
Historical background

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The persisting ancient view of cancer as a contagious disease ended with 19th century scientific investigations which seemed to show it was not. The resulting dogma against an infectious cause for cancer produced great prejudice in the scientific community against the first report of an oncogenic virus by Rous early in the 20th century and, even in the 1950s, against Gross's finding of a murine leukaemia virus and a murine virus causing solid tumours. The Lucké frog renal carcinoma virus was the first cancer-associated herpesvirus. Intriguingly, an environmental factor, ambient temperature, determines virus genome expression in the poikilothermic frog cells. Although an α -herpesvirus, Marek's disease virus of chickens shares some aspects of biological behaviour with Epstein–Barr virus (EBV) of man. Very significantly, its lymphomas are the first naturally occurring malignancy to be controlled by an antiviral vaccine, with implications for human virus-associated cancers. The circumstances and climate of opinion in which successive γ -herpesviruses were discovered are described. The identification of EBV involved two unconventionalities: its finding in cultured Burkitt's lymphoma cells when no human lymphoid cell had ever been maintained *in vitro*, and its recognition in the absence of biological activity by the then new technique of electron microscopy. These factors engendered hostility to its acceptance as a new human tumour-associated virus. The EBV-like agents of Old World apes and monkeys and the T-lymphotropic γ -herpesviruses of New World monkeys were found at about the same time, not long after the discovery of EBV. For many years these were thought to be the only γ -herpesviruses of non-human primates; however, very recently B-lymphotropic EBV-like agents have been identified in New World species as well. Mouse herpesvirus 68 came to light by chance during a search for arboviruses and has become important as a laboratory model because of its close genetic relatedness to EBV and its comparable biological behaviour. The discovery of Kaposi's sarcoma-associated herpesvirus six years ago was made using unconventional new methods, but, unlike with EBV 30 years before, this did not hinder its acceptance. This contrast is discussed in the context of the great progress in human tumour virology which has been made in recent years.

Keywords: γ -herpesviruses; tumour virus history; human cancer viruses

1. INTRODUCTION

Discussion of the mechanisms whereby viruses of several families play a role in the causation of various human cancers is currently a legitimate and important topic. Indeed, recent progress in human tumour virology has ensured that this field occupies a place high on the biomedical agenda; we are even witnessing at the present time preliminary success in the control of a human cancer, hepatocellular carcinoma, by means of a vaccine programme against the causative agent, hepatitis B virus (Chang *et al.* 1997). Yet only three or four decades ago the idea of cancer viruses of man would have been considered eccentric or even cranky.

This change of perception has come about slowly and painfully, driven by discoveries from many areas of virology and molecular biology, but it is the family of γ -herpesviruses which has provided the latest and most unexpected new evidence for viral carcinogenesis. All members of the family are spread horizontally and infect their hosts at an early age, usually without disease manifestations, but thereafter they set up lifelong latent infections and possess the power to drive *in vivo* proliferation of the latently infected cells, incurring thereby an attendant

risk of oncogenic change. For a long time Epstein–Barr virus (EBV) was the sole known representative of the family; it was subsequently joined by related B-lymphotropic herpesviruses of Old World monkeys and by the T-lymphotropic herpesviruses of New World monkeys, which proved to be tumour-inducing experimentally in alien hosts. Later it was realized that a murine herpesvirus genetically close to EBV can also cause tumours and belongs in the group, whilst two rather new findings have added the human herpesvirus responsible for Kaposi's sarcoma and EBV-related B-lymphotropic herpesviruses of New World monkeys to the list.

The historical background in this paper provides a survey of the vicissitudes which have beset tumour virology in the past, and of the circumstances and climate of scientific opinion in which each of the oncogenic γ -herpesviruses discussed in this issue came to light.

2. ORIGINS OF THE IDEA THAT VIRUSES CAUSE CANCER

Belief in the contagious nature of cancers arose in classical times and persisted for many centuries, sustained by anecdotes of supposed cancer houses in which many

inhabitants became suffers, by the alleged occurrence of *cancer à deux* affecting married couples, and by apparent instances of cancer passing from mother to child or from patient to attendant. But when scientific methods were applied to cancer as the 19th century progressed, extensive study failed to demonstrate that contagion actually played a part in cancer causation.

During the last quarter of the 19th century, after the dramatic findings of Pasteur and Koch had revealed the microbial aetiology of many diseases, the idea of an infectious origin for cancer was revived on a plausible modernized basis. As a result, a great deal of work appeared in the years before the start of the last century claiming to incriminate as the cause of diverse cancers various kinds of bacteria, yeasts, fungi, protozoa, spirochaetes and coccidia, but rigorous investigations of these suspects did not reveal a carcinogenic role for any of them. Such consistently negative results rapidly led to the wide acceptance of the dogma that cancer did not, and could not, have an infectious agent as its cause. However, though little noticed at the time, Borrel (1903), following the reasoning adopted by Pasteur in the case of rabies, concluded that the inability to detect a micro-organismal cause of cancer did not mean that the 'parasitic theory' was untenable, but rather that the causal parasite was being sought amongst the wrong type of organism. If a micro-organism could not be found, then the cause should be looked for amongst the submicroscopic, namely the viruses; but only a few independently minded workers proceeded to do just that.

In fact, the very first demonstration of a virus capable of causing a malignant tumour came about in this way but was unrecognized for what it was because current knowledge was insufficiently advanced. Thus, when Ellermann & Bang (1908) reported that a cell-free filtrate of chicken leukaemia cells passed on the disease on inoculation into healthy chickens, little interest was aroused since the nature of leukaemia was not understood, the condition only being generally accepted as cancer of bone marrow cells some 40 years later.

3. DISBELIEF IN THE FIRST DISCOVERIES OF TUMOUR VIRUSES

In 1910 Peyton Rous, at the Rockefeller Institute in New York (now the Rockefeller University), succeeded in transplanting a spontaneous spindle cell sarcoma from a Plymouth Rock chicken into further chickens in series (Rous 1910). Extensive morphological studies, histological investigations, and close observation of the behaviour of the sarcoma established that this was a neoplasm exactly similar to the malignant tumours of mammals (Rous 1910), but following the reasoning propounded by Borrel (1903), Rous (1911) was able to go on and show conclusively that transmission could also be achieved using cell-free filtered tumour extracts.

Cell-free transmission was the only property which distinguished the Rous fowl sarcoma from the generality of cancers and because of this most authorities regarded it merely as some kind of infective granuloma, or argued that the tumour-inducing filtrates were not cell free. It was even suggested that cell fragments or diminutive submicroscopic cells passed through the filters and could

grow into daughter cells capable of forming new tumours (Nakahara 1926; Fujinami 1930; Lockhart-Mummery 1932). The climate of hostile prejudice was exemplified by Ewing (1931) when he bracketed the Rous sarcoma with rabbit myxomatosis and wrote, 20 years after Rous reported, 'the nature of the diseases and the relation to specific micro-organisms is *sub judice*'. Indeed, so disheartened was Rous by the rejection of his fully confirmed findings that after a few years he ceased working on the sarcoma and, had he not lived to the age of 86, he would not have received the Nobel prize, which was only awarded to him in 1966—exactly 55 years after the publication of his first paper on the classic oncogenic virus which bears his name.

Dogma rejecting the possibility of viruses playing a part in cancer causation persisted into the 1940s and early 1950s and affected important contemporary discoveries. Thus, when an extrachromosomal, maternal milk-borne influence was shown to increase the development of breast cancer in mice, Bittner (1936, 1940, 1942) felt constrained to designate it as the 'milk factor' or 'milk agent', and it was only later that its true nature was actually accepted as mouse mammary tumour virus.

Perhaps because of Bittner's positive findings a great many attempts were made over the next decade to transmit numerous types of murine cancers by means of cell-free tumour-derived preparations, but none succeeded. Ideas on carcinogenic viruses remained therefore pretty much as before; if it was now conceded that chicken tumours might be induced by such agents, this was deemed to be a special category without implications for cancers of other animals, especially mammals. The pioneering discoveries by Gross of the first mouse leukaemia virus (Gross 1951) and a murine virus causing various solid tumours, which he called parotid tumour virus (Gross 1953), were treated with disbelief and even derision. For many experts Gross was a charlatan who must have falsified his results since so many leading workers had found nothing in similar experiments—the crucial difference, namely the use by Gross of newborn mice to test tumorigenicity, being overlooked. Gross remained marginalized and slighted for several years and this only changed when many others began to publish on mouse leukaemia viruses—Graffi (1957), Friend (1957), Moloney (1960) to name only the first of these. His work on parotid tumour virus was also confirmed in 1957 by Stewart, Eddy and their co-workers (Stewart *et al.* 1957) who showed their disdain for Gross by omitting to refer to his paper and subsequently renaming the agent 'SE' polyoma virus (Stewart *et al.* 1958). But at least Gross did not have to wait for acceptance as long as Peyton Rous.

4. THE FIRST HERPESVIRUS ASSOCIATED WITH CANCER

Lucké (1934) was the first to report that leopard frogs (*Rana pipiens*) caught in the north-east United States and neighbouring Canada had a remarkable incidence of renal adenocarcinomas, which in some areas reached as high as 9%. The presence of eosinophilic intranuclear inclusions in both primary and metastatic tumour cells prompted Lucké to suggest (1934, 1938) that this cancer might be caused by a herpesvirus. Confirmation of the

presence of the latter was only obtained when electron microscopy showed that the inclusion bodies were often filled with typical herpesvirus particles (Fawcett 1956), but it took some time before the virus was finally incriminated in the aetiology of the disease. A number of other frog viruses were found and had to be shown not to be involved, and various difficulties had to be overcome before it could be demonstrated that filtered tumour extracts and purified Lucké herpesvirus preparations caused adenocarcinomas on injection into frog kidneys and tadpoles (Tweedell 1967; Mizell *et al.* 1969; Naegele *et al.* 1974).

An interesting and unusual feature of the Lucké herpesvirus is the temperature dependence of its activities in the host carcinoma cells and the way resulting differences in viral biological behaviour change the pathological behaviour of the tumours. Thus, during warm summer weather there is a non-productive infection of the poikilothermic tumour cells and the virus appears to drive the malignant transformation; summer tumours are free of virus particles, grow rapidly, metastasize and often lead to the death of the host. In those frogs which survive into autumn and winter, the cold ambient temperature activates virus replication leading to cell death and consequent regression of tumour growth (for a review, see Rafferty 1964). With the return of warm summer weather, virus replication is switched off again and the viral genome promotes the resumption of rapid tumour growth (Rafferty 1964; McKinnell & Ellis 1972). These temperature-dependent activities can be changed at will in either direction merely by experimental manipulation of the temperature at which tumour-bearing frogs are kept in the laboratory (Zambernard & Vatter 1966).

5. THE IMPLICATIONS OF MAREK'S DISEASE HERPESVIRUS AND ITS VACCINE

Marek's disease of chickens has been recognized for nearly a century (Marek 1907). It consists of a generalized lymphomatosis with nerve infiltration causing paralysis, and in more acute cases polyclonal malignant lymphomas are the most prominent feature. It is very common, highly contagious, and until the advent of vaccines had a steady mortality in commercial flocks of 20 to 30%, with occasional explosive outbreaks which killed up to 70% of the birds (Biggs 1970). Yet despite the obvious high infectivity of Marek's disease it took many decades before the causative agent was isolated (Churchill & Biggs 1967) and identified as a herpesvirus (Churchill & Biggs 1967; Epstein *et al.* 1968; Nazarian *et al.* 1968).

Although Marek's disease herpesvirus (MDHV) is an α -herpesvirus (Biggs 2001), it is important in the context of the γ -herpesviruses because: it is lymphotropic and oncogenic; its epidemiology and biological behaviour share some striking similarities with those of EBV; and the implications of its control by a vaccine. Thus like EBV (Crawford, this issue; Bornkamm & Hammerschmidt, this issue; Moss *et al.*, this issue), MDHV is ubiquitous and almost all adult chickens are infected (Chubb & Churchill 1968), lifelong infection being acquired in early life after maternally derived antibodies have disappeared; and to add to the general parallelism, most infections are subclinical. In contrast to EBV, MDHV infects T lympho-

cytes not B cells, but both viral genomes are latent in their respective host lymphocytes. Production of MDHV *in vivo* takes place in the squamous epithelial cells of feather follicles and infection is spread horizontally by virions in shed follicular squames—in a manner comparable to the horizontal spreading of EBV in salivary droplets containing infectious particles produced in the oropharynx.

As for the cofactors which influence MDHV to cause infected T lymphocytes to give rise to lymphomatosis and lymphomas, some—especially stress—have long been recognized (Biggs 1970); although of course different from those like hyperendemic malaria or immunosuppression, which ultimately lead EBV to oncogenic activity (Crawford, this issue; Bornkamm & Hammerschmidt, this issue), they are just as crucial for tumour induction. Again as with EBV, the MDHV genome is latent in every tumour cell but when the cells are put into tissue culture a productive infection is turned on in a proportion of the cells, MDHV antigens are expressed, and infectious virus can be detected (Kato & Akiyama 1975). This change of virus genome function resulting from a change in environment can be considered analogous to the temperature-dependent switch seen with the Lucké herpesvirus.

Over and above the great importance of MDHV for the poultry industry worldwide, Marek's disease is of notable significance because it is the first naturally occurring malignant tumour to have been controlled by an antiviral vaccine. The original vaccine against MDHV made use of live, attenuated MDHV preparations (Churchill *et al.* 1969) and this was quickly followed by a live vaccine containing a herpesvirus of turkeys (Okazaki *et al.* 1970), which is antigenically closely related to MDHV but apathogenic for chickens. Commercial vaccines arising from this work have been of huge economic benefit for poultry production and the success of the MDHV vaccine project, together with some of the principles underlying it, provided much of the rationale for a programme to develop a vaccine against EBV (see § 6(a)).

6. HISTORICAL NOTES ON THE γ -HERPESVIRUSES

(a) Epstein–Barr virus

The events, ideas and investigations which led to the discovery of EBV (Epstein *et al.* 1964a) have recently been described in a definitive and comprehensive review (Epstein 1999). Repetition is therefore unnecessary, but since the virus was found by methods deemed unconventional and controversial at the time, it might be instructive to consider the climate of opinion which prevailed when the virus was first reported and in the years immediately afterwards.

It is well known that the virus was sought from the outset in biopsy samples from East African Burkitt's lymphomas, and that all the usual methods then available for virus detection and isolation gave uniformly negative results when applied to cells from a large number of tumours. The crucial next step was to try and grow lymphoma cells *in vitro* away from host defences (Epstein *et al.* 1964b) and then see what could be found in the cultures by then current isolation methods and, if need be, by electron microscopy. However, there were two

main difficulties: (i) culture of any kind of human lymphoid cell had never been achieved; and (ii) biological electron microscopy was in its infancy, confined to very few laboratories and generally little understood.

Thus, when cultures of Burkitt's lymphoma cells were established, the results and details of the unusual methods (Epstein & Barr 1965), i.e. suspension culture now used routinely everywhere, encountered serious problems when submitted for publication from reviewers who were unwilling to believe that human lymphoid cells could be grown in culture at all. Furthermore, when the then conventional techniques for virus detection failed to show anything in the cultures of lymphoma cells, yet electron microscopy—used daily in our laboratory—clearly revealed the presence of a morphologically typical herpesvirus, even greater scepticism was engendered. Dogma required that the presence of viruses must be demonstrated by their effects on test animals, embryonated eggs or tissue cultures, or by finding of the specific antibodies they induced. It was not credited that they could be distinguished by their morphology, even though this had been acceptable with bacteria using the light microscope for nearly 100 years. Indeed, for many at the time the images obtained of any biological material with the electron microscope were considered as artefacts of fixation and processing; as for hitherto unknown viruses without apparent biological activity, that they might be 'virus-like particles' was the best which was said—whatever that was thought to mean.

The successful finding of EBV after culturing Burkitt's lymphoma cells *in vitro* resulted from the changed environment activating the genome from its latent state in the tumour cells *in vivo* into a productive replicative cycle. Many of the pathways involved are now coming to be understood (Bornkamm & Hammerschmidt, this issue), but even before this new knowledge there were clear resonances with the effects of environment on the genomic function of the Lucké herpesvirus and MDHV.

However, in the years after EBV was first reported, such considerations were of little interest. Even after it was reluctantly accepted as a virus it was usually discounted as a banal contaminant despite having been shown to be biologically, immunologically and biochemically unique. As for EBV's relevance as a human tumour virus, that recognition took very much longer and only began very slowly once a great body of information had built up on it and its behaviour (for reviews, see Epstein & Achong 1979) and after the outstanding seven-year prospective study on 42 000 children in the West Nile district of Uganda carried out by World Health Organization's International Agency for Research on Cancer (de Thé *et al.* 1978).

Two aspects of EBV which are still with us today arose as a direct result of some of the earliest work. Thus, when infectious virus was first found in the buccal fluid of infected persons (Golden *et al.* 1973; Chang *et al.* 1973) a clear indication was provided of a productive infection in the oropharynx, which was in marked contrast to the latency of EBV in Burkitt's tumour cells *in vivo* along with the seemingly similar latency of the virus in circulating lymphocytes. The whereabouts of the permissive cells producing the infectious virions soon became a long continuing controversy (Allday & Crawford 1988; Niedobitek & Young 1994) which has only now seemingly

been resolved (Crawford, this issue). Reports of replicating EBV in shed oropharyngeal epithelial cells were not confirmed and it is currently thought that it is intra-epithelial permissive lymphocytes which produce the virus in saliva. An epithelial cell source would nevertheless be an attractive idea because of analogies with the well-documented production of MDHV in feather follicle epithelium. However, even if EBV is not now thought to replicate in the squamous epithelial cells of the mouth and pharynx, it should not be forgotten that such cells are perfectly able to provide a milieu for this. The squamous cells of nasopharyngeal carcinoma carry a latent EBV infection which can be activated into a virus replicative cycle *in vitro* (Trumper *et al.* 1976) with the release of infectious EBV (Trumper *et al.* 1977) and EBV particles are regularly produced in the squamous epithelial cells of oral hairy leucoplakia (Greenspan *et al.* 1985).

The question of an EBV vaccine also has old roots. The example afforded by successful vaccination against MDHV has already been mentioned. It is also worth recalling that in the course of laboratory studies into the immunological mechanisms of protection by the vaccines it was demonstrated early on that plasma membranes from cells infected by MDHV markedly reduced the incidence of Marek's lymphomas when used as an experimental vaccine (Kaaden & Dietzschold 1974) and that even soluble antigens from such cell membranes protected in the same way (Lesnick & Ross 1975). It was these findings, in conjunction with the fact that the virus-determined membrane antigen (MA) on cells infected with EBV elicits powerful EBV-neutralizing antibodies (Pearson *et al.* 1970; De Schryver *et al.* 1974), that led to the suggestion that a vaccine against EBV should be developed (Epstein 1976), and formed the rationale for an extended programme of work on an MA-based anti-EBV subunit vaccine (Epstein 1986, 1994). An indication of the time taken for such projects to go forward is given by the successful completion only during last year of a phase I human trial of a vaccine of this type (Gilbert 1999).

Another event in EBV research whose history merits recording relates to the recognition of the causal relationship between the virus and infectious mononucleosis (IM). Not only did this come about entirely by chance, but the circumstances provide a perfect example of Pasteur's classic 1854 affirmation that '*dans les champs de l'observation le hasard ne favorise que les esprits préparés*' ('Where observation is concerned, chance favours only the prepared mind'). Shortly after the discovery of EBV the virus was sent for study to a number of laboratories, including, for reasons already explained (Epstein 1999), that of Werner and Brigitte Henle in Philadelphia. There, sera from many different sources were being screened for antibodies to EBV in a search for disease associations and a young female technician was using samples of her own serum as a negative control in the tests. At this stage she developed IM and, when she returned to work a few weeks later, her serum was found no longer to be negative but to have developed antibodies to EBV. This hint was rapidly followed up by the Henles who drew on the resources of a serum bank collected over many years from students with IM at Yale University. It did not take long to work out that all were sero-negative to EBV before their attacks of IM and developed specific antibodies to

EBV during the disease, indicating causation by the virus (Henle *et al.* 1968; Crawford, this issue).

A further point in the context of EBV and IM relates to the very rare occurrence of fatal IM in families. This X-linked condition, leading to death from lymphoproliferative disorders (XLP syndrome or 'Duncan's syndrome' after one of the early families to be identified), was worked on extensively by Purtilo (Purtilo *et al.* 1975) and he is almost invariably described, erroneously, as its discoverer. In fact, the first such family was reported by Bar *et al.* (1974), who not only demonstrated the role of EBV but also suggested that an X-linked defect in immunological responses to primary EBV infection was responsible for the disease.

(b) γ -Herpesviruses of non-human primates

The γ -herpesviruses of apes and monkeys fall into two long-recognized groups together with a third group that has only recently come to light. All, however, persist for life in host lymphoid cells, can produce lymphoproliferation and are transmitted horizontally by shed infectious virus. The group of agents affecting Old World non-human primates are B lymphotropic and EBV-like, while those of the second group confined to New World monkeys are T lymphotropic; the third and very new group consists of B-lymphotropic viruses of New World species (Wang *et al.*, this issue; Damania & Desrosiers, this issue; Fickenscher & Fleckenstein, this issue). At least some aspects of the first discoveries regarding these viruses have unusual features and are therefore worth recalling.

(i) Old World non-human primate γ -herpesviruses

The first suggestion that apes and Old World monkeys carried EBV-like viruses arose from the finding of antibodies to EBV in captive animals of various kinds. Antibodies to EBV were subsequently detected in newly caught rhesus monkeys and chimpanzees; an EBV-like herpesvirus antigenically close to EBV was then actually demonstrated in chimpanzee cell lines by Landon *et al.* (1968). And there the matter rested until an extraordinary event at the Institute of Experimental Pathology and Therapy, a vast primate centre set up in Sukhumi, Georgia, by the USSR Academy of Medical Sciences primarily to support the Soviet space programme. Most of the animals were baboons (*Papio hamadryas*) kept in large groups in open pens. After the introduction into the pens of a few individuals previously inoculated with human leukaemic blood an outbreak of leukaemia and/or lymphoma occurred with over 30 cases diagnosed (Lapin 1974). Cell lines were established that carried an EBV-like herpesvirus (Agrba *et al.* 1975), designated *Herpesvirus papio* (HVP), which was again antigenically close to EBV, and cross-reacting antibodies were found in many baboon colonies elsewhere. Although the Sukhumi baboon lymphoma outbreak was never fully investigated, much work was stimulated by it into the non-human primate agents of this group. It was soon determined that the herpesvirus of chimpanzees (*Herpesvirus pan*) had about 40% homology with EBV, HVP about 35% homology, and that related viruses were carried as silent natural infections in many species of Old World monkeys (Wang *et al.*, this issue).

It is greatly to be regretted that the Sukhumi Institute was almost totally destroyed during the fighting between Georgians and Abkhazians after the break-up of the Soviet Union, and all material and records were lost.

(ii) New World non-human primate γ -herpesviruses

The classic T-lymphotropic New World primate γ -herpesviruses also owed much to a primate research centre for their first identification. Each of the Regional Primate Research Centers set up in the United States was given a specific remit and that run by Harvard Medical School for New England was assigned the study of South American species for use in biomedical research. In the 1960s a young Chilean, Luis Meléndez, was working there and made a number of virus isolates from squirrel monkeys (*Saimiri sciureus*) when he noticed that kidney cultures from these animals underwent spontaneous cytopathic effects of a type associated with herpesviruses (Meléndez *et al.* 1968). In order to see whether all the isolates were of a single virus or several new ones, antibodies were needed for cross-neutralization tests; these would normally have been raised in rabbits but at a primate centre it was simpler to use monkeys, and when this was done the results were dramatic—both inoculated owl monkeys and cotton-top tamarins developed rapidly fatal malignant T lymphomas (Meléndez *et al.* 1969a). The isolates were in fact of a single virus designated *Herpesvirus saimiri* (HVS) (Meléndez *et al.* 1969b) and its herpes morphology was soon confirmed by electron microscopy (Morgan *et al.* 1970). A virus with similar oncogenic properties was discovered shortly afterwards in a primary kidney culture from a healthy black spider monkey (*Ateles geoffroyi*) and was named in turn *Herpesvirus ateles* (HVA) (Meléndez *et al.* 1972). HVS and HVA are non-pathogenic in their natural hosts, share many biological and molecular properties (Fickenscher & Fleckenstein, this issue) but are quite distinct from the newly discovered group of New World monkey γ -herpesviruses which, as already mentioned above, are B lymphotropic and EBV-like (Wang *et al.*, this issue).

(c) Mouse herpesvirus 68

Mouse herpesvirus 68 (MHV-68) provides another example of an agent found in the course of investigations aimed at something altogether different. In 1974 a survey was initiated in then Czechoslovakia into the ecology of arboviruses in the country. During fieldwork for this project round Bratislava in Slovakia, wild rodents of two species—bank voles (*Chlethrionomys glareolus*) and yellow-necked field mice (*Apodemus flavicollis*)—were trapped to determine the possibility of a rodent arbovirus reservoir. When virus isolation tests were applied, five herpesvirus strains were identified in tissue culture (Blaskovic *et al.* 1980) and when checked for herpesvirus morphology by electron microscopy, these were found to be one single new agent, and shown to be able to grow in a wide range of mammalian and even avian cell cultures (Svobodova *et al.* 1982). The virus, subsequently named mouse herpesvirus 68 (MHV-68), was readily adapted to laboratory mice. Since it causes an acute productive infection in these animals on primary exposure followed by latency in B cells with suspected late lymphoproliferative and/or lymphomatous complications in 10% of cases, MHV-68

has been proposed as a useful animal model for EBV, to which it is genetically related (Nash *et al.*, this issue; Doherty *et al.*, this issue).

(d) *Kaposi's sarcoma-associated herpesvirus*

Kaposi's sarcoma-associated herpesvirus (KSHV), or human herpesvirus 8 (HHV-8) as it is also called, was, like EBV, discovered in an unconventional way. Apart from the rather rare but long recognized classical form of Kaposi's sarcoma (KS), an upsurge of a new variant of this tumour accompanied the start of the acquired immune deficiency syndrome (AIDS) epidemic in the 1980s; careful epidemiological studies soon showed that the huge increase in KS cases followed the pattern expected of a sexually transmitted disease caused by an agent additional to human immunodeficiency virus (Beral *et al.* 1990). In an effort to identify such an agent Patrick Moore and Yuan Chang (Mrs Moore) applied a then quite new molecular technique, representational difference analysis (RDA) (Lisitsin *et al.* 1993), to KS material in order to search for non-human unique DNA sequences resident specifically in the lesions. In this way they found solid evidence for the presence of a herpesvirus (Chang *et al.* 1994) that we now know as KSHV. Molecular and epidemiological studies soon made it clear that this virus is causally involved both with KS and AIDS-related primary effusion lymphoma (Moore & Chang, this issue; Boshoff & Weiss, this issue).

The experimental steps and innovations which led to the discovery of KSHV have been described in detail elsewhere (Moore & Chang 1998, this issue) and therefore need not be repeated here.

7. CONCLUDING COMMENTS

The change in attitudes to human tumour viruses over the past few decades is clearly exemplified by the reception given to the discovery of EBV in 1964, in contrast to that of KSHV in 1994. Each was found by methods deemed unorthodox for virus identification at the time; EBV, seen first by the then new technique of electron microscopy, took several years before it was even accepted as a virus and it was only with the later advent of the unequivocally oncogenic herpesviruses of Marek's disease and New World primates that its possible function as a human tumour virus received the first tentative consideration. Even after the accumulation of abundant data on the powerful transforming ability of EBV (for reviews, see Epstein & Achong 1979) and the persuasive evidence for a causative role in Burkitt's lymphoma that resulted from the massive prospective study of Ugandan children (De Thé *et al.* 1978), the reluctance to believe that there could be a carcinogenic herpesvirus of man persisted.

It is a pleasing measure of current progress in human tumour virology that when KSHV was found by an unconventional technique (RDA), first reported barely a year before, the evidence was judged credible immediately and KSHV was viewed as a human tumour virus shortly afterwards.

In fact, between the finding of EBV and KSHV the changed perception of viruses and cancer in man was driven by progress outside the field of herpesvirology. The important role of hepatitis B virus in the induction of

liver cancer, the similar role recognized later for hepatitis C virus, the close involvement of various genotypes of papilloma virus in genital and other epithelial cancers, and the clear participation of human T-cell leukaemia virus in the induction of a certain leukaemia, have all contributed to the changing of ideas. For a long time EBV stood alone as the only putative human cancer virus; the discovery of the others in this category just listed, and of KSHV, now make it evident that at least 20–25% of human cancer is virus-associated in origin, and there are surely other such agents as yet unrecognized.

Of course, none of the currently known human tumour viruses is directly and immediately carcinogenic, but each appears for its cancer to be a necessary, but not on its own sufficient, link in a complicated chain of events leading to malignancy. Exploration of the molecular phenomena involved, as illustrated in this present issue, will surely reveal the exact mechanisms of viral carcinogenesis sooner rather than later.

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