:
**Susceptibility**

If antibodies to the viral capsid antigen are not detected, the patient is susceptible to EBV infection.

**Primary Infection**

Primary EBV infection is indicated if IgM antibody to the viral capsid antigen is present and antibody to EBV nuclear antigen, or EBNA, is absent. A rising or high IgG antibody to the viral capsid antigen and negative antibody to EBNA after at least 4 weeks of illness is also strongly suggestive of primary infection. In addition, 80% of patients with active EBV infection produce antibody to early antigen.

**Past Infection**

If antibodies to both the viral capsid antigen and EBNA are present, then past infection (from 4 to 6 months to years earlier) is indicated. Since 95% of adults have been infected with EBV, most adults will show antibodies to EBV from infection years earlier. High or elevated antibody levels may be present for years and are not diagnostic of recent infection.

**Reactivation**

In the presence of antibodies to EBNA, an elevation of antibodies to early antigen suggests reactivation. However, when EBV antibody to the early antigen test is present, this result does not automatically indicate that a patient's current medical condition is caused by EBV. A number of healthy people with no symptoms have antibodies to the EBV early antigen for years after their initial EBV infection. Many times reactivation occurs subclinically.

**Chronic EBV Infection**

Reliable laboratory evidence for continued active EBV infection is very seldom found in patients who have been ill for more than 4 months. When the illness lasts more than 6 months, it should be investigated to see if other causes of chronic illness or CFS are present. ("Epstein-Barr", para. 18)
DEFENSE AGAINST THE VIRUS

As is the case with most viruses, vaccination serves as the best chance of defense. The search for the vaccine has been somewhat partially fruitful—mostly due to the fact that the virus is extremely good at hiding. For the cases of Burkitt’s lymphoma and nasopharyngeal carcinoma prevention, a vaccine would need to develop 100 percent immunity or have the capability to establish a population of T lymphocytes that can recognize EBNA1. These tasks have proven themselves extremely difficult to carry out. However, a greater possibility lies at creating vaccines against glandular fever (infectious mononucleosis) and post-transplant lymphoproliferative disease, since they both involve antigens that are recognizable by T-cells (“Kissing EBV”, 1997).

The principle behind this vaccination would be artificially introducing the identified antigen behind a particular disease—or at least the most important parts of it (the antigen)—into the system to trigger the manufacture of T-cells in response to these foreign ‘markers’. By doing so, the body is ‘tricked’ into producing T-cells that are well acquainted with these antigens and be able to recognize the real ones should infection take place (“Kissing EBV”, 1997).

Researchers have so far been able to produce a peptide identical to part of the antigen EBNA3, which forms the basis of a new vaccine that is hoped to ‘arm’ the body against the real EBNA3 under the circumstance of infection.

Having completed the first phase of the trial which led them to the confidence that the vaccine possess no harmful effects on the patients, the researchers are now moving on to the next phase which aims to determine the effectiveness of this vaccine in preventing diseases brought about by the virus (“Kissing EBV”, 1997).

Aside from that, there are also attempts made on growing and expanding T-cells in the laboratory to help treat various forms of EBV-induced cancers which would be extremely beneficial for treating patients suffering from post-transplant lymphoproliferative disease (“Kissing EBV”, 1997).
The prospect of better and more powerful vaccination in the future has long been the topic of discussion and research. Much focus has been put on coming up with a vaccine that is as much appealing as it is effective—as is being done with the search for the EBV vaccine.

The Australian Academy of Science (1997), in an article titled “Kissing the Epstein-Barr Virus Goodbye?” pointed out several new and possible innovations in this strategy of combating diseases down to the cellular, if not molecular level by means of vaccination:

**Gene guns and golden bullets**

One such technique uses DNA to produce what are known as nucleic acid vaccines (sometimes called ‘naked DNA’ vaccines). Scientists isolate the genes from disease-causing bacteria or viruses that provide information to make specific antigens. These genes are then inserted into a plasmid (which is a genetic element capable of replicating independently of the chromosomal DNA) which, in turn, is injected into the patient. This can be done in the usual way with a hypodermic syringe into muscle tissue, or with a ‘gene gun’, which fires tiny gold particles coated with the DNA into the surface layers of the skin.

Once inside the body the plasmids penetrate host cells, where they start manufacturing antigens. These antigens, released over a long period, induce an active immune response by the body, including the production of both antibodies and specialised white blood cells (cytotoxic T lymphocytes).

The technique may be used to produce vaccines for both viral and bacterial infections; it shows such promise that clinical investigation of a possible AIDS vaccine has already begun.

**‘Antigen factory’ vaccines**

A similar technique involves the insertion of selected genes from a disease into benign bacteria or viruses. These are then administered to the patient and serve as
a sort of antigen factory, using the inserted genes to churn out antigens of the disease-causing organism. These antigens invoke an immune response that will help protect the patient from a subsequent infection. Several ‘antigen factory’ organisms have been tested and are under development as vaccines.

**Making vaccines more effective**

Conventional vaccines often use weakened or killed cells of the disease itself. While this has proved effective against many diseases, some vaccines developed by this technique produce occasional side-effects in patients. New methods using recombinant DNA technology have led to the development of many vaccines that use only a small part of the disease-causing organism.

These methods produce extremely safe vaccines, but they are often less effective than whole-cell vaccines. A major area of vaccine research seeks ways of making such ‘subunit’ vaccines more effective in producing an immune response. This usually involves the use of what are called adjuvants, which are substances added to the vaccine to aid its operation. Conventional vaccines mostly use aluminium salt as an adjuvant, but recent work has tested oil-based emulsions that contain biodegradable material.

**User-friendly vaccines**

Vaccine development agencies recognise the importance of increasing the rate of childhood immunisation. One way of achieving this would be to develop vaccines that could be taken orally or nasally, rather than by injection.

With this in mind, researchers have investigated the use of what are called microcapsules. These consist of an inner reservoir of antigen surrounded by an outer, biodegradable polymer wall, through which the antigen is released slowly. Vaccines administered in this way have been shown to produce strong, sustained immune responses for some antigens. One advantage of microcapsules is that refrigeration is not required, making them suitable for remote regions.
Scientists are currently investigating the possible safety implications of having microcapsules in the body for extended periods. If the method proves to be safe, it may become widely used for vaccine administration.

**Combining vaccines**

Another way of boosting the rate of childhood immunisation would be to combine vaccines so that patients could be vaccinated against several diseases at one time. Some combinations are already available (the diptheria-tetanus-pertussis vaccine is one example), and researchers continue to seek ways of combining vaccines without reducing their effectiveness.

**Linking chains**

Armed with an understanding of the molecular structure of antigens for a particular disease, scientists are often able to replicate certain peptides of the antigen in the laboratory. These peptides show promise as vaccines because they can produce an immune response in patients. Indeed, the new Epstein-Barr virus vaccine currently under clinical trial is based on a peptide found on one of the virus’s antigens.

Nevertheless, despite considerable promise, there has been surprisingly little progress in the development of synthetic peptide vaccines. One reason for this might be that the peptides are too small and unstable to provoke an effective immune response.

Australian scientists at the Cooperative Research Centre for Vaccine Technology are pioneering work to polymerise (join together) small peptides. Early results suggest that the polymerisation process aids the potency of the peptides as antigens, and may also allow peptides against more than one disease to be included in the same molecular structure. (para. 31)
CONCLUSION

Clearly with all the extensive amounts of research and scientific studies going into the elucidation of microscopic agents—such as viruses, bacteria, protists and fungi alike—we are slowly but surely heading towards grasping the solutions behind the world’s diseases. As for the case of this interestingly elusive and pathologically versatile virus, we hope to acquire the panacea to maladies that it holds responsibility for, and perhaps with better understanding of the currently befuddling nature of EBV and other viruses for that matter, we might just be able to manipulate, if not re-create this biological uniqueness for our positive benefits. As of present, the results yielded have been promising enough to keep the rates of EBV-induced diseases at bay, largely thanks to the leading figures in the research especially those behind the materialization of this virus in our virological database.

(3,611 words)
Reference:


http://www.extension.umn.edu/distribution/horticulture/DG1152.html
