Retroviruses and cancer

Robin A. Weiss

Wohl Virion Centre, Department of Immunology and Molecular Pathology, University College London, 46 Cleveland Street, London W1T 4JF, UK

The study of animal retroviruses has yielded fundamental insight into molecular pathways of carcinogenesis, while human retroviruses predispose infected persons to develop malignant disease. Human T-cell lymphotropic virus type I (HTLV-I) causes adult T-cell leukaemia. Human immunodeficiency virus types 1 and 2 (HIV-1, HIV-2) indirectly cause lymphoma, Kaposi's sarcoma and cervical carcinoma by allowing the DNA viruses underlying these tumours to exert their oncogenic effects in the immunosuppressed host. Human endogenous retroviral genomes may be linked with myeloproliferative disease. Retroviruses may also be put to use in gene therapy for human cancer.

ONCOGENIC viruses are important in cancer research for two distinct reasons. Firstly, approximately 20% of human cancer incidence worldwide is attributable to virus infection[1,2], and further cancers may yet be revealed to have a viral component to their aetiology[3]. A substantial proportion of these infections may one day be prevented by immunization, significantly lowering the global cancer burden, especially in developing countries.

Secondly, the experimental study of tumour viruses in animals and humans has illuminated our understanding of cancer more generally. In particular, oncogenes and tumour-suppressor genes were initially identified through the analysis of oncogenic animal viruses. Indeed, the study of retroviruses in relation to cancer has been recognized in the award of three separate sets of Nobel Prizes: to Peyton Rous in 1966 for the discovery of his eponymous sarcoma virus in chickens; to David Baltimore and Howard M. Temin in 1975 for their discovery in 1970 of reverse transcriptase; and to J. Michael Bishop and Harold E. Varmus in 1989 for their demonstration in 1976 that retroviral oncogenes originate from cellular genes in the host.

Retroviruses were first associated with malignant disease in animals more than ninety years ago[4]. In 1908 the Danish veterinarians Ellerman and Bang observed that erythroleukaemia is infectiously transmissible in chickens. Then in 1911, Rous in USA and in 1914, Fujinami in Japan showed that some avian sarcomas could be transmitted by inoculation of cell-free filtrates. In those days nothing was known about the causative agents other than that they passed through filters which held back bacteria. These findings were largely ignored by mainstream cancer research, so Rous' meticulous studies of what became known as Rous sarcoma virus had to wait 55 years before he was awarded the Nobel Prize. In 1936 Bittner observed that the mammary carcinoma in C3H mice was due to a transmissible factor in the milk, and in 1951 Gross found that the thymic lymphoma characteristic of inbred AKR mice could be passed to C3H mice by infection of neonatal animals. Each of these malignancies is now known to be caused by viruses classified as retroviruses. Since the 1960s retroviruses have been shown to cause leukaemia, lymphoma and other forms of cancer in a wide variety of vertebrate animals ranging from fish to apes. The first oncogenic human retrovirus[5] was isolated in 1980.

A simplified replication cycle of retrovirus is shown in Figure 1. Retroviruses carry diploid, single-stranded RNA genomes in the virion and replicate by forming one double-stranded DNA copy in the infected cell, by means of the viral enzyme, reverse transcriptase[4]. This genome becomes integrated as a DNA 'provirus' into the chromosomal DNA of the host cell and thus persists for the lifetime of the infected cell and its progeny. The proviral genome carries its own promoter and enhancer elements in sequences duplicated at each end of the genome, known as long terminal repeats (LTR). Expression of the provirus yields full length RNA transcripts that are packaged to become the genomes of progeny virus particles, and mRNA that is translated to provide the viral proteins.

Current classification of retroviruses delineates seven genera in this virus family[6], as shown in Table 1. These genera can be broadly grouped into 'simple' and 'complex' retroviruses. The genome of simple retroviruses

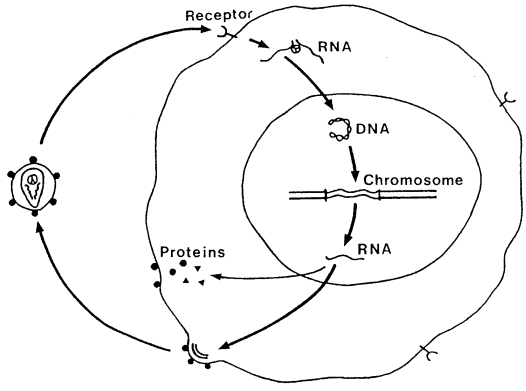

Figure 1. Replication cycle of a retrovirus.

e-mail: r.weiss@ucl.ac.uk

carries the *gag*, *pol* and *env* genes common to all retroviruses, and in some types an extra gene such as an oncogene or the superantigen gene of murine mammary tumour virus (MMTV). The complex retrovirus genomes carry regulatory genes such as *tax* and *rex* of HLTV, *tat* and *rev* of HIV and *bel* and *bet* of spumaviruses. The lentiviruses, including HIV, bear accessory genes, *nef*, *vif*, and *vpr*, which help them to infect non-dividing cells such as macrophages.

On many occasions during vertebrate evolution, retroviruses have infected cells of the host's germ-line, destined to become the eggs and sperm. In this way the integrated DNA provirus can be passed on to the next generation without undergoing further viral replication. Such genetically transmitted retroviral genomes are called endogenous retroviruses (ERV) to distinguish them from exogenous, infectiously transmitted retroviruses. There are many types of human endogenous retrovirus (HERV) belonging to the beta and gamma retrovirus genera[7,8]. With the annotation of the human genome it appears that more than 1% of human DNA sequences represent HERV and related retrotransposons.

Cell transformation by retroviruses

Figure 2 illustrates three distinct ways in which retroviruses can lead to malignant transformation of cells. In Figure 2 *a*, the most frequent means of animal retrovirus oncogenesis, integration of a provirus adjacent to a cellular oncogene can cause its ectopic activation by regulatory sequences in the LTR. An active promoter sequence causes downstream activation, as shown in Figure 2 *a*, whereas an active enhancer may activate oncogenes upstream or downstream as seen for *int 1* and *int 2* oncogenes switched on in mammary tumours by MMTV.

In Figure 2 *b*, the oncogene has become incorporated within the retroviral genome. This can occur when transcription from the 5′ LTR reads through the packaging signal of viral RNA at the beginning of *gag* and onwards, often spliced, into cellular sequences. This usually results in a defective viral genome in which *v-onc* is substituted for essential viral genes. Therefore, replication defective, *v-onc* containing retroviruses can only be replicated by complementation with a replication-competent 'helper' virus.

In Figure 2 *c*, characteristic of deltaviruses, such as HTLV and bovine leukosis virus, the Tax protein acts in *trans*, not only to activate the viral LTR, but also a set of cellular genes, including proto-oncogenes that drive cell division. A number of host genes are upregulated (and some downregulated) by Tax in this way, and the effect of Tax on the overall cellular transcriptome is currently being elucidated using DNA arrays.

Table 2 shows many of the oncogenes that were first elucidated by studying retroviruses bearing *v-onc* genes. Others were found by reading through integration loci to identify adjacent cellular genes (Table 3). Often these were re-discoveries of homologues of previously known *v-onc* genes, e.g. c-*myc* and v-*myc* in avian, murine and feline retroviral tumours. In other cases, like MMTV int-1 (Wnt-1) and int-2 (fibroblast growth factor), the genes were first characterized as integration sites.

Certain oncogenes are frequently implicated in viral and non-viral tumours. Thus *myc* was first characterized as the v-*onc* of the avian myelocytoma virus, MC-29. Then it was found to be the most frequent *cis*-activated cellular oncogene of B-cell lymphoid leukaemia induced by avian leukosis viruses[4]. In human Burkitt's lymphoma, c-*myc* is also activated, in this case by transposition of the c-*myc* locus on chromosome 8 to become adjacent to active immunoglobulin promotors on chromosome 14 (Ig heavy chain), chromosome 2 (Ig *k* light chain), or chromosome 22 (Ig *l* light chain). Later, a related gene, N-*myc*, was identified in neuroblastoma. Thus integration of a retroviral LTR and chromosome translocations have the same net effect in activating cellular oncogenes.

Oncogene-bearing retroviruses (Figure 2 *b*) usually cause tumours with a short latent period. Oncogenic retro-

Table 1. Retrovirus genera

Genus	Example	Genome structure
Alpharetrovirus	RSV, ALV	Simple
Betaretrovirus	MMTV, SRV	Simple
Gammaretrovirus	MLV, FeLV	Simple
Deltaretrovirus	HTLV, BLV	Complex
Epsilonretrovirus	WDSV	Simple
Lentivirus	MVV, HIV	Complex
Spumavirus	SFV	Complex

ALV, avian leukosis virus; BLV, bovine leukosis virus; FeLV, feline leukaemia virus; HIV, human immunodeficiency virus, HTLV, human T-lymphotropic virus; MLV, murine leukaemia virus; MMTV, murine mammary tumour virus; MVV, Maedi-visna virus; SFV, simian foamy virus; SRV, simian retrovirus D-type; WDSV, Walleye dermal sarcoma virus of fish.

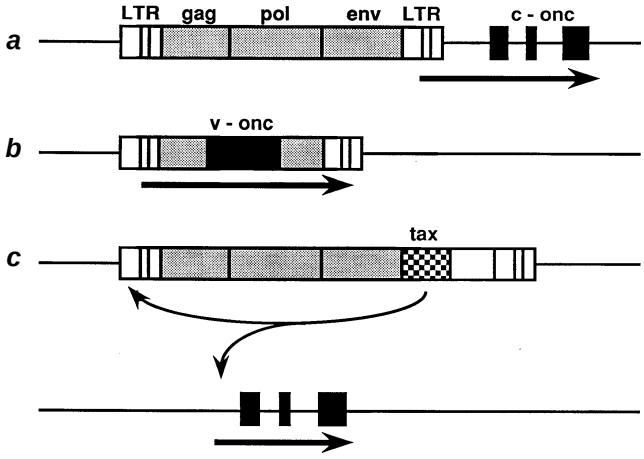

Figure 2. Three molecular mechanisms of retroviral transformation. ***a***, Insertional activation of oncogene; ***b***, Transduction of oncogene; ***c***, Trans-activation of oncogene[43].

Table 2. Oncogenes originally identified in transforming retroviruses

Oncogene	Protein function	Host species	Tumour
abl	Kinase	Mouse, cat	Pre-B-cell leukaemia
akt	Kinase	Mouse	T-cell lymphoma
crk	Kinase activator	Chicken	Sarcoma
erb-a	TH-R	Chicken	Erythroleukaemia
erb-b	EGF-R	Chicken	Erythroleukaemia
ets	TF	Chicken	Myeloid leukaemia
fes/fps	Kinase	Chicken/cat	Sarcoma
fgr	Kinase	Cat	Sarcoma
fms	Kinase	Cat	Sarcoma
fos	TF	Mouse	Osteosarcoma
jun	TF	Chicken	Fibrosarcoma
kit	Kinase	Cat	Sarcoma
mil/raf	Kinase	Chicken/mouse	Sarcoma
mos	Kinase	Mouse	Sarcoma
myb	TF	Chicken	Myeloid leukaemia
myc	TF	Chicken	Myelocytoma, lymphoma, carcinoma
h-ras	G-protein	Rat	Sarcoma
k-ras	G-protein	Rat	Sarcoma
rel	TF	Turkey	Reticuloendotheliosis
ros	Kinase	Chicken	Sarcoma
sea	Kinase	Chicken	Sarcoma, leukaemia
sis	PDGF	Monkey	Sarcoma
ski	TF	Chicken	Carcinoma
src	Kinase	Chicken	Sarcoma
yes	Kinase	Chicken	Sarcoma

EGF-R, epidermal growth factor receptor; PDGF, platelet derived growth factor; TH-R, thyroid hormone receptor; TF, nuclear transcription factor. Adapted from ref. 45.

Table 3. Examples of oncogenes activated by proviral insertion

Oncogene	Protein function	Host species	Tumour
abl	Kinase	Mouse	Myeloma
erb-b	EGFR	Chicken	Erythroblastosis
fis-1	Cyclin D1	Mouse	T-cell lymphoma
fos	TF	Chicken	Nephroblastoma
int1/wnt1	GF	Mouse	Mammary carcinoma
int2	FGF	Mouse	Mammary carcinoma
myc	TF	Chicken Cat, Mouse	T-cell leukaemia T-cell leukaemia
N-myc	TF	Mouse	T-cell leukaemia
notch1	Receptor	Mouse	T-cell leukaemia
pim1/2	Kinase	Mouse	Lymphomas
tpl2	Kinase	Rat	T-cell lymphoma

GF, growth factor; FGF, fibroblast GF. Adapted from ref. 4.

viruses that lack oncogenes (Figure 2 *b*) induce tumours with a much longer latent period. Only a minute proportion of the infected cells will acquire proviruses integrated adjacent to c-*onc* genes, and these cell clones will require further mutations to evolve full malignancy. Moreover, several of the infectiously transmissible retroviruses such as murine and feline leukaemia viruses do not exert an oncogenic effect until they recombine with ERV *env* sequences to generate retroviruses with a changed cell tropism.

Human T-cell leukaemia viruses

Human T-cell leukaemia virus type I (HLTV-I) was first isolated in 1980 in R. C. Gallo's laboratory at the National Cancer Institute in USA[5]. It was detected in a T-lymphocyte cell line derived from a black American patient described as having an aggressive form of 'mycosis fungoides' cutaneous T-cell lymphoma. The same group made a second isolate from a White American sailor described as having the leukemic form of the lymphoma, 'Sezary syndrome'. In Japan, HTLV-I was soon linked with a T-cell leukaemia-lymphoma called Adult T-cell Leukaemia (ATL)[9,10]. This specific type of leukaemia had earlier been distinguished as being the most prevalent lymphoid leukaemia of adults in Japan. In the UK, Catovsky noted a leukaemia resembling ATL in West Indian immigrants, and it soon became evident that the occurrence of the malignancy correlated geographically and ethnically with a relatively high frequency of HTLV-I infection, particularly in South-West Japan and among blacks in the Americas[10,11]. With hindsight, one can rediagnose the index American patients as suffering from ATL. ATL is a malignancy of a mature type of T-cell expressing the CD4 antigen; it overexpresses the CD25 antigen which is the *b*-chain of the interleukin-2 receptor (Figure 3).

In 1985, HLTV-I became linked to an entirely distinct disease, tropical spastic paraparesis (TSP), which was first recognized in Jamaica 25 years earlier[12]. In Japan TSP is called HTLV-I associated myelopathy (HAM)[13]. HAM/TSP presents as a progressive, debilitating disease associated with demyelination. It does not appear that oligodendrocytes or neurones are themselves infected by HTLV-I; rather the virus is confined to T-lymphocytes that invade the nervous system. HAM/TSP is character-

ized by the infiltration of CD8 positive cytotoxic T-lymphocytes (CTL) with a specificity for a peptide epitope of the HTLV-I Tax protein[14]. Many asymptomatic HTLV-I infected individuals carry CTLs with the same specificity[15], so the underlying immunobiology of HAM/TSP is unclear. The outward similarity of HAM/TSP to multiple sclerosis has led several groups to search for related retroviruses in MS. Apart from some claims which have not been upheld, it seems unlikely at present that a virus closely related to HTLV is responsible for MS. One implicated retrovirus[16] is related to HERV-W and is probably endogenous itself. HTLV-I is associated with a number of other symptoms, including polymyositis and immune deficiency, which like HAM/TSP might arise from autoimmune consequences of virus infection. These require much more study before a clear picture will emerge.

With further serological surveys, it became clear that HTLV-I infects human populations at low prevalence worldwide[17,18], though it is infrequent in India. In Japan, it is present among Ainu aborigines in Hokkaido as well as in the main reservoir in Kyushu and Shikoko. It has been found in Melanesian island populations, and in West Africa, whence it probably travelled with the slave trade to the New World. HTLV-I isolates have been made from Romanians, from a large Jewish kindred of Iranian descent now living in Italy and USA, and sporadically from other ethnic groups.

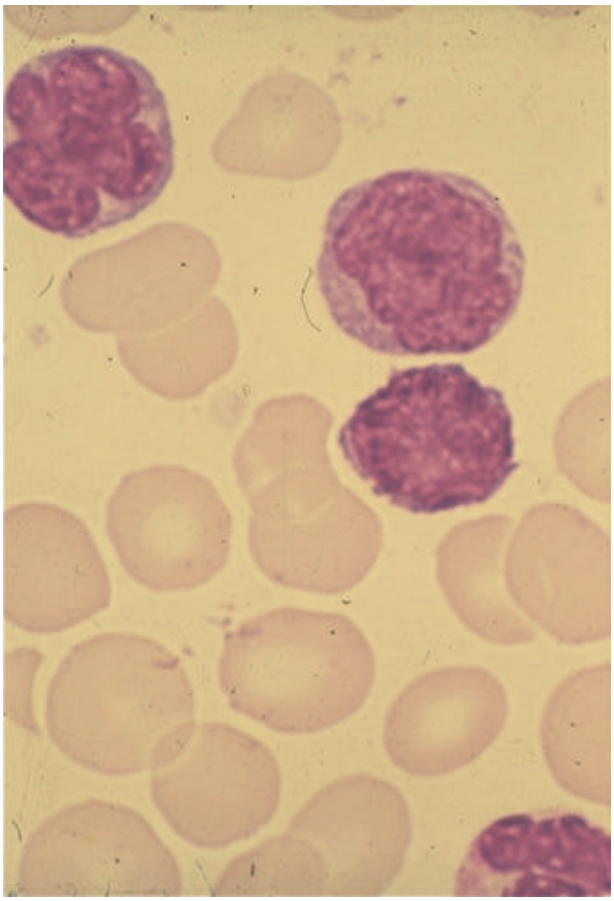

Figure 3. Adult T-cell leukaemia blood smear showing a typical 'flower cell' at top left.

HAM/TSP appears to be more prevalent in individuals who have become infected through contaminated blood transfusions than those who have acquired HTLV-I naturally. Wherever HTLV-I infection occurs, it seems that the majority of persons carrying life-long infection are unlikely to develop HTLV-I associated diseases. It is estimated[17] that less than 5% infected individuals develop either ATL or TSP. However, a broader spectrum of haematological disorders is becoming linked with HTLV-I, so the overall incidence of disease may need correction.

Several studies, mainly in Japan, have shown that the major means of HTLV-I transmission is from mother to infant via the milk[17]. A small proportion of children may also acquire infection pre- or peri-natally. The virus is sexually transmissible with a low risk of transmission and a bias of male to female spread. More than 70% of seronegative recipients of HTLV-positive blood become infected, but plasma and other cell-free blood products are not infectious. This contrasts the transmission of HTLV-I to that of HIV, but is consistent with the relative difficulty of achieving cell-free transmission of this virus experimentally in culture. Thus the infected cell appears to be the infectious unit. In Japan, blood donations have been universally screened for HTLV-I antibodies since 1985, which has drastically diminished the iatrogenic transmission rate. Pregnant mothers are also screened for HTLV-I and if positive, they are advised not to breastfeed their infant[19]. While this preventive health policy may be apt for a developed country like Japan, its introduction is debatable for less developed countries with a high prevalence of infantile infection by both HTLV-I and enteric pathogens.

A second strain of human T-cell leukaemia virus, named HTLV-II, was isolated from a white American man diagnosed as having an indolent form of hairy cell leukaemia[20]. In fact, an immortalized T-cell line was derived from this patient, and the virus was detected by chance reverse transcriptase assay of this cell line some four years after it was established. The patient, however, had antibodies specific to HTLV-II, and virus was re-isolated from a fresh blood sample. For many years it was not known in which human populations HTLV-II was naturally endemic. We had noted that 6% of intravenous drug users in London were seropositive for this virus[21] and it subsequently became apparent that HTLV-II has an even higher prevalence among injecting drug users in the USA. Later, it was found that HTLV-II infection occurs in several native American population groups in North, Central and South America, e.g. among the Guaymi Indians in Panama[22]. The distribution of HTLV-II is far too widespread among native Americans to be accounted for by injecting drug habits; rather, this virus has probably spread to the drug using groups through its long-term

endemicity in indigenous Americans. To date, no disease has been ascribed to HTLV-II although there may be neurological symptoms milder than HAM/TSP.

Both HTLV-1 and HTLV-II can immortalize[4,9] T-lymphocytes grown in culture after lectin and interleukin-2 (IL2) stimulation, rather like the immortalization of B-lymphocytes by Epstein-Barr virus. Many of these cell lines and those derived from ATL patients eventually become independent of IL 2 *in vitro*. In culture, there appears to be no discernable difference between *in vitro* transformed cells and cells from ATL blood samples, in contrast to EBV-lymphoblastoid cell lines. However, it is likely that the cells growing from ATL blood are in fact not derived from the malignant clone and they may be newly immortalized by HTLV-I. *In vitro*, HTLV-I genes do not appear to be expressed in the typical ATL 'flower' cells of the peripheral blood (Figure 3), but presumably the precursor cells expressed at least the gene that determines transformation. This is believed to be the *tax* gene which acts as a transcriptional activator of the HTLV LTR. Because Tax elicits cytotoxic T-cell responses, it tends to be shut-off in the majority of tumour cells.

From the foregoing discussion, it would appear that HTLV-I acts early in the complex process of oncogenic transformation, but other events besides Tax-transactivation appear to be required for the emergence of a malignant clone in the infected individual. Oligoclonal and monoclonal premalignant T-lymphoblast populations representing up to 10% of the leukocyte count are sometimes found in otherwise asymptomatic individuals, only some of whom later progress to full malignancy. This condition is sometimes called 'smouldering leukaemia'. Although numerous chromosome anomalies, often involving chromosome 7, have been observed in ATL cells, precise and consistent changes like the 8;14 translocation in Burkitt's lymphoma have not been found. Neither did an analysis of integration sites by the HTLV-I provirus in different ATL patients reveal a common chromosomal site[23]. Overall, we are only at the beginning of our understanding of HTLV-I oncogenesis at the molecular level.

HIV and cancer

There are two major types of human immunodeficiency virus, HIV-1 and HIV-2. HIV-1 infection is unfortunately now pandemic. HIV-2 is still largely confined to West Africa, although HIV-2 occurs in India. Some 37 million people currently harbour HIV, not counting the 22 million who have already died from AIDS[24]. India is second only to South Africa in encompassing the greatest number of people infected by HIV. HIV-1 and HIV-2 do not appear to be directly oncogenic, and neither have animal lentiviruses been linked with cancer. Nevertheless, together with *Pneumocystis carinii* pneumonia, Kaposi's sarcoma (KS) was the salient attribute of AIDS when it was first recognized in the USA in 1981.

Kaposi's sarcoma

Until the AIDS pandemic, Kaposi's sarcoma was considered to be a rare tumour in certain Eastern European and Mediterranean populations and to be endemic in parts of central and East Africa[1,25]. There is a strong predominance of KS in males, except in African children, and outside Africa non-AIDS KS occurs mainly in men over sixty years old. There is also a 400-fold relative risk (RR) of KS in immunosuppressed transplant patients[26], although owing to the extreme rarity of KS in the Western population the absolute incidence is still low. The relative risk of KS in HIV-infected, adult male American homosexuals less than sixty years old is some 10,000-fold higher than that of their counterparts in the general population[1]. With the advent of AIDS, KS has become the most common of all tumours in sub-Saharan Africa[27].

The epidemiology of KS before and after the advent of AIDS suggested that a transmissible agent may underlie the tumour[1], and several candidate agents including cytomegalovirus and papilloma viruses were put forward. However, a novel virus, Kaposi's sarcoma-associated herpesvirus[28] or human herpesvirus 8 (KSHV or HHV-8) was discovered in 1994 and proved to be the aetiological agent. KSHV is a g2-herpesvirus related to herpesvirus saimiri of squirrel monkeys, and more distantly related to Epstein-Barr virus (EBV).

KSHV DNA is found in the great majority of KS biopsies (Table 4). The viral genome and latent nuclear antigen are present in the spindle cells which represent tumour cells in KS[29,30]. KSHV detection in the blood of HIV-positive patients presages the development of KS[31]. Serological surveys indicate that KSHV is more prevalent in geographic regions and in risk groups where KS incidence is high[32,33]. Unlike EBV, therefore, KSHV is not an ubiquitous herpesvirus. Like EBV, it is mainly transmitted by saliva[34] and tracks vertically within families[35].

Both KSHV and HIV infection are independent and highly significant risk factors in the development of KS in AIDS[36]. The major effect of HIV is immune suppression; the lower the CD4 T-helper lymphocyte, the higher the risk of KS in KSHV-positive patients[31]. However, it has been suggested that the Tat protein of HIV-1, but not of HIV-2, may play a more direct role in KS pathogenesis by acting synergistically with cellular growth factors[37]. This

Table 4. Detection of KSHV DNA by PCR in Kaposi's sarcoma biopsies

Type of KS	No. tested	% Positive
AIDS	259	97
Classic mediterranean	175	91
Endemic African	80	89
Post-transplant	13	100
Other tumours	743	1.8

Adapted from ref. 25.

is in accord with the observation that KS occurs more frequently in KSHV-positive patients with HIV-1 than with HIV-2 infection[38].

AIDS lymphomas

The major tumour associated with HIV infection is non-Hodgkin lymphoma (NHL)[1,2]. Most AIDS-associated lymphomas are B-cell immunoblastic lymphomas, but Burkitt's lymphoma (BL) is also seen at a much higher frequency in AIDS cases than in matched HIV-negative persons. These lymphomas are frequently but not always positive for Epstein-Barr virus. EBV-negative NHL in AIDS has been noted, in particular, in patients who acquired HIV infection by an intravenous route[39]. NHL in AIDS frequently occurs in the brain and BL in the gut, presenting relatively early in immunosuppression. Overall, AIDS-associated NHLs represent a diverse group of B-lymphoid tumours. Although HTLV-I and HTLV-II infections are prevalent among HIV-infected intravenous drug users, an increase in T-cell lymphoma has not been recorded. The CD4-positive, HTLV-transformed cells in these individuals would, of course, be susceptible to HIV infection.

In HIV-positive patients, KSHV is also associated with two distinct forms of lymphoproliferative disease[25]: primary effusion lymphoma[40] and plasmablastic multicentric Castleman's disease[30,41]. Just as EBV causes Burkitt's lymphoma, immunoblastic lymphoma in immune deficiency, and nasopharyngeal carcinoma, so this herpesvirus underlies several types of tumour.

Other cancers

Further, neoplasms have an increased incidence in AIDS patients[2], most notably premalignant warts and cervical and ano-genital squamous carcinomas. But it is not yet clear to what extent HIV infection is a specific risk, because the risk of infection by genital human papilloma viruses (HPV) and by HIV coincide in sexual transmission. There is also some indication of a more aggressive course and possibly higher incidence of Hodgkin lymphoma, liver cancer and testicular seminoma and teratocarcinoma[1]. The immunosuppression induced by HIV may exacerbate the progression to malignancy of HPV-infected epithelial cells. In view of the increased incidence of other tumours in immunosuppressed transplant patients, such as skin cancers including melanoma as well as KS and NHL[1,2], it will be interesting to ascertain whether these tumours also occur at a higher frequency in AIDS patients. Preliminary evidence indicates that this is so, but care must be taken that the increase is not simply accounted due to an increased exposure to other carcinogens by the same patients. As the subset of tumours in humans and animals that develop in immunosuppression tend to have a viral aetiology, AIDS is providing a window on immunosuppression and cancer more generally. In India, it will be important to determine whether oral cancer is increased in AIDS.

Thus the cancers associated with AIDS may be considered to be 'opportunistic neoplasms' in the same way as certain opportunistic infections by viruses, bacteria, mycobacteria, fungi, and protozoa are characteristic of AIDS. In the case of KS, as discussed above, the immunosuppression may allow proliferative lesions driven by KSHV to develop, which would otherwise be kept under immunological control. In the case of B-cell lymphoma, immunosuppression clearly plays a role, but it is noteworthy that a salient feature of the early immune dysregulation in AIDS is a hyperplasia of B-cells. The continuous activation of B-cell proliferation in HIV-infected persons may allow premalignant clones to expand, thus increasing the probability of lymphoma emerging. This model is analogous to the role of holo-endemic malarial infestation promoting children's BL in Africa.

Human endogenous retroviruses

Human chromosomes contain numerous 'endogenous' retrovirus genomes which are part of our normal genetic constitution, shared with other primates[7,8]. Most of these genomes are defective and do not possess open reading frames. It is conceivable, however, that the endogenous LTRs might be active, and might become transposed in rare cases of human cancer. Because so many genomes exist, it has been difficult to discern any specific link with disease from the background of endogenous genome polymorphism. Some of the endogenous genomes can express proteins, and there is renewed interest in determining whether they may be linked with disease. Reverse transcriptase activity derived from endogenous retrovirus has been detected in preleukaemic states such as polycythaemia vera and essential thrombocythaemia[42]. Amplification of retroviral cDNA by the polymerase chain reaction is now permitting the characterization of the endogenous genomes specifically expressed in these precancerous cells. It remains to be elucidated whether these viral genes will be causally associated with disease. In mice, the minor lymphocyte antigens that act as superantigens have been identified as endogenous retroviral proteins[4]; the significance, if any, of their human counterparts has only begun to attract interest.

HERV-K genomes are expressed in testicular teratocarcinoma and seminoma[7]. Similar genomes are expressed in certain breast cancer cell lines[8]. In testicular tumour patients antibodies to HERV-K often are detectable. However, it remains difficult to determine whether HERV-K is involved in pathogenesis, or whether it is simply a tumour marker.

Retroviruses and cancer gene therapy

Gene therapy is opening up the prospect of new treatment for some otherwise intractable diseases. While considerable effort has been focused on inherited disorders caused to single gene defects, cancer treatment represents the majority of clinical trials involving gene therapy. For example, the introduction of genes encoding cytokines is being explored in the treatment of melanoma and other malignancies and prodrug therapy by activating enzymes (e.g. herpesvirus thymidine kinase) in glioblastoma and other tumours. Among the vehicles to be used for gene therapy in human cancer, gene therapy are retroviral vectors[4]. In this way retroviruses may prove to play a useful role in the treatment of human disease as well as having a pathological role in its causation. Retroviral vectors, however, carry an inherent risk of promoting cancer as well as controlling it by the nature of their integration into chromosomal DNA (Figure 1).

For example, replication-competent recombinants of Moloney leukaemia virus vectors have caused tumours in experimentally infected monkeys[43]. Third generation retroviral vectors have been constructed to minimize the chance of recombination and rescue of infectious genomes, but this will not obviate integration of the proviral form of the infecting vector, which is after all the purpose of utilizing a retroviral vector in the first place. It will therefore be important to monitor patients who have received gene therapy via retroviral vectors for increased cancer incidence, though such an untoward consequence of treatment may take decades to become manifest.

Concluding remarks

The identification of viral causes of cancer should lead to more precise diagnosis, better treatment and prevention of disease. Prevention is already evident for ATL, since HTLV-I can be screened diagnostically and vaccine tests are protective in animals experimentally challenged with the virus. Cancer is a complex, multifactorial set of diseases involving many steps in oncogenesis. Moreover, virus-associated cancers develop only in a small proportion of infected individuals. Thus proper methods and criteria for causality[44] can guide our understanding of virus-associated cancers.

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