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THE KISSING VIRUS

THE Epstein-Barr virus, also called "The Kissing Virus", is perhaps the commonest of all viruses that infect human beings. It usually causes no ill-effects whatsoever, but it does cause glandular fever or infectious mononucleosis, and there is substantial evidence linking it with two human cancers, not usually found in this part of the world. It was discovered through the co-operation of surgeons, physicians and pathologists and is involved in many branches of medicine. In recent years it has been implicated in a wide variety of clinical conditions.

This virus belongs to the Herpesvirus Group, and while each member of the group has absolutely similar appearance when examined by the electron microscope, each is distinguishable by the different disease or diseases which it causes and by the different antibodies which it produces. For instance, herpes simplex causes cold sores, varicella zoster causes chicken pox and shingles, and cytomegalovirus causes both congenital infection and infection in renal transplant patients.

Epstein-Barr (EB) virus causes glandular fever, an acute and self limited disease usually in young adults, which is associated with severe fatigue and which can last for up to six weeks. The main symptoms are skin rash, pharyngitis, tonsillitis, enlarged lymph glands and enlarged spleen, and the peak incidence of the illness is around twenty years of age. Convalescence can be prolonged, and relapses and complications are both rare. Diagnosis is made clinically and by examination of the blood, which initially shows a diminished number of white blood cells, followed by a striking increase in their number with a large proportion of mononuclear cells, some of which are atypical lymphocytes. These cells have a hyperbasophilic and vacuolated cytoplasm, and kidney shaped nuclei. A serological test, the Paul-Bunnell-Davidsohn or heterophil antibody test is usually positive, though false positive reactions do occur. Foolproof diagnosis can now be made by showing certain specific antibodies to EB virus, and these will be discussed later.

THE ASSOCIATED CANCERS

The two human cancers closely associated with EB virus are Burkitt's lymphoma and nasopharyngeal carcinoma; the virus was originally found in the former. Mr. Dennis Burkitt, an Ulsterman and a surgeon, working in Uganda, recognised a jaw tumour commonly occurring in youngsters, with a peak incidence around five to seven years of age, and in investigating this he undertook many landrover "Tumour Safaris" in Uganda and throughout Africa, and made many exciting observations. He first reported his pioneer work in the Middlesex Hospital Medical School in London in 1961. There is a restricted geographical distribution of these tumours with clusters of cases in the area of Africa where malaria is endemic. Sporadic cases occur in other parts of the world. The histopathology of this lymphoma has a "starry sky" pattern, due to the large pale macrophages with their abundant cytoplasm, and the much smaller, closely packed tumour cells, which are B lymphocytes.

Nasopharyngeal carcinoma occurs primarily in the posterior and lateral walls of the nasopharynx.

With this cancer there is also a restricted geographical distribution, with a very high incidence in some populations of Chinese descent. In the very high risk area around Singapore there is a particularly high incidence in the Cantonese Chinese; this also appears in these Cantonese people who have emigrated to San Francisco and Hawaii. On the other hand, there is a lower incidence in a number of countries around the Mediterranean and in East Africa. The peak age of the cancer is between 45 and 55 years, with males being affected about three times more than females, and in Tunisia there is an additional age peak of frequency at between ten and twenty years.

THE VIRUS AND ITS DISCOVERY

Dr. Tony Epstein, a pathologist, at the Middlesex Hospital, became interested in Burkitt's lymphoma, and in 1964 he and Yvonne Barr, a Dublin Graduate, cultured cells from tumour material flown from Uganda. Shortly after with Dr. Achong, another Dublin graduate, they first discovered the virus itself by electron microscopic examination of the cultured cells. This explains how the virus got its name—the Epstein-Barr virus.

The virus is divided into three main parts, a central core, a capsid and an envelope. The central core, consists of viral DNA. The capsid, which is icosohedral in shape, consists of hollow tubular protein sub-units called capsomeres, which are the "building blocks" which give the virus its shape. The virus acquires its outer protective coat, or "envelope" from part of the cell in which it grows. All three parts can be seen microscopically.

While all the other herpesviruses have similar appearance and grow easily in tissue culture cells used conventionally, EB virus behaves very differently in the laboratory and is more difficult to propagate.

METHODS OF STUDYING THE VIRUS

Three main methods are used in the study of this virus. *First*, cells from African Burkitt's lymphoma, the B lymphocytes which carry EB virus, can be grown and cell cultures derived from these have been used in laboratories all over the world as standard sources of virus. *Secondly*, certain white blood cells, in our circulation, the B lymphocytes, whose main function is the production of antibody, can be infected with EB virus. For example, if we take throat washings from a patient with glandular fever which contain a lot of virus at the acute stage of illness and mix these washings up with B lymphocytes, the virus will stick to these cells which possess the appropriate receptors. The protein coat of the virus will be shed and the viral DNA will be incorporated into the nucleic acid of the B lymphocytes, which will become "transformed". When this event takes place the cells will proliferate indefinitely provided they are suitably fed, and we say they are "immortalized". At this stage each cell contains in its nucleus EB virus nuclear antigen, known as EBNA. Thus, we use our second method of artifically infecting B lymphocytes either to find out if an individual is excreting virus, or to measure the amount of virus we are using in our laboratory tests. In the *third* method of studying the virus, we estimate the amount of antibody, and in infections with this particular virus we get maximal information about the illness if we look for and measure antibody to the different viral antigens. We do this by means of the immunofluorescent technique, using as antigen in our tests suitable preparations of standard Burkitt lymphoma cultured cells showing each particular antigen. Approximately 10 per cent of the cells of the producer cell line, P_3HRI, show capsid antigen, while 100 per cent of the nuclei of the non-producer line, Raji, contain EBNA. Early antigen is obtained by superinfecting Raji cells with enveloped virus prepared from cultured P_3HRI cells, and the two types, restricted and diffuse, are obtained by using different fixatives.

We use our antibody tests to determine whether or not individuals have previously been infected with the virus. The easiest way is to test sera for the presence or absence of antibody to the viral capsid. While EB virus is extremely common, serological surveys have shown that primary infection takes place at different ages in different communities. Most infants in all communities inherit maternal antibody, which declines rapidly after birth. In East Africa, where Burkitt's lymphoma is found, by two years of age almost 100 per cent of infants have produced their own antibody to EB virus as proof of early primary infection. By contrast, in the United States of America 80 per cent of individuals of a high socio-economic level have developed antibody only by seventeen years of age. In both these immune groups, immunity has been acquired by a "silent" or asymptomatic infection. The 20 per cent who are susceptible in the second group are likely to become infected later, and about half of those infected experience the clinical illness, glandular fever.

SPECTRA OF ANTIBODIES IN DIFFERENT SITUATIONS

The susceptible individual does not have antibody to any of the different antigens, while the immune individual has antibody in immunoglobulin class G to viral capsid and also antibody to EBNA. At an early

stage of primary infection in childhood, such as occurs in Uganda, antibody to virus capsid is present in both IgG and IgM classes, and there is antibody to one component of the early antigen complex, the restricted component. In glandular fever, again there is antibody to virus capsid in both IgG and IgM classes, but the antibody to early antigen is directed against the diffuse component. Antibody to EBNA develops slowly, while heterophil antibody is present at an early stage. Very distinctive and different spectra of antibodies exist in both Burkitt's lymphoma and nasopharyngeal carcinoma. In both conditions antibodies to capsid antigen in IgG class and EBNA are of high titre, but heterophil antibody is absent; in Burkitt's lymphoma there is antibody to the restricted component of the early antigen, while in nasopharyngeal carcinoma the antibody is to the diffuse component, and antibody to this antigen and to the capsid antigen are found also in IgA class. Heterophil antibody is not present in either of these conditions.

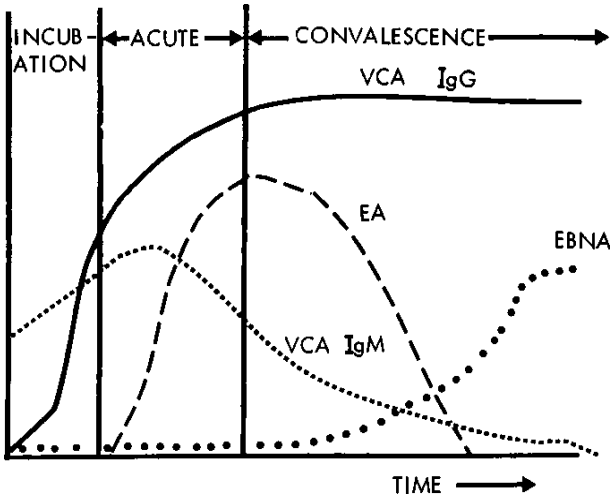

Figure EB virus antibody response during the course of glandular fever. VCA = *Virus capsid antigen*, EA = *Early antigen*, EBNA = *Epstein-Barr nuclear antigen.*

The figure shows the sequence of the appearance of antibodies to the different antigens of EB virus in glandular fever, and it is obvious that the transitory antibody to early antigen and the transitory antibody to capsid antigen in IgM class are indicative of active early infection. From the above observations, it is evident how important detection and measurement of the different antibodies can be in diagnosis.

PROOF THAT EPSTEIN-BARR VIRUS CAUSES GLANDULAR FEVER

Drs. Gertrude and Werner Henle in Philadelphia showed conclusively that EB virus causes glandular fever and they did so when they were testing very many sera in an attempt to unravel the mystery of the virus found in Burkitt's lymphoma. By a quite extraordinary coincidence a junior laboratory technician developed glandular fever and they found that her serum, frozen away in the laboratory some time before her illness, had no antibodies, while these appeared in her serum during and after her illness. Confirmation in a large number of patients was possible because stored samples of serum from Yale University students were available as part of a prospective clinical and laboratory study of glandular fever. Also, it was shown that virus was freely shed from the throat during illness.

Regarding transmission of the virus, interesting studies have shown that a large and direct inoculum of virus is needed to transmit the disease—indeed, intimate oral contact labelled the virus "The Kissing Virus". The illness is characteristically associated with kissing in young adults and affects the more susceptible upper socioeconomic classes. Indeed, a peak incidence in late winter, four-six weeks after Christmas, with probable increased intimate exposure to virus-containing saliva at that time has been reported.

This is well illustrated from an article by Dr. Robert J. Hoagland in the American Journal of Medical Sciences, 1955. I quote: "One of my patients with infectious mononucleosis stated that on December 23, 1950, he was in a train and spent about twelve hours in the company of a female medical student whom he had never seen before and whom he never saw again, but with whom he corresponded. These two individuals kissed frequently and, more important, in a way to allow mingling of saliva . . . The patient learned, by letter, from his train acquaintance, that she was a patient in a university hospital with a disease diagnosed as infectious mononucleosis". This story speaks for itself.

RELATIONSHIP OF EPSTEIN-BARR VIRUS TO BURKITT'S LYMPHOMA AND NASOPHARYNGEAL CARCINOMA

Extensive and difficult investigations have not proved conclusively that Epstein- Barr virus is the sole cause of Burkitt's lymphoma and nasopharyngeal carcinoma.

First I shall discuss findings in relation to the 98 per cent of cases of Burkitt's lymphoma which contain virus and which occur in the high incidence area in Africa. The proof of the virus association in endemic Burkitt's lymphoma is —*firstly*, that there is a very "special" spectrum of antibodies of a very high titre, and the antibody to the early antigen complex points to active ongoing viral activity. *Secondly*, after surgery or chemotherapy, a drop in antibody indicates a

favourable prognosis, whereas a rise in antibody points to tumour recurrences. *Thirdly*, cells from the tumour can be grown in culture outside the body and are what we call monoclonal: that is, growth occurs from the multiplication of a single cell. *Fourthly*, the virus from the tumour itself has the ability to "transform" B lymphocytes and the molecular biologists have been able to show us that Epstein-Barr virus DNA is in each tumour cell, as well as EBNA. When the tumour cells are injected into New World monkeys they cause tumours.

A rather similar set of findings occurs in nasopharyngeal carcinoma. There is a distinctive pattern of antibodies of high titre—with specific antibody in immunoglobulin class A—probably locally stimulated in the pharynx. Further, a rise in antibody has been shown to be associated with increased tumour size and with secondaries—a fall occurs with successful treatment. Viral DNA and EBNA have been shown in the epithelial carcinomatous cells in all classical cases of nasopharyngeal carcinoma, and while these cells have not been cultured outside the body, it has been possible to transform B lymphocytes with virus particles from the tumours.

In summary, very high levels of antibody to EB virus occur in both Burkitt's lymphoma and nasopharyngeal carcinoma, multiple copies of viral DNA occur in both tumours and in the case of Burkitt's lymphoma whose cells can be cultured, the tumour is monoclonal.

Distinct differences exist between the cultured cells of Burkitt's lymphoma and cultured B lymphocytes from healthy individuals previously infected with EB virus, whose cells will grow due to reactivation of latent virus. In Burkitt's lymphoma the tumour cells are monoclonal, while cultured cells from a healthy person are polyclonal, several clones of cells having multiplied. The lymphoma cells are aneuploid with the chromosomal marker $14q^{+}$, while the healthy cells are diploid and do not possess a special chromosomal marker. The lymphoma cells have the ability to induce tumours when injected into certain monkeys, while the healthy cells will not. There are different surface glycoproteins on the two cell types.

While Burkitt's lymphoma is a monoclonal tumour with Epstein-Barr virus in every cell indicating that the original B lymphocyte undergoing malignant transformation must have been infected at the outset, nasopharyngeal carcinoma is the proliferation of a clone of carcinomatous epithelial cells, containing EB virus. In both conditions the serological profile is consistent with patients carrying a heavy load of the virus.

But it is difficult to reconcile the ubiquitous presence of the virus with the rarity of these tumours. A prospective study of sera from 42,000 children in Uganda showed that normal patterns of antibody to EB virus were present long before the development of the tumour; in other words the children had their primary viral infection before the tumour began to grow. It has been argued that malaria is the crucial cofactor which triggers off the malignancy, and a clear parallel has been shown between a high incidence of Burkitt's lymphoma, a high level of malarial infection and, to a lesser degree, high temperature and high rainfall. Evidence points to the EB virus infecting infants in the neo-natal period when the lymphocytes and indeed the entire immune system is immature, while heavy malarial burden and possibly other environmental and host factors are also involved in the pathogenesis of Burkitt's lymphoma. The fact that malaria infection has recently been shown to stimulate the growth and proliferation of B lymphocytes, and also to suppress T lymphocytes, the regulatory cells which control the immune responses of the body, supports the view that environmental factors are closely implicated.

In areas where nasopharyngeal carcinoma occurs it has been shown that primary EB virus infection also occurs very early in life, and again the long interval between virus infection and tumour development suggest participation of co-factors. In South East Asia persons affected are usually over 40 years. Multiple cases, with a preponderance in males, occur in Chinese families through several generations, and a certain histocompatibility antigen has been found in high frequency in these Asian cases. Indeed this antigen appears to be a marker for a very high risk of developing nasopharyngeal carcinoma. This would point to the importance of genetic factors in the pathogenesis of this cancer, though there may also be environmental cases. For instance, consumption by Cantonese Chinese of salted fish, known to contain a carcinogenic compound, may precipitate the cancer.

Thus, there is strong evidence that EB virus is a causative factor in these two human cancers. Professor Epstein believes that the perfecting of a preventative vaccine, and its subsequent use, will give the final answer as to whether or not EB virus is a cause in both of the conditions. Regarding Burkitt's lymphoma—many pin their faith in the successful eradication of malaria, to eliminate the tumour.

What are we to say about *anti-viral drugs* in this respect? Undoubtedly the new anti-viral agent Acyclovir or acycloguanosine, has attracted much attention in relation to the herpesvirus group. Clinicians have been most enthusiastic in its preliminary

use, for instance in shingles in immunosuppressed patients, in whom it is being carefully evaluated. However, it has been shown that EB virus is only very slightly sensitive to this drug, so it looks as if we will have to wait for another antiviral drug to turn up to cope with this virus.

IMMUNOLOGICAL ASPECTS OF EPSTEIN-BARR VIRUS INFECTION

Immunologists have learnt a lot about how the immune system works by examining both antibodies to EB virus, and also the reactions of the cells of the immune system, both in patients with infectious mononucleosis, and also in patients with a wide variety of disorders of the immune system.

In a healthy immune person, neutralising antibodies will block any further infecting virus from infecting a B lymphocyte. If a virus does pass this block and infect such a cell, suppressor T lymphocytes will prevent the B lymphocyte from transforming. If any B lymphocyte does transform, it will be prevented from proliferating by further suppression by T lymphocytes.

However, if a susceptible adult does become infected, these first three mechanisms may be insufficient to stop proliferation of the B cells, now EBNA—-positive from growing; therefore the more powerful killer T cells come into play. This is what happens in infectious mononucleosis. Indeed the majority of the atypical lymphocytes in the blood of patients with infectious mononucleosis are T cells of different types—some are probably cytotoxic, some are suppressor, and others are immature T cells. George Klein describes infectious mononucleosis as a "Civil War" between a small number of virus-transformed cells, and a large number of T cells. Most primary infections are not manifested as infectious mononucleosis —in children and in subclinical infection, infection is controlled without the large scale T cell response, seen in infectious mononucleosis.

The patient recovers after infection and the virus establishes itself in a persistent carrier state. When the lymphocytes are cultured the virus can be recovered indefinitely, and in addition the virus is shed intermittently into the saliva. However, in immunosuppressed patients, such as patients following renal transplant, virus is shed much more freely. One immunosuppressive agent, Cyclosporin A, has been shown to inhibit the controlling effect of the T cells, and thus causes B cell lymphomas containing EB virus. Such lymphomas and also widespread growth of virus have been found in rare instances where there is a loss of immunoregulatory control, or a cytogenetic defect in B cells.

Raised antibody titres to EB virus have been found in disease like sarcoidosis and systemic lupus erythematosus, which have in themselves an immunosuppressive effect, and it is well documented that those Hodgkin's disease patients, who have very high levels of antibody to EB virus, have depressed cell mediated immunity, or lack of control of antibody production due to failure of suppressor T cells. Recent findings in this and in other situations have prompted the suggestion that "patterns" of antibody to EB virus might be a more sensitive measure of defective cell mediated immunity than more conventional tests of immune function.

SUMMARY

I have given a panoramic view of the EB virus and the diseases associated with it. On the one hand the virus has been shown to cause the infectious disease, infectious mononucleosis—on the other hand it has been shown to be closely associated with two human cancers of two distinct cell types, B lymphocytes in Burkitt's lymphoma, and epithelial cells in nasopharyngeal carcinoma. This virus persists harmlessly in the vast majority of individuals. However, it is now recognised that undesired effects of the virus are connected with alterations of the immune system; some of these have a genetic origin, and some result from immunosuppressive therapy now frequently employed. Therefore I believe firmly that this virus has to be reckoned with, and indeed has to be considered as a candidate virus which may be involved in pathogenesis in a wide variety of diseases of unknown aetiology.

The remarkable story of this virus will continue, and we can understand Roizman's comment: "Not since the heyday of poliomyelitis research has a single agent attracted so much interest as Epstein-Barr virus".