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News & Views

Tribute to Anthony Epstein who discovered the first human cancer-causing Epstein-Barr virus: The subject in precise on his demise

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Oncovirus: avian and human

Invariably, cancer is not contagious except transplant-related cancer. However, virus induced cancer in man and animal is infectious. During undergraduate professional course, we have come across two important viruses one is Epstein-Barr virus (EBV) and another Rous sarcoma virus (RSV). Those are tumor inducing viruses. RSV causes tumor in avian species, and EBV is responsible for Burkitt's lymphoma in human. Surprisingly, we have never shown any interest to know how these two viruses have been named. Long back, in 1910, a woman brought a hen (Plymouth Barred Rock) having a large tumor to Rockefeller Institute, New York (now Rockefeller University). At that time, a researcher, Francis Peyton Rous working at Rockefeller Institute, showed interest to investigate the reason behind the tumor. He used cell-free tumor extract, devoid of bacterial contaminant, to confirm the transmissibility of tumor production in healthy chicken and observed that tumor inducing agent was no other than a virus (Rous, 1910; Rous, 1911). Initially, critics were reluctant to accept the oncogenic character of the virus. Once several reports substantially proved that transmission of murine cancer could be developed following inoculation of cell-free extract of tumor tissue, gradually viral oncogenicity received scientific support (Gross, 1951). According to the name of Francis Peyton Rous, the virus had been named as Rous sarcoma virus (RSV) that could induce soft tissue tumor, called sarcoma. For his discovery, Rous claimed fame as a Nobel Prize recipient in 1966. During early 1960, a British pathologist, Michael Anthony Epstein at Middlesex Hospital in London, UK was also working on the Rous sarcoma virus. Subsequently, Epstein, along with his student Yvonne Barr, isolated the first human oncogenic virus from Burkitt's lymphoma, a cancer prevalent in African children (Epstein *et al*., 1964). According to their name, the virus had been named as Epstein-Barr virus. They also recorded that the virus was capable to transform normal lymphocytes to lymphoblastoid cell line. So, for the first time, a virus that could cause cancer in humans was identified by Epstein. The lifelong effort of Epstein to develop a vaccine against this virus was unsuccessful. His discovery of the Epstein-Barr virus initially rocked the scientific world. The purpose of the manuscript is to be precise about the contribution of Epstein to pay tribute to his demise. He passed away on 6th February 2024 at his home in London.

Career of cancer virus hunter

On 18th May 1921, Michael Anthony Epstein was born in London. His father, Mortimer Epstein, was a well-known writer and translator who edited "The Statesman's Yearbook" for Macmillan from 1924 until his death in 1946. His mother, Olga Oppenheimer, was involved in charitable work in the Jewish community. After initial education at St Paul's School, Anthony Epstein joined Trinity College in Cambridge and finally obtained a degree in medicine from Middlesex Hospital in London. After serving two years (1945-47) for the Royal Army Medical Corps in India, following World War II, as per his own interest, he returned to Middlesex Hospital and joined as an Assistant Pathologist, as retrieved from one of his publications (Epstein, 2015). In 1982, Epstein received the award for Distinguished Achievement in Cancer Research. From 1968-1985, he served as Professor of Pathology at the University of Bristol, UK. In 1991, he was knighted by Queen Elizabeth II. After completion of his tenure, he remained as a fellow at the Wolfson College (Oxford, UK) till 2001.

Epstein:1921-2024

Switch from sarcoma to lymphoma

Since the early 1950s, Epstein was involved with the

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Rous sarcoma virus in his lab, but his interest was something else. In 1961, he got a lucky break in his scientific career. The event was like this: In 1957, Northern Ireland-born surgeon Dennis Burkitt, a British Colonial Service medical officer based in Uganda, was serving at a university teaching hospital in Kampala, the capital city of Uganda (Africa). While Burkitt was on home town leave on 22nd March 1961, he delivered a lecture in the Surgical Department of Middlesex Hospital in London; the subject was "The Commonest Children's Cancer in Tropical Africa: a hitherto unrecognized syndrome", an unusual form of cancer mostly prevalent in African population. Burkitt was not so much renowned. However, a case report on sarcoma involving the jaws of African children had already been published by him in a surgery journal (Burkitt, 1958). However, that received minimal attention as many oncologists were not reading surgery journals in those days. Thus, the kind of tumor what he described in his lecture was not designated as Burkitt's lymphoma in medical terminology (Burkitt, 1958). It was a chance of occurrence for Dr. Epstein to occupy a back seat in that lecture hall. Burkitt described a particular type of tumor growth on the jaw of children predominantly inhabiting certain geographical regions of Africa receiving heavy rainfall and high temperature. Burkitt already observed that children with facial tumor were also having tumor at abdominal site. Pathological confirmation directed the jaw lesions as sarcoma but unrelated to tumors in abdominal site. A strange but undeniable fact of climate dependence of tumor was providing some clue for vector borne biological agents as felon, but precise evidence to blame any specific biological agent was not traceable. African countries have more malaria infections with a high insect population; therefore, the possibility of malarial parasites and Anopheles mosquito naturally came to their mind. In 1962, Gilbert Dalldorf first suggested malaria parasite may induce tumor in association with climatic condition. Ultimately this hypothesis was reversed (Dalldorf, 1962). Burkitt was equally clueless, not sure whether genetic elements of African race or involvement of pathogen for tumor progression is contributing factor or not. Successive hypotheses of etiology were raised as well as erased. Epstein was inquisitive to explore the uniqueness of the site of tumor lesion and its geographical restriction to equatorial Africa. He decided to switch from Rous sarcoma and approached Burkitt to provide a clinical sample so that he could search for a virus in his lab at his own expense. Dialogue between Epstein and Burkitt moved in a positive direction. Subsequently, Epstein managed funding from the British Empire Cancer Campaign (later Cancer Research UK) to travel to Uganda for sample collection on a regular basis. From his earlier experience working with RSV, it was in his mind that if certain animal tumors are virus-induced, so why not for human tumor? Additionally, Epstein was in an advantageous position, having one year of training in electron microscopy in 1956 under Nobel Prize winner George Palade to detect and categorise viruses. Over the next three years, Epstein's lab made all efforts to grow the tumor cell in his lab following traditional approach of *in-vitro* culture technique. During the early 1960s, *in-vitro* culture of human lymphocytes was in the beginning stage. Much expertise in this area was unavailable to grow human lymphocytes in suspension culture. Despite countless efforts to adapt several modifications in their experimental protocols, they failed to achieve any success. Similarly, electron microscopy of biological sample was also in infancy. Therefore, Epstein lab consumed much time to get success in virus isolation and its confirmation. The delegates who attended international cancer control meeting held at Paris in January 1963, were initially reluctant to designate this tumor as lymphoma. However, after a gap of few months, it was settled; accordingly, its nomenclature became Burkitt's lymphoma (BL) (Burkitt,1983).

Delayed shipment brought achievement

In a regular manner, biopsy samples collected from Burkitt's lymphoma patient were instantly frozen, followed by airlifting from Uganda to London to minimize spoilage during transit. On arrival at Epstein lab, it was immediately thawed and cell suspension prepared in several ways was inoculated to newborn mice, embryonated eggs, and tissue culture system as available in their hand to isolate a virus. All these attempts were proved to be futile. Till early 1963 almost twenty such attempts were made by Epstein group but end results were uniformly negative to detect presence of any viral agent in the BL (Burkitt's lymphoma) samples (Rickinson, 2024). Bert Geoffrey Achong, having expertise in electron microscopy, was hired to support electron microscopy in Epstein Lab. Due to failure in standard biological isolation procedures, they decided to direct examination of tissue samples under electron microscope that too did not yield anything good (Epstein and Achong, 1979). From clinical signs of tumor, Epstein was partly sure that a virus must be the culprit but unable to get luxuriant growth of tumor cells to track the evidence. Once Yvonne Margaret

Barr a 31-year-old research assistant joined Epstein lab, her expertise in cell culture technique to grow cells under controlled condition, brought an ease to overcome trouble sustaining the growth of cells in their lab. All these three were equally contributed in this field.

It was 5th December, 1963, a fresh sample from Uganda was shipped to London bound BOAC flight, but due to fog, the flight was diverted to Manchester, so sample delivery was delayed. On arrival, the sample in transport medium was found to be unusually turbid suspected for bacterial contamination and to their mind sample was of no use. Yvonne Barr applied her own technique to grow the cells in a culture vessel, and every few days, the cells were washed and supplemented with a new cell culture medium, as she mentioned in one of her interviews (Winter, 2022). By good fortune, the sample was bacteria free, and the cloudiness was due to free-floating huge number of viable cells visible under microscope. To their surprise, the viable cells when suspended in fresh culture medium, the cells grew optimally within a weeks' time and that was the beginning of suspension culture from a lymphoma. Epstein and his research assistant Yvonne Barr equally contributed in this tedious journey and the cells derived from Burkitt's lymphoma (BL) was named as EB-1 cell line in accordance to the name of Epstein and Barr. Once enough EB-1 cells were grown, it was subjected to electron microscopy. They could able to recognize the virus like particle showing resemblance with herpesvirus, however, unaware of which herpes virus was involved. They quickly published their findings in 1964, and the virus was named EBV, that has paved the foundation for sweeping studies on human oncoviruses. Unlike other herpes virus, the EBV caused non-lytic infection and was considerably inert, which they published in Lancet (Epstein *et al*., 1964). As of now, it has received 3908 citations in research journals. Yvonne Barr was born on March 11th, 1932 at Carlow town, Ireland. In 1966, she received her doctorate degree from the University of London. Barr died on 13th February 2016 in Melbourne after developing multiple medical problems. Another major contributor in Epstein's lab was Dr. Bert Geoffrey Achong (1928–1996). He was born in Port of Spain, Trinidad; graduated in medicine in 1953 from University College Dublin (UCD). He was skilled in electron microscopy, and in late 1963, Achong joined Epstein team for electron microscopy (Winter, 2022). Subsequently, the transforming ability of EBV to transform normal lymphocytes to lymphoblastoid cell lines was observed. Thus, the first human oncogenic virus identified was EBV. Cells derived from tumor of jaw as well from ovarian tumor were found to have continuous dividing ability in artificial medium, revealing the character of transformed cell lines (Epstein and Barr, 1965; Epstein *et al*., 1965).

Yvonne Barr:1932-2016

Scientific proof under distance roof

Shortly after identification of the virus, Epstein decided that another laboratory should repeat his finding for confirmation. Therefore, the EBV was sent to the Henle laboratory in Philadelphia, USA, for further studies. As early as 1967, Werner Henle and Gertrude Henle, husband and wife, both virologists, established the oncogenic potential of EBV in their laboratory. Co-cultivation of irradiated BL cells (irradiation causes cell lysis and releases the virus) with healthy human leukocytes resulted in the transformation of healthy leukocytes to acquire immortality (unlimited *in-vitro* proliferating ability), indicating the oncogenic potential of EBV (Henle *et al*., 1967). Henle laboratory was serious about screening sera from their serum bank against the EB virus. In their lab, an interesting event happened. A lady technician of Henle's laboratory with confirmed seronegative status for EBV was regularly donating her own blood as a source of control lymphocytes for EBV-induced transformation test, and her lymphocyte never survived without the presence of virus indicative of virus-free lymphocyte property. In August 1967, she became ill and remained out of lab for 5 days, when she resumed, her lymphocytes grew as self-replicating immortal cells. Once tested, she was found to be positive for EBV specific antibody (Henle *et al*., 1985). Lymphocytes obtained from subhuman primates were equally susceptible to being transformed and became lymphoblastoid cell lines when co-cultivated with EBV in a different lab (Miller *et al*., 1972). Since 1966, Henle's lab had claimed to detect high level of antibody against EBV from BL and nasopharyngeal carcinoma patients as well as from healthy individuals

(Levy and Henle, 1966). In 1880, Nil Filatov, a Russian pediatrician, recognized infectious mononucleosis (IM) and named it idiopathic adenitis syndrome (Filatov and Earle, 1904), but undeniably, the etiology of IM remained hidden till the findings from Epstein lab disclosed the EBV the prime offender for IM. The EB cells were dispatched to Henle's laboratory at the Children's Hospital in Philadelphia to test those cells against human sera of infectious mononucleosis patients. As per their findings, all the patients showed high levels of anti-EBV in their acute stage sera, that had given an implication that EBV is etiologically related to infectious mononucleosis (Henle *et al*., 1968). Henle lab demonstrated that EBV was present in cells of many Americans who had no evidence of tumor. It is now clear enough that EBV can immortalize B lymphocytes but the intrinsic mechanism governing the oncogenic potential is yet to be disclosed (Spector, 2022). Detection of EBV and its confirmation in Burkitt's lymphoma cells remained tough since the beginning. However, small amounts of viral DNA had been revealed by hybridization with DNA from cells of the Raji line of the tumor (Zur Hausen and Schulte-Holthausen, 1970). Previously, it was speculated that the malaria parasite may be the real offender to induce lymphoma. Later, it was disapproved due to a lack of concrete evidence. Nevertheless, intensive survey conducted by Burkitt in lymphoma belt of Africa which happens to be endemic zone for malaria, gave an indication that EBV infection and malaria parasite *Plasmodium falciparum* were two known cofactors in the etiology of endemic Burkitt's lymphoma. As per published reports, numbers of EBV carrying B cells are five times higher during any acute malaria infection. EBV may cause cytogenetic abnormality in virus-invaded B cells, resulting in progressive Burkitt's lymphoma (Lam *et al*., 1991). Due to the geographical restriction of BL, an insect-vectored virus was suspected as an etiological agent. Eventually, this was disapproved once the transmissibility of EBV through saliva and not by insects was established (Klein, 2009). Nevertheless, due to chronic malaria, the immune system remains suppressed to fight against EBV, thereby, it favors survival of virus ending in tumor initiation. Information collected from various labs has now established that EBV has evolved to evade host immune system. EBV causes persistent infection without showing any symptoms. Young ones, who have not been exposed to EBV early in life, are prone to develop IM. Infectious mononucleosis is also called "kissing disease", as transmission of disease takes place due to direct contact through saliva during kissing and young children sharing toys. Following primary infection, EBV remains in latency in the peripheral B cell in circulation; periodic reactivation facilitates spreading of disease (Yu and Robertson, 2013). This virus has an association with Hodgkin lymphoma and certain B-cell lymphomas, and nasopharyngeal carcinoma and to a lesser degree for gastric cancers.

Conclusion

The uncontrolled growth of cells due to mutation in DNA is the most acceptable concept for cancer growth. It is arguably true that either environmental factors or some agent may enhance the mutation in DNA so that normal cells become malignant. Direct involvement of EBV to develop cancer has not been proved. However, it can enhance the mutation in healthy cells, and this possibility has not been ruled out (Spector, 2022). The Epstein-Barr virus is the most ubiquitous DNA virus. It infects more than 90% of the human population. Two major EBV types have been detected in humans, viz. EBV-1 and EBV-2. These differ in their gene sequence, also known as Epstein-Barr virus types A and B. As per recent taxonomy of viruses, EBV is grouped under family *Herpesviridae*, subfamily *Gamaherpesvirinae*, genus *Lymphocryptovirus* and species Human herpesvirus 4 (Gequelin *et al*., 2011). As of now, all efforts to develop a vaccine against EBV remain in the dark. Epstein was extremely humble and never claimed credit in his favor for the discovery of EBV. In one of the interviews, he told "Everyone is putting a brick in the wall," and "It is the accumulation of bricks which makes the building" (Murphy, 2024). The above narration should never be considered as a mini-review either on EBV or about the biography of Michael Anthony Epstein. We are precise to pay tribute to his demise, and we acknowledge his contribution that has enriched scientific knowledge.

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