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Viruses in Human Cancers*

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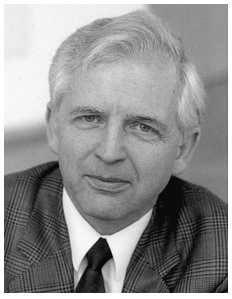

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IT HAS been one of the surprising aspects, particularly of the past 2 decades, that widespread chronic diseases, previously thought to be due to metabolic imbalances or genetic modifications, are increasingly linked to infectious events. Besides atherosclerosis, now suspected to be caused by chlamydial infections, approximately 15% of global cancer incidence is aetiologically related to specific infections. Bacteria (*Helicobacter pylori*) and helminths (*Schistosoma*, *Opistorchis*, *Clonorchis*) contribute to the development of gastric, bladder and rectal cancers and to cholangiocarcinomas. Viruses, however, emerge as major causal cancer factors. In addition to hepatocellular carcinomas, linked to hepatitis B and C virus infections, specific types of papillomaviruses have been shown to cause a major human cancer, cancer of the cervix. They have also been implicated in a number of other anogenital, oropharyngeal, and cutaneous cancers. Epstein–Barr virus (EBV), the longest known human tumour virus (since 1964), human herpes virus types 8 (HHV-8), and human T-lymphotropic retrovirus type 1 (HTLV-1) represent additional identified human tumour viruses.

Although difficult to predict, there are still a number of types of tumours with a possible infectious aetiology, including lymphomas and leukaemias, but also some epithelial tumours. Several human tumour viruses are ubiquitous and only a low rate of infected individuals eventually develop the respective form of cancer. Malignant conversion occurs either as the consequence of additional genetic modifications in the latently infected cells or under conditions of severe immunosuppression. It is likely that epidemiological studies may not point to the existence of as yet unknown additional tumour viruses if these are ubiquitous. Their future discovery will probably depend on molecular, biological or immunological approaches.

DISCOVERY OF HUMAN TUMOUR VIRUSES

When the original discoveries of currently known human tumour viruses (EBV, Hepatitis B, HTLV-1, human papillomaviruses, Hepatitis C and HHV-8) are considered, observations pointing to their relationship to specific human tumours resulted from remarkably different approaches (summarised in Table 1).

Epstein–Barr Virus (EBV) was initially observed electron microscopically in cell cultures derived from Burkitt's lymphoma [1]. This tumour had been suspected to be caused by an infectious agent in view of its peculiar geographically limited occurrence [2]. Seroepidemiological studies performed with virus-positive Burkitt's lymphoma cell lines revealed a relationship of this infection with Burkitt's lymphomas [3] and nasopharyngeal cancer [4]. This was emphasised by nucleic acid hybridisation tests revealing viral latency in an

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Table 1. *Methods used for the initial discovery of human tumour viruses and early data relating to their carcinogenic potential*

Virus type	Method of initial discovery [Ref.]	Early data relating to carcinogenicity [Ref.]
Epstein–Barr virus	Electron microscopy [1]	Seroepidemiology [3, 4] nucleic acid hybridisation [5, 6]
Hepatitis B	Immunology [7, 8]	Seroepidemiology [9, 10], animal models [11]
Human 'high risk' HPV (HPV 16, 18, etc.)	DNA cloning [17, 18]	DNA hybridisation [19, 20]
HTLV-1	Tissue culture [12]	Seroepidemiology [13]
Hepatitis C	cDNA cloning [21]	Seroepidemiology [22]
Human herpes-virus type 8	DNA cloning [23]	Nucleic acid hybridisation, serology [23, 24]

HPV, human papillomavirus.

'EBV-free' culture of Burkitt's tumour cells, Raji [5] and in the vast majority of Burkitt's lymphoma and nasopharyngeal tumour biopsies [6].

It is much more difficult to trace early attempts to link Hepatitis B virus (HBV) infections to hepatocellular carcinomas. Viral antigens were originally discovered by Blumberg who recognised their relationship to hepatitis infections in 1967 [7]. More specifically Prince and colleagues pointed out that 'serum-hepatitis' was related to these antigens [8]. A large number of seroepidemiological studies, particularly those of Szmuness [9] and Beasley and colleagues [10], clearly showed a link between this infection and hepatocellular cancer. This was further emphasised by the isolation of a closely related virus from woodchucks which efficiently caused liver cancer in these animals [11].

In 1980 Poiesz and colleagues reported the isolation of a retrovirus, now termed human T lymphotropic retrovirus-1 (HTLV-1), from a patient suffering from a condition at that time considered as mycosis fungoids [12]. This isolation was the consequence of a large number of previous attempts to isolate retroviruses from human lymphomas and leukaemias based on a retroviral aetiology of similar proliferative diseases in animals. Simultaneously, tissue culture lines derived from Japanese patients with adult T cell leukaemia revealed the presence of a retrovirus that turned out to be identical with the Poiesz isolate. Seroepidemiological studies quickly revealed its relationship to this in southern Japan endemic, but otherwise rare form of human leukaemia [13].

Speculations suspecting the carcinogenic potential of human papillomavirus (HPV) infections for a rare hereditary condition, epidermodysplasia verruciformis, date back to the 1950s [14], and for a common human cancer, carcinoma of the cervix, to the period between 1974 and 1976 [15, 16]. In squamous cell cancers of the skin of epidermodysplasia verruciformis patients, Orth and Jablonska demonstrated in 1978 the presence of a specific HPV type, HPV 5. First isolations of novel virus types (HPV 16 and 18) directly from cervical cancer were reported in 1983 and 1984 [17, 18]. The same group revealed these virus types also in genital precursor lesions of malignant tumours [19] and revealed the expression of specific viral genes in malignant tissues [20].

In 1989 Choo and colleagues cloned a new hepatitis virus, Hepatitis C (HBC), from the peripheral blood of an experimentally infected chimpanzee [21]. One year later Kiyosawa and colleagues seroepidemiologically revealed a relationship of HBC to hepatocellular carcinomas [22]. This relationship has been subsequently confirmed in numerous additional studies.

The most recent tumour virus isolate, Human Herpesvirus Type 8 (HHV-8), was obtained by representational differential hybridisation from Kaposi tumours [23], most frequently found in AIDS patients, occasionally also under other conditions of severe immunosuppression. Here, nucleic acid hybridisation studies, but also seroepidemiology, established a role for HHV-8 in the aetiology of Kaposi's sarcoma [23–25].

HOW CAN A VIRUS INFECTION BE LINKED WITH A HUMAN TUMOUR?

The mere presence of viral DNA within a human tumour represents a hint but clearly not proof for an aetiological relationship. The same accounts for seroepidemiological studies revealing elevated antibody titres against the respective infection.

The characteristics of tumour emergence as a consequence of virus infections add to the difficulties in identifying the causative agent. Most of these cancers arise only after latency periods of several decades between the primary infection and the development of the respective neoplasm. The tumours uniformly arise from single cells, their monoclonality can frequently be deduced from the integration pattern of viral DNA. In addition, most of the infected individuals either clear the respective infection by immunological intervention or may harbour viral DNA for a lifetime within specific cells without any clinical symptoms. There exists no known virus infection in humans where the exposure of human cells results in immediate malignant transformation. Thus, these infections may be necessary for specific tumours but they are in no instance sufficient to induce those neoplasms.

In virus-positive tumours the viral DNA frequently persists in subgenomic fragments which are unable to give rise to infectious progeny. For all these reasons, Koch's postulates to prove that a bacterial infection is the cause of a specific disease, based on the isolation of the infectious agent, its *in vitro* propagation, the re-inoculation into a susceptible animal host and the induction of symptoms analogous to those observed in the diseased patient, cannot be applied to tumour viruses.

A number of attempts have been made to overcome these difficulties and to define new criteria linking virus infections to human cancer, most frequently involving epidemiological and seroepidemiological data [26]. The different modes of virus-mediated cell transformation, including their role as causal factors or as cofactors render such efforts extremely difficult.

Taking these limitations into account, the four criteria [27] shown in Table 2 can only be considered as valid for those tumour virus infections that permit the identification of a *trans*- or *cis*-acting viral gene or DNA-fragment and exclude all indirect contributions to cancerogenicity, as for instance by continuing immunosuppression (e.g. HIV infections).

Particularly the fourth point could be taken as a most stringent criterion to pinpoint a causal role of an infection and to differentiate it from cofactors.

Table 2. Criteria for defining a causal role for an infection in cancer

1. Epidemiological plausibility and evidence that a virus infection represents a risk factor for the development of a specific tumour;
2. Regular presence and persistence of the nucleic acid of the respective agent in cells of the specific tumour;
3. Stimulation of cell proliferation upon transfection of the respective genome or parts therefrom in corresponding tissue culture cells;
4. Demonstration that the induction of proliferation and the malignant phenotype of specific tumour cells depend on functions exerted by the persisting nucleic acid of the respective agent.

MECHANISMS OF VIRUS-MEDIATED CELL TRANSFORMATION

Several human tumour viruses induce unlimited cell proliferation ('immortalisation') of specific human tissue culture cells. Under such *in vitro* conditions immortalisation proceeds malignant conversion which cannot be achieved in a single step. Malignant growth is here defined as invasive proliferation after heterografting the cells into immunocompromised animals (nude mice). It becomes more and more apparent that the individual steps resulting in immortalisation and eventually in malignant growth include modifications of specific cellular genes, part of them engaged in the control of the persisting viral genome (see the following section). Here some known functions of viral oncogenes will be discussed. Table 3 lists human tumour viruses and other human virus infections, the latter are potentially carcinogenic in experimental animal systems without a similar role in humans.

Although prospective epidemiological studies have emphasised the role of Hepatitis B and C viruses in the aetiology of hepatocellular carcinomas, these viruses are as yet unable to immortalise human cells in tissue culture. In spite of the definition of *trans*-activating functions of specific HBV and HBC proteins (see below), their contribution to malignant conversion is presently unknown.

Similarly, little is known of viral functions contributing to the malignant conversion of lesions associated with papillomavirus types (HPV 5, 8, 14, 17, 20 and a few others) in epidermodysplasia verruciformis patients. In contrast to anogenital malignant tumours, carcinomas in epidermodysplasia verruciformis patients only exceptionally seem to contain integrated viral DNA [28]. The preservation of E6 and E7 genes under these conditions may suggest a similar important role as demonstrated for high risk anogenital HPV infections.

Table 3. Established human tumour viruses and human viruses that induce tumours thus far exclusively under experimental conditions

Established human tumour viruses
- 'Direct' carcinogens:
  - Epstein–Barr virus
  - Human herpesvirus type 8
  - Several anogenital and cutaneous human papillomavirus types
  - HTLV-1
  - Hepatitis B (?)
- 'Indirect' carcinogens:
  - HIV
  - Hepatitis B (?) and hepatitis C

Human viruses causing tumours thus far exclusively in experimental animal systems:
- Human polyomaviruses BK and JC
- Several types of human adenoviruses

The human polyomavirus types BK and JC, as well as various types of human pathogenic adenoviruses have not been consistently found in any human tumour, although they are carcinogenic upon infection of newborn rodents. They will not be discussed subsequently. Under specific circumstances they are able to immortalise human cells, though very inefficiently.

EBV, HPVs, HTLV-1 and HHV-8 possess defined oncogenes that stimulate proliferation of specific human cells. Although *trans*-activating properties have been defined for the HBV X and pre-S antigens [29–31] and mice transgenic for these genes and those transgenic for the HBC core antigen develop hepatocellular cancers [32], it is currently unknown whether and to what extent the same genes contribute to human liver cancers.

In vitro EBV infection of B lymphocytes requires the activity of at least 6 viral genes (EBNAs 1, 2, 3A, 3C, LP, LMP1) for cell immortalisation [33]. Following *in vivo* infections, that may lead initially to infectious mononucleosis [34], three modes of EBV latency have been defined [35, 36]. Specifically in EBV-positive Burkitt's lymphomas, latency programme I is expressed, permitting only the synthesis of the EBV nuclear antigen (EBNA)-1, transcripts clustered around one other open reading frame, BARF-0, and a small abundant non-translated RNA, EBER. EBNA-1 is required for EBV DNA replication and to permit the persistence of episomal viral DNA, the role of transcripts in the BARF-0 region is still not elucidated. Interestingly, recent data seem to point to a specific role of EBER transcripts in preventing apoptosis (Takada; Cancer Institute, Sapporo, Japan). In most other EBV containing malignant tumours (nasopharyngeal cancer, EBV-positive Hodgkin's lymphomas and gastric cancers), latency programme II is expressed, corresponding to the previous one, but expressing in addition to a varying degree the latency membrane proteins (LMP) 1 and 2. Finally, latency programme III is observed in EBV-induced lymphoblastoid proliferation and in EBV-positive B cell lymphomas arising under conditions of immunosuppression. In this case gene regulation occurs from a different promoter (Cp/Wp) and results in the expression of 6 different EBNA proteins, three latent membrane proteins and EBER RNA. The EBNA-2 protein represents a specific transactivator of cellular and viral genes. It binds to the cellular protein RBP-Jκ which acts downstream of the Notch-signalling pathway and transforms this protein from a repressor into an activator [37, 38].

The mechanism by which EBV proteins contribute to malignant tumours is still poorly understood. This accounts, in particular, for tumours expressing latency programmes I or II. EBV-positive lymphomas developing in immunosuppressed patients emerge as the result of a failing immune system and the potent transactivating and growth-stimulating activity of EBNA-2.

HHV-8 is the most recently identified human tumour virus. Its genome contains a number of genes whose products are related to cellular cyclins (cyclin D), to cytokines (Il-6) and to interferon responsive factors (IRF-2). Their accurate contribution to growth-stimulation and cell transformation is presently under intensive study [24, 39, 40].

HTLV-I and also a related retrovirus, not yet linked to human tumours, HTLV-II, are both able to immortalise

human T lymphocytes [41, 42]. This property seems to relate to a specific viral gene *tax* that has been identified as a transforming factor [43], possessing strong transactivating properties [44, 45].

High risk human papillomavirus, particularly well studied HPV 16 and 18, code for three viral oncoproteins. One of them, E5, is obviously not required to initiate and maintain the malignant phenotype of cervical carcinoma cells. However, it seems to play a role in early growth stimulation of cells infected by these viruses. The two other oncogenes, E6 and E7, of high risk types are able to immortalise human keratinocytes in contrast to low risk types. They contain all the necessary information for cell immortalisation [46], although infection of susceptible cells or transfection of these genes *per se* is not sufficient for the induction of an unlimited *in vitro* lifespan (see below). The E7 protein interacts with the retinoblastoma susceptibility gene product pRb [47] and other pRb related proteins. As one consequence it interrupts a complex between pRb and E2F, releasing the E2F transcription factor which activates genes engaged in cell cycle progression. Additional binding activities have been described for E7 whose functional importance has not yet been clearly established. The E6 protein binds p53 and abrogates its tumour suppressive and transcriptional activation properties [48]. It promotes ubiquination of p53 and its subsequent proteolysis through interaction with the E6AP ubiquitin-protein ligase [49, 50]. E6 and E7 are able to immortalise human keratinocytes independently, although both genes cooperate effectively in immortalisation events. As observed for E7, E6 also targets other proteins: the focal adhesion protein paxillin [51] and the interferon regulatory factor 3 (IRF-3), blocking the induction of interferon β mRNA after viral infection [52]. These data indicate the multifunctionality of viral oncoproteins, modifying a multitude of cellular functions.

PROTECTIVE MECHANISMS OF THE HOST AGAINST POTENTIAL TUMOUR VIRUSES—THE CIF-CONCEPT

Several virus-induced malignant tumours occur mainly or almost exclusively under conditions of severe immunosuppression. This points to an effective immunological control of the respective infections. EBV-positive B cell lymphomas and Kaposi's sarcomas containing HHV-8 are the most prominent tumours under these conditions. Both types of viral infections are tightly controlled by cell-mediated immune reactions permitting, however, a probably life-long viral genome persistence in specific cell types, commonly without any symptoms. The role of viral and cellular genes in regulating this state of persistence is still not clarified. In latent EBV infections the switch to a programme, probably corresponding to latency programme I, is poorly understood. EBNA-1 positive cells are not recognised by the immune system of the host, apparently due to repetitive glycine and alanine repeats which are also interspersed in several cellular proteins [53].

The role of cellular modifications in the development of nasopharyngeal cancer (NPC), EBV-positive Hodgkin's disease and gastric cancers remains to be clarified. The peculiar geographic distribution of NPC with high incidence areas in Southeast Asia strongly suggests that additional factors should be involved. Speculations range from variations in virus strains, genetic factors (overrepresentation of the HLA haplotypes A2Bw46 and A19B17), salted fish consumption containing volatile nitrosamines, selective exposure to tumour-promoting phorbol esters and specific chromosomal aberrations (particularly deletions of the short arm of chromosome 3) reviewed in [35].

Burkitt's lymphomas reveal at least one consistent feature, the reciprocal c-*myc* translocation at 8q24 with either the immunoglobulin heavy chain locus on chromosome 14 or with one of the light chain loci on chromosomes 2 or 22. This translocation is uniformly present, even in EBV-negative Burkitt's tumours. In the so-called Burkitt's lymphoma belt in equatorial Africa, almost all of these lymphomas contain EBV DNA, sporadic Burkitt's lymphoma cases range between 15 and 25% in EBV positivity. BL emerging rarely in AIDS patients reveal a slightly higher percentage of EBV positivity. The regularity of the c-*myc* translocation resulting in dysregulation of the gene and its enhanced expression in affected B cells emerges as one dominant factor in the aetiology of Burkitt's lymphoma.

The discussion of the role of EBV in Burkitt's lymphoma has been controversial for more than 30 years. Recently some direct evidence has been reported pointing to an EB viral contribution to the malignant phenotype of these cells. Loss of EBV genomes have been noted in the Burkitt's lymphoma line Akata [54]. EBV negative clones lost the tumorigenic phenotype which was reconstituted by re-introduction of EBV DNA.

Recent evidence was obtained for the host regulation of persistent EBV infections by the genetic analysis of a rare EBV-linked condition, the X chromosome-linked lymphoproliferative syndrome (XLP) [55]. In XLP patients the host is unable to cope with the B cell proliferation which characterises the initial stage of infectious mononucleosis, a disease caused by acute EBV infection. XLP patients succumb from an enormous lymphoproliferation. The condition affects exclusively young males and has been linked to deletions of the X chromosome. Recently mutations have been identified in an inhibitor of a protein (SLAM) that regulates T/B cell interactions. The gene for the inhibitor protein, SLAM-associated protein or SAP, was identified at the site of the X chromosome deleted in XLP patients. A subsequent analysis demonstrated its mutation specifically in this group of patients. It is currently not understood how this disturbance in T/B cell relatively selectively affects the T cell control of EBV infections.

Probably the best evidence for host defence mechanisms against a family of tumour viruses, even beyond mere immunological control, has been derived recently from studies on human papillomavirus infections. The papillomavirus family reveals an enormous complexity with 85 fully analysed genotypes and more than 120 additional putative genotypes which are only partially characterised to date [56, 57]. It seems that the peculiar mode of papillomavirus propagation at cutaneous and mucosal surfaces only marginally exposes these viruses to the immune system of the host. This may release these infections from immunological constraints acting on other systemic infections and may in part account for the puzzling multitude of HPV genotypes.

Papillomavirus propagation depends on the induction of cell proliferation in infected basal layer cells in order to generate clones where the suprabasal differentiating layers produce infectious progeny. The expression pattern of viral genes is tightly regulated, permitting at best transcription of early genes in the basal layer, but expression of all genes as

soon as the infected cells start to differentiate. The regulatory pattern in basal layer cells could be determined by viral or host cell factors. Although integration of viral DNA into the host cell genome partially dysregulates E6/E7 gene expression in proliferating cells due to a disturbance in viral intragenomic regulation, reviewed in [58], emerging evidence points to an important role of cellular control mechanisms.

Previous studies have shown that E6/E7 gene expression of high risk HPV types is necessary for the initiation and maintenance of the immortalised [59] but also for the malignant phenotype of HPV DNA-carrying cells [60, 61]. These findings, combined with additional observations, have demonstrated that the expression of viral oncoproteins and transcription of viral oncogenes is necessary but not sufficient for the immortalised and malignant state of HPV-infected cells. Somatic cell hybrids between HPV-immortalised clones or between SV40-immortalised clones revealed the existence of complementary groups complementing each other to cellular senescence in spite of continuing E6/E7 mRNA or SV40 T-antigen synthesis [62, 63]. Therefore, a hypothesis was put forward postulating a cellular control of viral oncogene transcription or viral oncoprotein function, preventing, in proliferating cells of the natural host, their potential transforming and thus deleterious effect on the host [64]. For high risk HPV infections, a possible transcriptional control received experimental support from data showing suppression of HPV transcription upon inoculation of immortalised cells into immuno-compromised animals [65–67] and from a barely detectable level of E6/E7 transcripts in most low grade cervical intra-epithelial lesions in contrast to high grade dysplasia [68]. The initial suspicion of one cellular interfering factor (CIF) exhibiting an intracellular control beyond an immunological surveillance [69] had to be modified into a CIF-concept [70].

Today there exists good evidence in support of the CIF-concept: at least two signalling cascades emerge regulating the transcription of high risk HPV oncogenes and interfering with the function of viral oncoprotein, reviewed in [71]. A functional control can be deduced from somatic cell hybridisation studies revealing continued HPV mRNA synthesis in senescent hybrids of initially immortalised clones. Transfection of human keratinocytes with the HPV 16 E6 oncogene only results in immortalised clones that generally contain mutated or methylation-silenced sequences of the $p16^{ink4}$ cyclin-dependent kinase inhibitor [72] (Whitaker and zur Hausen, Deutsches Krebsforschungszentrum, Germany). This suggests an important role of p16 in the control of E6-mediated cell immortalisaton. The regulatory steps engaged here are still not understood. For E7 as well as for E6 and E7 immortalised cells the obviously existing functional interference is even less understood. A high level of $p21^{KIP-1}$ and $p27^{CIP-1}$ may negatively interfere with a low level of E7 expression, [71], at least dysregulated E7 expression in turn is able to inactivate these cyclin-dependent kinase inhibitors reciprocally [73–75].

The CIF-cascade interfering with the transcription of persisting high risk HPV is somewhat better understood. Exposure of HPV 16- or 18-immortalised cells to macrophages or treatment with tumour necrosis factor (TNF) α leads to a selective suppression of HPV transcription [76]. The effect is absent in HPV-containing malignant cervical cancer cells. Suppression in HPV transcription is accompanied by a remarkable shift in the composition of the AP-1 transcription factor as one of the important regulators of the HPV genome activity. In immortalised cells in tissue culture predominantly *c-jun*/*c-jun* homodimers form this complex, whereas in most malignant lines *c-jun*/*c-fos* heterodimers prevail. TNFα treatment results in the induction of a *c-fos* analogon, *Fra-1*, which now heterodimerises with a phosphorylated form of *c-jun*. Malignant cells neither reveal *Fra-1* induction nor an increase in *c-jun*/*Fra-1* heterodimers. Although not directly proven, the available data suggest that the change in the AP-1 composition is responsible for the observed selective inhibition of HPV transcription in immortalised cells.

It is interesting to note that other AP-1 regulated genes seem to react differently under the same type of treatment: the macrophage chemoattractant protein-1 (MCP-1) as well as the collagenase gene become selectively induced by TNFα treatment [77]. A similar induction of MCP-1 after TNFα treatment has been initially observed in endothelial cells, reviewed in [78]. In this system it has been shown that the MKK6/p38 stress kinase cascade is critical for the induced expression of MCP-1 [79]. It remains to be seen whether a similar mechanism causes the induction of *Fra-1* and the suppression of HPV transcription. It is obvious, however, that the TNFα-mediated regulation of HPV transcription and of MCP-1 induction is interrupted in HPV-positive cervical cancer cells.

Presently a tentative CIF-cascade can be deduced from data available in the literature: there exists evidence that the protein phosphatase 2A (PP2A) plays an important role in the regulation of HPV transcription. This is derived from data published by Smits and colleagues, who demonstrated that human cells carrying a deletion in the short arm of chromosome 11 revealed an upregulation of the regulatory component of PP2A, the PR55β protein [80]. The resulting down-regulation of PP2A function that was also achieved by ocadaic acid treatment or by the introduction of SV40 small t-antigen led to increased transformability of these cells by high risk HPV DNA transfection and high levels of E6/E7 gene transcription. Preliminary data from our laboratory suggest that conditional upregulation of E7 expression in turn activates the PR55β gene, pointing to a positive feedback mechanism (Hoffmann and zur Hausen, Deutsches Krebsforschungszentrum, Germany). The activation of MKK6/p38 kinases by TNFα [79] may point to an important role of these kinases in the downstream events resulting in upregulation of FRA-I and *c-jun*.

The data reported here reveal a control of persisting natural tumour virus infections that are not readily reached by the immune system by intra- and intercellular signalling cascades. In these instances cancer represents an accident resulting from modifications of host cell genes involved in the control of viral oncogenes and oncoproteins. In high risk HPV infections the viral oncoproteins, in addition to their gene regulatory functions, appear to act as mutagens and may thus act as solitary carcinogens, reviewed in [71].

CAN WE STILL EXPECT THE DISCOVERY OF NEW TUMOUR VIRUSES?

Currently approximately 15% of global cancer incidence can be linked to infections, with a substantial contribution of members of different virus families. Does this cover the spectrum of cancers that may be caused by infectious events? This question is difficult to answer.

Cancers and precursor lesions arising under immunosuppression (lymphomas, Kaposi's sarcoma, non-melanoma skin

cancers, cervical high-grade intra-epithelial neoplasias) have been shown to be at least in part to be virus-linked. This seems to support suggestions that virus-associated tumours are commonly well controlled by the immune system and more readily identified in those neoplasms arising under immunosuppressive conditions. The development of hepatocellular carcinomas, of Burkitt's lymphomas and nasopharyngeal cancers does not seem to follow this rule.

By considering only tumours arising under immunosuppression, there exists still a remarkable subset of lymphomas not yet linked to EBV or other virus infections. Approximately 50–60% of Hodgkin's lymphomas are thus far virus-negative. In spite of the finding of papillomavirus types in non-melanoma skin cancers [81,82], their aetiological involvement remains to be clarified.

In addition, ubiquitous virus infections which may rarely lead to malignant tumours would be difficult to trace epidemiologically. Clusters of childhood leukaemias have been suspected to be due to various environmental factors, but also to infections reviewed in [83]. It is still worthwhile to continue the search for potential human tumour viruses, particularly for agents that do not fall into well known patterns of DNA-virus and retrovirus tumour biology. The emerging link between HBC virus infections and hepatocellular carcinomas and possibly also lymphomas emphasises this suggestion. There currently exists no evidence for HBC sequences integrated into the host cell genome, and members of the RNA-containing Flavivirus family have previously not been linked to proliferative diseases.

PERSPECTIVES FOR CANCER PREVENTION AND CANCER THERAPY

The discovery of infectious agents as causative factors for specific human cancers has important consequences for cancer prevention. This is currently apparent from vaccination studies performed in Taiwan and The Gambia [84, 85]. The Taiwan vaccination programme of newborn children against Hepatitis B infections, introduced in 1986, not only drastically reduced the percentage of persistently HBV-infected children, but also resulted in a first measurable decrease in liver cancer incidence [85]. Current clinical trials will determine the efficacy of vaccines directed against high risk papillomavirus infections. Preceding tests in animal papillomavirus infections, based on analogous vaccine preparations revealed a remarkable efficiency [86–88]. Provided the vaccines against human high risk HPVs prove to be similarly effective, one can estimate that a global application of HPV and HBV vaccines could theoretically reduce the cancer risk in women by approximately 15%.

Clinical trials are also currently being conducted to test the therapeutic potential of viral vaccines directed against antigenic domains of viral oncoproteins [89]. Their chances for effectiveness are more likely to concern pre-malignant lesions rather than fully invasive tumours, although this is currently difficult to predict.

Finally, the identification of viral oncoproteins involved in cancerogenesis, our detailed knowledge of viral replication cycles, of functions of early and late genes expressed in the viral life cycle, but also the knowledge of host cell functions engaged in the control of viral infections, may lead to novel approaches in the therapeutic control of these infections. Tumour virology had a long way to go but is now reaching the point of practical importance.

1. Epstein MA, Achong BG, Barr YM. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet* 1964, **1**, 702–703.
2. Burkitt D. A children's cancer dependent on climatic factors. *Nature* 1962, **194**, 232–234.
3. Henle G, Henle W. Immunofluorescence in cells derived from Burkitt lymphoma. *J Bacteriol* 1966, **91**, 1248–1256.
4. Old LJ, Boyse E, Oettgen HF, *et al.* Precipitating antibodies in human serum to an antigen present in cultured Burkitt's lymphoma cells. *Proc Nat Acad Sci USA* 1966, **56**, 1699–1704.
5. zur Hausen H, Schulte-Holthausen H, Klein G, *et al.* EBV DNA in biopsies of Burkitt tumours and anaplastic carcinoma of the nasopharynx. *Nature* 1970, **228**, 1056–1058.
6. zur Hausen H, Schulte-Holthausen H. Presence of EB virus nucleic acid homology in a "virus-free" line of Burkitt tumor cells. *Nature* 1970, **227**, 245–248.
7. Blumberg BS, Gerstley BJ, Hungerford DA, London WT, Sutnick A. A serum antigen (Australia antigen) in Down's syndrome, leukemia, and hepatitis. *Ann Intern Med* 1967, **66**, 924–931.
8. Prince AM. An antigen detected in the blood during the incubation period of serum hepatitis. *Proc Nat Acad Sci USA* 1968, **60**, 614–621.
9. Szmuness W. Hepatocellular carcinoma and the hepatitis B virus: evidence for a causal association. *Prog Med Virol* 1978, **24**, 40–69.
10. Beasley RP, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. *Lancet* 1981, **2**, 1129–1133.
11. Summers J, Smolec JM, Snyder R. A virus similar to hepatitis B virus associated with hepatitis and hepatoma in woodchucks. *Proc Nat Acad Sci USA* 1978, **75**, 4533–4537.
12. Poiesz BJ, Ruscetti FW, Gazdor AF, *et al.* Detection and isolation of type C retrovirus from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Nat Acad Sci USA* 1980, **77**, 7415–7419.
13. Hinuma Y, Nagata K, Hanaoka M. Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc Nat Acad Sci USA* 1981, **78**, 6476–6480.
14. Jablonska S, Millewski B. Zur Kenntnis der epidermodysplasia verruciformis Lewandowsky-Lutz. *Dermatologica* 1957, **115**, 1–22.
15. zur Hausen H, Meinhof W, Scheiber W, Bornkamm GW. Attempts to detect virus-specific DNA sequences in human tumors: I. Nucleic acid hybridizations with complementary RNA of human wart virus. *Int J Cancer* 1974, **13**, 650–656.
16. zur Hausen H. Condylomata acuminata and human genital cancer. *Cancer Res* 1976, **36**, 530.
17. Dürst M, Gissmann L, Ikenberg H, zur Hausen H. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc Nat Acad Sci USA* 1983, **80**, 3812–3815.
18. Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, zur Hausen H. A new type of papillomavirus DNA, its presence in genital cancer and in cell lines derived from genital cancer. *EMBO J* 1984, **3**, 1151–1156.
19. Ikenberg H, Gissmann L, Gross G, Grussendorf-Conen E-I, zur Hausen H. Human papillomavirus type 16 related DNA in genital Bowen's disease and in Bowenoid papulosis. *Int J Cancer* 1983, **32**, 563–564.
20. Schwarz E, Freese UK, Gissmann L, Mayer W, Roggenbuck B, zur Hausen H. Structure and transcription of human papillomavirus type 18 and 16 sequences in cervical carcinoma cells. *Nature* 1985, **314**, 111–114.
21. Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989, **244**, 359–362.
22. Kiyosawa K, Sodeyama T, Tanaka E, *et al.* Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990, **12**, 671–675.
23. Chang Y, Cesarman E, Pessin MS, *et al.* Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's soma. *Science* 1994, **266**, 1865–1869.
24. Moore PS, Gao SJ, Dominguez G, *et al.* Primary characterization of a herpesvirus agent associated with Kaposi's sarcoma. *J Virol* 1996, **70**, 549–558.

25. Gao SJ, Kingsley L, Hoover DR, *et al*. Seroconversion to antibodies against Kaposi's sarcoma-associated herpesvirus-related latent nuclear antigens before the development of Kaposi's sarcoma. *N Engl J Med* 1996, **335**, 233–241.
26. Evans AS. *Viral infections of humans. Epidemiology and control*. New York Chapter 1: Epidemiological concepts and methods, Plenum Publishing Corporation, 1976, 1–32.
27. zur Hausen H. Molecular pathogenesis of cancer of the cervix and its causation by specific human papillomavirus types. *Curr Topics Microbiol Immunol* 1994, **186**, 131–156.
28. Yabe Y, Sakai A, Hitsumoto T, Hanafusa H, Jitsumori Y, Ogura H. Human papillomavirus-5b DNA integrated in a metastatic tumor: cloning, nucleotide sequence and genomic organization. *Int J Cancer* 1999, **80**, 334–335.
29. Zahm P, Hofschneider PH, Koshy R. The HBV X-ORF encodes a transactivator: a potential factor in viral hepatocarcinogenesis. *Oncogene* 1988, **3**, 169–177.
30. Meyer M, Caselmann WH, Schlüter V, Schreck R, Hofschneider PH, Baeuerle PA. Hepatitis B virus transactivator MHBst: activation of NF-kappa B, selective inhibition by antioxidants and integral membrane localization. *EMBO J* 1992, **11**, 2991–3001.
31. Natoli G, Avantaggiati ML, Balsano C, *et al.* Characterization of the Hepatitis B virus preS/S region encoded transcriptional transactivator. *Virology* 1992, **187**, 663–670.
32. Moriya K, Fujie H, Shintani Y, *et al.* The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. *Nature Med* 1998, **4**, 1065–1067.
33. Farrell PJ, Cludts I, Stuhler A. Epstein–Barr virus genes and cancer cells. *Biomed Pharmacother* 1997, **51**, 258–267.
34. Henle G, Henle W, Diehl V. Relation of Burkitt's tumor-associated herpes-type virus to infectious mononucleosis. *Proc Nat Acad Sci USA* 1968, **59**, 94–101.
35. Rickinson AB, Kieff E. Epstein–Barr virus. In Fields BN, Knipe DM, Howley PM, eds. *Fields Virology*. Philadelphia, New York, Lippincott-Raven, 1996, 2397–2446.
36. Thorley-Lawson DA, Miyashita EM, Khan G. *Trends Microbiol* 1996, **4**, 204–208.
37. Strobl I, Hoefelmayr H, Stein C, *et al*. Both Epstein–Barr viral nuclear antigen 2 (EBNA2) and activated Notch1 transactivate genes by interacting with the cellular protein RBP-Jκ. *Immunobiology* 1997, **198**, 299–306.
38. Sakai T, Taniguchi Y, Tamura K, *et al*. Functional replacement of the intracellular region of the Notch1 receptor by Epstein–Barr virus nuclear antigen 2. *J Virol* 1998, **72**, 6034–6039.
39. Cesarman E, Nador RG, Bai F, *et al.* Kaposi's sarcoma-associated herpesvirus contains G-protein-coupled receptor and cyclin D homologs which are expressed in Kaposi's sarcoma and malignant lymphoma. *J Virol* 1996, **70**, 8218–8223.
40. Neipel F, Albrecht JC, Fleckenstein B. Cell-homologous genes in the Kaposi's sarcoma-associated rhadinovirus human herpesvirus 8: determinants of its pathogenicity? *J Virol* 1997, **71**, 4187–4192.
41. Miyoshi I, Yoshimoto S, Kubonishi I, *et al*. Transformation of normal human cord lymphocytes by co-cultivation with a lethally irradiated human T-cell carrying type C virus. *GANN* 1981, **72**, 997–998.
42. Yamamoto Y, Okada M, Koyanagi Y, Kannagi M, Hinuma Y. Transformation of human leukocytes by cocultivation with an adult T cell leukemia virus producer cell line. *Science* 1982, **217**, 737–739.
43. Grassmann R, Dengler C, Müller-Fleckenstein I, *et al.* Transformation to continuous growth of primary human T lymphocytes by human T-cell leukemia virus type I X-region genes transduced by a herpesvirus saimiri vector. *Proc Nat Acad Sci USA* 1989, **86**, 3351–3355.
44. Fujii M, Niki T, Mori T, *et al.* HTLV-I tax induces expression of various immediate early serum response genes. *Oncogene* 1991, **6**, 1023–1029.
45. Fujii M, Tsuchya H, Chuhjo T, Akizawa T, Seiki M. Interaction of HTLV-I tax-1 with p67 (SRF) causes the aberrant induction of cellular immediate early genes through CArG boxes. *Genes Dev* 1992, **6**, 2066–2076.
46. Hawley-Nelson P, Vousden KH, Hubbert NL, Lowy DR, Schiller JT. HPV 16 E6 and E7 proteins cooperate to immortalise human foreskin keratinocytes. *EMBO J* 1989, **8**, 3905–3910.
47. Dyson N, Howley PM, Münger K, Harlow E. The human papillomavirus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 1989, **243**, 934–937.
48. Werness BA, Levine AJ, Howley PM. Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science* 1990, **248**, 76–79.
49. Scheffner M, Werness BA, Huibregtse JM, Levine JM, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 1990, **63**, 1129–1136.
50. Scheffner M, Huibregtse JM, Vierstra RD, Howley PM. The HPV 16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquination of p53. *Cell* 1993, **75**, 495–505.
51. Van de Pol SB, Brown MC, Turner CE. Association of bovine papillomavirus type 1 E6 oncoprotein with the focal adhesion protein paxillin through a conserved protein interaction motif. *Oncogene* 1998, **16**, 43–52.
52. Ronco L, Karpova AY, Vidal M, Howley PM. The human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. *Genes Dev* 1998, **12**, 2061–2072.
53. Hennessy K, Kieff E. One of two Epstein–Barr virus nuclear antigens contains a glycine-alanine copolymer domain. *Proc Nat Acad Sci USA* 1983, **80**, 5665–5669.
54. Shimizu N, Tanabe-Tochikura A, Kuroiwa Y, Takada K. Isolation of Epstein–Barr virus-negative cell clones from the EBV-positive Burkitt's lymphoma line Akata: malignant phenotype of BL cells are dependent on EBV. *J Virol* 1994, **68**, 6069–6073.
55. Sayos J, Wu C, Morra M, *et al.* The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature* 1998, **395**, 462–469.
56. de Villiers E-M. Heterogeneity of the human papillomavirus group. *J Virol* 1989, **63**, 4898–4903.
57. de Villiers E-M. *Human Pathogenic Papillomaviruses: An Update. Curr Topics Microbiol Immunol.* Berlin-Heidelberg, Springer Verlag, 1994, **86**, 1-12.
58. Shah KV, Howley PM. Papillomaviruses. In Fields BN, Knipe DM, Howley PM, eds. *Fields Virology*. Philadelphia, New York, Lippincott-Raven, 1996, 2077–2109.
59. Münger K, Phelps WC, Bubb V, Howley PM, Schlegel R. The E6 and E7 genes of human papillomavirus type 16 are necessary and sufficient for transformation of primary human keratinocytes. *J Virol* 1989, **63**, 4417–4423.
60. von Knebel Doeberitz M, Rittmüller C, zur Hausen H, Dürst M. Inhibition of tumorigenicity of cervical cancer cells in nude mice by HPV E6-E7 antisense RNA. *Int J Cancer* 1992, **51**, 831–834.
61. von Knebel Doeberitz M, Rittmüller C, Aengeneyndt F, Jansen-Dürr P, Spitkovsky D. Reversible repression of papillomavirus oncogene expression in cervical carcinoma cells: consequences for the phenotype and E6-p53 and E7-pRB interactions. *J Virol* 1994, **68**, 2811–2821.
62. Pereira-Smith OM, Smith JR. Expression of SV40 antigen in finite lifespan hybrids of normal and SV40-transformed fibroblasts. *Somatic Cell Genetics* 1981, **7**, 411–421.
63. Chen T-M, Pecoraro G, Defendi V. Genetic analysis of *in vitro* progression of human papillomavirus-transfected human cervical cells. *Cancer Res* 1993, **53**, 1167–1171.
64. zur Hausen H. Intracellular surveillance of persisting viral infections: human genital cancer resulting from failing cellular control of papillomavirus gene expression. *Lancet* 1986, **2**, 489–491.
65. Bosch F, Schwarz E, Boukamp P, Fusenig NE, Bartsch D, zur Hausen H. Suppression *in vivo* of human papillomavirus type 18 E6-E7 gene expression in nontumorigenic HeLa-fibroblast hybrid cells. *J Virol* 1990, **64**, 4743–4754.
66. Dürst M, Bosch FX, Glitz D, Schneider A, zur Hausen H. Inverse relationship between human papillomavirus (HPV) type 16 early gene expression and cell differentiation in nude mouse epithelial cysts and tumors induced by HPV positive human cell lines. *J Virol* 1991, **65**, 796–804.
67. Stoler MH, Rhodes CR, Whitbeck A, Wolinsky SM, Chow LT, Broker TR. Human papillomavirus type 16 and 18 gene expression in cervical neoplasias. *Hum Pathol* 1992, **23**, 117–128.
68. Dürst M, Glitz D, Schneider A, zur Hausen H. Human papillomavirus type 16 (HPV 16) gene expression and DNA

replication in cervical neoplasia: analysis by *in situ* hybridization. *Virology* 1992, **189**, 132–140.
69. zur Hausen H. Cell-virus gene balance hypothesis of carcinogenesis. *Behring Inst Mitt* 1977, **61**, 23–30.
70. zur Hausen H. Disrupted dichotomous intracellular control of human papillomavirus infection in cancer of the cervix. *Lancet* 1994, **343**, 955–957.
71. zur Hausen H. Papillomavirus infections—a major cause of human cancers. *Biochem Biophys Acta Rev on Cancer* 1996, **1288**, F55–F78.
72. Reznikoff CA, Yeager TR, Belair CD, Savelieva E, Puthenveettil JA, Stadler WM. Elevated p16 at senescence and loss of p16 at immortalization in human papillomavirus 16 E6, but not E7, transformed human epithelial cells. *Cancer Res* 1996, **56**, 2886–2890.
73. Zerfass-Thome K, Zwerschke W, Mannhardt B, Tindle R, Botz JW, Jansen-Dürr P. Inactivation of the cdk inhibitor p27KIP1 by the human papillomavirus type 16 E7 oncoprotein. *Oncogene* 1996, **13**, 2323–2330.
74. Funk JO, Waga S, Harry JB, Espling E, Stillman B, Galloway DA. Inhibition of CDK activity and PCNA-dependent DNA replication by p21 is blocked by interaction with the HPV-16 E7 protein. *Genes Dev* 1997, **11**, 2090–2100.
75. Jones DL, Alani RM, Münger K. The human papillomavirus E7 oncoprotein can uncouple cellular differentiation and proliferation in human keratinocytes by abrogating p21Cip1-mediated inhibition of cdk2. *Genes Dev* 1997, **11**, 2101–2111.
76. Soto U, Das BC, Lengert M, Finzer P, zur Hausen H, Rösl F. Conversion of HPV 18 positive non-tumorigenic HeLa-fibroblast hybrids to invasive growth involves loss of TNF-α mediated repression of viral transcription and modification of the AP-1 transcription complex. *Oncogene* 1999, **18**, 3187–3198.
77. Rösl F, Das BC, Lengert M, Geletneky K, zur Hausen H. Antioxidant-induced changes in AP-1 composition result in a selective suppression of human papillomavirus transcription. *J Virol* 1997, **71**, 362–370.
78. Mantovani A, Bussolino A, Introna M. Cytokine regulation of endothelial cell function: from molecular level to the bedside. *Immunol Today* 1997, **18**, 231–240.
79. Goebeler M, Kilian K, Gillitzer R, *et al*. The MKK6/p38 stress kinase cascade is critical for tumor necrosis factor-α-induced expression of monocyte-chemoattractant protein-1 in endothelial cells. *Blood* 1999, **93**, 857–865.
80. Smits PHM, Smits HL, Minnaar R, *et al*. The trans-activation of the HPV 16 long control region in human cells with a deletion in the short arm of chromosome 11 is mediated by the 55 kDa regulatory subunit of protein phosphatase 2A. *EMBO J* 1992, **11**, 4601–4606.
81. Berkhout RJM, Tieben LM, Smits HL, Bouwes-Bavinck JN, Vermeer BJ, ter Schegget J. Detection and typing of epidermodysplasia verruciformis-associated human papillomavirus types in cutaneous cancers from renal transplant recipients: a nested approach. *J Clin Microbiol* 1995, **33**, 690–695.
82. Shamanin V, zur Hausen H, Lavergne D, *et al*. Human papillomavirus infections in non-melanoma skin cancers from renal transplant recipients and nonimmunosuppressed patients. *J Natl Cancer Inst* 1996, **88**, 802–811.
83. Kinlén LJ. Infection and childhood leukemia. *Cancer Causes Control* 1998, **9**, 237–239.
84. Whittle HC, Maine N, Pilkinton J, *et al*. Long-term efficacy of continuing hepatitis B vaccination in infancy in two Gambian villages. *Lancet* 1995, **345**, 1089–1092.
85. Chang MH, Chen CJ, Lai MS, *et al*. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan childhood hepatoma study group. *N Engl J Med* 1997, **336**, 1855–1859.
86. Suzich JA, Ghim SJ, Palmer-Hill FJ, *et al*. Systemic immunization with papillomavirus L1 protein completely prevents the development of viral mucosal papillomas. *Proc Nat Acad Sci US* 1995, **92**, 11553–11557.
87. Breitburd F, Kirnbauer R, Hubbert NL, *et al*. Immunization with virus-like particles from cottontail rabbit papillomavirus (CRPV) can protect against experimental CRPV infection. *J Virol* 1995, **69**, 3959–3963.
88. Campo MS. Vaccination against papillomavirus in cattle. *Clin Dermatol* 1997, **15**, 275–283.
89. Borysiewicz LK, Fiander A, Nimako M, *et al*. A recombinant vaccinia virus encoding human papillomavirus types 16 and 18, E6 and E7 proteins as immunotherapy for cervical cancer. *Lancet* 1996, **347**, 1523–1527.