Hypothesis

Proliferation-inducing viruses in non-permissive systems as possible causes of human cancers

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Animal viruses, some of which are probably unable to replicate in human cells, could be transmitted to people where they may be linked to tumours currently not attributed to viruses. Several human virus types have oncogenic potential in animals. A potential risk for acquiring such infections by handling and preparation of animal products was analysed against the background of available epidemiological reports. Human tumours should be systematically assessed for proliferation-inducing viruses in non-permissive systems.

Many animal viruses may be transmitted to human beings causing in most instances symptoms of acute disease; this accounts, for example, for rabies, haemorrhagic fevers, influenza, equine Hendravirus and porcine Nipahvirus infections, and cowpox. Equine Borna disease is suspected to cause a persistent infection in human beings accompanied by psychotic alterations after transmission from horses.[1] Serological evidence has been obtained for usually symptom-free lymphocytic choriomeningitis virus infections in people keeping Syrian hamsters as pets.[2] Symptom-free spumavirus infections have been noted in zoo keepers, resulting from transmission of persistently infected African green monkeys.[3] Thus we are exposed to a substantial risk of acquiring such infections, although, due to our selection procedures, such transmissions from our farm animals and pets appear to be rare.

Current concern is mainly directed towards the recognition of sources of the more or less acute infections and their eradication wherever they appear. Do we possibly overlook a whole group of additional animal-transmitted pathogens that are able to infect but unable to replicate in human cells? Do we overlook viruses that may still express proliferation-inducing proteins (oncoproteins) in human cells despite a stringent replicative restriction within this heterologous host. If we look at this question initially from the opposite point of view, we need to ask whether human virus infections exist that cause tumours in animals.

Human viruses as animal carcinogens

Several human pathogenic virus families can induce tumours in animals. Usually this is the consequence of artificial neonatal infections, followed by tumour development after several months or 1 year subsequent to virus inoculation (panel 1). Sometimes tumours are induced even after infection of adult animals, for example after intracerebral inoculation of owl monkeys with JC virus,[4] or after infection of cottontop marmosets, or owl monkeys with Epstein-Barr virus.[5,6] In all these systems the cancer develops without previous immuno-suppression. The resulting tumour cells harbour viral DNA and express oncoproteins of the respective virus. For the polyoma family viruses BK and JC, and also after inoculation with adenoviruses, their T-antigens are expressed. For lymphoma induction by Epstein-Barr virus in cottontop marmosets or owl monkeys, EBNA antigens are expressed.

Tumour development in such systems is usually the result of a non-productive infection. The animal host cells are usually non-permissive for those human viruses; they remain, however, susceptible to infections and permit the expression of early viral antigens, as seen in cells of the arising tumours.

Some of these viruses, like BK and adenoviruses, are not known to cause tumours within their human host. But all of them, on inoculation into tissue culture cells of tumour-susceptible animals, are transforming viruses, and immortalise those cells with some efficiency. Transgenic mice that carry the oncogenes of these viruses frequently develop tumours during later life. When the expression of viral oncogenes is targeted to specific organs, abnormal cell proliferation usually results at these sites.

Thus we can define a large group of human pathogenic viruses that may cause tumours in animal systems without measurable virus production. No natural infections of animals with human viruses have been recorded that resulted in tumour formation. All data have been obtained in experimental systems.

Animal viruses as carcinogens

Heterologous animal hosts

Similar to the human viruses that can cause tumours in animals, many animal viruses have been defined—some non-tumorigenic in their natural hosts—that cause tumours in other species (panel 2). Although many of these viruses cause tumours only after neonatal infections, some of them, such as herpesvirus saimiri and herpesvirus ateles, induce lymphoproliferative tumours with surprising efficiency even after infection of some adult primates.

The question of transmission of these viruses to heterologous species under natural conditions has been poorly investigated. One example is the development of sarcoids in horses and donkeys after infections with bovine papillomavirus types 1 or 2.[7,8]

Are animal viruses carcinogens for human beings?

Some investigators describe the presence of the rhesus monkey polyomavirus SV40 in human mesotheliomas and

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Panel 1: **Human viruses as animal carcinogens**

Virus family	Human virus	Tumour induction in human beings	Tumour induction in animals	Tumour types induced in animals
Polyomavirus	BK	—	Hamster, mouse, rat	Fibrosarcoma, brain tumours, lymphomas, carcinomas
	JC	?	Hamster, mouse, owl monkey	Brain tumours, astrocytomas, neuroblastomas
Papillomavirus	HPV 16	+++	Mouse, rat	Various types of carcinomas in transgenic animals
	HPV 18	+++		
Adenoviruses	Types 12, 18, and several others	—	Mouse, rat, hamster	Fibrosarcomas, carcinomas, ependymomas
Herpesviruses	EBV	+++	Cottontop marmoset, owl monkey	Lymphoblastoses, lymphomas
	HHV-1	++	?	?
Retroviruses	HTLV-1	+++	Rabbit, mouse	Leukaemia, lymphoma, neurofibromatosis

brain tumours.[9] The implication is that these infections came about because of contamination of poliomyelitis vaccines used in the 1950s and early 1960s. Our laboratory has been unable to confirm these observations. The widespread use of SV40 sequences in common vector systems, at least in some of the reported positive findings, could have led to misinterpretation.

Proliferation-inducing animal parapoxvirus infections, however, are often recorded in human beings. Orf virus infections of sheep and bovine papular stomatitis virus infections serve here as examples;[10] they induce benign epitheliomas or occasionally histocytomas.

Butchers frequently develop extensive verrucosis on their hands, which was initially thought to be due to the transmission of an animal papillomavirus. Subsequent studies identified human papillomavirus type 7 in most of these lesions.[11] No evidence for human papillomavirus infections, however, has yet been found in farm or other animals.

Apart from studies on SV40, there are few investigations that address potential transmission and tumour causation by animal proliferation-inducing viruses in non-permissive systems in human beings.

Sources of animal viruses

We are widely exposed to animal materials and animals that are potentially contaminated or infected by proliferation-inducing viruses in non-permissive systems. Three types of exposure emerge as the most likely candidates for a potential transmission (panel 3). The first risk originates from meat, egg, and dairy product handling and consumption. The second risk could result from agricultural exposures to farm animals, and occupational risks of butchers, meat cutters, dairy workers, and veterinarians. The third risk is from exposure to pets, mainly during childhood, to dogs, cats, rabbits, guineapigs, parrots, and canaries.

There are other potential exposures, such as in the wool and leather industry, in fish handling and consumption, and bone-meal production. These risks seem to be less relevant and restricted to specific regions.

Dietary risk

Substantial epidemiological evidence points to an increased risk for the induction of common human cancers in conjunction with meat and fat consumption; in particular cancers of the colon, breast, and prostate.[12] This risk is commonly attributed to the formation of chemical carcinogens in the frying process, during smoking or air-drying of meat, or during marinating or other preserving procedures. Transmission of infectious agents, however, is usually thought much less likely.

Nonetheless, most people handle meat during the preparation of meals, produce aerosols in the early frying process, or consume air-dried, smoked, medium-cooked, or even raw meat. Many also consume daily milk and other dairy products. Soft-boiled or raw-boiled eggs are common ingredients of meals.

Which kinds of viruses can we anticipate as possible contaminants of these foods? Members of at least seven virus families are often found in domestic animals as persistent infections in cells of the peripheral blood, or of skin and mucosa, or are found as viral particles within these sites (panel 3). These viruses are therefore highly likely to contaminate meat. Endogenous retroviruses exist in all species studied; they have been more intensively analysed in pigs as a consequence of plans to use porcine organs for xenotransplantation. Retroviruses cross the species barrier and can infect human tissue-culture cells.[13] Thus far, no pathogenicity has been attributed to these

Panel 2: **Animal viruses as carcinogens in heterologous animal hosts**

Virus family	Representative type	Natural host	Tumour in natural host	Susceptible foreign species	Tumour types
Polyomavirus	SV 40	Rhesus monkey	–	Newborn rodent	Fibromas, carcinomas, brain tumours
Papillomavirus	BPV-1	Cow	±	Horse, donkey, newborn rodent	Sarcoid tumours, fibrosarcomas
Adenovirus	Bovine adenovirus 3, Canine adenovirus, Chicken adenovirus	Cow, dog, chicken	–	Newborn rodent	Fibrosarcomas
Herpesvirus	Herpesvirus saimiri	Squirrel, monkey	–	Marmoset, owl monkey	Leukaemias, lymphomas
Poxviruses	Yaba virus, orf virus	Monkey, sheep	±	Human	Histiocytoma, epithelioma
Retrovirus	Murine, feline, hamster, and chicken leukaemia viruses	Rodents, chicken	+	Rodents, quail, others	Leukaemias, lymphomas, sarcomas

Panel 3: **Viruses in domestic animals as persistent infections**

Virus family	Virus type
Polyomaviruses	Bovine, porcine
Papillomaviruses	Bovine 1–8, canine, feline, porcine
Herpesviruses	Two types bovine, two types porcine, lymphotrophic
Retroviruses	Chicken, endogenous in cat, dog, bovine leukaemia virus
Circinoviruses	TTV-like viruses in cattle, pigs, and chicken
Circoviruses	Porcine, chicken anaemia virus
Adenoviruses	Specific serotypes in all domestic animals
Parvoviruses	Porcine

infections. Due to their structural properties retroviruses are probably sensitive to handling procedures and therefore easily inactivated.

Lymphotropic gammaherpesviruses have also been identified in pigs, cattle, and rabbits. Two types of porcine,[14] two types of bovine, and one type of ovine gammaherpesviruses[15] have so far been identified. At least some of these infections are acquired during the neonatal period, probably resulting from excretion in saliva of persistently infected mothers or from contamination of the mother's milk. These viruses share structural and some biological properties with Epstein-Barr virus and human herpesvirus type 8; all are likely to possess transforming properties and may pose a risk to human beings, particularly when we take into account the broad spectrum of tumour types linked to Epstein-Barr virus infections, and the oncogenicity of two other gammaherpesviruses, H saimiri and H ateles, when inoculated into specific heterologous hosts. With E-M de Villiers, I have screened a broad spectrum of human tumours by a sensitive PCR and herpesvirus consensus primers, and we could not find any of these viruses in the cancer biopsy specimens tested.

Papillomaviruses have been identified in almost all species that have been carefully analysed, and appear as ubiquitous infections persisting exclusively in cutaneous and mucosal surfaces. Since even macroscopically unaffected skin harbours these viruses,[16] they should give rise to frequent contaminations in butchering procedures. The enormous heterogeneity of this virus group rendered their identification difficult. Members of this virus family have great species specificity, although some bovine papillomavirus types induce malignant tumours in rodents after neonatal infection.

Polyomaviruses have been identified in widespread infections in cattle and pigs. Bovine polyomavirus has been identified as a contaminant in tissue-culture cells resulting from bovine serum supplementation.[17] At least in experimental infection they are tumorigenic for newborn rodents and transform the respective host cells.

In a similar way to human adenoviruses, animal adenovirus infections are non-tumorigenic in their natural hosts. But some types might induce tumours after inoculation into newborn rodents. Most reside in their natural hosts in the oral and respiratory tract, as well as in lymphatic tissues of the Waldeyer ring; some types are found as gastrointestinal infections. Occasionally adenovirus viraemias have been found.

Circoviruses have been identified in pigs, parrots, and chickens and appear to be widespread.[18] Circoviruses are small single-standard DNA viruses that have attracted increasing attention in the past decade. Their species specificity and their potential pathogenicity for other species require further investigations. One report suggests a transforming ability of porcine circovirus infections for primary pig-kidney cells.[19]

Finally, the recently discovered TT viruses[20] should remind us that there probably exist many more hitherto unknown viral infections in domestic animals (as well as in human beings), some of which may be oncogenic. Circinoviruses also contain a single-stranded DNA genome with up to 3900 nucleotides and emerge in many genotypes, persisting in the peripheral blood of many healthy human beings as well as in some domestic animals. Nothing is known about how these viruses escape immune surveillance mechanisms and whether they are pathogenic.

Dairy products in the more-developed world are generally pasteurised before consumption. It remains to be seen whether any members of the virus families mentioned above can survive pasteurisation. This concern has been analysed for bovine papillomaviruses by Meischke,[21] who came to the conclusion that this virus is not inactivated under these conditions. Structural properties of at least four, but possibly also five, of the virus families listed in panel 3 suggest that they have a reasonable chance of surviving pasteurisation (papillomaviruses, polyomaviruses, circoviruses, and circinoviruses). Although adenoviruses are inactivated by prolonged exposure to heat of 60°C proteins and fats in milk could protect members of this virus family during 10 min at 80°C.

Occupational risk

Many people are exposed to potentially virus-contaminated meat and dairy products. Specific occupational groups, such as farm workers, butchers, veterinarians, and employees in dairies are even more consistently exposed. It would be interesting, therefore, to analyse cancer incidence in these groups.

There is evidence of an increased risk in these groups for specific cancer types. In agricultural and dairy workers this is mainly an increased risk of haematological disorders, such as Hodgkin's disease, multiple myeloma, lymphoma, and leukaemia, but also brain and prostate cancers.[22–24] The argument that this results from pesticide use in farm workers is less convincing for the other occupational groups. In butchers and abbatoir workers a greater risk of lung cancer has been reported.[25–27] In poultry-processing plants an excess risk of oesophageal cancer has been noted.[28] Although these data have not been confirmed in all studies there seems to be a general trend towards increased risks in these professional groups for those cancers. In rural areas of China, where oesophageal cancer is endemic, a high incidence of similar oesophageal cancers has been noted in chickens.[29–31] Agricultural workers have a lower frequency of lung and smoking-related cancers, and consume less tobacco than the general population.[32,33]

Pets as risk factors

The widespread habit of keeping pets, particularly during childhood, may represent an additional source of infections. Dogs, cats, rabbits, guineapigs, parrots, canaries, and other animals could harbour potentially oncogenic viruses. Canine papillomaviruses induce malignant tumours after inoculation into specific sites of their natural hosts.[34] Feline leukaemia viruses, gammaherpesvirus of rabbits, and polyomavirus of budgerigars may all pose as yet unknown risks. Thus far, epidemiological studies remain inconclusive, although some

reports try to link close contacts to pets, specifically to rabbits, to an increased incidence of Hodgkin's disease.[35,36]

In general, our laboratories carefully control our animal facilities to avoid viral and bacterial infections transmitted from human beings. By contrast, we care very little in the opposite direction. It would be surprising if acute viral infections, such as influenza, haemorrhagic fevers, rabies, and others were the sole existing viral zoonoses.

Testing

Various approaches can be devised to test for the presence of these viruses in human tumours, and here I outline three of the most promising ones. Representational differential analysis hybdridisation has been successfully applied to identify human herpesvirus type 8 in Kaposi's sarcoma of patients with AIDS,[37] as well as for the original identification of TT virus in human serum.[20] The method is laborious, time-consuming, and difficult, but clearly has potential for the detection of as yet unknown foreign sequences in human tumours. In a second approach, a systematic chain PCR analysis can be done with consensus/degenerate primer sequences derived from the nucleotide analysis of all members of known virus families. With this set of experiments most of the presently identified genotypes of human papillomaviruses have been found.[38] The genetic diversity of the circinovirus family has also been established by this technique.[39] The limitation of this system, however, is that specific viral families have to be preselected, thereby restricting detection to those virus families only.

With the completion of human genome sequencing a third approach should become feasible: the direct comparison of human sequences derived from normal and tumour tissue by computer analysis. Since the databank of human sequences contains sequences derived from normal as well as from various tumour tissues, some exogenous DNA sequences may have been incorrectly classified as human DNA, particularly in the absence of an apparent relation to known viral or other foreign nucleic acids. The rapid development of comparative data analysis should help resolve this concern.

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