

THE SEARCH FOR INFECTIOUS CAUSES OF HUMAN CANCERS: WHERE AND WHY

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by

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Slightly more than 20% of the global cancer burden can currently be linked to infectious agents, including viruses, bacteria and parasites. This manuscript analyses reasons for their relatively late discovery and highlights epidemiological observations that may point to the involvement of additional infectious agents in specific human cancers. Emphasis is placed on haematopoietic malignancies, breast and colorectal cancers, but also basal cell carcinomas of the skin and lung cancers in non-smokers.

INTRODUCTION

Present state of the global cancer burden

Currently a large number of infectious agents have been identified which either cause or contribute to specific human cancers (reviewed in zur Hausen, 2006). They include two members of the herpes virus family, Epstein-Barr virus and human herpes virus type 8, high risk and low risk human papillomaviruses (HPV), Hepatitis B and C viruses, a recently identified human polyomavirus, Merkel cell polyomavirus (Feng *et al.*, 2008), the human T-lymphotropic retrovirus type 1 (HTLV-1), and human immunodeficiency viruses (HIV) types 1 and 2. In addition, human endogenous retroviruses have been suspected to play a role in human cancers. Besides viruses, other pathogens have also been identified. They include the bacterium *Helicobacter pylori*, a major contributor to gastric cancer, and parasitic infections, here in particular *Schistosoma haematobium*, a major cause of bladder cancer in Egypt, and liver flukes. The latter, *Opisthorchis viverrini* and *Clonorchis sinensis*, are important factors for cholangiocarcinomas and hepatocellular carcinomas in South-eastern Thailand and Southern China. Figure 1 shows an estimate of the present contribution of infectious agents to global cancer incidence.

Cancers due to 5 infections correspond to 18.6% of total cancer incidence

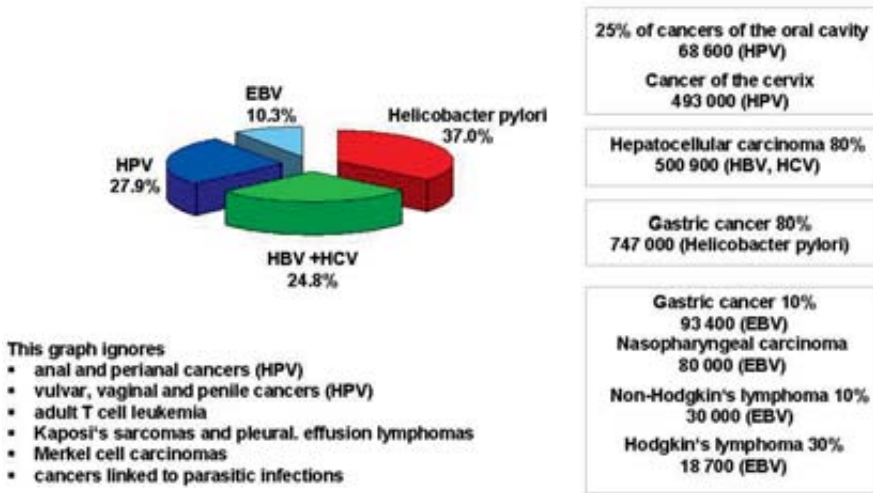


Figure 1. Estimated annual global cancer incidence due to infections (with inclusion of data from Parkin *et al.*, 2002; modified from zur Hausen, 2006).

It is important to note that there exist vast gender differences in the global role of papillomaviruses in human cancers. This is mainly due to the role of this virus family in the induction of cancer of the cervix. More than 50% of cancers linked to infections in females are caused by HPV infections. In males only approximately 4.3% of cancers have been linked to this virus family.

PROBLEMS IN IDENTIFYING INFECTIOUS AGENTS INVOLVED IN HUMAN CANCER INDUCTION

1. Why has it been so difficult to identify infectious agents as causative factors for human tumours?

The search for an infectious cause of at least some human cancers dates back to the second half of the nineteenth century (reviewed in zur Hausen, 2006). Yet the first hints for a role of infectious agents in human cancers date back to the beginning of the 20th century, when *Schistosoma* infections in Egypt and liver flukes in Eastern Europe and Asia were suspected to play a role in the development of bladder and liver cancers. In spite of intensive search, it took approximately 65 additional years before further evidence was obtained, namely by linking a specific virus, Epstein-Barr virus, to two human cancers, Burkitt's lymphoma and nasopharyngeal carcinoma. During the past three or four decades progress has been more rapid, currently linking about 20% of the global cancer incidence to infectious events.

Why has it been so difficult to identify infectious agents as causative factors for human cancers? Several reasons seem to provide an explanation:

1. Because no human cancer arises as the acute consequence of infection. The latency periods between primary infection and cancer development are frequently in the range of 15 to 40 years. The X-chromosome-linked lymphoproliferation (XLLP) represents a rare exception. Based on a specific host cell mutation, Epstein-Barr virus here causes an acute lymphoproliferative disease.
2. Besides some rare exceptions, no synthesis of the infectious agents occurs in cancer cells.
3. Most of the infections linked to human cancers are common in human populations; they are ubiquitous. They were present during the whole human evolution process. Yet only a small proportion of infected individuals develops the respective cancer type.
4. Mutations in host cell genes or within the viral genome are mandatory for malignant conversion.
5. Chemical (e.g. aflatoxin) and physical carcinogens (e.g. ultraviolet light in Epidermodysplasia verruciformis) usually act as mutagens. They facilitate the selection of specific mutations and frequently act synergistically with carcinogenic infectious agents.
6. Some infectious agents act as indirect carcinogens, without persistence of their genes within the respective cancer cells (HIV, *Helicobacter pylori*, *Schistosoma haematobium*, Hepatitis C and B).

Among all these factors, the ubiquity of most of these infections and the long time periods required for malignant transformation were the main reasons for the remarkable difficulties in identifying their carcinogenic functions.

2. *Epidemiology provided hints for a successful search*

a. *Geographic coincidence*

Geographic coincidence of a specific infection (Hepatitis B) and of liver cancer led to the original suspicion that this infection may predispose the patient to the subsequent development of hepatocellular carcinomas (reviewed in zur Hausen, 2006). The additional contribution of a chemical carcinogen was also suspected, based on similar observations. Figure 2 reveals the geographic distribution of Hepatitis B virus infections and hepatocellular carcinomas. Geographic clustering of specific cancers may, however, also result from other causes: Countries with a high rate of heavy smokers also experience a high incidence of lung cancer. The intensive solar exposure of Caucasian populations in Australia, South Africa and South America is responsible for a high percentage of skin cancer patients.



Figure 2. Geographic distribution of Hepatitis B infections (left) and of hepatocellular carcinomas (right). Modified from figures provided by CDC and Globocan 2002.

b. Regional clustering of cases

Regional clustering of specific cancer types triggered some investigations on a potential role of infectious agents in these malignant proliferations. Burkitt's lymphoma in equatorial Africa represents one of the most illustrative examples. Burkitt noted the apparent dependence of tumour incidence on climatic conditions and altitude and described the regional correlation with holoendemic *Plasmodium falciparum* infections (Burkitt, 1962). As a consequence he speculated that the tumour might be due to a viral infection, transmitted by an arthropod vector, possibly the same one carrying malaria parasites.

Nasopharyngeal carcinoma, occurring at high frequency in specific regions of South East Asia, represents another example. Adult T-cell leukaemia in the coastal regions of Southern Japan, cholangiocarcinomas in South East Thailand and bladder cancer in the Nile Delta or along the Nile River also raised early suspicions of an infectious origin. These observations resulted in speculations; they could not prove the underlying hypothesis by themselves.

c. Dependence on sexual contacts

If one disregards the occurrence of scrotum cancer in chimney sweepers, the early studies of Rigoni-Stern in Verona, Italy, pointing in 1842 to the role of sexual contacts in the causation of cervical cancer, represent a particularly interesting example of suspected contact transmission of a human cancer. It took another 140 years before viral infections were identified as causing this frequent cancer in women. These observations led to the identification of additional anogenital and oral cancers linked to the same virus infections.

d. Cancers arising under immunosuppression

Epidemiological surveys identified immunosuppression as a condition resulting in the appearance of remarkably specific forms of cancer. Many of those malignancies have by now been shown to be caused by reactivated viruses, whose oncogenic potential is usually suppressed by immunological reactions. The most prominent tumours arising here are Epstein-Barr virus caused B-cell lymphomas, Kaposi's sarcomas linked to human herpesvirus type 8 reactivation, and Merkel cell carcinomas of the skin associated with a novel

polyomavirus. The initial discovery of the viral origin of cervical cancer and its precursor lesions was not based on the moderately enhanced incidence under immunosuppression. Specific types of common warts as a non-malignant proliferative condition also occur at high frequency in immunosuppressed patients, mainly containing of genus-Beta papillomaviruses. The viral origin of basal and squamous cell carcinomas of the skin, frequently found in these patients, remains controversial.

MECHANISTIC ASPECTS OF CANCER INDUCTION BY INFECTIONS

Figure 3 lists identified mechanisms by which infections may contribute to cancer development. The expression of specific viral oncogenes as a mandatory precondition for the maintenance of the malignant phenotype has been identified as directly contributing to human carcinogenesis (reviewed in zur Hausen, 2006). A novel mode of direct viral carcinogenesis has probably been identified in Merkel cell carcinomas, where functional inactivation of the helicase part of the large T-antigen of the Merkel cell polyomavirus renders the viral DNA replication-incompetent (Shuda *et al.*, 2008). Viral DNA persisting in normal tissues seems to retain replication competence.

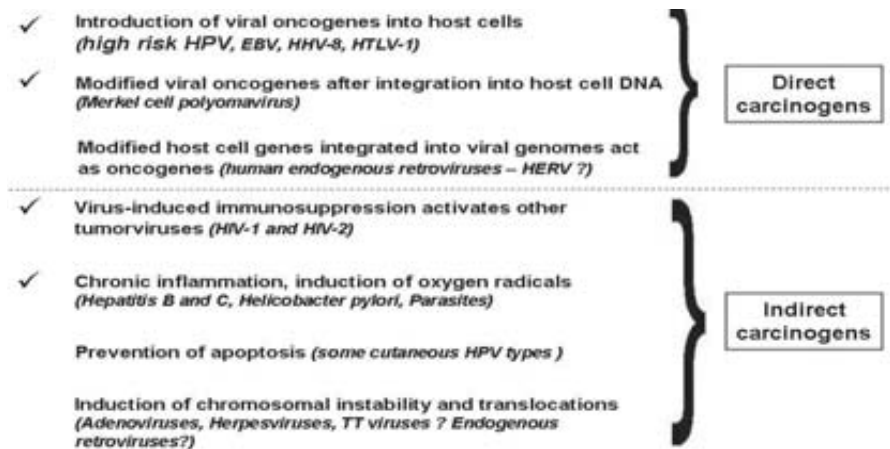


Figure 3. Summary of identified mechanisms by which infections either directly or indirectly contribute to carcinogenesis. Mechanistic contributions of infections to human cancers have been marked.

The most prominent indirect infectious carcinogens are agents causing immunosuppression or inducing reactive oxygen species via inflammatory reactions. Whereas the mechanism of immunosuppression induced by human immunodeficiency viruses (HIV) or after organ transplantation is reasonably well understood, the accurate mechanism by which Hepatitis B and C viruses, *Helicobacter pylori* and carcinogenic parasitic infections contribute to cancer still remains somewhat obscure.

WHERE IS IT WORTHWHILE TO SEARCH FOR AN INFECTIOUS ETIOLOGY OF HUMAN CANCERS NOT YET LINKED TO INFECTIONS?

When we summarise infectious agents that have been discovered during the past 15 years, it is interesting to note that several novel viruses belonging to potentially carcinogenic virus families have been identified even during the past two years (Figure 4). This raises the suspicion that additional links to novel or already identified infectious agents to cancers, hitherto not linked to infections, will become apparent. Thus it appears worthwhile to search for cancer-related epidemiologic observations that may point to the involvement of infectious agents in cancers hitherto not linked to infections. The following will summarise some hypotheses and considerations based on these reports.

Year	Virus	Symptoms	Natural Host
1994	Sabia virus	Hemorrhagic fever	Rodents
1994	Hum. Herpesvirus 8	Kaposi's sarcoma	Humans
1994	Hendravirus	Encephalitis	Bats, horses
1997	Influenza H5N1	Avian flue	Birds
1997	TT viruses	?	Humans
1998	Nipah virus	Encephalitis	Bats, pigs
2003	SARS Coronavirus	SARS	Chinese bushcat
2005	Bocavirus (parvovirus)	Acute wheezing	Humans
2005	New coronavirus	Respiratory symptoms	Humans
2007	KI-polyomavirus	?	Humans
2007	WU-polyomavirus	?	Humans
2008	MC-polyomavirus	Merkel-tumor	Humans
2008	Lymphotrop. polyomavirus	Periph. blood PML patients	Humans

Within the same time period at least 30 novel types of human papillomaviruses have been identified

Figure 4. "New" human pathogenic viruses, 1994–2008. The light arrows identify important human pathogens or a whole novel virus family (TT viruses). The dark arrows point to established or potentially oncogenic virus isolates.

1. Cancers occurring under immunosuppression

A review published in 2006 by Vajdic and colleagues demonstrates a large number of cancers occurring at increased frequency under immunosuppression after kidney transplantation. Kaposi's sarcoma, mainly found in HIV-infected patients, stands out and is found about 200 times more frequently in these patients compared to non-infected controls (Figure 5). The most interesting part of Figure 5 appears to be the 7–8 fold higher rate of vulvar and penile cancer in comparison to cancer of the cervix. The vast majority of cervical cancers are caused by high risk human papillomavirus (HPV) infections. In vulvar and penile cancers only 30–50% seems to be linked to the same HPV infections. The etiology of 50–70% of these cancers is unknown. Interestingly, the age distribution of HPV-positive and HPV-negative vulvar and penile cancers differs in that the negative tumours regularly occur in older age groups. Thus, the negative group should require attention as pos-

sible candidates for an unknown viral etiology. Unidentified types of HPVs or novel polyomaviruses may represent interesting candidates. Salivary gland, eye, thyroid and tongue cancers should also deserve attention.

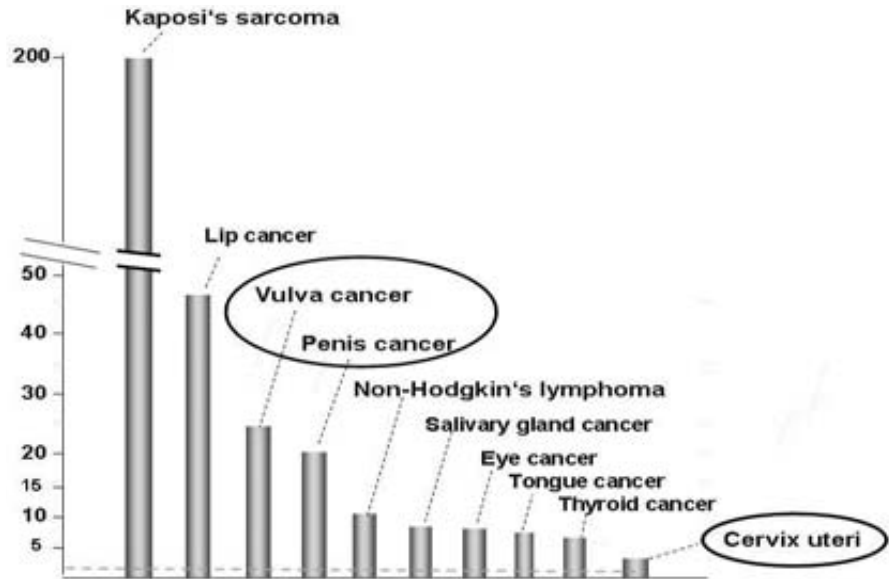


Figure 5. Some of the most frequently occurring cancers occurring after kidney transplantation (modified from Vajdic *et al.*, 2006). The dotted line indicates the incidence in immunocompetent patients.

2. Cancers not elevated or even reduced after immunosuppression

2a. Breast cancer as an example

Some cancers do not show an increased incidence during immunosuppression. Indeed, immunosuppression may even possess a protective effect against some of these tumours. Those cancers are shown in Figure 6. Besides prostate, rectal and brain tumours, human breast cancer represents a particularly intriguing malignancy, because murine mammary cancer is also not increased under immunosuppression. This latter tumour is caused by a retrovirus infection, murine mammary tumour virus (MMTV).

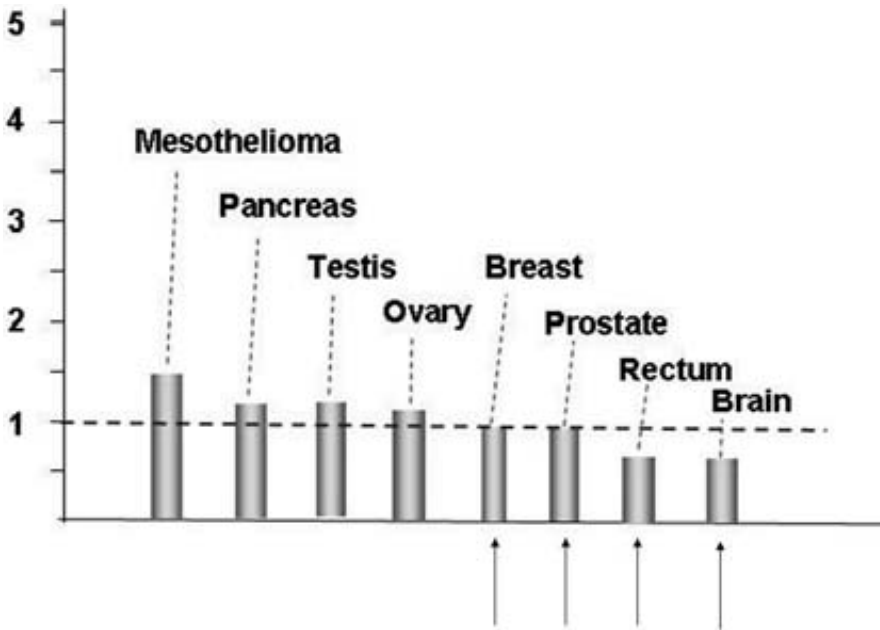


Figure 6. Cancer incidence marginally or not affected during immunosuppression after kidney transplantation (modified from Vajdic *et al.*, 2006).

In murine mammary tumours, the mechanism of a slightly protective effect exerted by immunosuppression is partially understood (see review zur Hausen, 2006). It is schematically outlined in Figure 7. The primary infection occurs via the milk of the infected mother. The virus reaches the Peyer's patches where it infects B- and T-lymphocytes. Superantigen induction in the infected cells leads to reactive T-cell depletion and immunotolerance. The superantigen expressing cells produce high quantities of infectious MMTV; this substantially increases the risk for the infection of mammary tissue. Specific integration of the MMTV proviral DNA in the mammary cells emerges as the prime risk factor for the resulting mammary carcinomas. Immunosuppression of such infected animals apparently interferes with the emergence of superantigen-producing T- and B-lymphocytes and, as a consequence, suppresses virus production which in turn decreases the risk for cancer development.

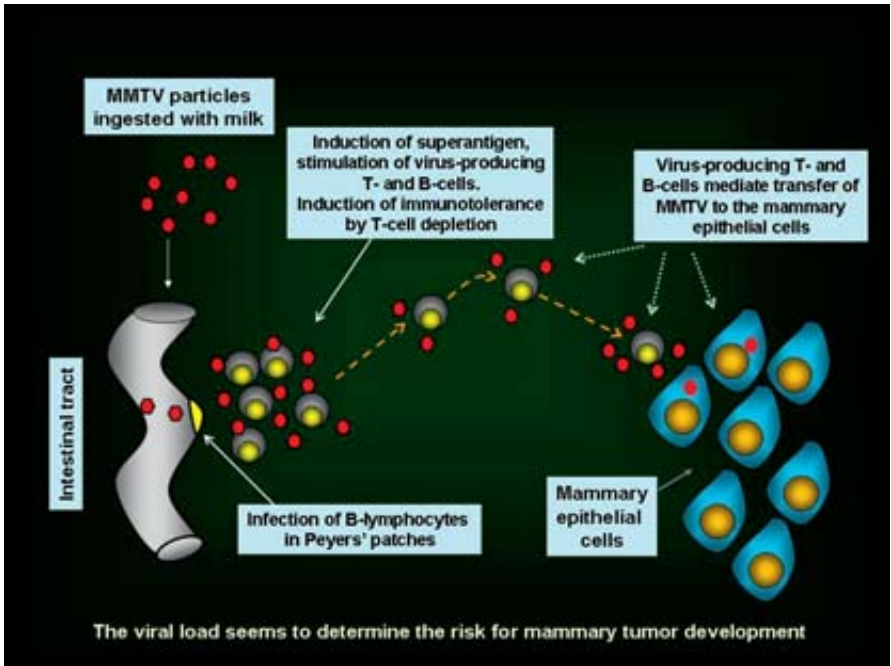


Figure 7. Schematic outline of events following infection of newborn mice with murine mammary tumour virus (modified from zur Hausen, 2006).

Is it possible that a similar mechanism contributes to human mammary cancer? A few data seem to support this notion. They may point to a possible involvement of a specific subgroup of human endogenous retroviruses (HERV) in this malignancy. At least 8% of our genome consists of retroviral sequences acquired in the course of human evolution. Although the vast majority of these sequences no longer reveal functional open reading frames, members of one subgroup HERV-K, which entered our germ line approximately 800,000 years ago, are still able to code for complete, though non-infectious virus particles. Retroviral gag and env transcripts of the 22q11.21 region are found in these particles (Ruprecht *et al.*, 2008). Correction of stop codons in HERV-K sequences resulted, moreover, in the re-constitution of infectious HERV-K viruses (Dewannieux *et al.*, 2006, Lee and Bieniasz, 2007). HERV-K expression also becomes activated by other virus infections: HIV infections activate HERV-K sequences (Laderoute *et al.*, 2007). Similarly Epstein-Barr virus infections result in the induction of HERV-K superantigen (Sutkowski *et al.*, 2004, Meylan *et al.*, 2005, Hsiao *et al.*, 2006). Epstein-Barr virus containing Burkitt's lymphoma cells occasionally reveal particles strongly resembling retroviral type A structures upon induction by the tumour-promoting phorbol-ester TPA (zur Hausen, unpublished). Typical structures are shown in Fig. 8.

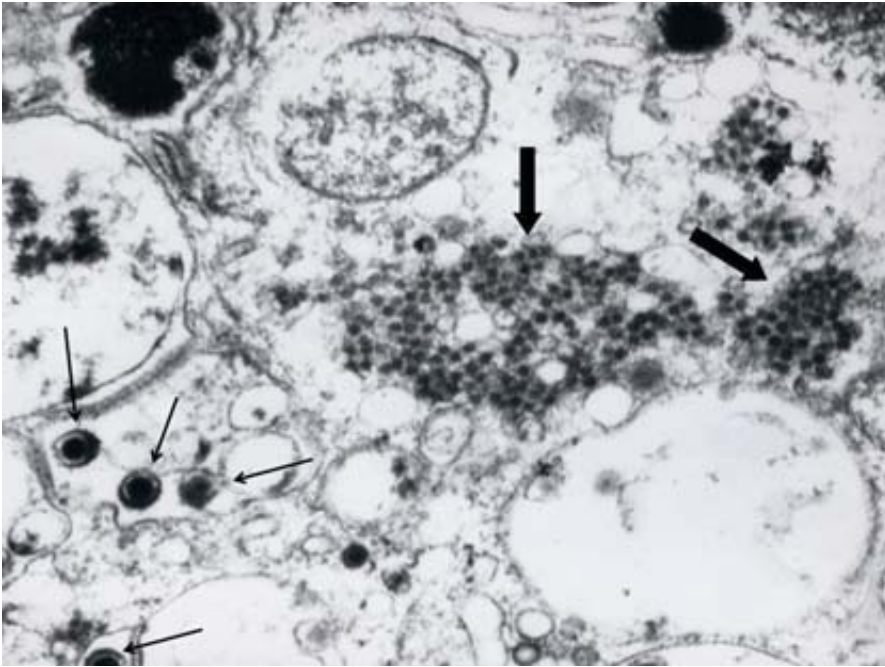


Figure 8. Epstein-Barr virus particles (small arrows) and two clusters of A-type particle-like structures (big arrows) in a TPA-treated Burkitt's lymphoma cell.

Some recent reports may further stress a potential role of reactivated HERV-K viruses in the pathogenesis of human breast cancer: an antigen-specific immune response was demonstrated in breast cancer patients (Wang-Johanning *et al.*, 2008). In addition, breast cancer patients, HIV-associated lymphomas, non-HIV-associated lymphomas and HIV-associated Hodgkin's lymphomas reveal about seven-fold elevated concentrations of HERV-K (HML-2) RNA in their plasma when compared to healthy controls. (Contreras-Galindo *et al.*, 2008). The RNA titres in lymphoma patients in remission returned to control values.

Although the available data seem to support a potential role of endogenous retroviruses in human breast cancer, they certainly do not prove it. Other agents may also contribute to at least a proportion of these cancers. A possible link between red meat consumption and breast cancer and a potential involvement of other viral factors will be discussed in connection with a subsequent paragraph. Nevertheless, human breast cancer remains an interesting candidate for a viral etiology.

3. Cancer incidence influenced by infections

The risk of some cancers seems to be influenced by other infections which neither directly contribute to carcinogenesis nor induce long-lasting immunosuppression.

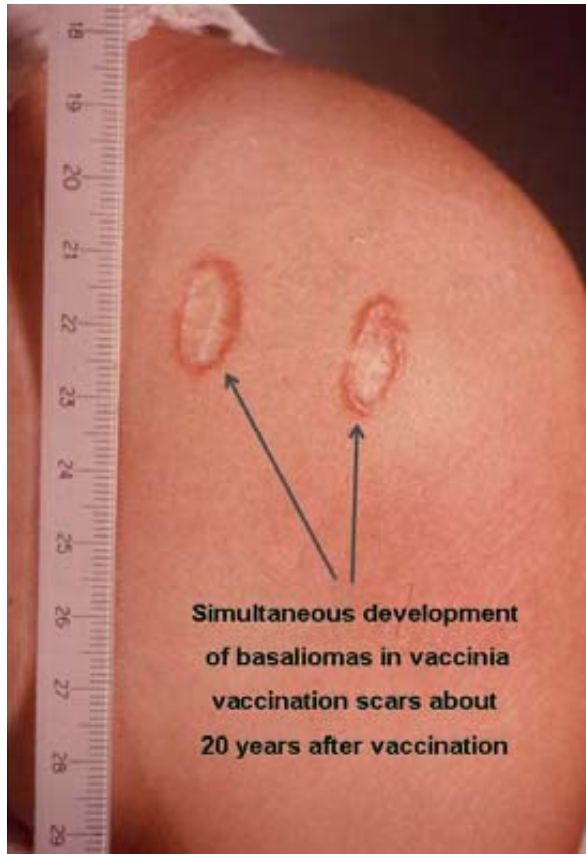


Figure 9. Multifocal basal cell carcinomas in small pox vaccination scars.

3a. Basal cell carcinomas in pox scars

Figure 9 reveals an example of multiple basal cell carcinomas arising after 20 years in a smallpox vaccination scar. This does not represent a solitary observation, since a large number of basal cell carcinomas, but also melanomas, squamous cell carcinomas, and a few more rarely occurring malignancies (dermatofibrosarcoma protuberans, fibrosarcoma and malignant fibrous histiocytes) have also been reported to occur in smallpox vaccination scars (Waibel and Walsh, 2006) They are summarised in Figure 10.

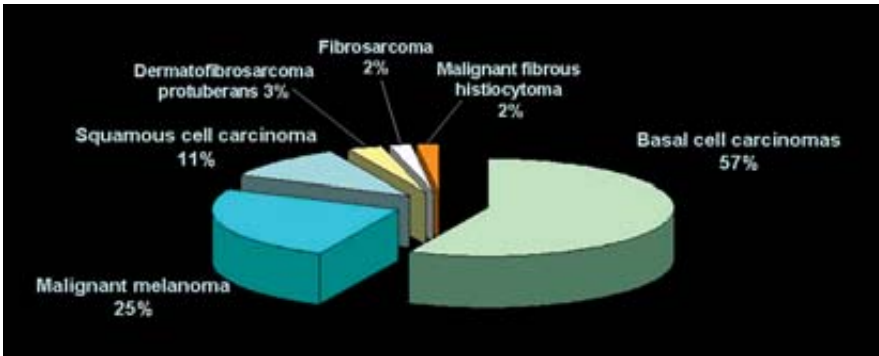


Figure 10. Malignant tumours arising in vaccinia virus vaccination scars (modified from Waibel and Walsh, 2006).

Prior to the eradication of smallpox infections, vaccines against these infections were prepared by inoculating vaccinia virus into the scarified skin of calves and harvesting the skin crusts containing the vaccinia virus particles. It is possible that these preparations contained contaminating bovine viruses. Previously it has been demonstrated that vaccinia virus infections cause amplification of persisting polyoma type virus genomes (Schlehofer *et al.*, 1986). This may increase the likelihood for contaminations with bovine members of this virus family. Persistent papillomavirus DNA would be also affected in cells replicating vaccinia virus (Schmitt *et al.*, 1989).

The published data permit several interpretations:

- Vaccinia virus infection of calf skin resulted in the activation of specific cattle viruses whose subsequent inoculation into humans as contamination represented a risk factor for subsequent local cancer development;
- Vaccinia virus infection of the human skin resulted in local activation of human potentially oncogenic viruses, increasing the risk of cancer development 20–60 years later;
- Early inflammatory reactions induced by this vaccination resulted in mutational events resulting in some cases in the simultaneous appearance of multifocal cancers.

Although still other interpretations remain possible, and basal cell carcinomas have also occasionally been observed in other non-vaccination scars, the observations described here should promote studies on a possible viral role in the initiation of these malignant proliferations.

3b. Haematopoietic malignancies

As shown in Figure 11, a number of human viruses turn out to be oncogenic when inoculated into newborn rodents. Intracerebral infections by JC virus are able to induce astrocytomas in adult owl monkeys (London *et al.*, 1978). For obvious reasons the reverse question, whether specific animal viruses

are also able to induce tumours in humans, has not yet been carefully investigated (zur Hausen, 2001). Yet we are living in close contact with domestic animals and regularly handle their products. This is particularly interesting because contact with cattle and consumption of red meat have been identified as risk factors for specific human malignancies. Contact with cattle has also frequently been considered as risk factor for haematopoietic malignancies, in particular childhood acute lymphocytic leukaemias (reviewed in zur Hausen, 2009).

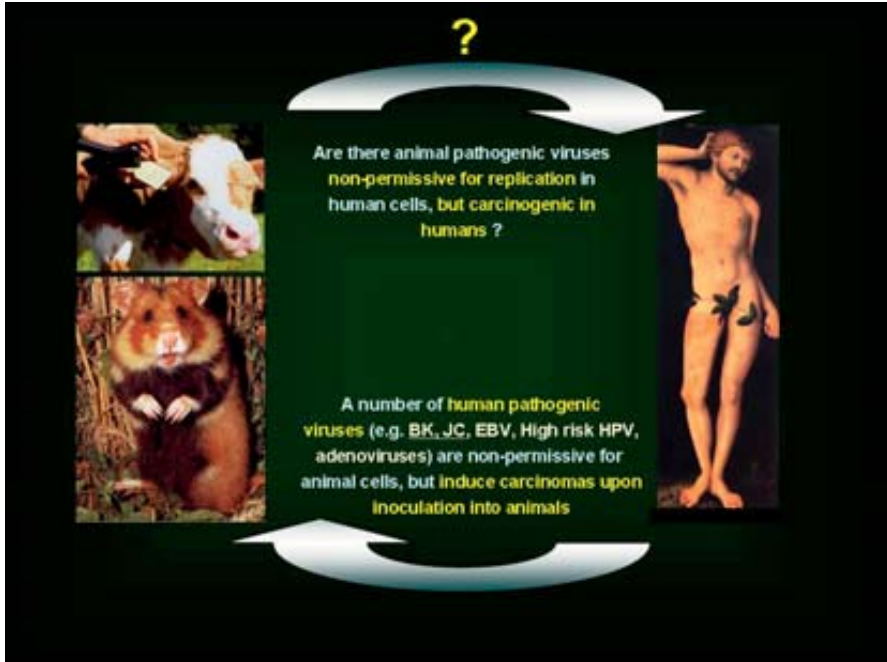


Figure 11. Some human viruses are carcinogenic for several animal species. Do animal viruses exist that are potentially carcinogenic in humans?

3b 1. Risk and protective factors

In the following, the reasons for considering childhood leukaemias as potential candidates for an infectious etiology will be briefly summarised. A more detailed account has been published recently (zur Hausen, 2009). Some protective factors as well as several risk factors for this malignancy are presented in Figure 12.

Repeatedly reported <u>protective factors</u> for childhood leukemias:	<u>Risk factors</u> for childhood leukemias
<div data-bbox="156 305 565 365" style="border: 1px solid black; padding: 2px; margin-bottom: 5px;">Multiple infections in early childhood</div> <p data-bbox="162 387 476 414">Underprivileged social status</p> <p data-bbox="162 444 543 471">Crowded household, many siblings</p> <p data-bbox="162 500 458 527">Inverse risk with birth order</p> <p data-bbox="162 556 558 584">More than 6 months of breastfeeding</p>	<div data-bbox="624 293 962 374" style="border: 2px solid red; padding: 2px; margin-bottom: 5px;">Rare infections during the first year of life</div> <p data-bbox="599 402 892 429">High socioeconomic status</p> <p data-bbox="599 480 1002 507">Prenatal chromosomal translocations</p> <p data-bbox="599 556 967 584">Agricultural occupation of parents</p>

Figure 12. Protective and risk factors for childhood acute lymphoblastic leukaemia.

Rare infections during the first year of life are frequently reported as risk factor for childhood leukaemias (reviewed in zur Hausen, 2009). Conversely, multiple infections during this period emerge as a protective factor. These observations are underlined by correlative data: a high socioeconomic state represents a risk factor, whereas crowded household conditions and many siblings emerge as protective factors. Cattle farming has been reported as an additional risk factor, whereas more than six months of breast feeding seem to reduce the risk.

Two additional sets of data deserve discussion: the frequent occurrence of specific chromosomal translocations in leukaemic cells, often observed already prenatally (Greaves, 2003). The same types of chromosomal alterations have also been found in healthy individuals, though here their frequency appears to be very low. Another striking observation originates from the description of occasional small clusters of leukaemic cases, specifically in regions where an influx of urban populations occurred in previously rural areas (reviewed in Kinlen, 2004).

3b. 2. Possible explanations

Three main hypotheses have been published to explain the epidemiological findings: Greaves (summarised in 2006) speculated that there exists an insufficient maturation state of the immune system in case of low exposure to infections. Preceding chromosomal translocations as the first event, followed by delayed infection “with an unspecified agent” should increase the risk for subsequent leukaemic conversion. Alternatively, Kinlen (1995) proposed that sudden mixing of a population of low exposure to a putative leukaemogenic agent (particularly in rural areas) with another population originating from urban areas previously highly exposed to the incriminated agent, could promote an epidemic of the relevant infection. These hypotheses were

supplemented by a further speculation, assuming that the protective effect of multiple infections during the first year of childhood was due to the reduction of the load with a putative leukaemogenic agent by interferon production as outlined in figure 13 (zur Hausen and de Villiers, 2005; zur Hausen, 2009).

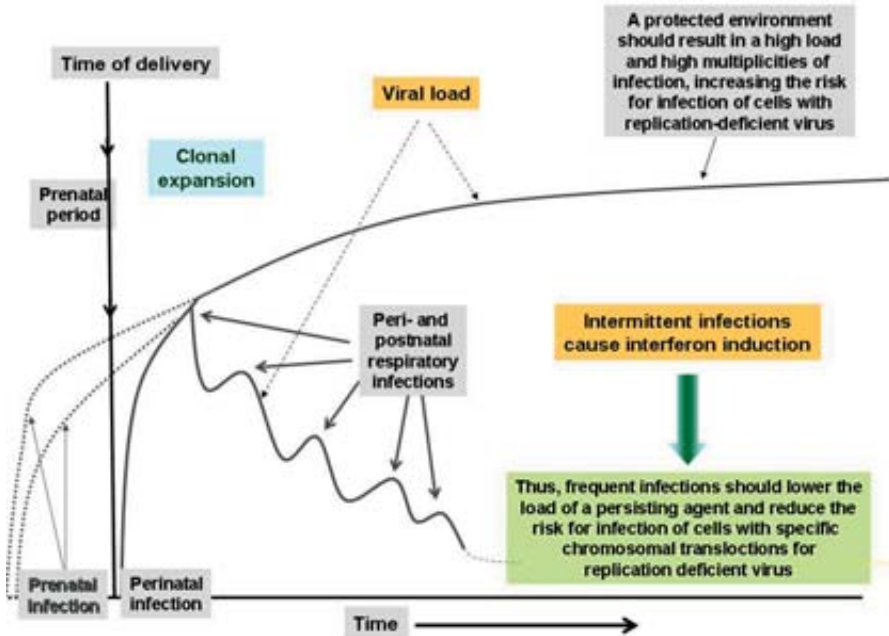


Figure 13. Schematic outline of the target cell conditioning hypothesis. Interferon synthesis resulting from multiple infections in early childhood reduces the load of a persisting potentially leukaemogenic agent and thus reduces the risk for malignant proliferation (adapted from zur Hausen and de Villiers, 2005).

Reports on supertransforming properties of specifically replication-incompetent SV40 and murine polyomaviruses (Small *et al.*, 1982; Roberge, C. and Bastin, 1988), in addition to the recent demonstration of replication-incompetent Merkel cell polyomavirus in Merkel cell carcinomas (Shuda *et al.*, 2008) resulted in an attempt to combine the three hypotheses, assuming that replication-incompetent polyoma-type viruses and high multiplicities at the time of initial infection represent an important precondition for an increased leukaemogenic risk. The generation of replication-incompetent viral progeny seems to depend on high multiplicities of infection and the co-infection of cells with both, replication-competent and incompetent genomes. The sole subsequent infection of a susceptible cell with a replication-incompetent genome may lead to the outgrowth of a leukaemic clone. Susceptibility of a cell to this malignant conversion would require the previous or subsequent acquisition of a specific chromosomal translocation. These translocations also occur in healthy individuals, though at low frequency (Liu *et al.*, 1994; Bell *et al.*, 1995; Fuscoe *et al.*, 1996; Roulland *et al.*, 2006). They represent

risk factors but are clearly not sufficient for cell transformation. They should activate the oncogene of the replication-incompetent virus. A synopsis of this hypothesis is presented in Figure 14.

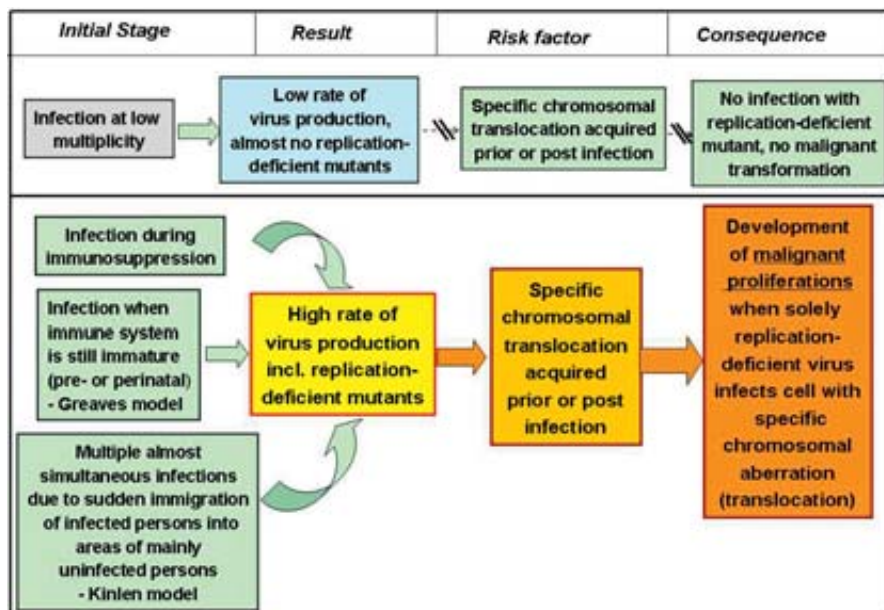


Figure 14. Synopsis of the target cell conditioning model for childhood leukaemia.

A polyoma-type virus infection would fit best for this model, although members of structurally related virus families might be also considered. Since a number of reports document elevated risks in families of cattle farmers and for individual in close contact with cattle (reviewed in zur Hausen, 2009), at least part of childhood leukaemias could be due to a native cattle virus. This virus should be replication-incompetent for human cells, but its oncogene may become activated in cells with specific chromosomal modifications. Since a number of reports also suggest human occupational risks of persons with communicative contacts (e.g. teachers, hairdressers), other types of similar infections may be spread by human-human contacts (reviewed in zur Hausen, 2009).

It remains an interesting question to which extent other haematopoietic malignancies, like acute and chronic myelogenous leukaemias, chronic lymphatic leukaemias, B- and T-cell non-Hodgkin lymphomas, Epstein-Barr virus-negative Hodgkin lymphomas, and multiple myelomas could be included in these considerations. Yet undefined polyomavirus-like particles have been electronmicroscopically demonstrated in trichodysplasia of a patient with non-Hodgkin's lymphoma (e.g. Osswald *et al.*, 2007).

4. Cancers potentially linked to animal – human transmission

Colorectal, breast and lung cancers

A large number of reports consistently describe an increased risk for colorectal cancers related to a high consumption of red meat (reviewed in Kuhnle *et al.*, 2007; Santarelli *et al.*, 2008). Recently this has also been noted for lung cancer in non-smokers (Cross *et al.*, 2007; Hu *et al.*, 2008; Lam *et al.*, 2009) and, to a more limited degree and less consistently, also for breast cancer (Taylor *et al.*, 2007; Egeberg *et al.*, 2008; Hu *et al.*, 2008; Linos *et al.*, 2008; Mignone *et al.*, 2009). A correlation seems to exist in countries with a high rate of red meat consumption and a high risk for colorectal and breast cancer. Common and frequently cited interpretations of these observations are dietary factors. Carcinogenic N-nitroso compounds, heterocyclic amines and heterocyclic aromatic hydrocarbons arise during cooking, broiling or meat curing. Some of these compounds require metabolic activation prior to conversion into a carcinogenic form, as initially described by Sugimura and colleagues (Ohgaki *et al.*, 1986). In addition, potentially carcinogenic nitrosyl haem and nitroso thiols have been reported to be significantly increased in faeces following a diet rich in red meat (Cross *et al.*, 2002).

In contrast to red meat, consumption of white meat, and here specifically chicken and other poultry meat, has not been found to be associated with an elevated risk of colorectal or other cancers. It has been reported, however, that fried, grilled or smoked chicken meat contains equally high concentrations of heterocyclic aromatic hydrocarbons and other carcinogens arising in the preparatory steps prior to consumption (Yano *et al.*, 1988; Kazerouni *et al.*, 2001; Reinik *et al.*, 2007). If this holds up and if no other hitherto unknown carcinogens are found specifically in red meat, these observations may require a fresh look at previous interpretations. In meat prepared medium or raw (Figure 15), temperatures in the central portions do not exceed 55 to 65° Celsius. At least members of the polyoma- and papillomavirus families readily survive these temperatures without significant loss of their infectivity (Lelie *et al.*, 1987; Sauerbrei and Wutzler, 2008). The only known bovine polyomavirus was initially identified as a contamination of foetal bovine sera, thus, it must have been present in the peripheral blood of yet unborn or newborn calves. Existing members of the polyomavirus family have been poorly studied in our domestic animals. These viruses are commonly non-oncogenic in their natural hosts, but reveal carcinogenicity only in heterologous tissues. At present, six different genotypes of polyomaviruses have been identified in humans, but only one in cattle.



Figure 15. Red meat cooked “raw”.

It is tempting to speculate that a hitherto unidentified bovine infectious agent with pronounced thermostability, replication-incompetent for human cells and possibly structurally related to the polyomavirus family, may play a role in colorectal cancer, potentially also in lung cancers of non-smokers and in breast cancer. This could be interpreted to mean that the described chemical carcinogens arising during cooking or curing processes are not sufficient for the induction of the respective cancers. In the case of red meat consumption they may, however, interact with viral agents, present in red, but not in white meat.

CONCLUSIONS

Although we know that at present, slightly more than 20% of global cancer incidence is linked to infectious events, some epidemiological observations suggest that this percentage will increase in the future. The recognition that no cancer linked to infections develops without additional modifications within the host cell genome permits the speculation that even cancers with well established chromosomal modifications deserve a careful analysis for additional involvement of infectious agents. Prime malignancies suggested here as candidates for potential links with infections are haematopoietic malignancies, particularly childhood lymphoblastic leukaemias, Epstein-Barr virus-negative Hodgkin's lymphomas, basal cell carcinomas of the skin, and breast, colorectal and a subgroup of lung cancers. Although still hypothetical, this proposal is accessible to experimental verification. Even if only one

of these speculations turns out to be correct, this would have profound implications for the prevention, diagnosis and hopefully also therapy of the respective malignancy.

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