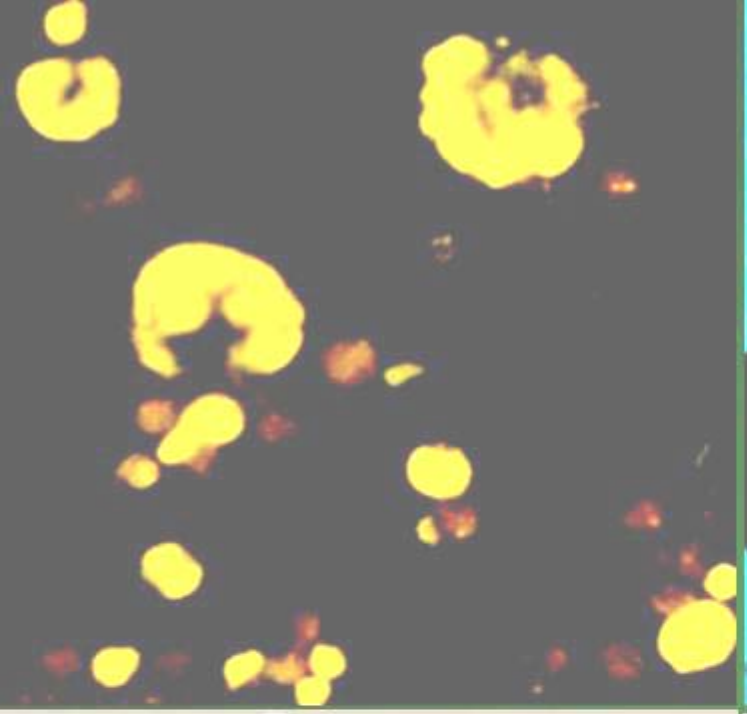
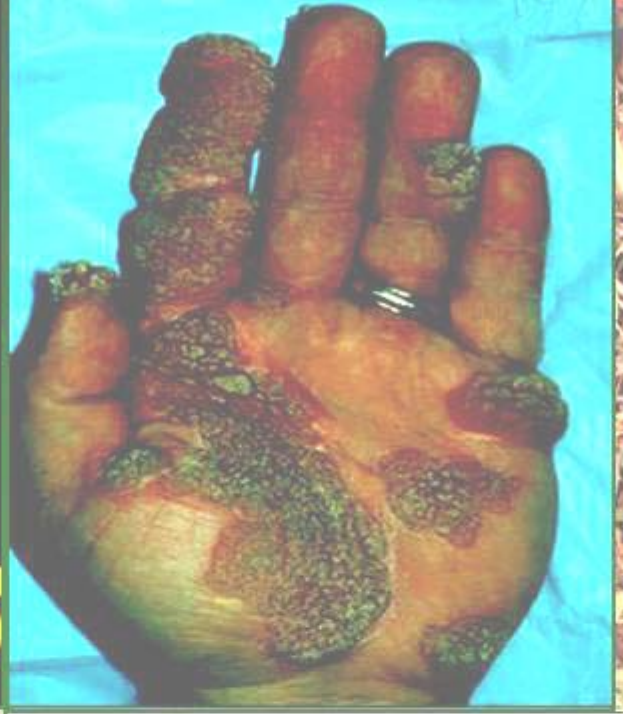
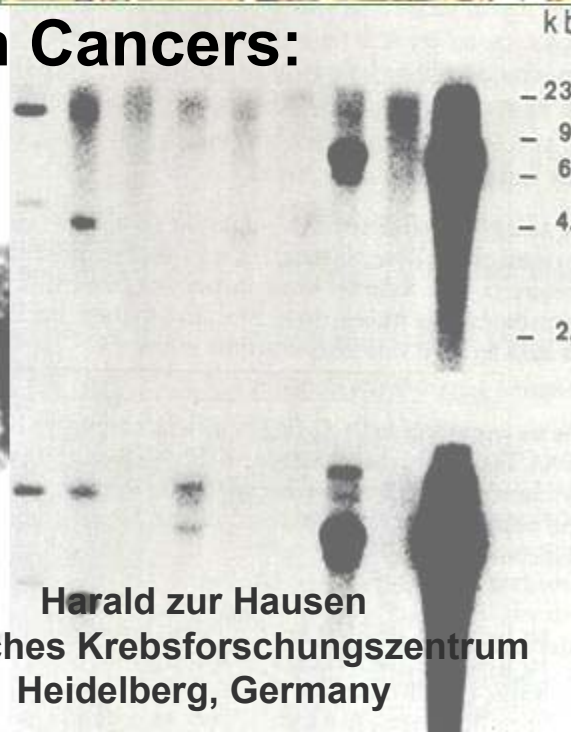




The Search for Infectious Causes of Human Cancers:

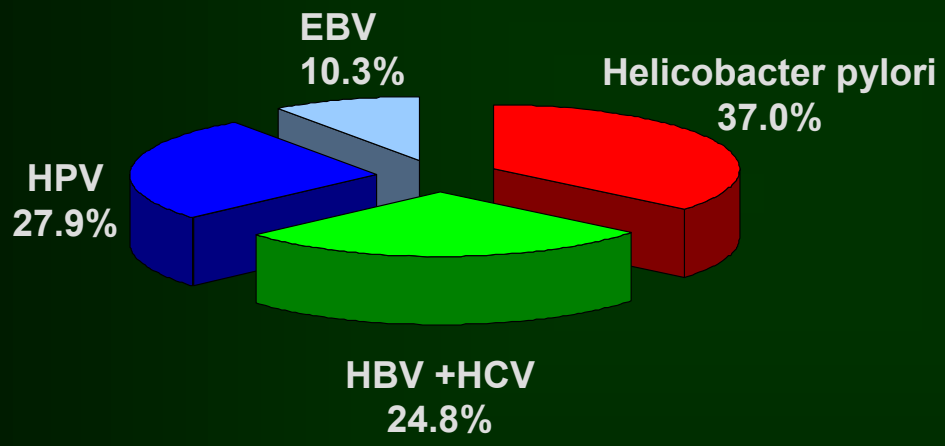
Where and Why?



Harald zur Hausen
Deutsches Krebsforschungszentrum
Heidelberg, Germany

Annual Global Cancer Incidence due to Infections

2 017 000 = 18.6% of total cancer incidence



25% of cancers of the oral cavity
68 600 (HPV)

Cancer of the cervix
493 000 (HPV)

Hepatocellular carcinoma 80%
500 900 (HBV, HCV)

Gastric cancer 80%
747 000 (Helicobacter pylori)

Gastric cancer 10%
93 400 (EBV)

Nasopharyngeal carcinoma
80 000 (EBV)

Non-Hodgkin‘s lymphoma 10%
30 000 (EBV)

Hodgkin‘s lymphoma 30%
18 700 (EBV)

This graph ignores

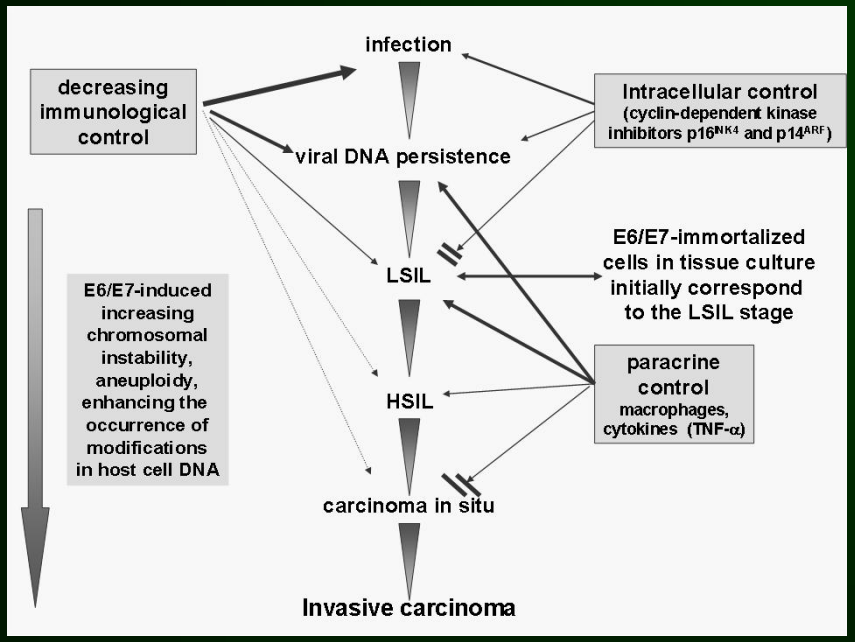
- anal and perianal cancers (HPV)
- vulvar, vaginal and penile cancers (HPV)
- adult T cell leukemia
- Kaposi‘s sarcomas and prim. effusion lymphomas
- cancers linked to parasitic infections

Modified from: Parkin et al. Global Cancer Statistics 2002
CA Cancer J. Clinicians 55: 74-106, 2002

I. First experimental evidence for the possible existence of human viral carcinogens emerged in the late 1960s

Why has it been so difficult to identify infectious agents as cancer-inducing factors in humans ?

- Because there is **no** human pathogenic infectious agent causing **cancer as the acute consequence of infection;**



- Infections linked to human cancers are common in human populations, most of them were present during the whole human evolution, and **only a small proportion of infected individuals develops the respective cancer type;**

- Except for rare germline mutations, *(XLLP)*, **cancers linked to infection commonly occur decades after primary infection**

Why has it been so difficult to identify infectious agents as cancer-inducing factors in humans ?

II.

- **Mutations in host cell genes**, in part detectable as chromosomal translocations, **or within the viral genome are mandatory for malignant conversion**;

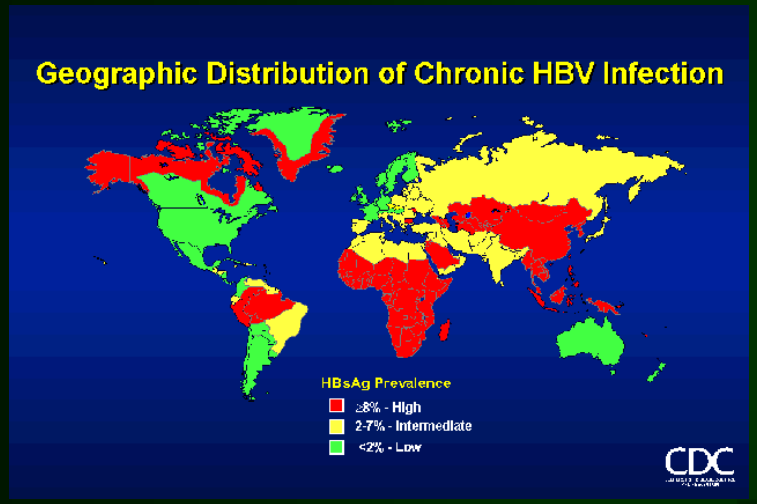
- **Chemical** ***(e.g. aflatoxin)*** **and physical carcinogens** ***(e.g. UV-light in EV)*** usually are mutagens. They facilitate the selection of specific mutations and **frequently act synergistically with carcinogenic infectious agents;**

- **Some infectious agents act as indirect carcinogens**, not persisting within the respective cancer cells ***(HIV, Helicobacter pylori, Schistosoma haematobium, Hepatitis C and B, Plasmodium falciparum?).***

Epidemiology commonly provided the first hints for an involvement of infectious agents in specific human cancers

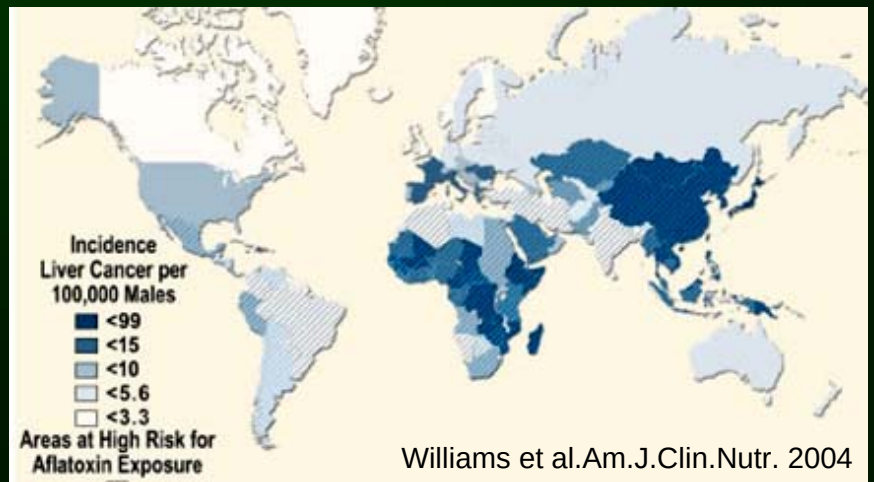
I. Geographic coincidence

Example:



Persisting hepatitis B virus infections and hepatocellular carcinoma *(Payet et al., 1956)* :

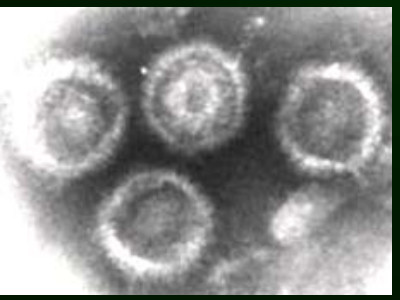
Possible interaction between a viral and a chemical carcinogen



Williams et al.Am.J.Clin.Nutr. 2004

Epidemiology commonly provided the first hints for an involvement of infectious agents in specific human cancers

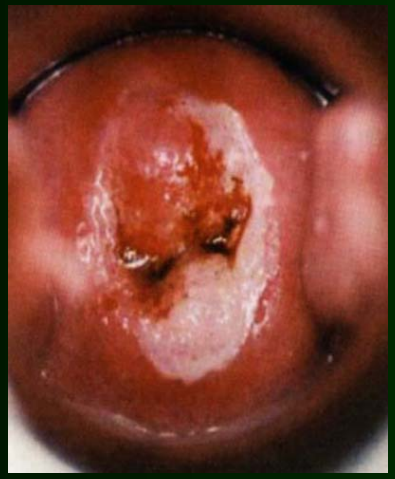
II. Geographic clustering of cases



- **Burkitt's lymphoma in equatorial Africa**
- Nasopharyngeal carcinoma in South-East Asia
- Adult T-cell leukemia in the coastal regions of Southern Japan
- Bladder cancer in the Nile delta and along the Nile river
- Cholangiocarcinoma in South-East Thailand

Epidemiology commonly provided the first hints for an involvement of infectious agents in specific human cancers.

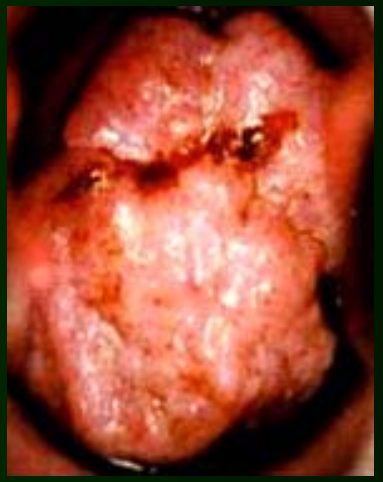
III. Dependence on sexual contacts



Cervical cancer and its precursor lesions

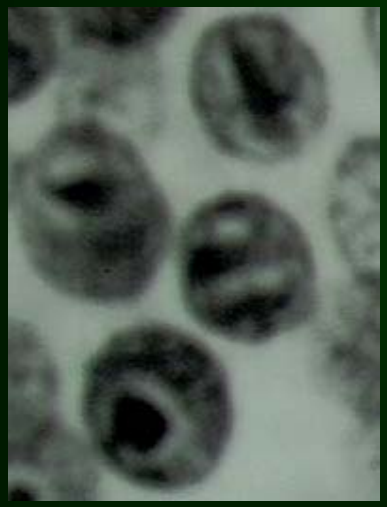
First observation by Rigoni-Stern
in Verona, 1842

Precursor lesions preceed cancer
development by 10-20 years



Epidemiology commonly provided the first hints for an involvement of infectious agents in specific human cancers.

IV. Cancers arising under immunosuppression *(HIV infection, organ transplantation)* are suspected to be linked to infectious events:



- **Kaposi‘s sarcomas**
- **Merkel cell carcinomas**
- **squamous cell carcinomas of the skin**
- **cervical cancer**
- **common warts**

Mechanisms by which infections contribute to human carcinogenesis

✓ **Introduction of viral oncogenes into host cells**
(high risk HPV, EBV, HHV-8, HTLV-1)

✓ **Modified viral oncogenes after integration into host cell DNA**
(Merkel cell polyomavirus)

Modified host cell genes integrated into viral genomes act as oncogenes *(human endogenous retroviruses – HERV ?)*

Direct carcinogens

✓ **Virus-induced immunosuppression activates other tumorviruses** *(HIV-1 and HIV-2)*

✓ **Chronic inflammation, induction of oxygen radicals**
(Hepatitis B and C, Helicobacter pylori, Parasites)

Prevention of apoptosis *(some cutaneous HPV types)*

Induction of chromosomal instability and translocations
(Adenoviruses, Herpesviruses, TT viruses ? Endogenous retroviruses?)

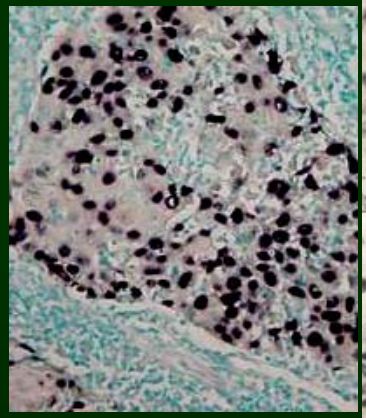
Indirect carcinogens

Identification of infectious agents as causative factors of human cancers depended on:

Molecular biology

Seroepidemiology and epidemiology

DNA sequencing



Tumor induction in animals

Cell transformation by specific subcomponents

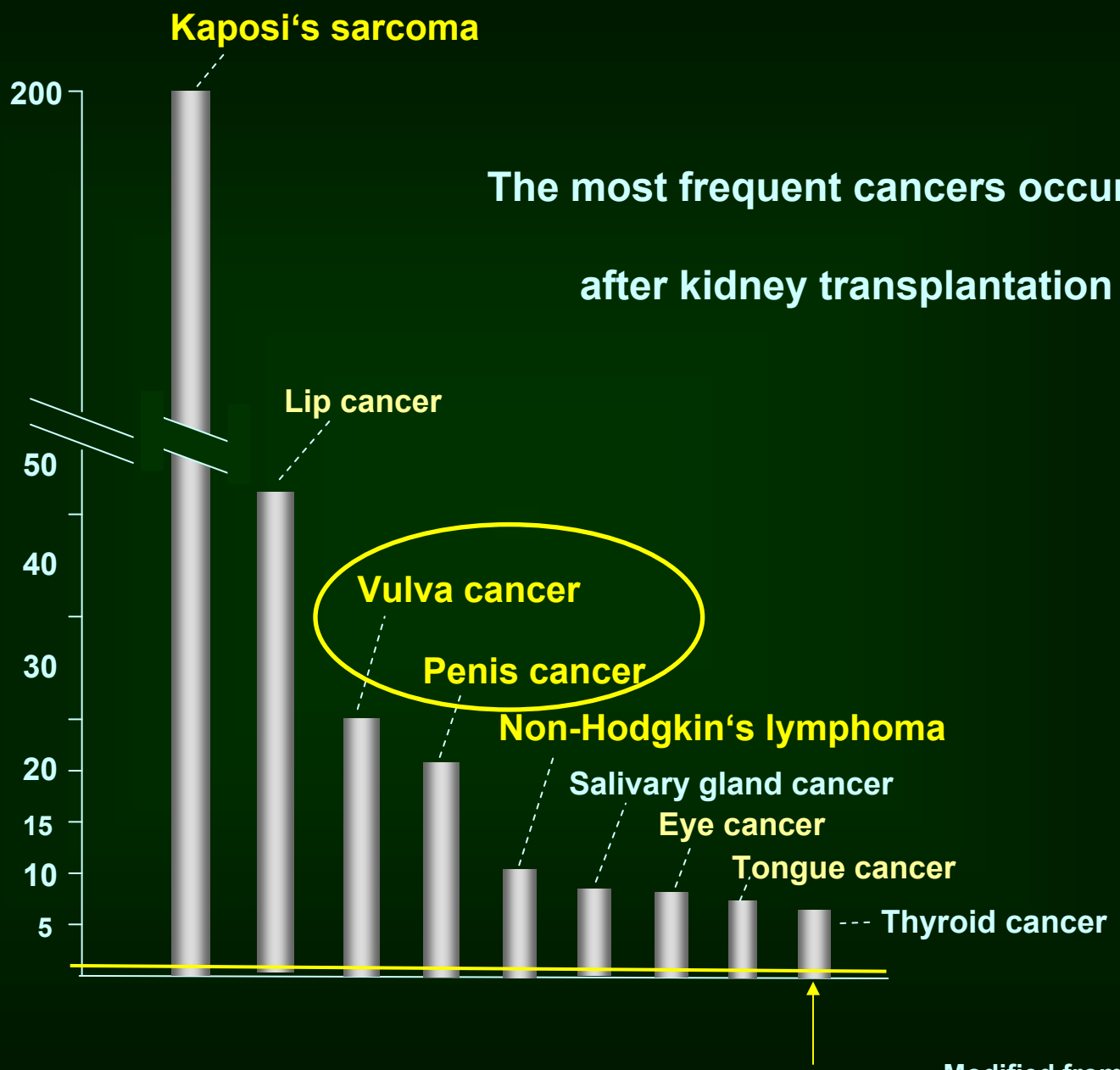
Where is it worthwile to search for an infectious etiology of human cancers not yet linked to infections?

I. Cancers occurring at increased frequency under immunosuppression

II. Cancers with reduced incidence under immunosuppression or not affected by immunosuppression

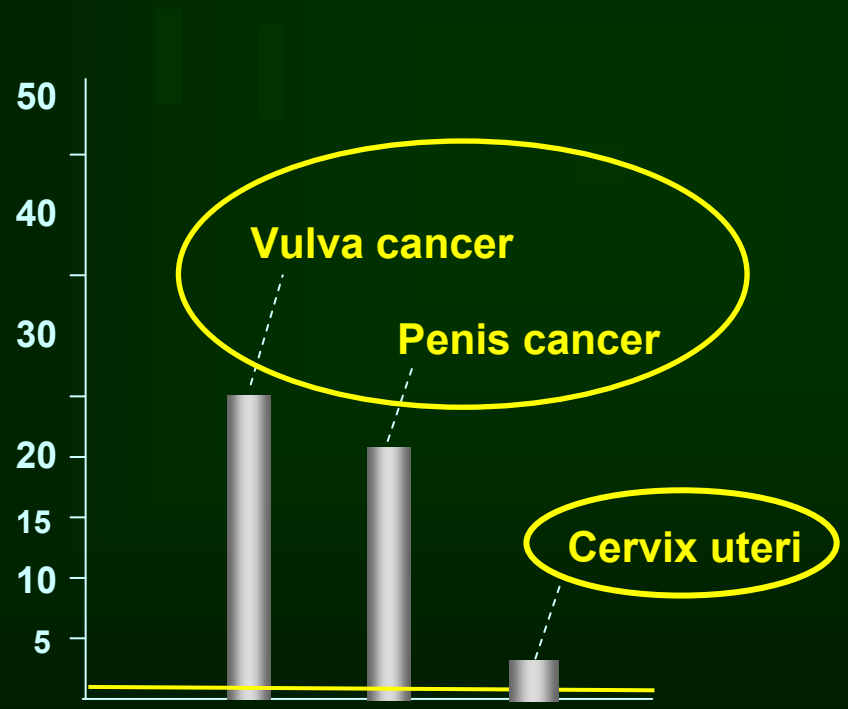
III. Cancer incidence influenced by infections

IV. Nutritional cancer risk factors possibly linked to infections



Modified from: Vajdic et al.
JAMA 295: 2823-2831, 2006

The most frequent cancers occurring after kidney transplantation

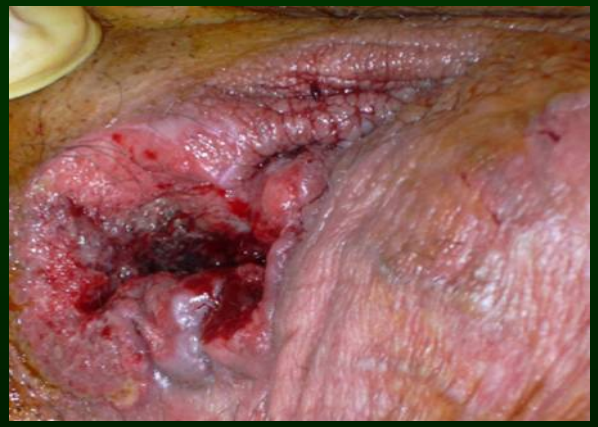


Modified from: Vajdic et al.
JAMA 295: 2823-2831, 2006

Only 30-50% of vulvar and penile cancers are presently being linked to high risk HPV infections (mainly HPV 16).

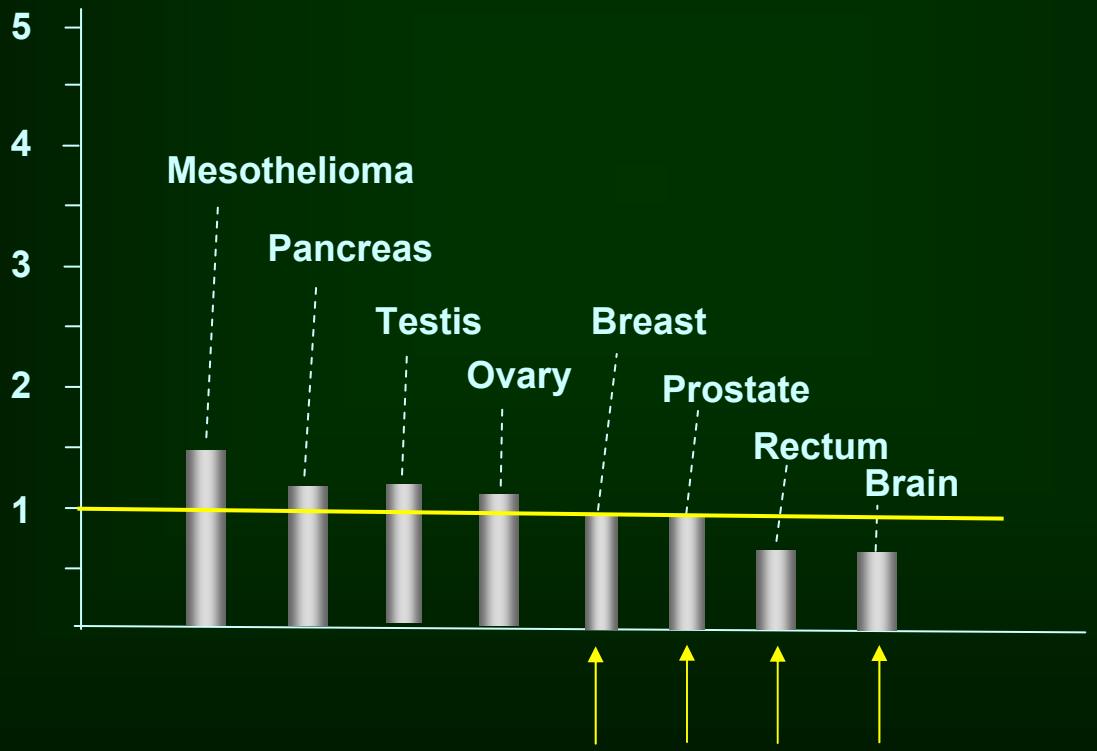
The histological pattern differs between HPV-positive and –negative cancers at these sites. In addition, the age distribution is also different.

Etiologic factors for the HPV-negative tumors are unknown



It might be worthwhile to study the negative tumors for other HPV or polyomavirus sequences

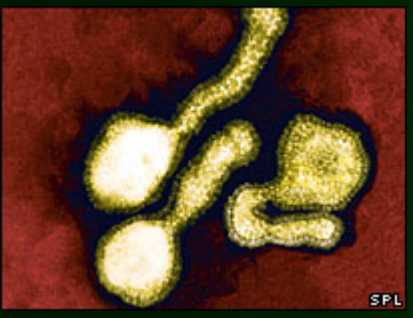
Cancers barely or not at all increased after kidney transplantation

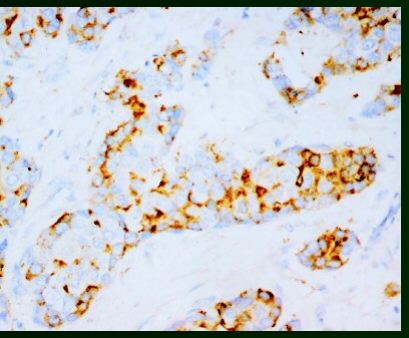


Modified from: Vajdic et al.
JAMA 295: 2823-2831, 2006

II. Cancers with reduced incidence under immunosuppression or not affected by immunosuppression

Example: Mouse Mammary Tumor virus (MMTV)



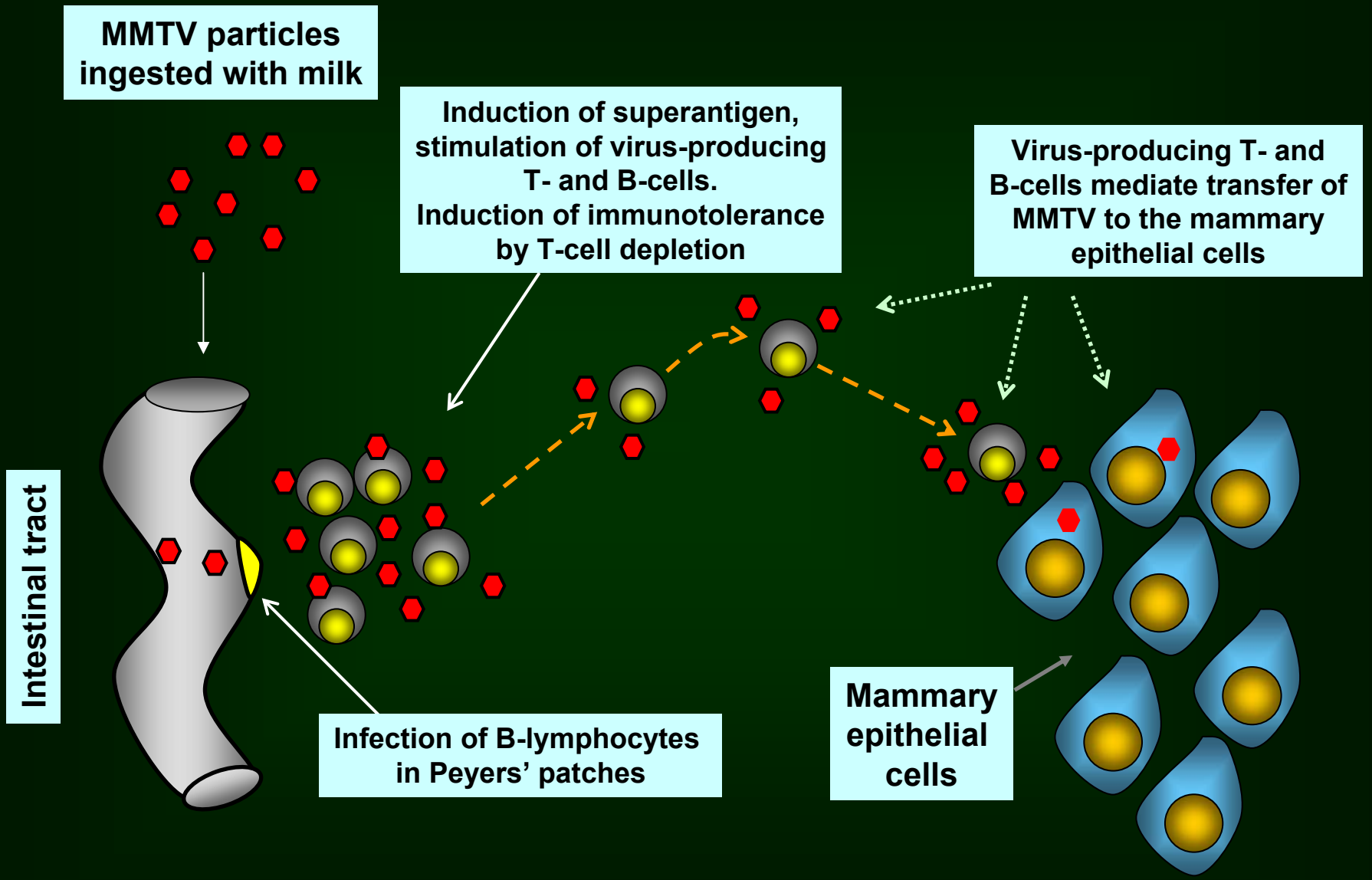


The MMTV 3' LTR sequence encodes a **superantigen orchestrating multiplication of T- and B-lymphocytes**. This results in amplification of virus-producing cells capable to deliver the infection to the mammary gland (reviewed in *Matsuzawa*, 1995, *Ross*, 1998).

Superantigen induction by MMTV leads to a clonal depletion of a subset of T-cells and immunotolerance

(Lobo-Yeo and Lamb, 1993, Abe et al., 1993 Le Bon et al. 1995)

Additional immunosuppression prevents multiplication of superantigen-induced in B- and T-lymphocytes which would produce large quantities of MMTV-particles (reduction of the viral load)



The viral load seems to determine the risk for mammary tumor development

Immunosuppression does not increase the rate of human breast cancer

Immunosuppression does not facilitate MMTV carcinogenesis but has a slight protective function. This corresponds to the effect of immunosuppression on human breast cancer.

The mechanism of no or even a slightly protective effect of immunosuppression in human mammary carcinogenesis is not understood: a parallel to the murine system?

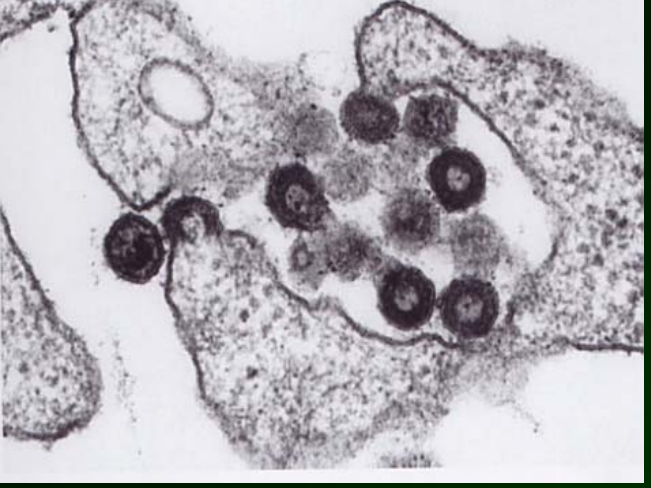
Breast Cancer:

A link between endogenous retrovirus activation and breast cancer?

Endogenous retroviral sequences account for ~8% of the human genome.

Some of the families entered the human germline >40 million to 800.000 years ago. Most of their open reading frames contain mutations and stop codons not permitting the expression of the respective proteins.

Specific members of one subfamily (HERV-K), distantly related to mouse mammary tumor virus are able to form complete, but non-infectious particles.



Retroviral particles produced in human germinal cell tumor lines are encoded by the HERV family HERV-K (HML-2).

HML-2 gag and env RNA transcripts are selectively packaged into these particles. They originate from the HML-2 provirus on chromosome 22q11.21.

(Ruprecht et al., J. Virol. 82: 10006-16, 2008)

Infectious HERV-K virus has been reconstituted from endogenous retrovirus

Elements by correcting stop codons in open reading frames.

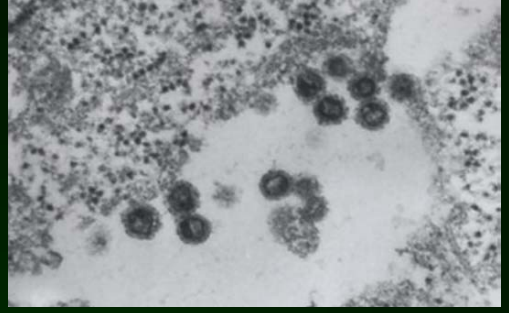
Dewannieux et al., Genome Res. 16: 1548-1556, 2006.

Lee and Bieniasz, PLOS Path. 3: e10, 2007

HERV-K(K102) expression becomes activated in AIDS patients

Laderoute et al. AIDS. 2007 Nov 30;21(18):2417-24

EBV infection activates endogenous retrovirus (HERV-K) and induces a superantigen:



Hsiao et al..

Epstein-Barr virus transactivates the HERV-K18 superantigen by docking to the human complement receptor 2 (CD21) on primary B cells.

J. Immunol.177: 2056-60, 2006

Meylan et al.

Negative thymocyte selection to HERV-K18 superantigens in humans.

Blood 105: 4377-4382, 2005.

Sutkowski et al.

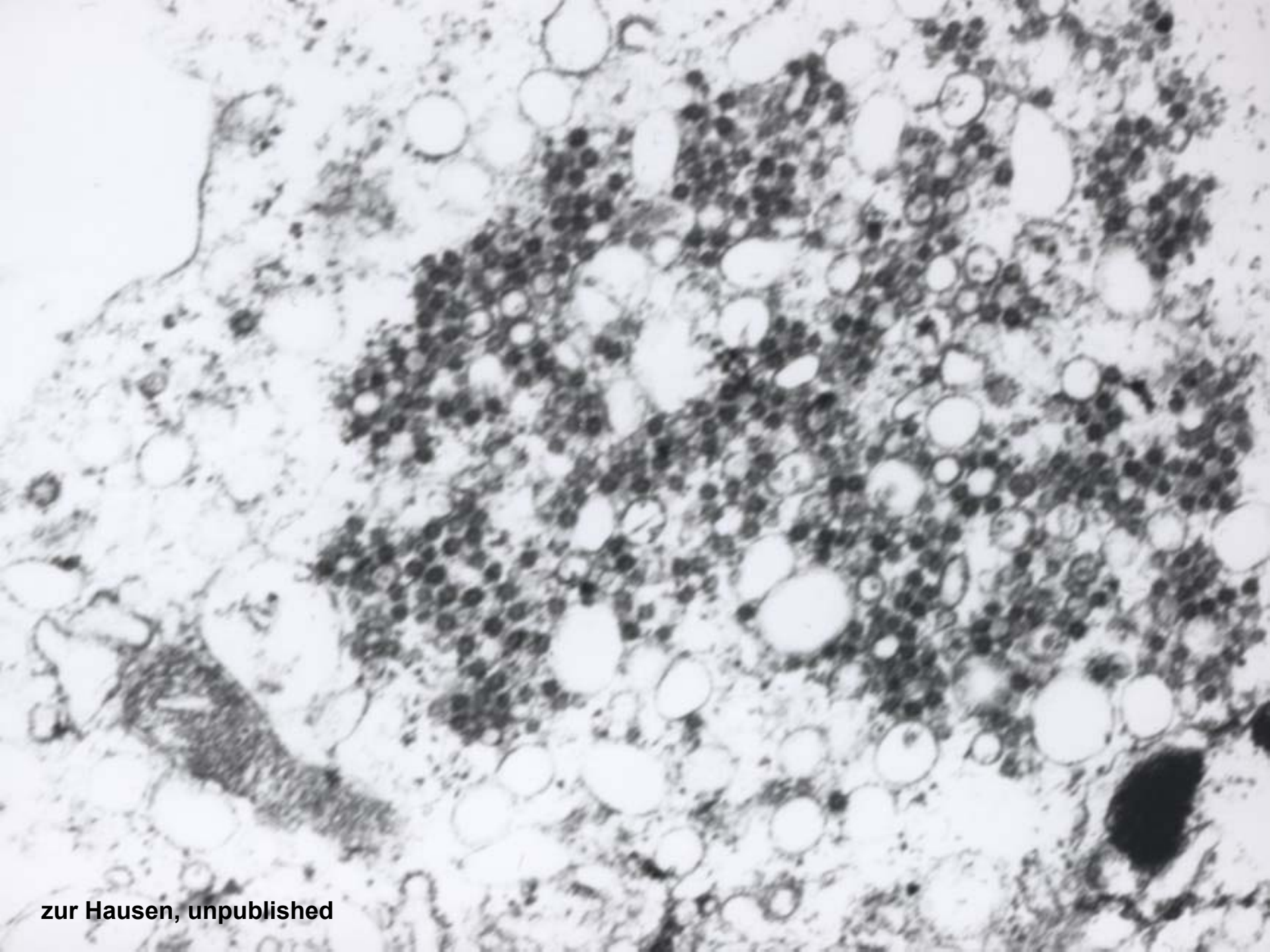
Epstein-Barr virus latent membrane protein LMP-2A is sufficient for transactivation of the human endogenous retrovirus HERV-K18 superantigen.

J. Virol. 78: 7852-7860, 2004.

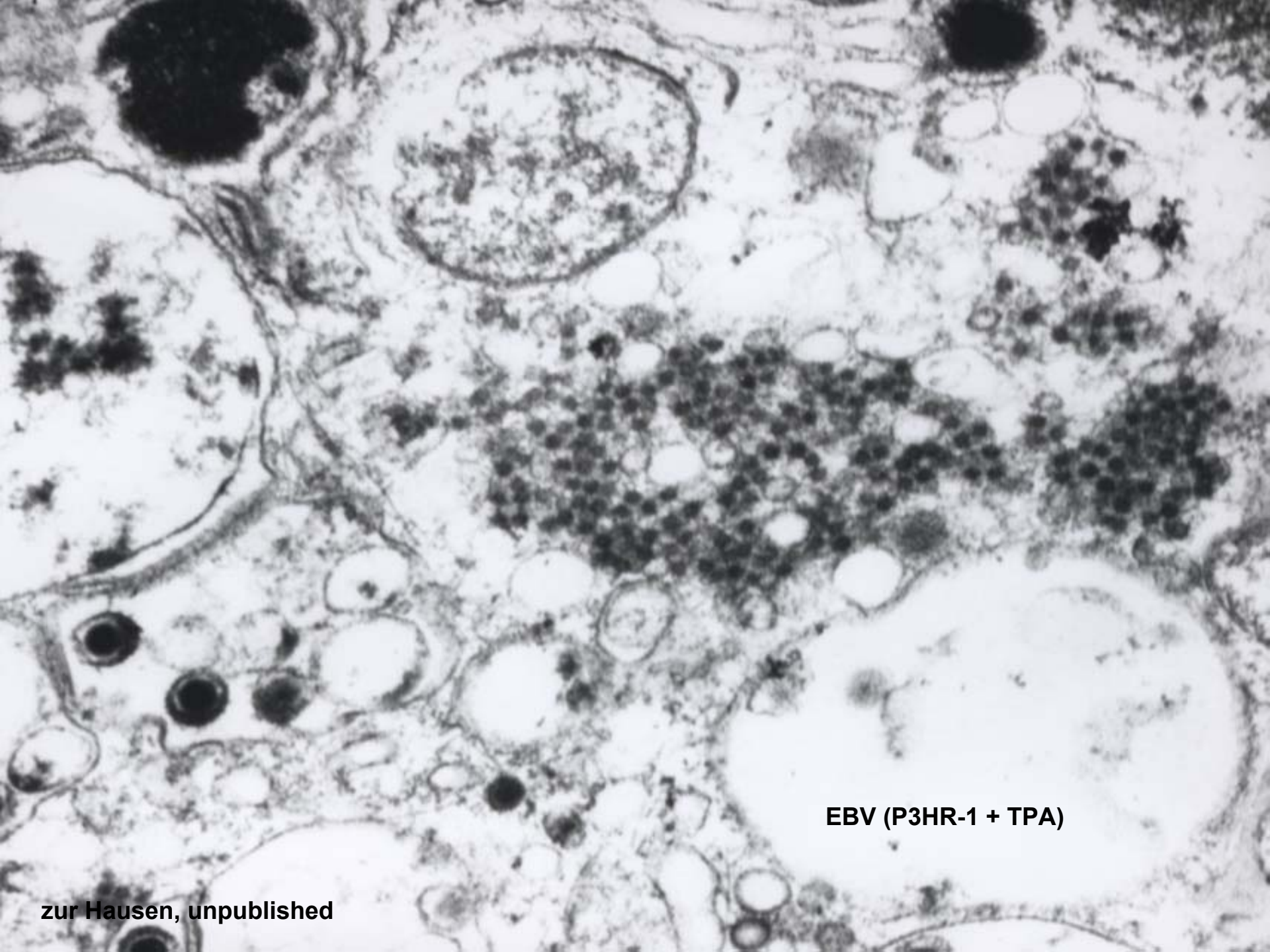
Sutkowski et al.

Epstein-Barr virus transactivates the human endogenous retrovirus HERV-K18 that encodes a superantigen.

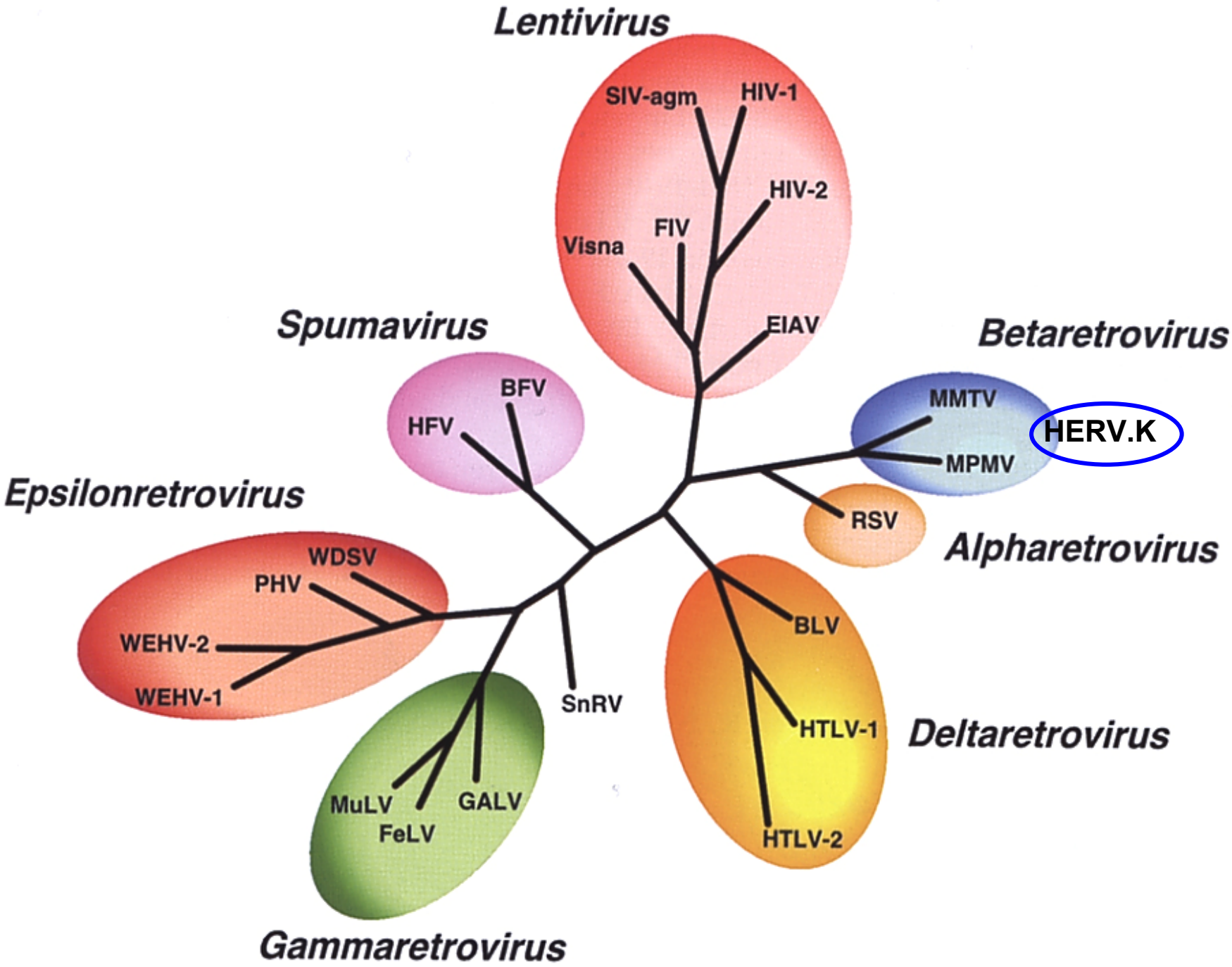
Immunity 15: 579-589, 2001.



zur Hausen, unpublished



zur Hausen, unpublished


Lentivirus
SIV-agm
HIV-1
HIV-2
FIV
Visna
EIAV
Spumavirus
BFV
HFV
Betaretrovirus
MMTV
HERV.K
MPMV
RSV
Alpharetrovirus
Epsilonretrovirus
WDSV
PHV
WEHV-2
WEHV-1
SnRV
BLV
HTLV-1
HTLV-2
Deltaretrovirus
MuLV
GALV
FeLV
Gammaretrovirus

Human endogenous retrovirus K triggers an antigen-specific immune response in breast cancer patients.

Wang-Johanning et al. **Cancer Res. 68: 5869- 5877, 2008.**

Human endogenous retrovirus K (HML-2) elements in the plasma of people with lymphoma and breast cancer.

Contreras-Falindo et al. **J. Virol. 82: 9329-9336, 2008**

- **Breast cancer patients, HIV-associated lymphomas, non-HIV-associated lymphomas, HIV-associated Hodgkin‘s lymphomas reveal about 7-fold elevated concentrations of HERV-K (HML-2) RNA in the plasma in comparison to healthy controls.**

- **The titers in lymphoma patients in remission returned to control values.**

In spite of a number of well known genetic modifications

In breast cancer cells, this tumor remains an interesting

candidate for further research into a

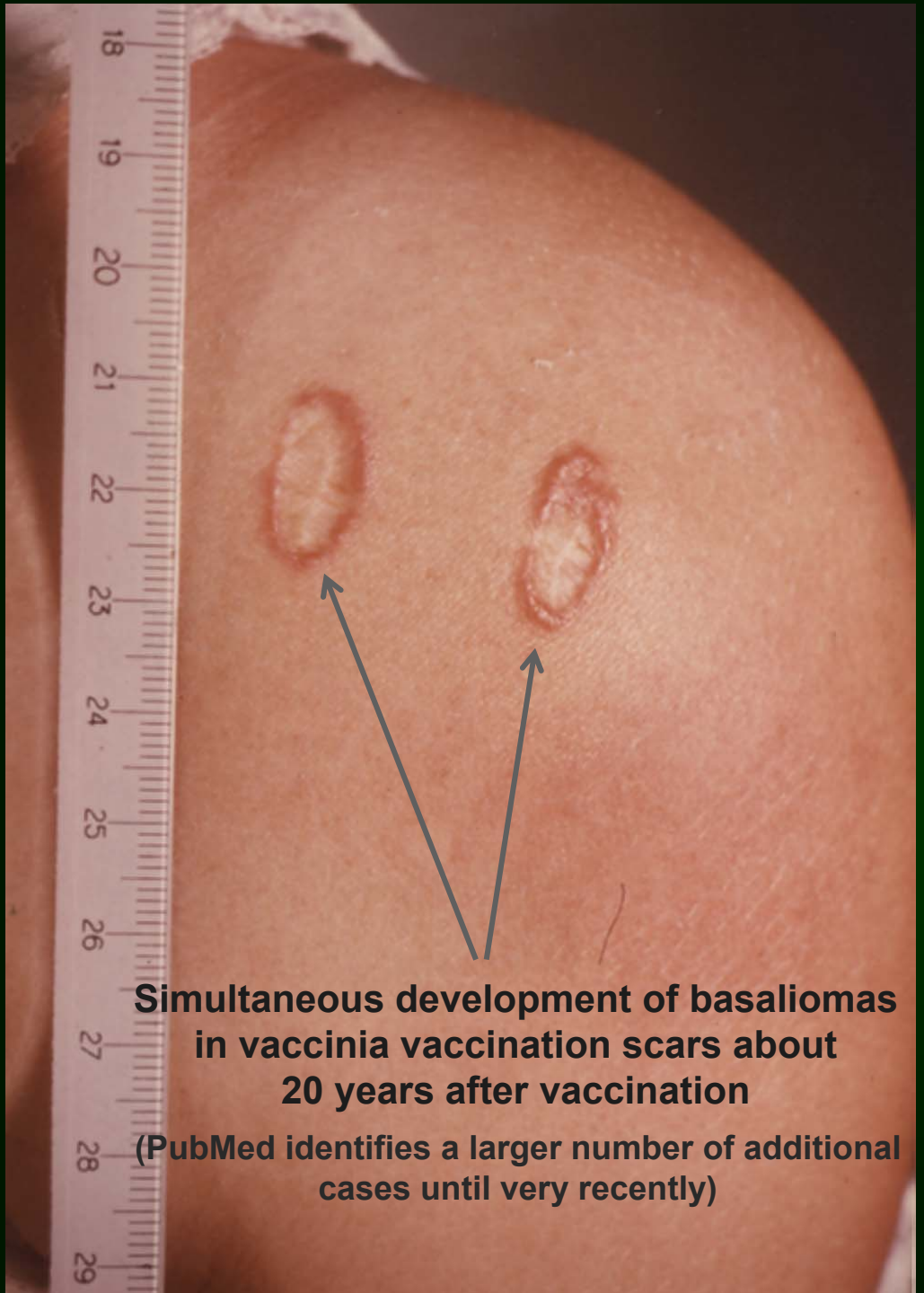
possible infectious etiology.

III. Cancer incidence influenced by infections

Vaccinia virus scars

Early preparations of vaccinia virus for pox vaccination were obtained after scarifying the skin of calves and harvesting the skin crusts containing the vaccinia virus particles

Childhood hematopoietic malignancies



Simultaneous development of basaliomas in vaccinia vaccination scars about 20 years after vaccination

(PubMed identifies a larger number of additional cases until very recently)

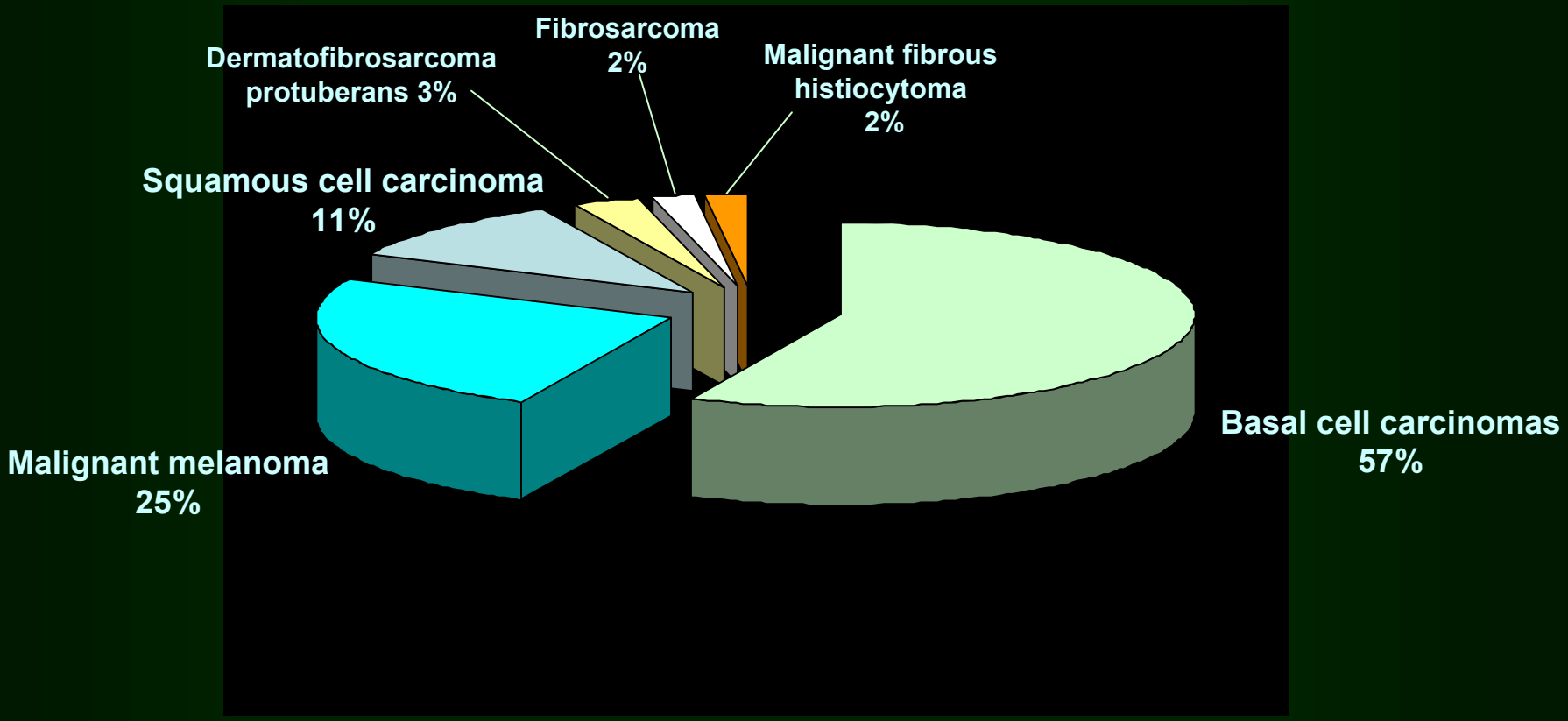
Vaccinia virus infection induces amplfication of persisting polyoma-type virus DNA

J.R. Schlehofer, M. Ehrbar, and H. zur Hausen:

Vaccinia virus, herpes simplex virus and carcinogens **induce DNA amplification in a human cell line** and support replication of a helper-dependent parvovirus.

Virology 152, 110-117, 1986

Malignant tumors arising in vaccinia virus vaccination scars



Modified from Waibel et al.
Int. J. Dermatol. 2006; 43: 685

Several interpretations of these results are possible:

- Vaccinia virus infection of calf skin resulted in the activation of specific cattle viruses whose inoculation into humans 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 for 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.

Others ??

?

Are there animal pathogenic viruses non-permissive for replication in human cells, but carcinogenic in humans ?

A number of human pathogenic viruses (e.g. BK, JC, EBV, High risk HPV, adenoviruses) are non-permissive for animal cells, but induce carcinomas upon inoculation into animals

zur Hausen, H.: Proliferation-inducing viruses in non-permissive systems as possible causes of human cancers. *Lancet 2001; 357: 381-384*

III. Cancer incidence influenced by infections

Vaccinia virus scars

Early preparations of vaccinia virus for pox vaccination were obtained after scarifying the skin of calves and harvesting the skin crusts containing the vaccinia virus particles

Childhood and other hematopoietic malignancies

Hematopoietic Malignancies

Leukemias, lymphomas, non-Hodgkin‘s disease, Hodgkin‘ disease, multiple myeloma:

- **Specific chromosomal modifications have been noted in most of these malignancies. These modifications are not sufficient for malignant proliferations.**

- **A larger number of reports exist demonstrating regional clustering of cases.**

- **Risk and protective factors suggestive for an infectious aetiology have been identified for childhood leukemias and lymphomas.**

Repeatedly reported **protective factors** for childhood leukemias:

- **Multiple infections in early childhood**
- Underprivileged social status
- Crowded household, many siblings
- Inverse risk with birth order

Risk factors for childhood leukemias

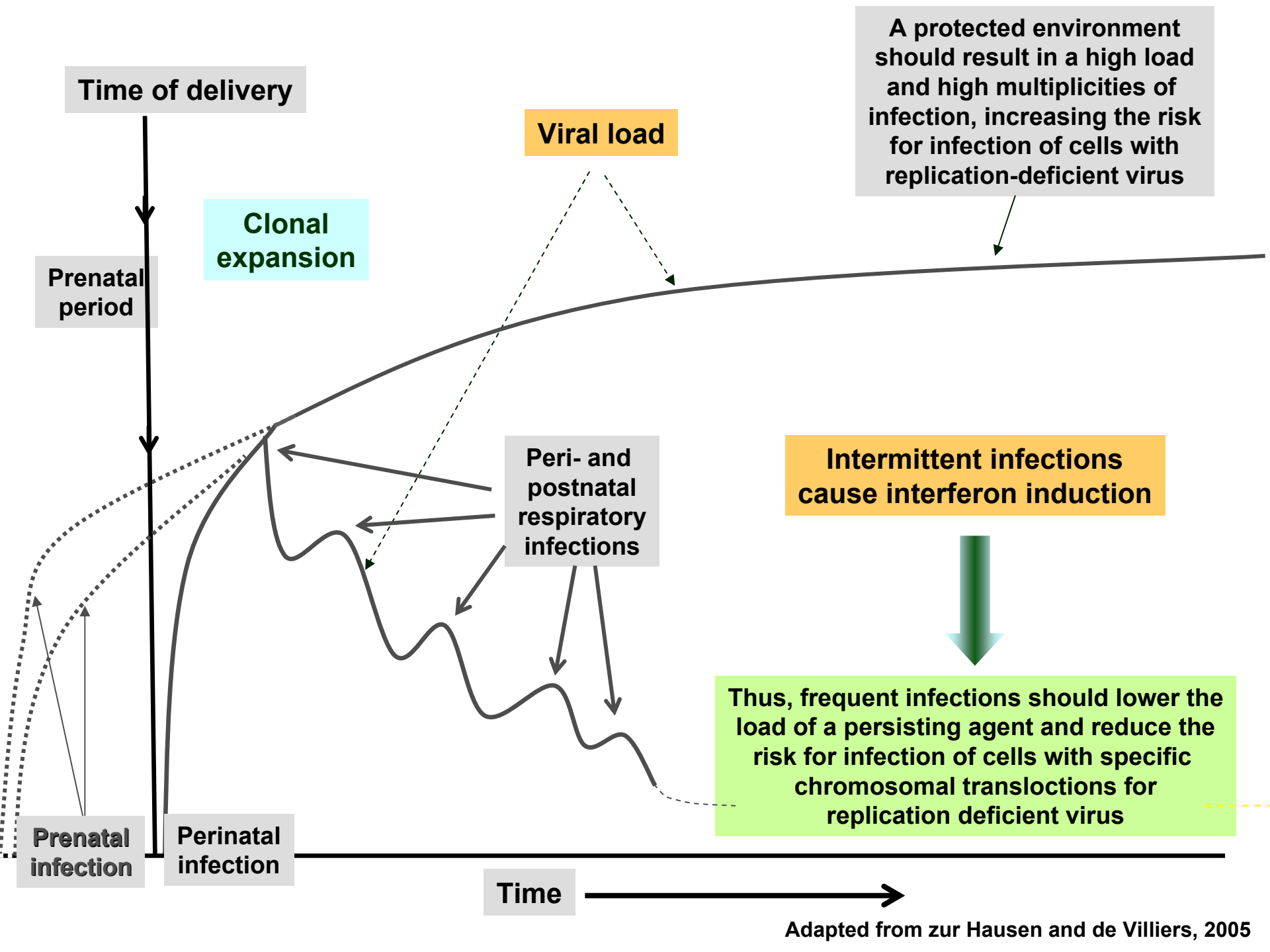
- **Rare infections during the first year of life**
- High socioeconomic status
- Prenatal chromosomal translocations
- Agricultural occupation of parents

The protective effect of infections during the first year of life has frequently been reported and is particularly striking.

It resulted in hypotheses implying:

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 leukemic conversion (*M. Greaves*, 2000).

Alternatively, sudden mixing of a population of low exposure to a putative leukemogenic 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 (*L.J. Kinlen*, 1995).


Time of delivery
Viral load
A protected environment should result in a high load and high multiplicities of infection, increasing the risk for infection of cells with replication-deficient virus
Clonal expansion
Prenatal period
Peri- and postnatal respiratory infections
Intermittent infections cause interferon induction
Thus, frequent infections should lower the load of a persisting agent and reduce the risk for infection of cells with specific chromosomal transloctions for replication deficient virus
Prenatal infection
Perinatal infection
Time
Adapted from zur Hausen and de Villiers, 2005

An interesting example exists in koala bears
(Tarlinton et al., 2005, 2006)

Koala bears **recently** acquired an endogenous retrovirus closely related to gibbon ape leukemia virus

This virus is readily reactivated in vitro and in vivo. Hematopoietic neoplasias in koalas are closely related to the viral load.

Human genomes contain an endogenous retrovirus, more distantly related to gibbon ape leukemia virus, also containing three open reading frames (gag, pro-pol, env) and both LTRs.

Polyoma-type viruses defective in the helicase region of Large T antigen may acquire supertransforming properties

Small, M.B., Gluzman, Y., and Ozer, H.L.
Enhanced transformation of human fibroblasts by origin-defective simian virus 40.
Nature 1982; 296: 671-672.

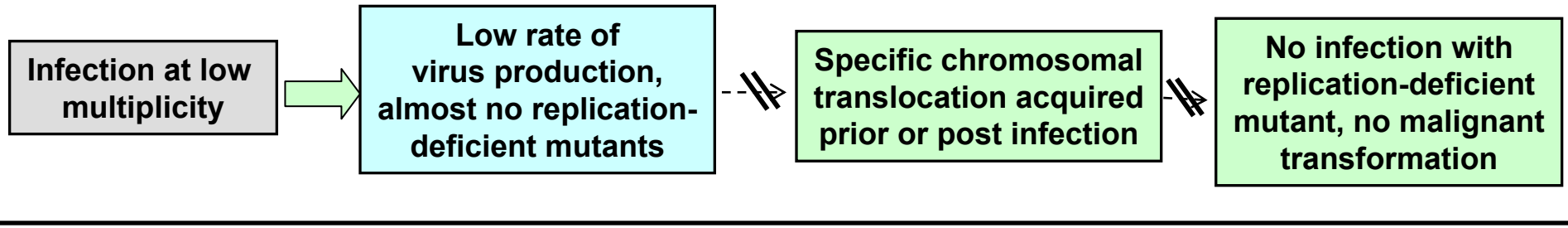
Roberge, C. and Bastin, M.
Site-directed mutagenesis of the polyomavirus genome: replication-defective large T mutants with increased immortalization potential.
Virology 1988; 162: 144-150.

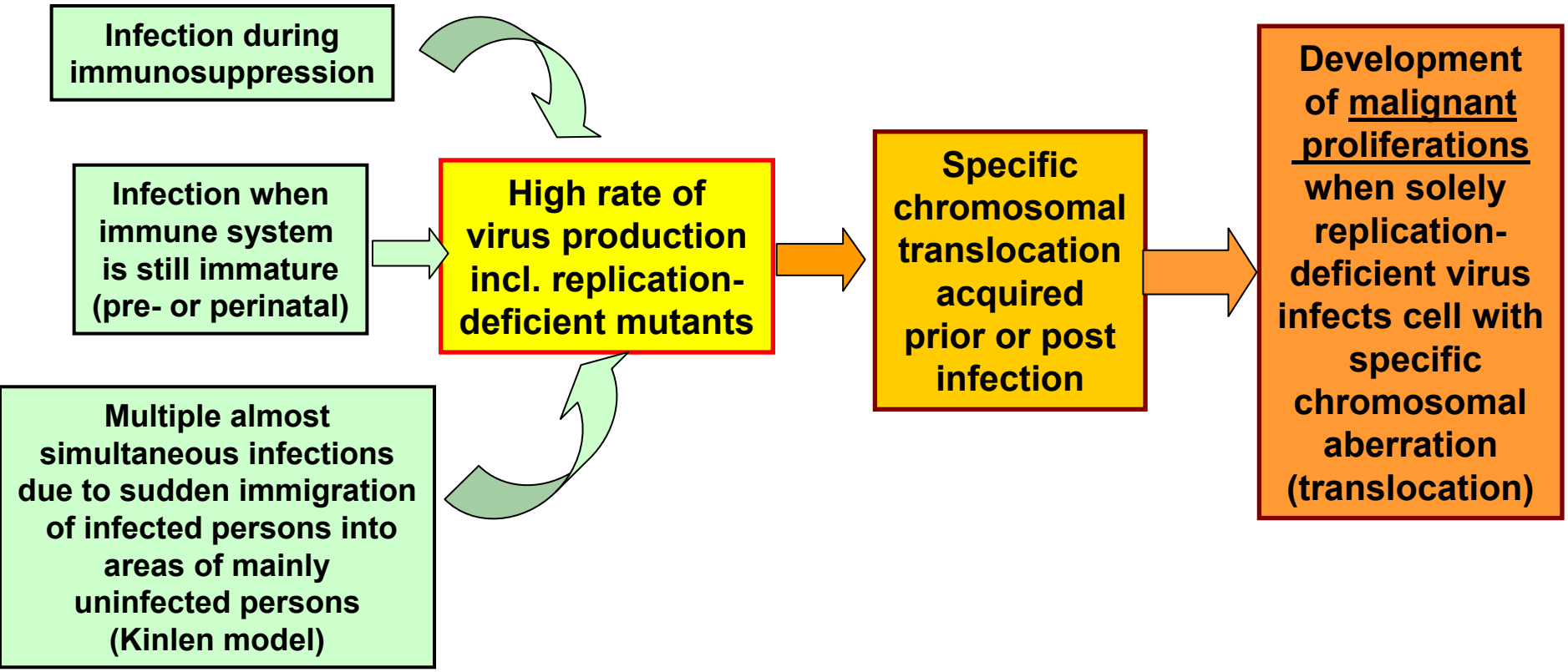
Feng, H., Shuda, M., Chang, Y. and Moore, P.S.
Clonal integration of polyomavirus in human Merkel cell carcinoma.
Science 2008; 319: 1096-1100.

High multiplicities of infection favour the production of defective viral genomes

Synopsis of the Hypothesis

Initial Stage	*Result*	*Risk factor*	*Consequence*



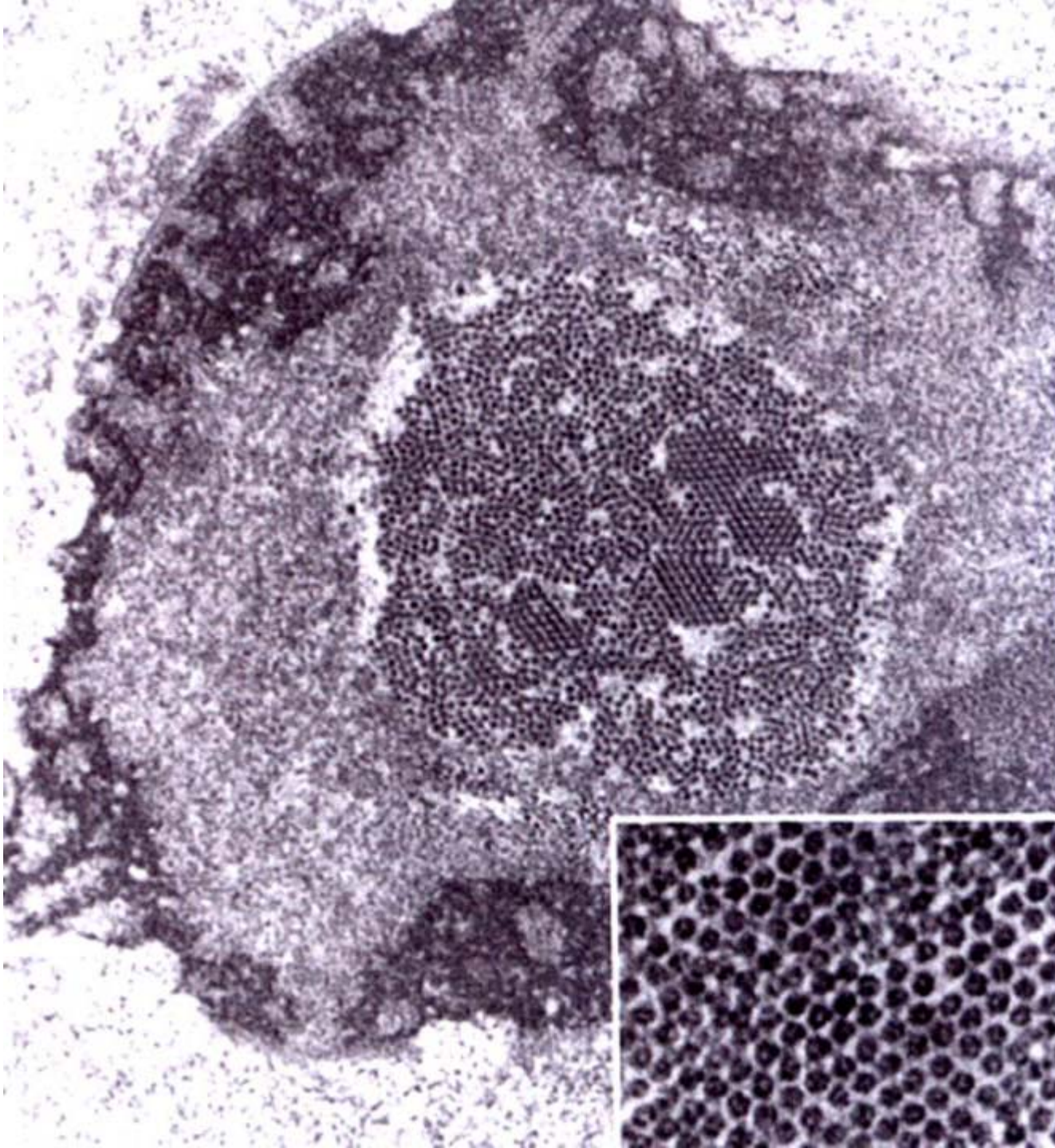


From:

Osswald, S.S., Kulick, K.B., Tomaszewski, M.M., and Sperling, L.C.

Viral-associated trichodysplasia in a patient with lymphoma: a case report and review.

J. Cutan. Pathol. 2007; 34: 721-725.



Although known human cancer viruses (e.g. high risk HPV, HTLV-1, etc.) emerge as necessary factors for the respective tumor types which they cause, **none of these infections leads directly to cancer development.**

Thus far, no human tumorvirus has been identified that does not require modifications of host cell or viral nucleic acids prior to tumor formation.

Commonly these modifications affect cellular pathways engaged in the control of these persisting virus infections.

For these reasons it is even worthwhile to analyze tumors with known hereditary components in their etiology (e.g. breast, colorectal cancers) for a possible etiological involvement of infectious components.

Why are some non-enveloped viruses (Polyoma- and Papilloma-type viruses) particularly interesting as potential candidates in the etiology of further human malignancies?

- Members of these virus families are relatively heat-stabile
- Polyomaviruses commonly transform cells which are non-permissive for viral replication.
- Polyomaviruses with mutations in the helicase part of Large T-antigen may gain super-transforming properties.

Colorectal Cancer

- A number of reports ascribe a **higher incidence rate of colorectal and breast cancer to the rate of consumption of red meat, in particular beef meat**
  (e.g.Santarelli et al., 2008, Hu et al., 21008, Egeberg et al. 2008, Taylor et al., 2007, Cross et al., 2007, Larsson and Wolk, 2006) .

- Countries with the **highest rate of red meat consumption** (e.g. Argentina, Uruguay,New Zealand) commonly reveal a **high rate of colorectal and breast cancer**
  (e.g. Bosetti et al., 2005, Matos and Brandani, 2002, Ferlay et al., 1998, Reif et a., 1989)

Polyomaviruses and papillomaviruses may survive in a protein environment temperatures of up to 80º C for 30 minutes or longer

These temperatures are not reached in central portions of roasted meat cooked "*medium*" or "*raw*"

Common and frequently cited interpretations are dietary factors

- N-nitroso compounds, heterocyclic amines and heterocyclic aromatic hydrocarbons, part of them requiring metabolic activation to convert into a carcinogenic form;

- nitrosyl haem and nitroso thiols have been reported to be significantly increased in feces following a diet rich in red meat.

Yet, "white" meat, specifically the consumption of fried, grilled or smoked chicken, is considered as relatively "safe". This in spite of the production of similarly high concentrations of heterocyclic aromatic hydrocarbons in the cooking process.

Yano, et al. Mutat. Res. 1988; 202: 119-123. Kazerouni et al. Food Chem. Toxicol. 2001; 39: 423-436.
Reinik et al. Food Addit. Contam. 2007; 24: 429-437.

A potential role of infectious agents has thus far barely been considered

Why is a potential role of polyoma-type viruses in human cancers difficult to discover?

- They may represent zoonoses, and human cells would be non-permissive for virus replication;
- They are not discovered by conventional polyomavirus consensus primers
- They become integrated into various chromosomal sites, regularly in low (single?) copy numbers;
- Their T-antigen expression is low and barely detectable by immunological methods,
- The respective DNA may already have been present in human sequences published up to today, but not identified as foreign DNA.

My conclusion:

Research on infectious causes of human cancers has a great potential for future surprises

Hereditary gene modifications as risk factors for cancers caused by infections:

X-linked lymphoproliferative syndrome	Epstein-Barr virus
Epidermodysplasia verruciformis	Genus β papillomaviruses
Cervical cancer	Genus α high risk HPV

The examples outlined here represent hypotheses

In none of the discussed cases an infectious cause has been proven.

The intention of this lecture is to raise interest in these topics and to stimulate interest and novel studies in the potential role of infectious agents in some common human cancers.

Even when only part of these considerations turn out to be correct, this would have profound implications for future strategies on cancer prevention, early diagnosis and therapy.