ETD EPA US Distinguished Lecture, July 25, 2006

The Role of Body Burden 2,3,7,8-TCDD in Triggering Malignancy-Associated Human Viruses: From Early Data to Mechanistic Concept

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The Role of Viruses in Human Cancer Development

- ✓ **Viruses are linked to at least 15 to 20% of human cancers[1-6]**
- ✓ **Viruses accepted as bona fide factors of cancer progression are: HBV, EBV, HPV, HTLV-1*, and a candidate CMV[7,8]**
- ✓ **The infectious nature of viruses distinguishes them from all other cancer-causing factors[1,2,3-5].**
- ✓ **Malignant forms of cancer arise in chronically inflamed tissue. A high frequency of virus genome and antigens in tumor cells is documented in persistent viral infection, which is necessary for formation of high-grade lesion and invasive cancer [2]**
- ✓ **Among factors influencing viral carcinogenesis, the synergy between viruses and environmental cofactors is suggested[3,6]**

References:

1. F. Hoppe-Seyler & K. Butz, Human Tumor Viruses. *Anticancer Res* **19**:4747-4758, 1999
2. L Kinlen, Infections and immune factors in cancer: the role of epidemiology. *Oncogene* **23**:6341-6348, 2004
3. J. Butel, Viral carcinogenesis: revelation of molecular mechanisms and etiology of human diseases. *Carcinogenesis* **21**:405-426, 2000
4. J. Kadow et al., The role of viruses in human cancer development and intiviral approaches for intervention. *Curr Opin Invest Drugs* **3**:1574-1579, 2002
5. J. Andersen Phelan, Viruses and neoplastic growth. *Dent Clin Am* **47**:533-543, 2003
6. Y. Aoki & G. Tosano, Neoplastic conditions in the context of HIV-1 infection. *Curr HIV Res* **2**:343-349, 2004
7. L. Harkins et al., Specific localization of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. *Lancet* **16:**1557-1563, 2002
8. C. Cobbs et al., Human cytomegalovirus infection and expression in human malignant glioma. *Cancer Res* **62**:3347-3350, 2002.

*Topics of the malignancy-linked HCV and HIV-1 viruses are beyond the scope of this presentation.

Immunosuppression is traditionally considered as predetermining condition for both chronic viral infections[1,2] and malignancies[1,3]

1. L. Kinlen, Oncogene 23:6341-6348, 2004
2. R. Luebke et al., J. Immunotoxicol 1:15-24, 2004
3. J. Butel, Carcinogenesis 21:405-426, 2000

§ **The introduction of highly active antiretroviral therapy (HAART) has dramatically decreased the incidence of AIDS-related malignancies associated with immunosuppression, such as Kaposi sarcoma[4-7]. At the same time, almost full restoration of immunocompetence in HIV-infected patients with the HAART has no effect on EBV-related non-Hodgkin's lymphomas[4-6], and HPV-related cervical cancer[7].**

§ **Malignant disease has been a major cause of death among immunocompetent HIV-infected patients in industrialized nations since the introduction of HAART[3].**

4 C.S. Rabkin, *Eur J Cancer* **37**:1316-1319, 2001
5 W.Y. Au et al., *Blood* **104**:243-249, 2004
6 F. Bonnet et al., *Cancer* **101**:317-324, 2004
7 Y. Aoki & G. Tosato, *Curr HIV Res* **2**:343-349, 2004

ARE HUMANS EXPOSED TO LOW DOSE TCDD AT RISK OF DEVELOPING CANCER OR, AT LEAST, IMMUNOSUPPRESSION?

Regarding TCDD as "a group 1 carcinogen" (IARC, 1997), all judgments derived from experimental animal data, and the data obtained in accidental and industrial cohorts heavily exposed to TCDD.

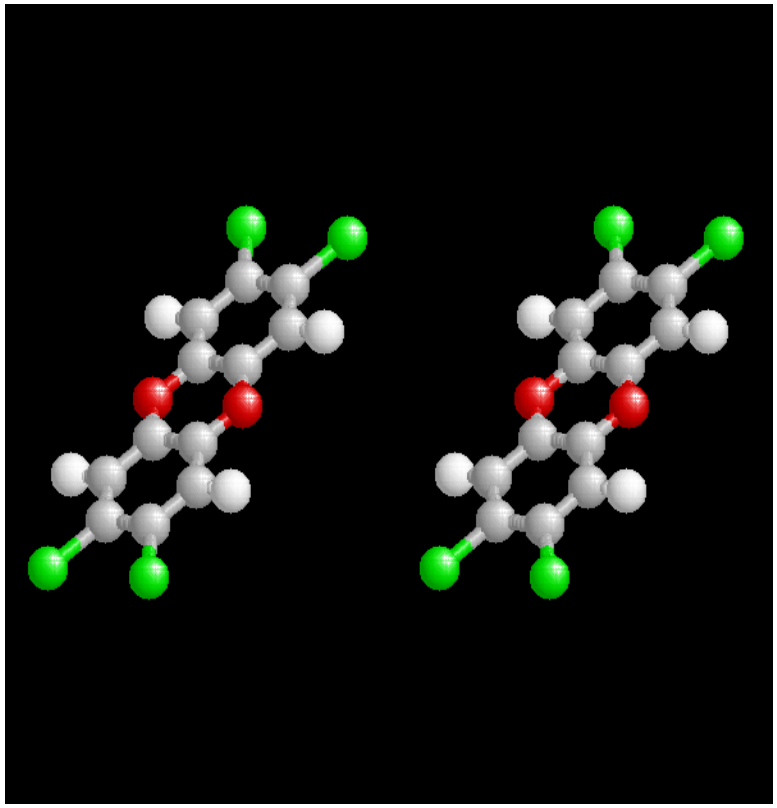
On the subject of human cancer risk at subchronic low exposure to TCDD, opinions are heterogeneous, from those accepting either a non-linear (IARC, 1997) or a linear[1] dose-response relationship, to those challenging any carcinogenic potential for TCDD body burden in humans, and stressing that current serum lipid-adjusted level of ~2-3 ppt is to low to pose a risk to human health[3].

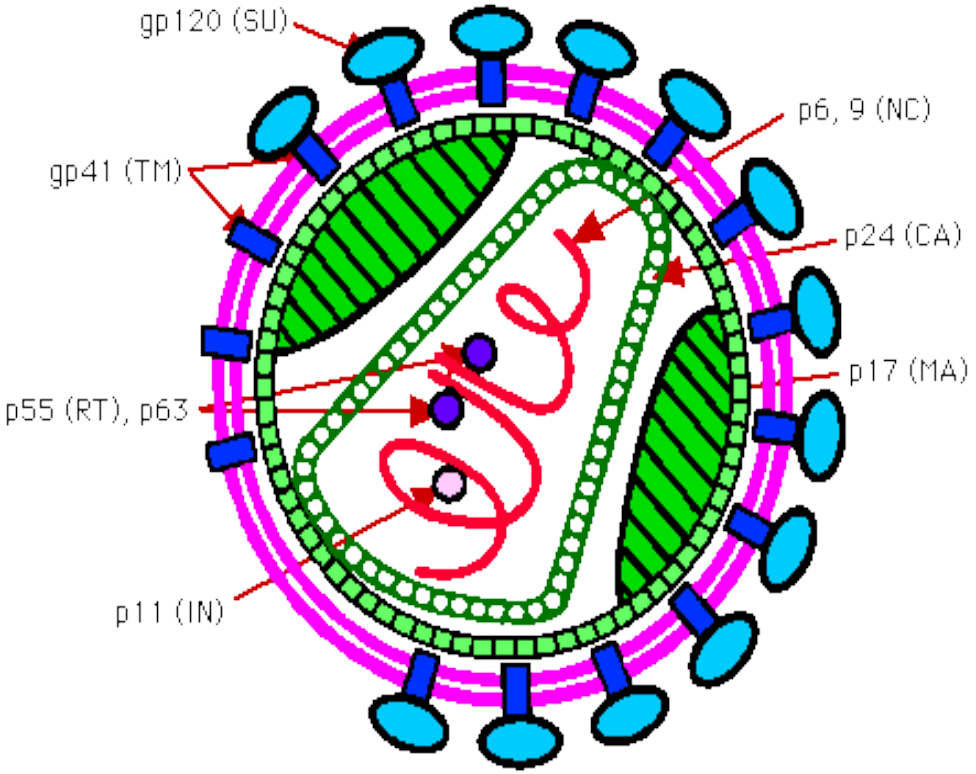
Immunotoxicity is commonly viewed an underlying cause of TCDD pro-infectious and carcinogenic effects in humans "at some dose level"[2,3], although there appeared to be too little information to suggest that TCDD at levels observed causes long-term adverse effects on the immune system in adult humans[3,4]

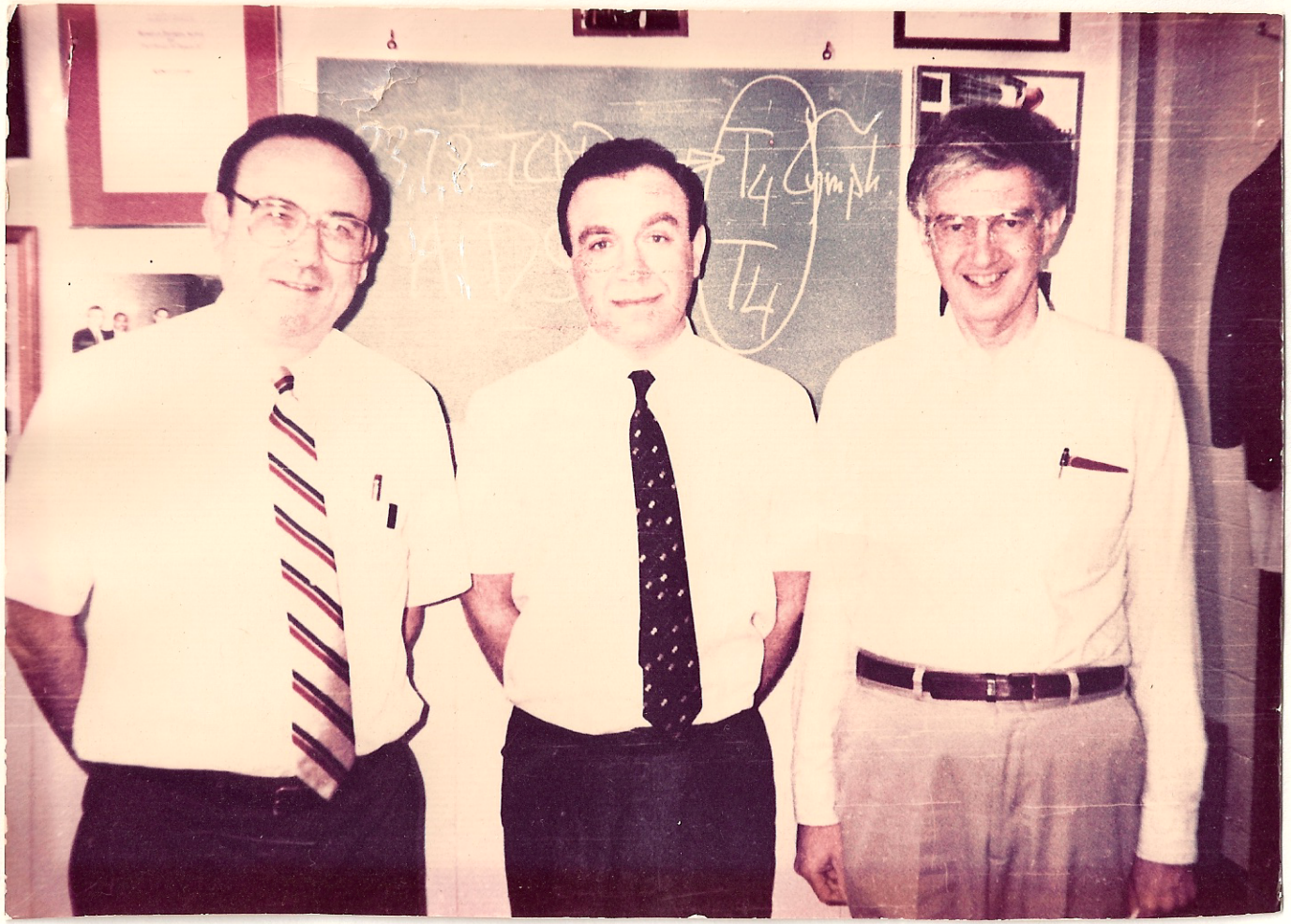
1. J. Diliberto et al., *Toxicol Sci* **61**:241-255, 2001
2. The *US EPA 2003 Draft* "Exposure and Human Health Reassessment of 2,3,7,8-TCDD and Related Compounds"
3. R. Luebke et al., *J. Toxicol Environm Health* Part B: Critical Reviews **9**:1-26, 2006
4. S. Hays & L. Aylward, *Regul Toxicol Pharmacol* **37**:202-217, 2003

HIV-1 and TCDD both target the same human CD4+ lymphocytes

(M. Popovic et al., *Science* **224**:497-500, 1984; R. Hoffman et al., *JAMA* **255**:2031-2038, 1986)







First presentation of the TCDD-HIV data at Dr. Alan Conney laboratory, Rutgers, 1989

- **2,3,7,8-Tetrachlorodibenzo-p-dioxin as a possible activator of HIV infection**
  A.G. Pokrovsky, A.I Chernykh, O.N. Yastrebova, and I.B. Tsyrlov
  Biochem. Biophys. Res. Commun. 179:46-51, 1991

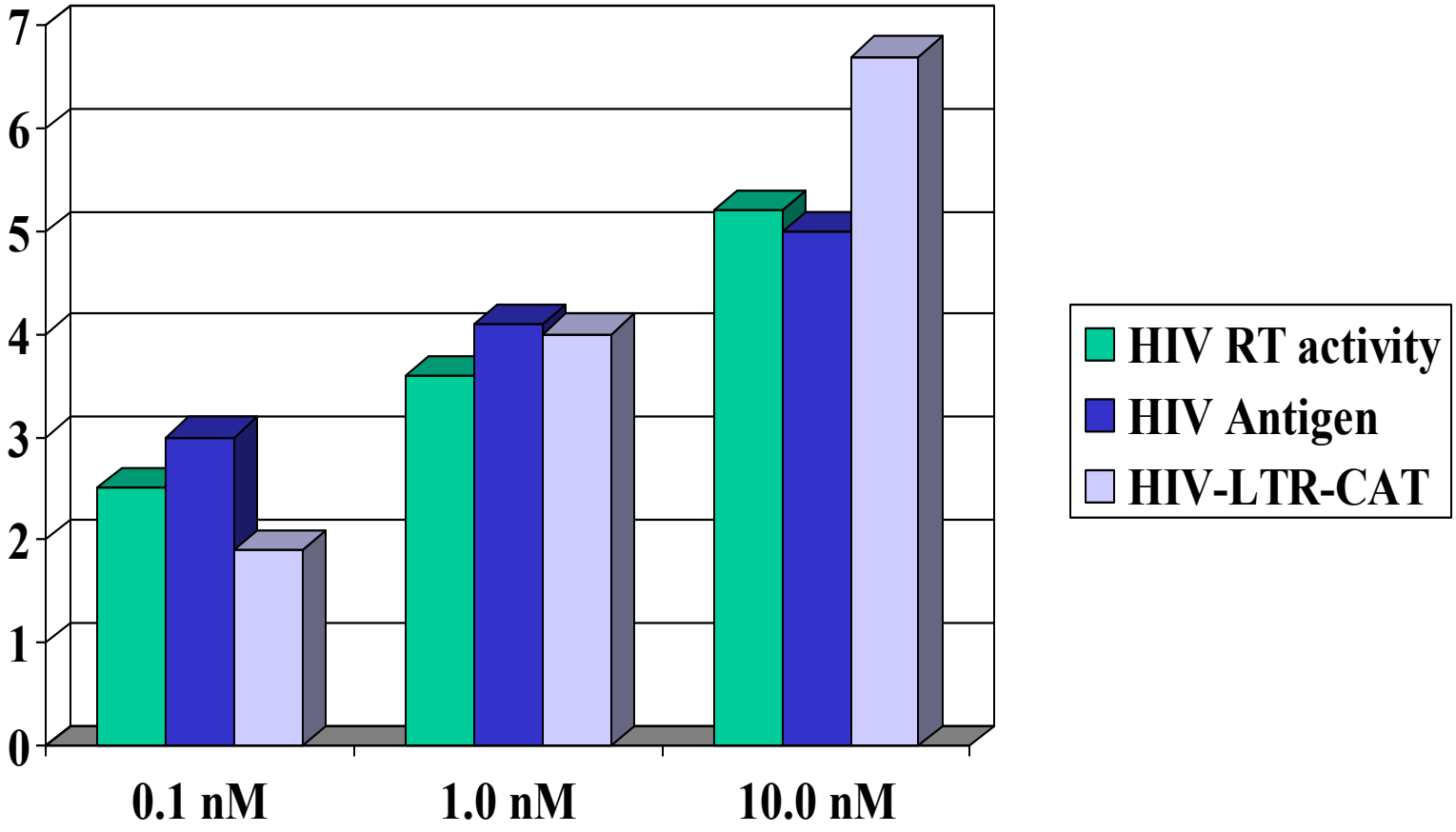
- **Stimulatory effect of the CYP1A1 inducer 2,3,7,8-tetrachlorodibenzo-p-dioxin on the reproduction of HIV-1 in human lymphoid cell culture**
  I.B. Tsyrlov and A.G. Pokrovsky. *Xenobiotica* 23:457-467, 1993

- **Activating effects of dioxin on HIV-1 in human CD4+ lymphoid cells**
  I.B. Tsyrlov and A.G. Pokrovsky. *Proceed. 10th Intern. Conf. AIDS* (Yokohama, Japan) 10:127, 1994

A nanomolar TCDD activates reproduction of HIV-1

Data on HIV RT and HIV antigen: Pokrovsky et al., *BBRC* 1991; Tsyrlov & Pokrovsky, *Xenobiotica* 1993
Gollapudi et al., *BBRC* 1996; Ohata et al., *Microbiol. Immunol*. 2003

Data on HIV-LTR-CAT: Yao et al., *Environ. Health Perspect*. 1995;
Gollapudi et al., *BBRC* 1996

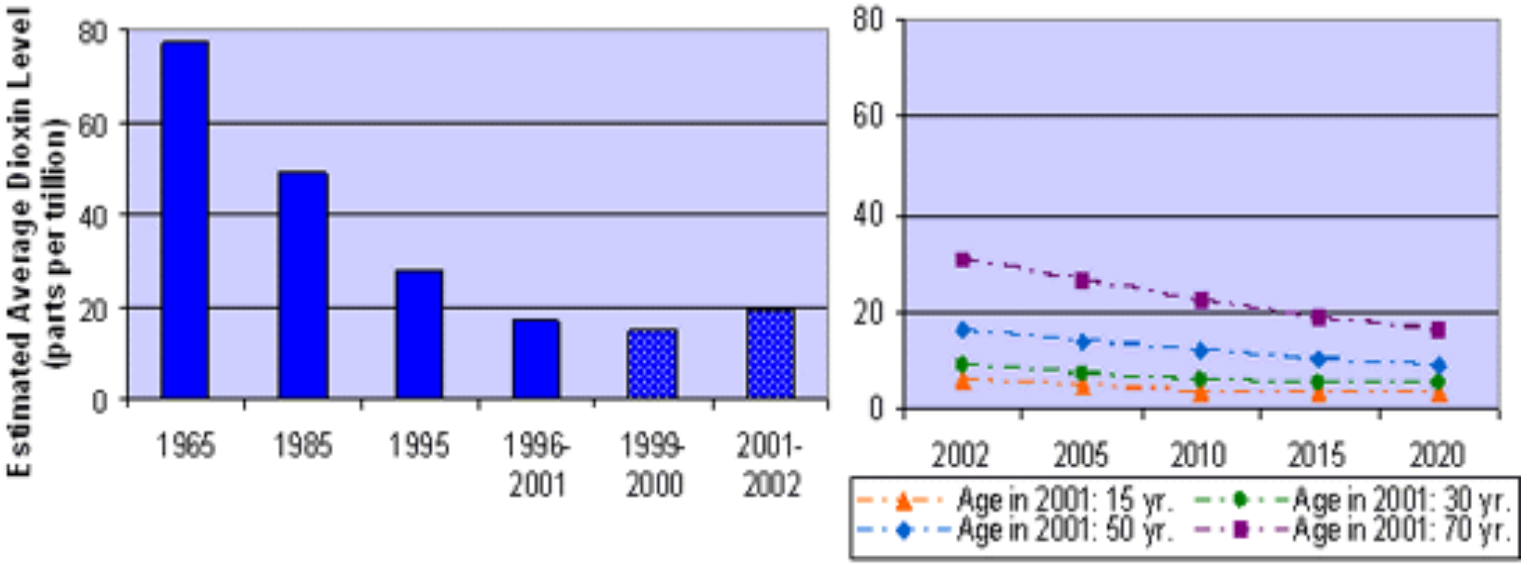


THE REAL QUESTION IS DO WE HAVE ANY IDEA WHAT LOCAL CONCENTRATION OF DIOXIN MIGHT TRIGGER VIRALLY DRIVEN MALIGNANCY IN CHRONICALLY INFLAMMED SPECIFIC TUSSUE?

D I O X I N L E V E L S :

A - Historically (US CDC)

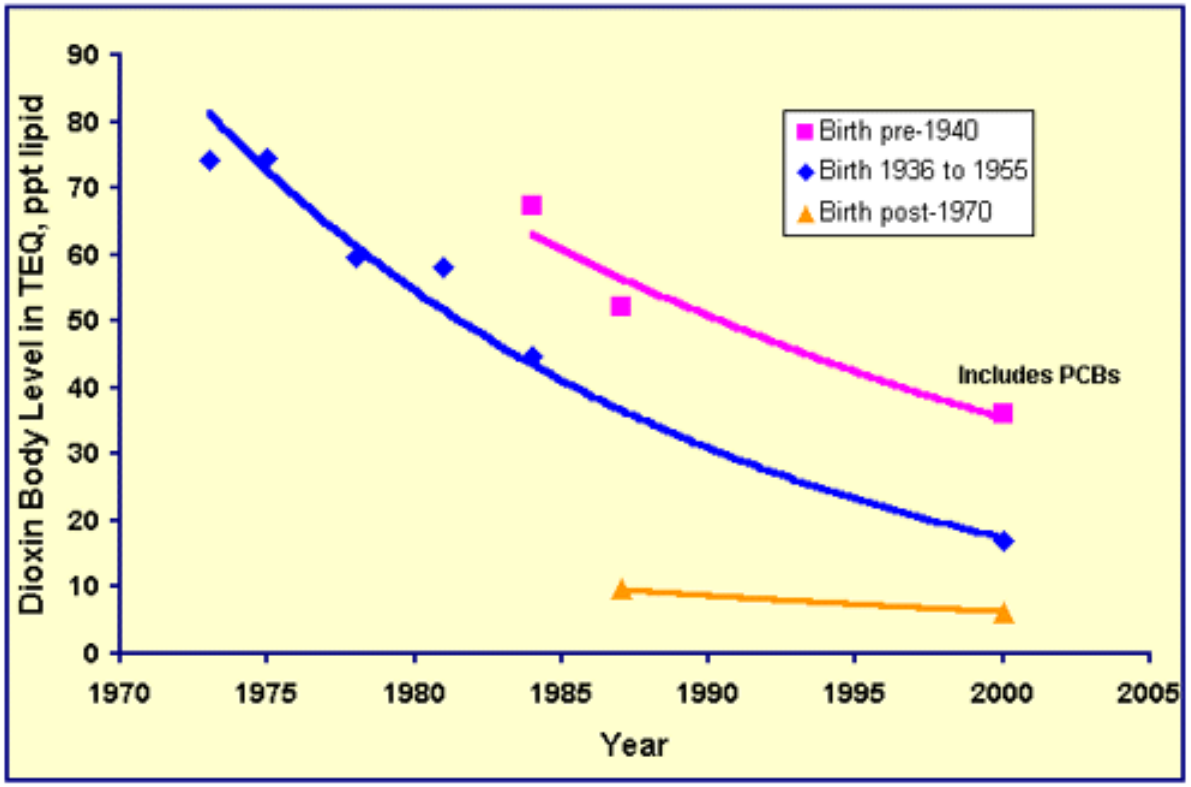
B - Looking Ahead (CCC)



A - Data for 1965, 1985 and 1995 are modeled average dioxin-TEQ for ages 20 to 70 (Lorber, 2002). Data for 1996-2001 are measured average TEQs from individuals age 15 to over 60 from LA, MO, NC, and NY (588 people) and includes 4 PCBs (Patterson et. al., 2004). Data for 1999-2000 are from CDC (2003) for people aged 20 and over. Data for 2001-2002 are from CDC (2005) for people aged 20 and over. Range for governmental exposure guidelines is 8 - 32 ppt, which includes dioxins and PCBs with TEFs. Limit of Detection (LOD): 1999-200 – 12.1 ppt; 2000-2002 – 5.8 ppt.

B - CCC modeled the future body levels (from 2002 to 2020) starting with the mid-point measurement for the average dioxin ppt-TEQ for each reported age group (20 years and older) reported in the Third National Exposure Report (2005). CCC based projections for the 15 year old age group on the 2001-2002 pooled blood samples (Needham, 2005).

Trends in Body Levels of Dioxins

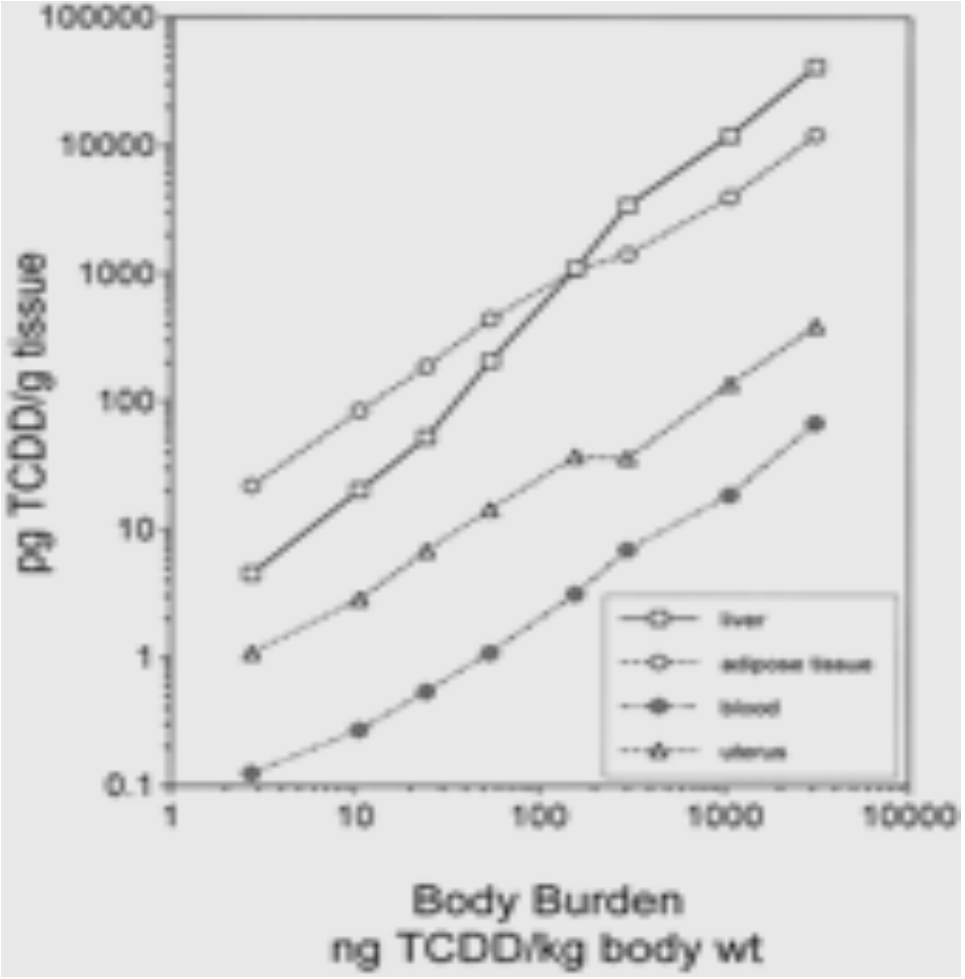


Probability of Developing Invasive Cancers Within Selected Age Intervals (in %, US, 2000-2002)

		Birth to 39	40 to 59	60 to 69	70 and older	Birth to Death
All sites	Male Female	**1.43** **1.99**	**8.57** **9.06**	**16.46** **10.54**	39.61 26.72	45.67 38.09
Colon & rectum	Male Female	0.07 0.06	0.90 0.70	1.66 1.16	4.94 4.61	5.84 5.51
Non-Hodgkin lymphoma	Male Female	0.14 0.09	0.47 0.31	0.56 0.42	1.57 1.29	2.18 1.82
Uterine cervix	Female	0.15	0.28	0.15	0.22	0.74

Relationship of body burden expressed as ng TCDD/kg body weight to dosimetry in liver, adipose tissue, blood, and uterus expressed as pg TCDD/g tissue following subchronic exposure over a 13-week period

(From J.J. Diliberto, M.J. DeVito, D.G. Ross, and L.S. Birnbaum, *Toxicological Sciences* **61**: 241-255, 2001)



Inflammation induces local tissue and intracellular lipid accumulation

Z. Xiong et al., Inflammation Modifies Lipid-Mediated Renal Injury. ***Nephrol Dial Transplant*** **18: 27-32, 2003**

CONCLUSION: Systemic or local inflammation could be an additional factor in anti-renal injury therapy.

CM Pond et al., Experimental Chronic Inflammation of Lymph Nodes Induced Formation of more Adipocytes in Contiguous Adipose Tissue. ***Antiviral Therapy*** **8:L25, 2003**

CONCLUSION: The formation of additional adipocytes may explain why HARS reverses very slowly during interruptions to antiviral therapy, often not at all.

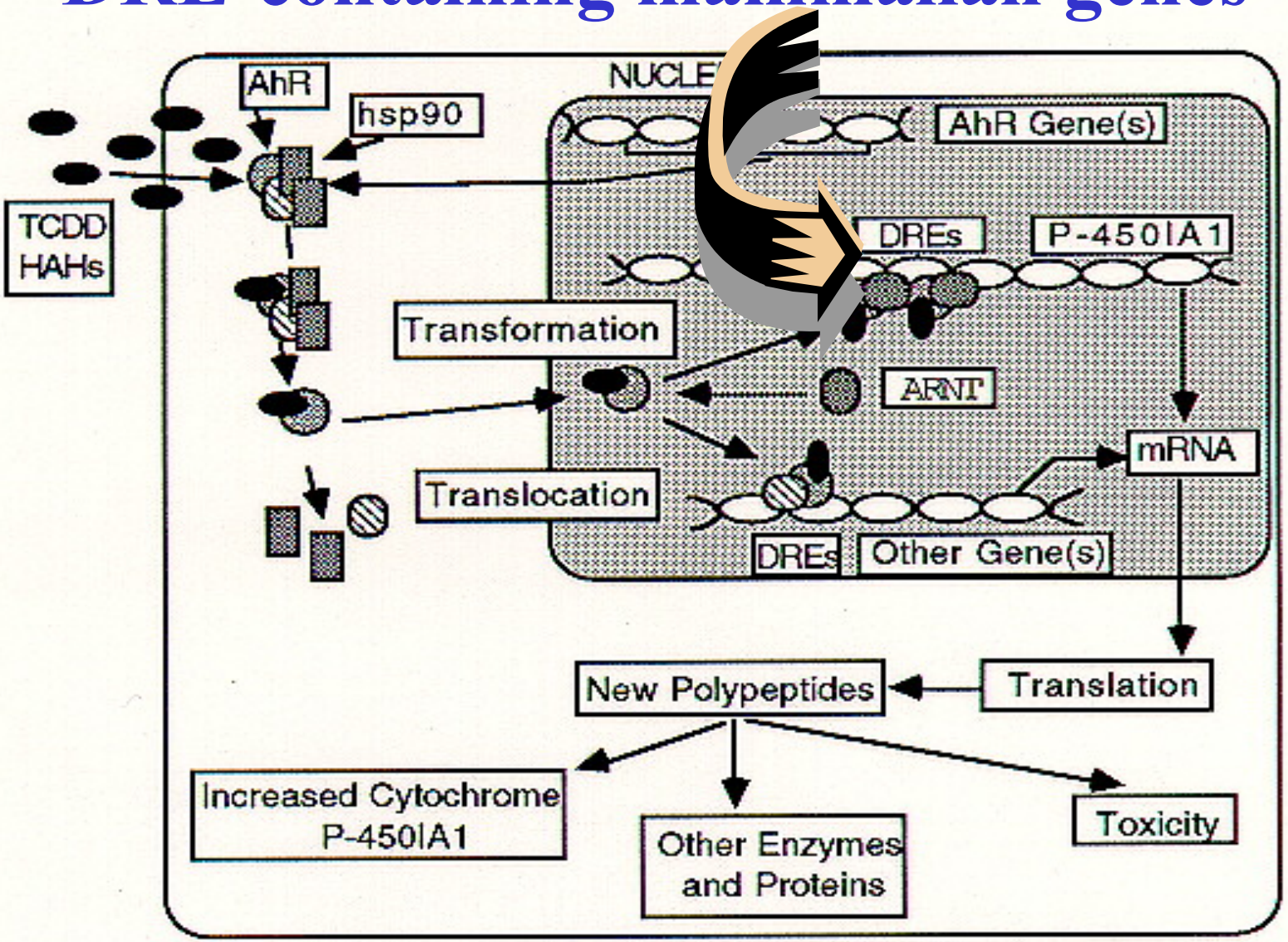
X. Z. Ruan et al, PPAR Agonists Protect Mesangial Cells from Interleukin 1β-Induced Intracellular Lipid Accumulation by Activating the ABCA1 Cholesterol Efflux Pathway. ***J Am Soc Nephrol*** **14:593-600, 2003**

CONCLUSION: These results suggest potential mechanisms whereby inflammation may exacerbate lipid-mediated cellular injury in the tissues.

R. Dickerson & C. Karwoski, Endotoxin-Mediated Hepatic Lipid Accumulation… ***J Am Coll Nutr*** **21:351-356, 2002**

CONCLUSION: The presence of inflammation or infection has been suggested to augment hepatic lipid accumulation in the patients.

Molecular mechanism of TCDD action on DRE-containing mammalian genes



Two major breakthroughs 2002

Organism summary of the dioxin response element (DRE) core sequence (5'-GCGTG-3') found in viral promoters in the Eukaryotic Promoter Database [from T. Zacharewski, 2002]

Species	# DREs Located	# Promoters Represented
Adenovirus		
Human adenovirus type 12	10	4
Human adenovirus type 2	36	9
Human adenovirus type 5	19	5
Human adenovirus type 7	12	5
Simian adenovirus (7P)	3	1
Epstein-Barr virus		
Human herpesvirus 4	154	22
Hepadnavirus		
Duck hepatitis B virus	6	2
Human hepatitis B virus	4	4
Herpes virus		
Human cytomegalovirus	102	10
Human herpes simplex virus type 1	345	30
Human herpes simplex virus type 2	38	8
Murine cytomegalovirus	1	1
Papilloma virus		
Bovine papillomavirus type 1	15	6
Human Papillomavirus type 16	3	1
Human Papillomavirus type 18	9	2
Papovavirus		
Mouse polyoma virus	1	1
Simian virus 40	5	3
Parvovirus		
(Murine) parvovirus H1	4	2
Adeno-associated virus 2	9	3
Lentivirus Oncovirus		
Human immunodeficiency virus type 1	1	1
Human immunodeficiency virus type 2	2	1
Simian AIDS retrovirus SRV-1	3	1
Mammalian Oncovirus		
(Avian) Rous sarcoma virus	7	1
Bovine leukemia virus	1	1
Gibbon ape leukemia virus	1	1
Human T-cell leukemia virus type I	4	1

A picomolar TCDD activates replication of human cytomegalovirus (CMV)

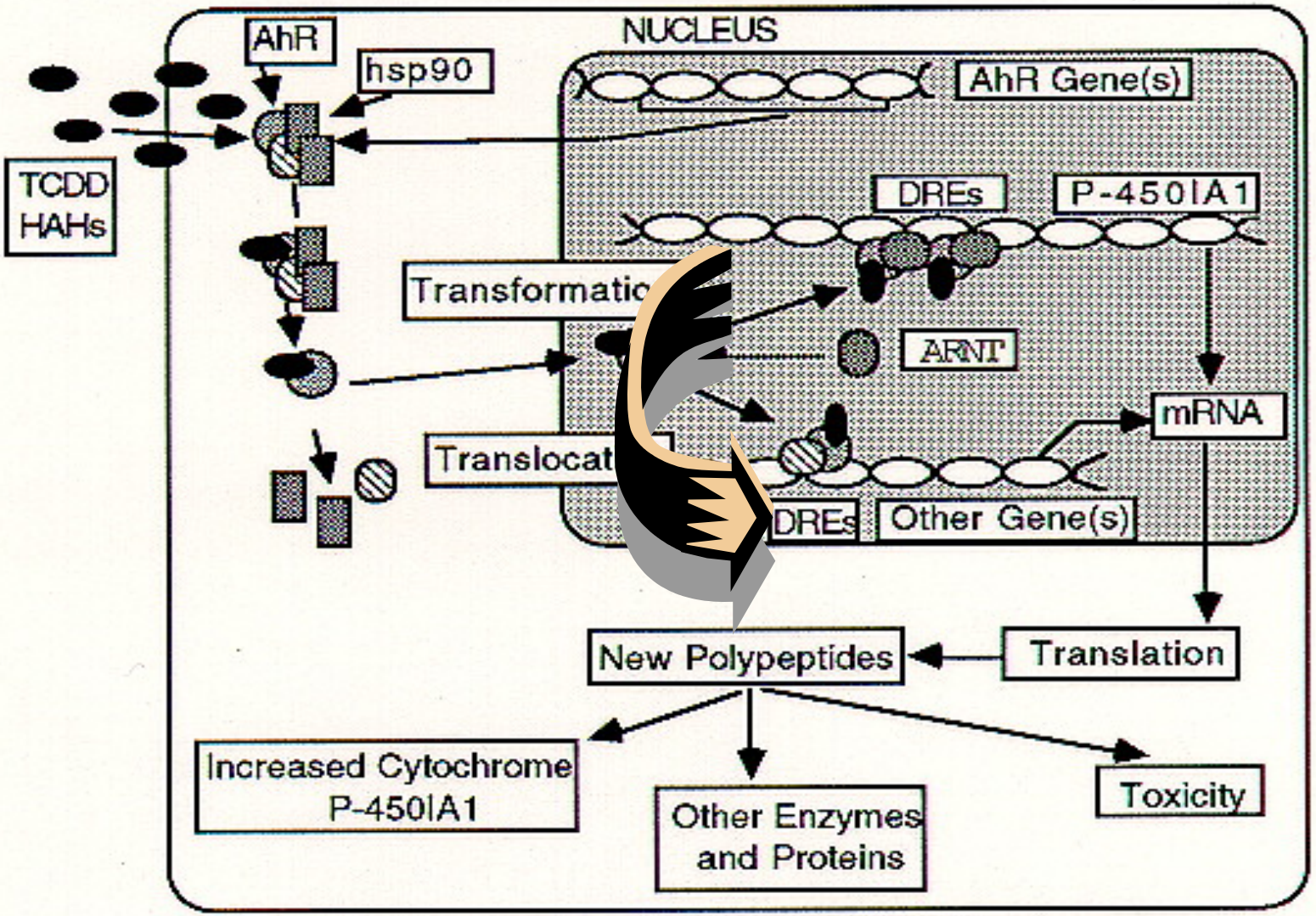
(From: T. Murayama et al., *BBRC* **296**:651-656, 2002)

- **About 4-fold enhancing effect on CMV production was determined in human fibroblast MRC-5 cell line treated with 0.0001 pg TCDD/ml**

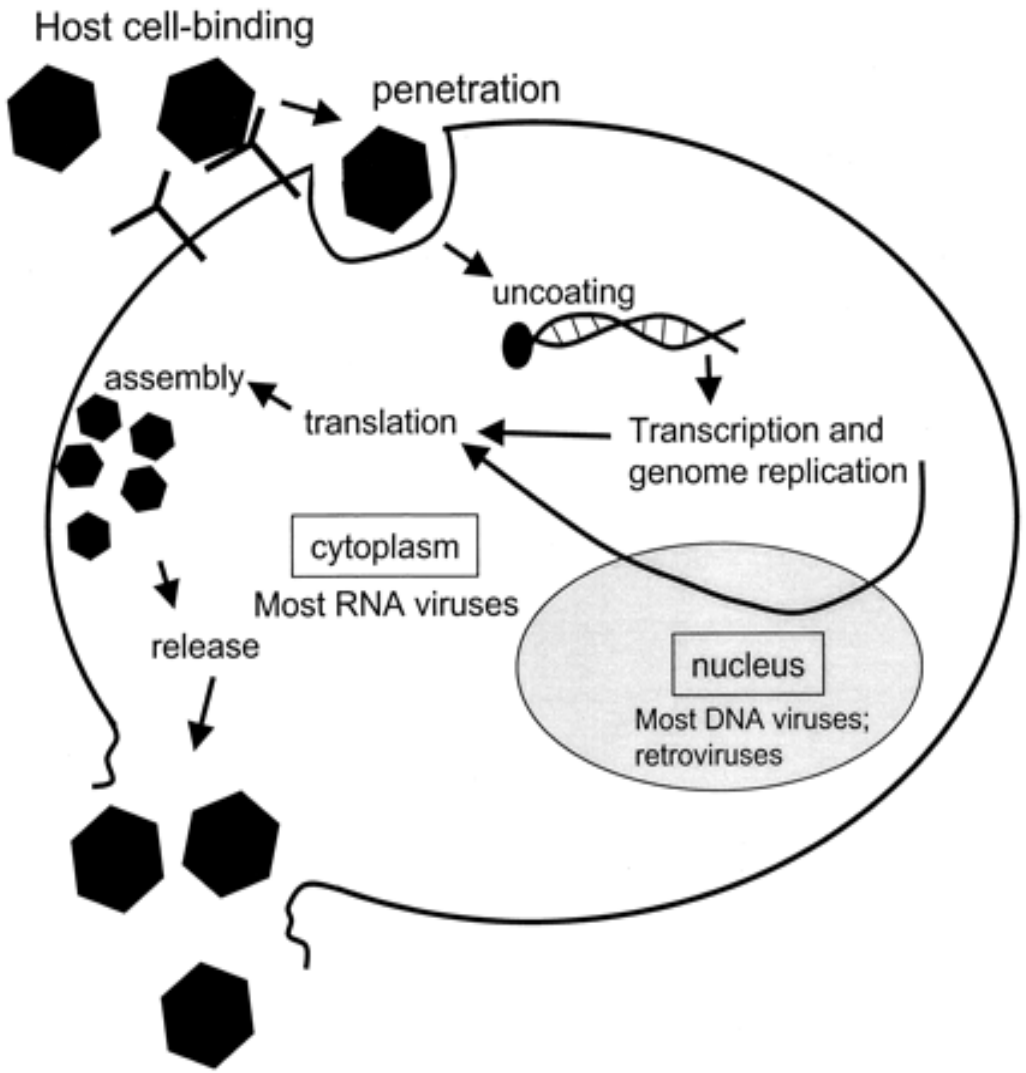
  (0.3 pM TCDD)

- **CMV-infected cells expressed transcripts of the AhR and AhR nuclear translocator. The anti-AhR antibody reduced TCDD-enhanced CMV replication to un-stimulated levels**

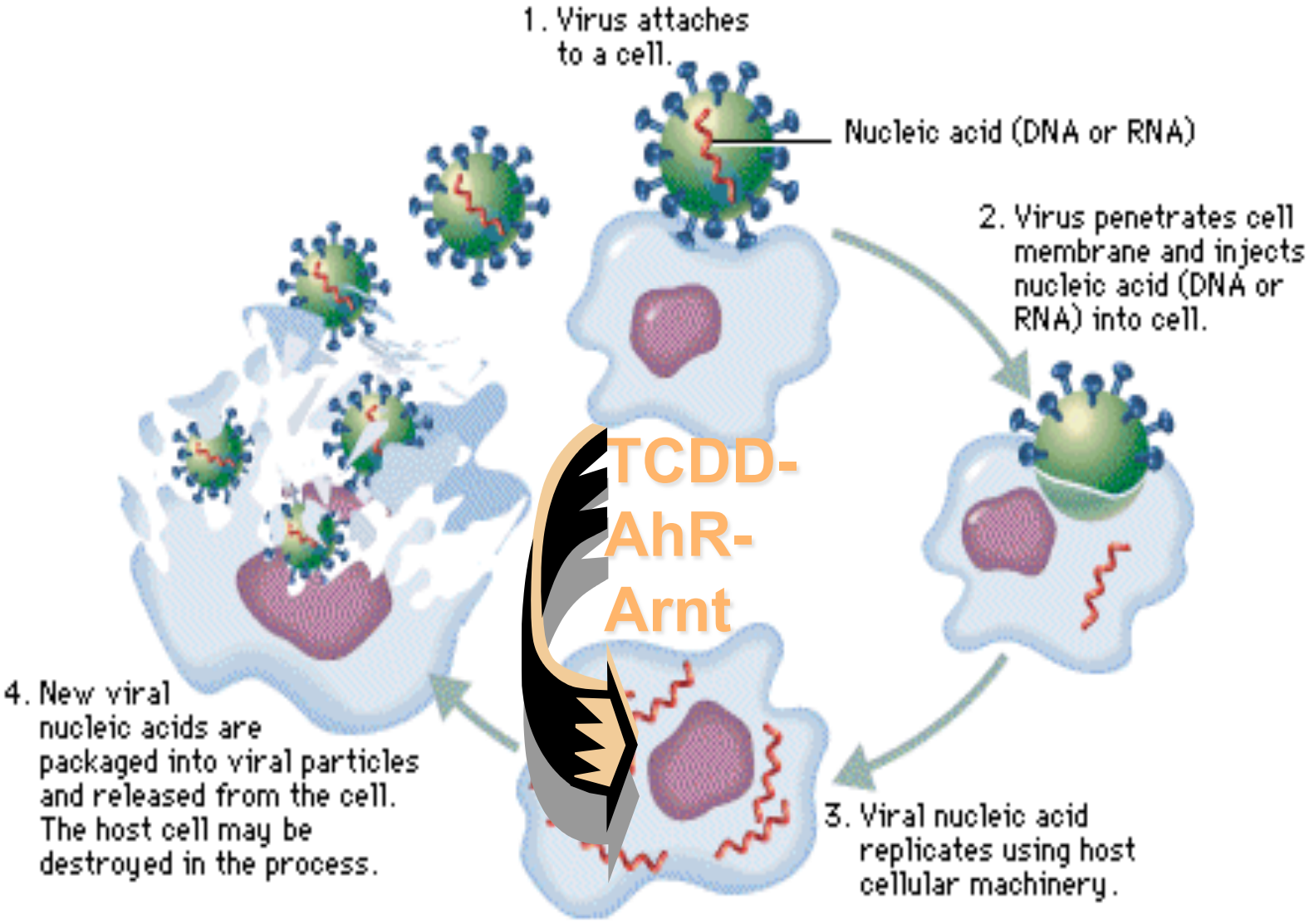
Molecular mechanism of TCDD action on viral DRE-containing genes ("Other Genes")



General model of eukaryotic viral replication



Dioxin Triggers the Ah Receptor-mediated Transcriptional Pathway to Facilitate HIV-1 and CMV Replication Cycle



Cancer-associated human viruses with multiple promoter DREs

Virus name	Promoter DREs (#)	TCDD/**(AhR overexpressed)	Virally derived cancers
Cytomegalovirus	10	**0.3 pM/****	Colon adenocarcinoma Colorectal polyps Congenital cancer Breast cancer in women < 40 yr
Epstein-Barr virus	22	**?/****	Non-Hodgkin's Lymphomas, Sarcomas Nasopharengeal sarcoma Burkett's lymphoma
Herpes simplex virus			
type 1	30	**?/?**	
type 2	8	**?/?**	Cervical Cancer?
Hepatitis B virus	4	**?/****	Hepatocellular carcinoma

Cancer-associated human viruses with a single promoter DRE

Virus name	Promoter DREs (#)	TCDD/**(AhR overexpressed)	Virally derived cancers
HIV type-1	1	**0.1-1.0 nM/****	Various malignancies in the context of HIV-1 infection
Papillomavirus type 16	1	/**	Invasive cervical cancer Skin cancer Oral & laryngeal cancers Anal cancer
T-lymphotropic virus type 1	1	/**	Adult T-cell leukemia (ATL)

Does cytomegalovirus play a causative role in the development of various inflammatory diseases and cancer?

Sadenberg-Naucler C. *J Intern Med* **259**: 219-46, 2006

Human cytomegalovirus (CMV) is a herpes virus that infects and is carried by 70-100% of the world's population, and it might survive in an immunocompetent host. For many years, CMV was not considered to be a major human pathogen.

CMV-mediated disease has highlighted the possible role of this virus in the development of other diseases, in particular inflammatory diseases, autoimmune diseases and, more recently, with certain forms of cancers. It either plays a causative role in these diseases or is merely an epiphenomenon of inflammation. Inflammation plays a central role in the pathogenesis of HCMV.

This virus might participate by influencing the regulation of various cellular processes including the cell cycle, apoptosis and migration as well as tumor invasiveness.

CMV May Modulate the Malignant Potential for Tumor Cells

CMV is able to reactivate other oncogenic human viruses:

EBV, after CMV superinfection of EBV positive cells

- R.C. Arcenas & R. Widen, *BMC Microbiol* **2**:20-41, 2002

HIV-1, through TNF-α in CMV-stimulated PBM cells

- P.K. Peterson et al., *J Clin Invest* 89:574-580, 1992

Modulatory effects of CMV on tumor cell biology:

Persistence in tumor cells is essential (shown in colon cancer, malignant glioma, prostatic intraepithelial neoplasia & carcinoma)

- J. Cinatl et al., Microbiol Rev 28:59-77, 2004
- M. Samanta et al., J. Urol 170:998-1002, 2003

Effects are mediated by activity of CMV IE proteins

- J. Cinatl et al., Intervirology 39:259-269, 1996

Mechanisms are associated with aberrant signaling pathways, transcriptional factors, tumor suppressor

- J. Cinatl et al., Trends Mol Med 10:19-23, 2004

CMV infection blocks apoptosis in cancer cells

- M. Michaelis et al., Cell Mol Life Sci 61:1307-1316, 2004

CMV IE72 and IE84 have the capacity to transactivate the COX-2 promoter

- A. Speir et al., Circ Res 83:210-216, 1998

"Malignancy dance" of CMV, IL-1β, NF-κB, and COX2 might be directed by body burden TCDD in chronically inflammated cells *via* the AhR-mediated transcriptional pathway

0.3 pM TCDD activates CMV in MRC-5 cells (T. Murayama et al., *BBRC* **296**:651-656, 2002)

1.5 pM TCDD induces COX2 expression and enhances malignant transformation of C3H/M2 cells (D. Wolfle et al., *Carcinogenesis* **21**:15-21, 2000)

IL-1β upregulates the CMV major IE promoter (J. Kline et al., *Exp Lung Res* **24**:3-14, 1998)

TCDD transcriptionally activates biosynthesis of IL-1β in human monocytes (A. Lozovatzky et al., *Organohalogen Comp* **1**:189-191, 1990)

Functional NF-κB site in the human IL-1β promoter: evidence for a positive autoregulatory loop (J. Hiscott et al., *Mol Cell Biol*. **13**: 6231–6240, 1993)

IL-1β upregulates HIF-1α via NF-κB/COX2 pathway thus linking between inflammation and oncogenesis (Y. Jung et al., *FASEB J* **17**: 2115-2117, 2003)

IL-1β is required for tumor invasiveness and angiogenesis (E. Voronov et al., *PNAS USA* **100**:2645-2650, 2003)

NF-κB links inflammation and immunity to cancer development and progression (M. Karin & F. Greten, *Nature Rev Immunol* **5**:749-759, 2005)

TCDD enhances NF-κB activity in chronically infected with HIV-1 U1 cells (S. Gollapudi et al., *BBRC* **226**:889-894, 1996)

The AhR and the Rel A NF-kB subunit cooperate to transactivate the c-myc oncogene promoter (D. Kim et al., *Oncogene* **19**:5498–5506, 2000)

At lower concentrations than body burden, TCDD might trigger malignancy-associated CMV and COX-2

¶ **TCDD at 0.3 pM is shown to augment replication of CMV in chronically infected human MRC-5 cells.**

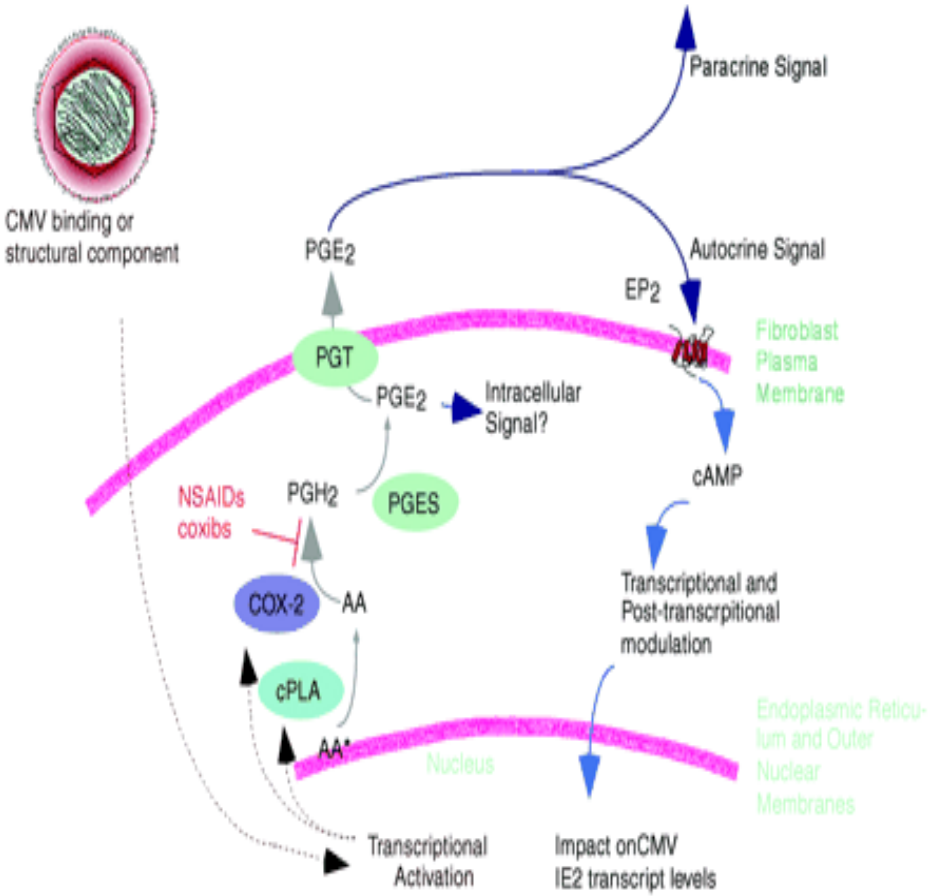
From: T. Murayama et al., *BBRC* **296**:651-656, 2002

¶ **TCDD at 0.15-1 .5 pM is shown to induced the expression of COX-2 in C3H/M2 fibroblasts.**

From: D. Wolfle et al., *Carcinogenesis* **21**:15-21, 2000

¶ **In human colon cancer cells, COX-2 is closely related to liver metastasis and survival.**

From: T. Yamauchi et al., *Anticancer Res* **23**:245-249, 2003

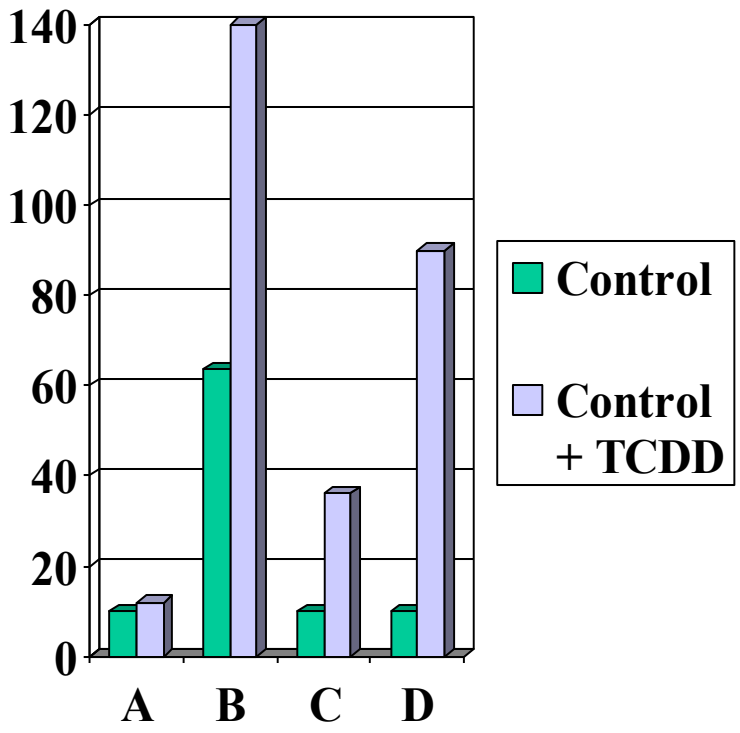


¶ **A model for increased prostaglandin E2 synthesis in CMV replication. Cell activation via binding or other effects of viral structural proteins leads to rapid transcriptional activation (in the nucleus) of two key cellular genes, encoding cytoplasmic hospholipase (cPLA) and cyclooxygenase-2 (COX-2).**

From: E. Mocarski, *PNAS USA* **99**:3362-3364, 2002

Stimulatory Effect of TCDD on Concanavalin A Induced PBM Proliferation and IL-1ß Synthesis

(From: A. Lozovatzky, V. Mordvinov, O. Kuprianova, and I. Tsyrlov, *Organohalogen Compounds* **1**:189-191, 1990)



1. Peripheral blood mononuclear cells (PBM) were preincubated with 0.1-1.0 nM TCDD for 24 h prior to concanavalin A - induced co-culture period. While having no effect on PBM spontaneous proliferation (**A**), TCDD caused a 2.2-fold increase of mitogen-induced proliferation of PBM (**B**).

2. Dot-hybridization assay showed a 3.5-fold increase of IL-1ß mRNA in human monocytes after 4 h incubation with TCDD (**C**). A 24 h incubation (**D**) caused 9-fold augmentation of IL-1ß synthesis.

Target viral genes for NF-kappaB

[The transcriptional factor NF-κB is a critical regulator of IL-1ß-induced gene expression]

•Gene // *promoter DREs	•Function	•Reference
•Adenovirus (E3 region) // *4-9	•Adenovirus	•Williams et al, 1990
•Avian Leukosis Virus	•Causes avian leukosis	•Bowers et al, 1996
•Bovine Leukemia Virus	•Causes bovine leukemia	•Brooks et al, 1998
•CMV // *10	•Cytomegalovirus	•Sambucetti et al, 1989
•EBV (Wp promoter) // *22	•Epstein-Barr virus	•Sugano et al, 1997
•HBV (pregenomic promoter) // *4	•Hepatitis B virus	•Kwon & Rho, 2002
•HIV-1 // *1	•Human immunodeficiency virus	•Nabel & Baltimore, 1987; Griffin et al, 1989
•HSV // *8	•Herpes simplex virus	•Rong et al, 1992
•JC Virus	•Polyoma virus	•Ranganathan & Khalili, 1993
•HPV type 16 // *1	•Human Papillomavirus	•Fontaine et al, 2000
•SIV // *1	•Simian immunodeficiency virus	•Bellas et al, 1993
•SV-40 // *3	•Simian virus 40	•Kanno et al, 1989

Overexpression of the AhR in human malignant cells

♦ **In human colon adenocarcinoma cell line LS180**
(P. Harper et al., *Arch Biochem Biphys* **290**:27-36, 1991)

♦ **In human adult T-cell leukemia cell lines**
(T. Hayashibara et al., *BBRC* **300**:128-134, 2003)

♦ **In malignant prostatic epithelial cells**
(M. Kashani et al., *Prostate* **37**:98-108, 1998)

♦**In human lung adenocarcinoma tissues and cell lines**
(P. Lin et al., *Toxicol Pathol* **31**:22-30, 2003)

♦ **In human breast carcinoma cell line Hs578T**
(T. Murray et al., *Breast Cancer Res* **8**:R17, 2006)

♦ **In human pancreatic cancer cells**
(A. Koliopanos et al., *Oncogene* **21**:6059-6070, 2002)

The overexpressed AhR: transcriptional activation by proteins of the DRE-containing human viruses

The etiological agent of adult T-cell leukemia (ATL), human T-cell leukemia virus type 1, caused elevated constitutive expression of the AhR in ATL cell lines, which is in part attributable to the action of the viral transactivation protein, Tax
(T. Hayashibara et al., *BBRC* **300**:128-134, 2003)

The HBV-associated XAP2 protein is a subunit of the unliganded AhR core complex, and has been demonstrated to enhance the transcriptional activation of endogenous AhR and increase cytosolic AhR levels when overexpressed
(B. Meyer et al., *Mol Cell Biol* **18**:978-988, 1998)

A Dual-Use FACTOR: XAP2

¶ **XAP2, a novel hepatitis B virus (HBV) X-associated protein that inhibits X protein transactivation effects. The X protein-XAP2 interaction may play a role in HBV pathology.** (Kuzandaivelu et al., *Nucleic Acids Res* 24:4741-50, 1996)

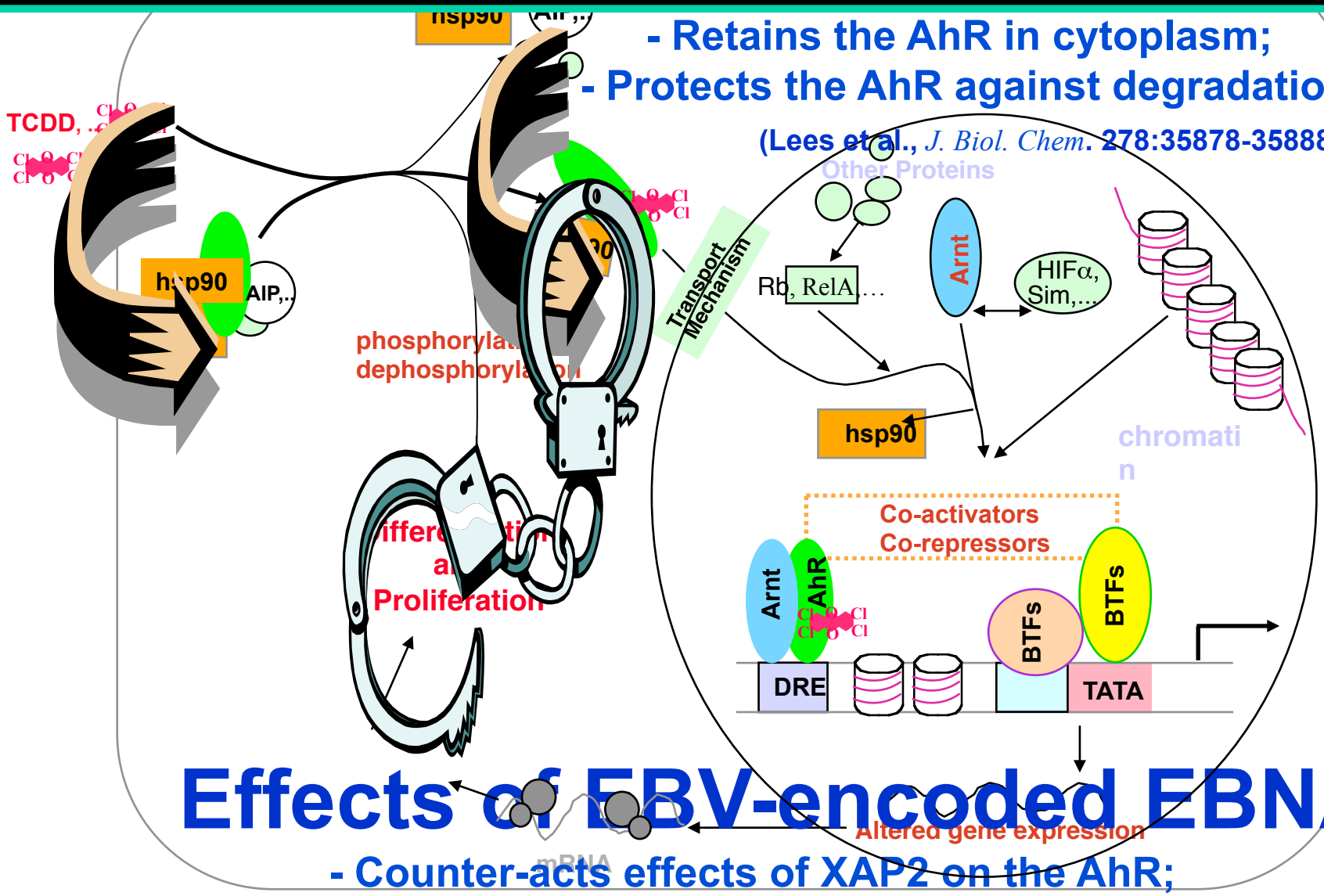
¶ **XAP2 is a stable member of the unliganded AhR core complex, until TCDD binds the AhR. Thus XAP2 interacts with both X-protein from HBV and human AhR.** (Meyer et al., *Mol Cell Biol* 18:978-988, 1998)

¶ **The presence of XAP2 in the ligand-bound AhR complex enhanced the rate of nuclear translocation but repressed transcriptional activity. XAP2 is able to protect ligand-free AhR against ubiquitination, and inhibit ligand-independent nuclear import of AhR.** (Petrulis et al., *J Biol Chem* 275:37448-53, 2000)

Effects of HBV-associated XAP2

- Retains the AhR in cytoplasm;
- Protects the AhR against degradation.

(Lees et al., *J. Biol. Chem*. 278:35878-35888, 2003)



Effects of EBV-encoded EBNA-3

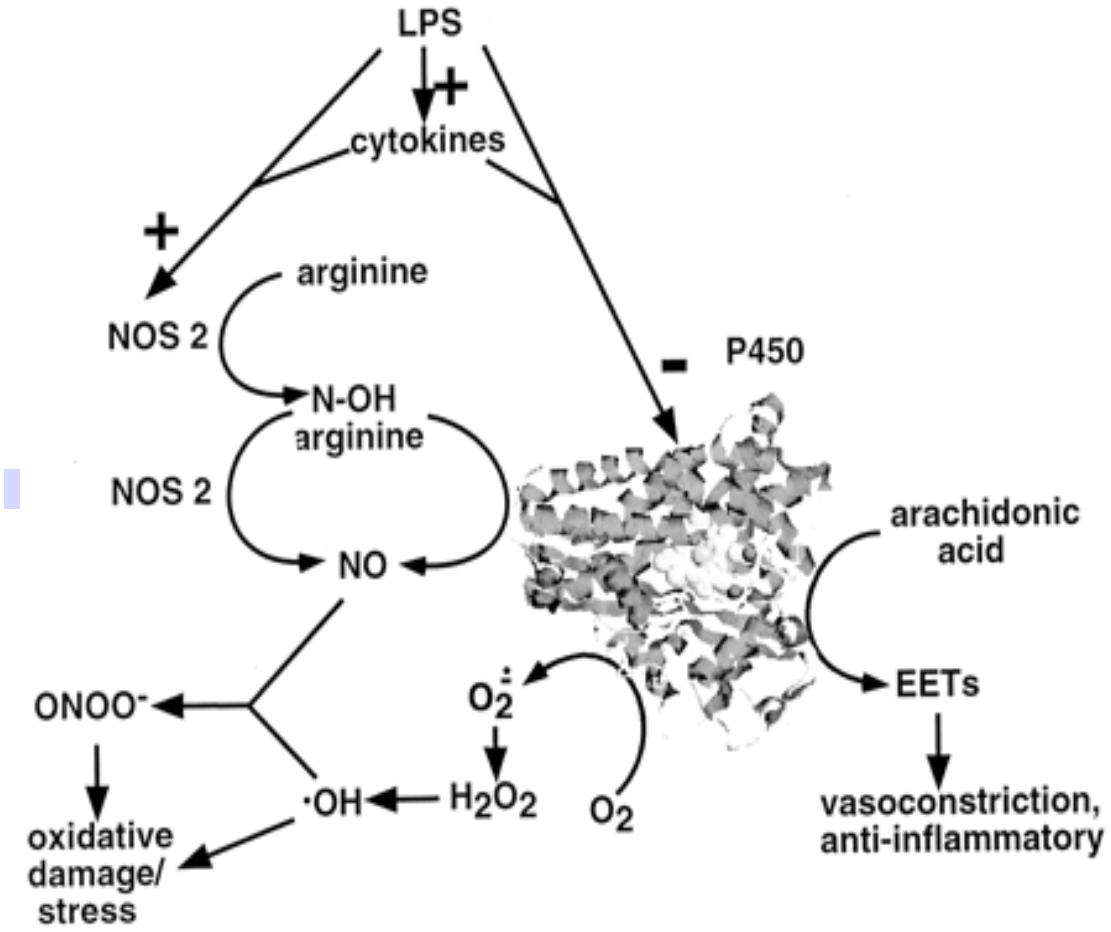
- Counter-acts effects of XAP2 on the AhR;
- Enhances trans-activation activity of AhR-Arnt complex.

(Kashuba et al., *J. Biol. Chem.* 281:1215-1223, 2006)

Putative functions of P450 enzymes during cellular responses to pro-inflammation cytokines

E.T. Morgan, Regulation of Cytochrome P450 by Inflammatory Mediators: Why and How? *Drug Metab Dispos* **29**:207-212, 2001

S. Ke et al., Mechanism of Suppression of Cytochrome P-450 1A1 Expression by Tumor Necrosis Factor- and Lipopolysaccharide *J Biol Chem* **276**:39638-39644, 2001



Virus-associated cancers in the Arctic

✓ **Nasopharyngeal carcinoma encounters exclusively among Eskimos and other Arctic natives. The Epstein-Barr virus (EBV) DNA was detected in plasma/serum of 60% patients with this tumor** [Shotelersuk et al., *Clin Cancer Res* 2000; McDermott et al., *Clin Otolaryngol Allied Sci* 2001]

✓ **Undifferentiated salivary gland lymphoepithelial carcinomas are endemic in the Arctic regions. All cases of these tumors are associated with the EBV** [Herbst et al., *Pathologe* 2004]

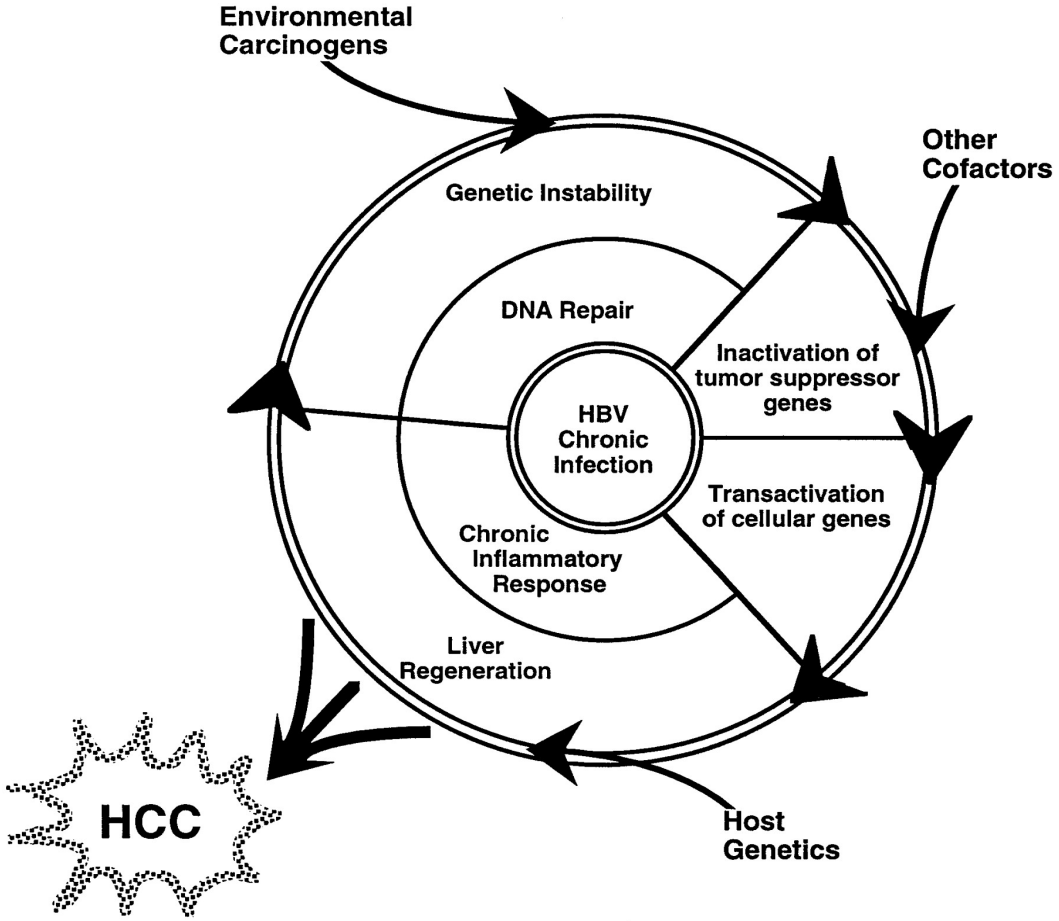
✓ **The papillomavirus-associated invasive cervical carcinoma is the second leading cause of death in Canadian Inuit women, and the incidence ratio in this population is 3.1 times the Canadian average** [Martin et al., *Int J Circum Health* 1998]

HBV-associated HCC in Arctic

✓ **According to the US CDC, the rate of hepatitis B lesions has been high among Alaska Natives, and the annual incidence of hepatocellular carcinoma (HCC) among Eskimo males was five times that of white males in the United States.** [McMahon et al., *Hepatology* 2000]

✓ **Among 1,400 Alaska Native the Hepatitis B virus (HBV) carriers, the relative risk factor of developing HCC was 148 compared to the general population.** [McMahon et al., *Ann Intern Med* 2001]

✓ **Gaps also exist in our knowledge of the incidence of Hepatitis B in circumpolar countries, the distribution of HBV genotypes, risk factors for hepatocellular carcinoma.** [McMahon, *Notes from the IUCH Infectious Disease Working Group Meeting* 2003]

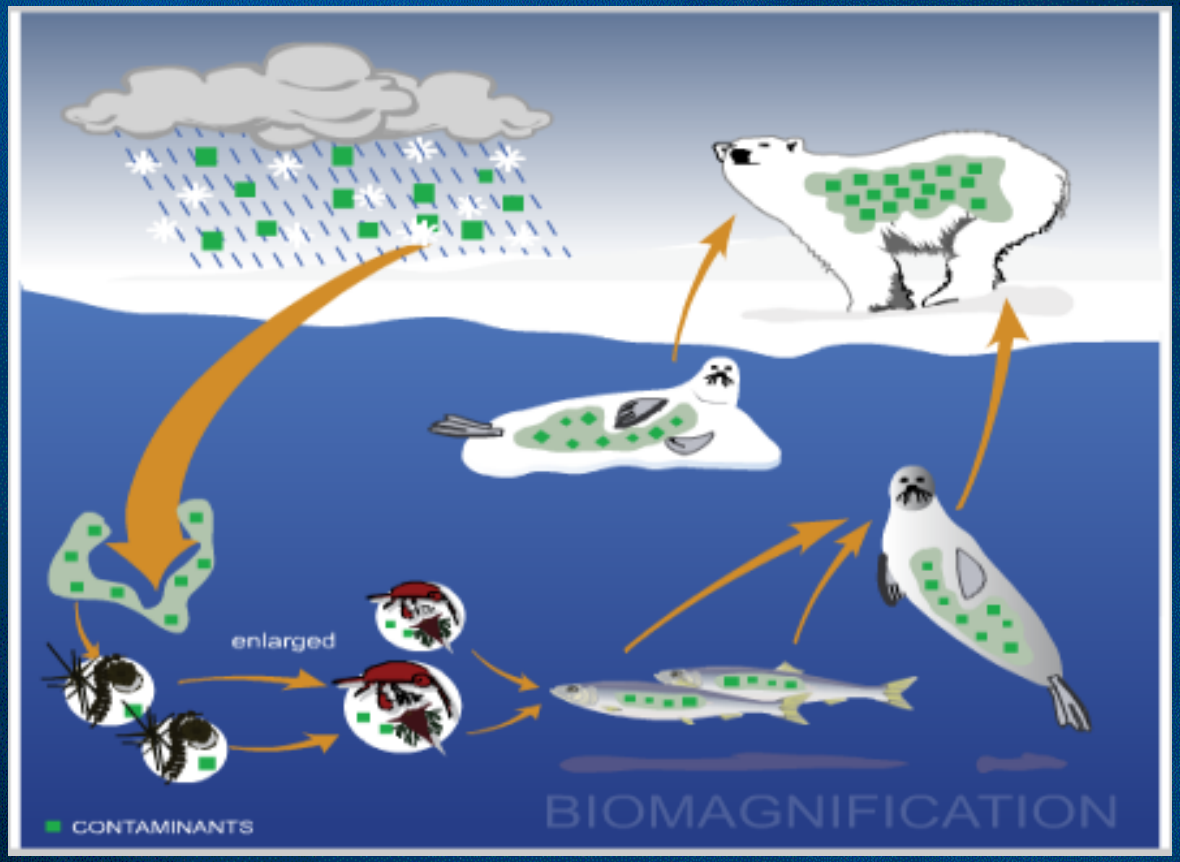


Model for the role of HBV in the development of HCC

Chronic infection by HBV and resulting inflammatory response introduce the potential for errors during DNA replication. The HBV X protein interacts with DDB1, a cellular DNA repair protein.. Also the X protein can trans-activate cellular genes, which might contribute to carcinogenesis. Environmental carcinogens are important cofactors in certain areas of the world. Reproduced, with permission, from Butel et al. (*Trends Microbiol*. 4:119-124, 1996)

Airborne Long Range Transport of Dioxin-like POPs

Highest risks for top predators



Dioxins are soluble in lipids. Marine food chain: rich in lipids

Dioxins in the Arctic diet

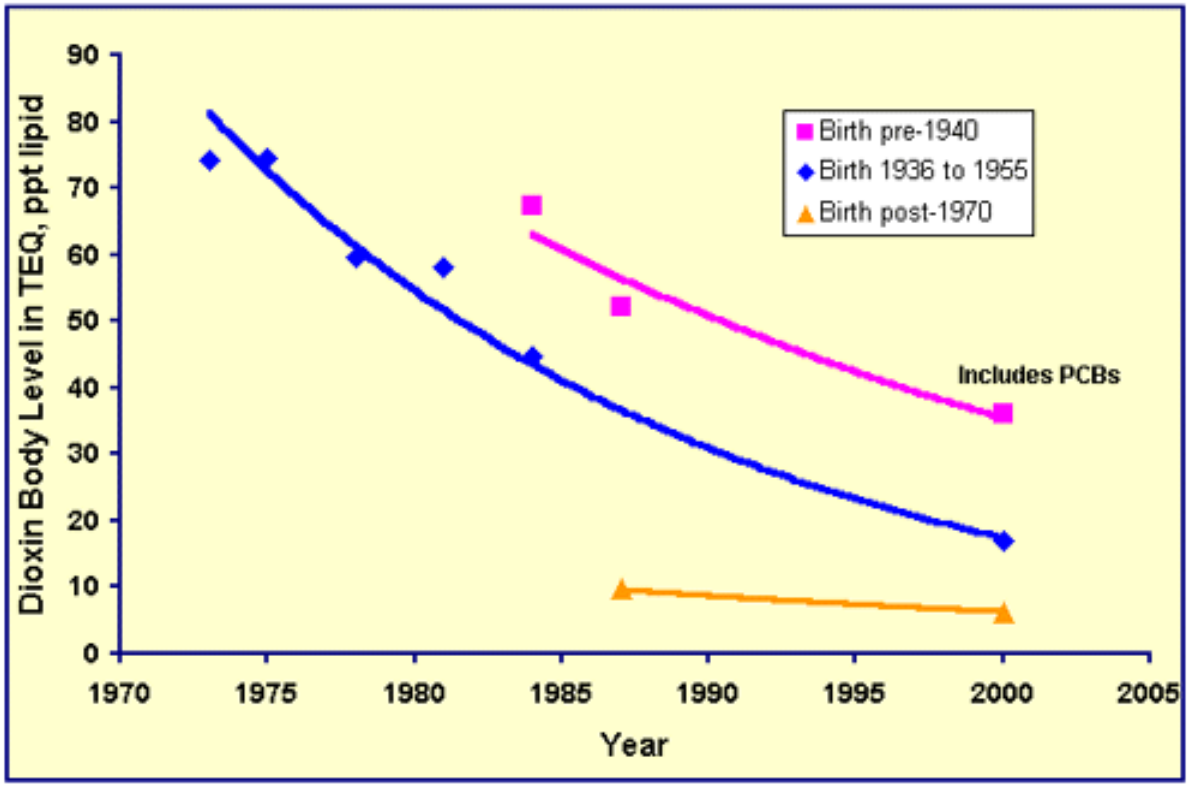
✓ **The importance of diet on exposure and health effects of dioxin-like compounds in the Arctic has been recently reviewed** [Odland et al., *Acta Paediatr* 2003]

✓ **The mean total body burden (concentration of dioxin-like compounds expressed in 2,3,7,8-TCDD toxic equivalents) in Inuit people of Arctic Quebec is 7 times of that in people of South Quebec, whereas among fishermen it might reach 25 times of controls. However, "although the body burden of dioxin-like compounds are close to those induced adverse effects in laboratory animals, dietary benefits from sea-food based diet outweigh the hypothetical health risks"** [Dewailly et al., *Envir Health Perspect* 1994; Ayotte et al., *Chemosphere* 1997]

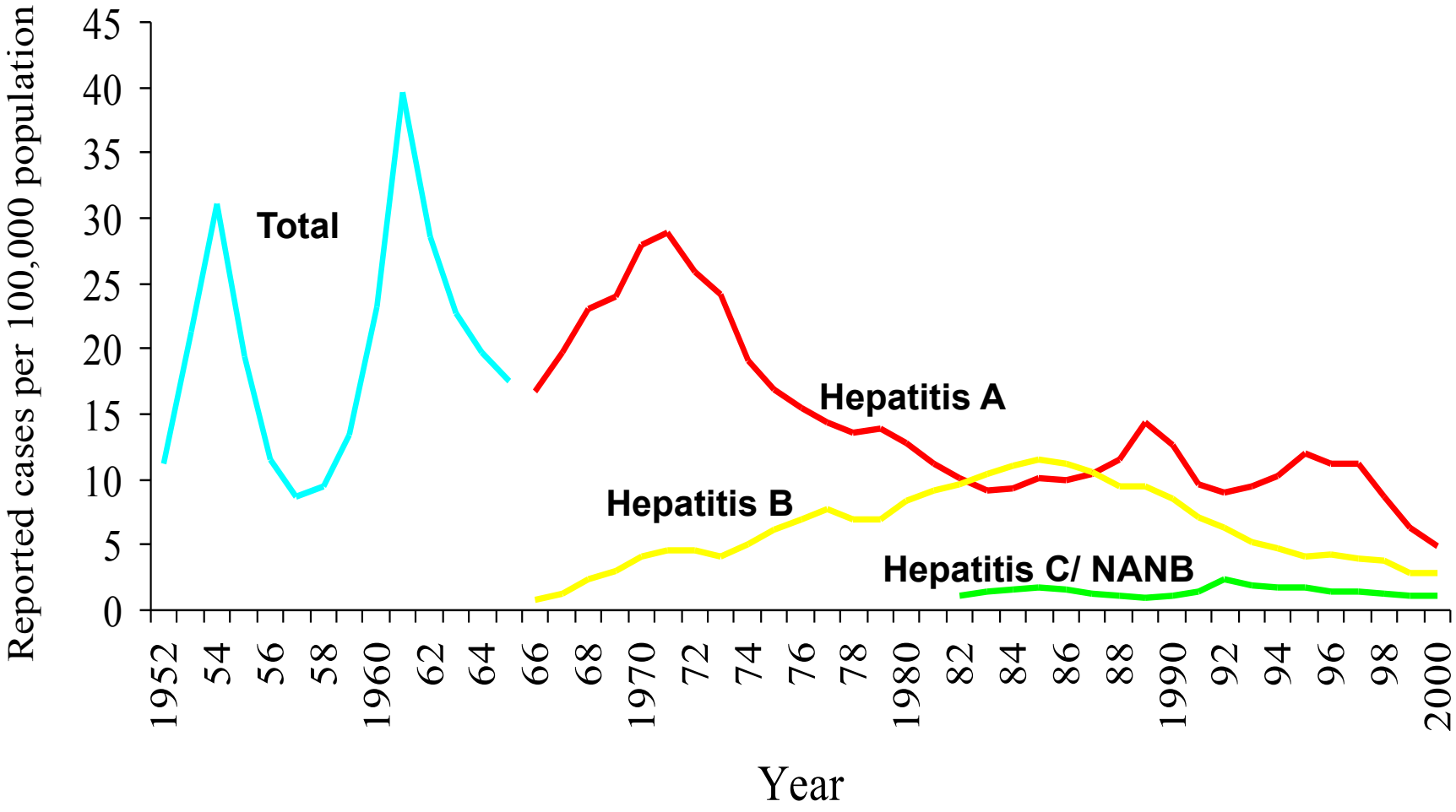
ESTIMATED RELATIVE SUSCEPTIBILITY to TCDD of HBV *vs* HIV-1 and CMV

Structural and functional parameters of the virus	**HIV-1**	**HBV**	**CMV**
Quantity of DRE(s) in gene enhancer	**1**	**4**	**10**
Minimal gene-activating concentration of TCDD	0.1 nM (32 ng/kg)	**?**	0.3 pM (0.1 ng/kg)

Trends in Body Levels of Dioxins



Viral Hepatitis A, B and C/NANB by Year, United States, 1952-2000



What Arctic Natives have in common with Southern Vietnamese?

1) Increased body burden TCDD due to consumption of contaminated marine products

2) Increased frequency of chronic inflammation caused by the HBV, and HBV-associated HCC

■ Viral infections and chemical exposures as risk factors for hepatocellular carcinoma in Vietnam

Cordier et al., *Int J Cancer* 55:196-201, 1993

A case-control study investigating risk factors for hepatocellular carcinoma (HCC) was conducted in Hanoi, North Vietnam. Positivity for hepatitis B surface antigen (HBsAg) was the main risk factor for HCC in this sample. Alcohol drinking was associated with HCC and interacted with HBsAg positivity. Agricultural use of pesticides and military service in the south of Vietnam for 10 years or more were also associated with an increased risk of HCC. This study confirms the major role played by HBV infection and its association with HCC in south-east Asia. It also suggests how other factors such as exposure to chemicals may interact with HBV.

■ Differences in Cancer Risks in the South and North of Viet Nam

Ngaon L.T. et al., *Asian Pac J Cancer Prev* 2:193-198, 2001

For males, significantly higher [than in Hanoi, North Vietnam] incidences in Ho Chi Minh (South Vietnam) were observed for cancers of the oesophagus (RR = 1.66, 95% CI = 1.19-2.32), liver (RR = 1.22, 95% CI = 1.09-1.36), gall bladder (RR = 5.95, 95% CI = 2.49-14.23), and larynx (RR = 3.54, 95% CI = 2.26-5.55).

- **Effects of dietary habits and CYP1A1 polymorphisms on blood dioxin concentrations in Japanese men**

  Tsuchiya, Y. et al., *Chemosphere* 52:213-219, 2003

  The environmental and genetic factors that influence blood dioxin concentration, and investigated the relationship among dioxin concentrations, dietary habits and CYP1A1 polymorphisms in Japanese fishermen and farmers, as compared to a group of office workers as controls. Elevated dioxin concentration with dioxins, furans, and coplanar-PCBs found in the fishermen may be due to the frequent consumption of fish; no such relationship was found either in the farmers or the controls. It is likely that the primary route of dioxin exposure in the Japanese population is through the food chain via fish consumption, regardless of occupation.

- **Estimated cancer risk of dioxins to humans using a bioassay and physiologically based pharmacokinetic model**

  Maruyma, W., Aoki, Y. *Toxicol Appl Pharmacol,* 2006

  The relative risk of excess liver cancer for Japanese people in general is 1.7-6.5 x 10(-7) by TCDD only, and 2.9-11 x 10(-7) by the three dioxins at the present level of contamination.

From the CDC Third National Report on Human Exposure to Environmental Chemicals, 2005

People consuming fish from the Great Lakes had mean concentrations of dioxins and furans that are several times background values in the population

- C. Falk et al., *The Great Lakes Consortium Environ Res* 80:S19-S25, 1999
- H.A. Anderson et al., *The Great Lakes Consortium Environ Health Persp* 106:279-289, 1998
- L.P. Hanrahan et al., *The Great Lakes Consortium Environ Res* 80: S26-S37, 1999

A Dual-Use Factor: α-Fetoprotein

- **Generally, studies of HBsAg-positive HCC patients have shown that serum α-fetoprotein levels were raised as early as 2 years before clinical presentation of tumor**

- **Detection of α-fetoprotein and hepatitis B surface antigen in blood spotted on filter paper is generally used as a screen for HCC, including HCC in Alaska Natives**
[A. Parkinson et al., *Arctic Med Res* 55,1996]

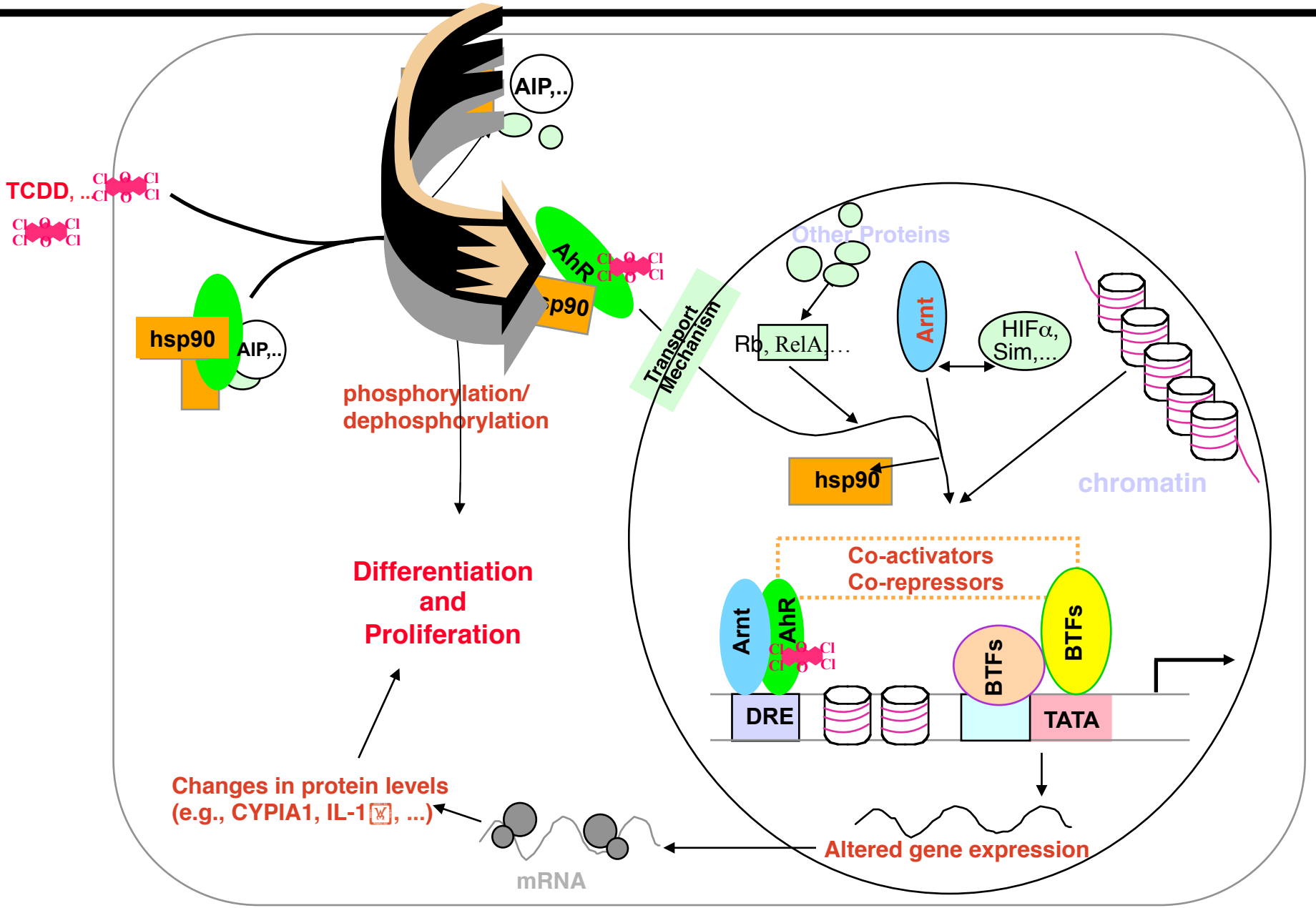
- **X-gene product of the HBV transactivates human α-fetoprotein gene in human hepatoma cells**
[T. Arima et al., *Intern J Mol Med* **9**:397-400, 2002]

Water-soluble 2,3,7,8-TCDD complex with human α-fetoprotein

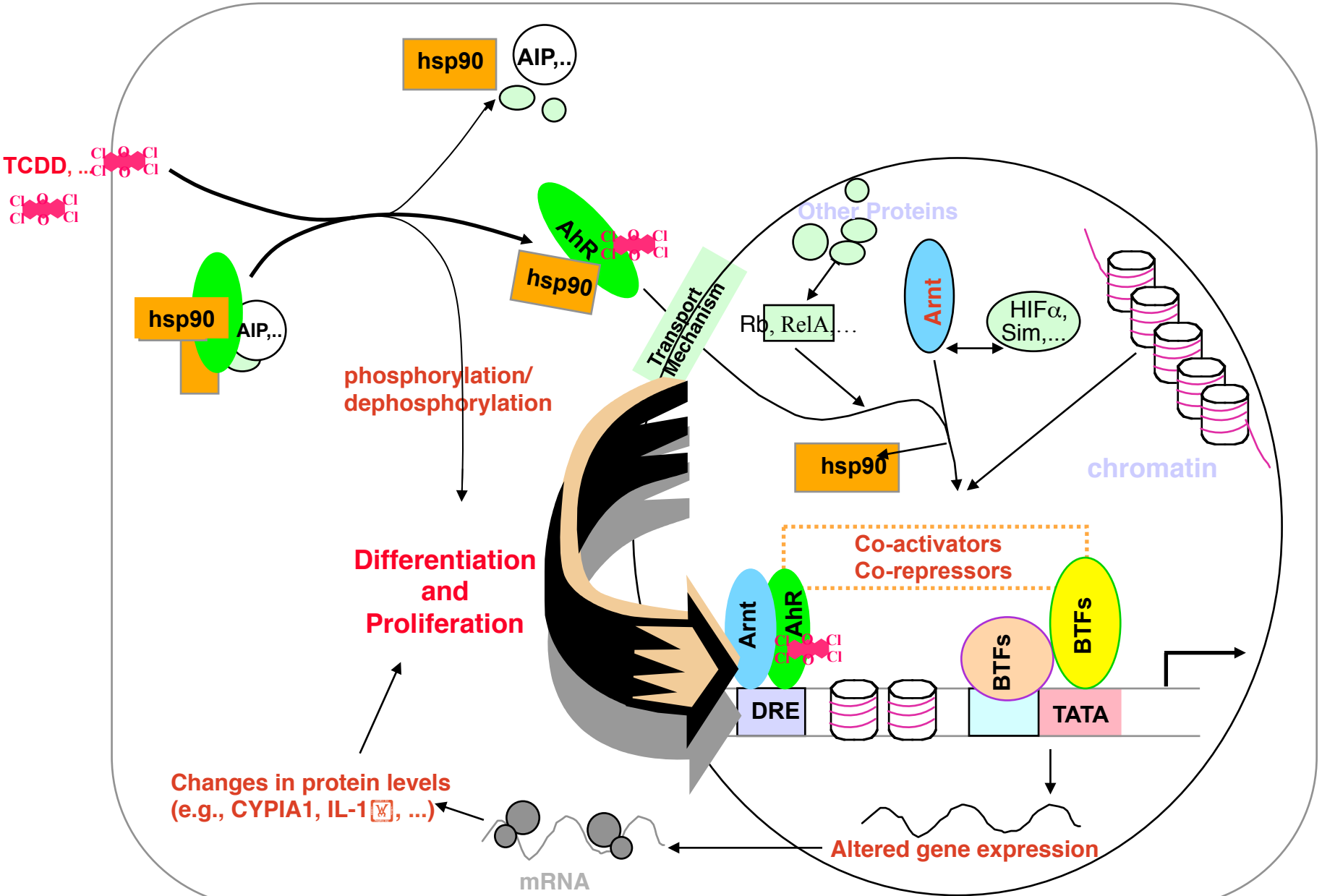
A. Sotnichenko et al., *FEBS Letters*, **450**:49-51, 1999

The conditions for the formation of a non-covalent complex between TCDD and the human transport fetal protein, α-FP have been studied. TCDD has been shown to form a stable complex with α-FP in a 2:1 (TCDD:α-FP) ratio. The apparent solubility of TCDD in water increases 10^5-fold after complex formation. The toxicity of the TCDD:α-FP complex injected into mice by the intravenous route is comparable with that of free TCDD administered in oil solution *per os*. The complex manifests very much higher toxicity (200–1400 times) in vitro and surpasses TCDD in selectivity. α-FP may facilitate TCDD transport in embryonic tissues and enhance its toxic effects.

The AhR as a treatment target of virus-associated human cancers with bioflavonoids or indole-3-carbinol



Viral DRE as a potential target for the treatment of virus-associated human malignancies with salycilamide



Conclusions

- **A molecular mechanism of trans-activation by body-burden TCDD of DRE-containing viral gene is postulated**

- **The fraction of human cancers attributable to common viruses infection may now need to be epidemiologically revised in light of the fact that new TCDD-viral associations have been discovered**

- **In addition to vaccine against viruses, new antiviral therapy (targeting the host cell Ah receptor, and viral gene promoter DREs) for virally-driven inflammation and malignancy might be developed to treat chronically infected and cancer patients**

XENOTOX Inc. is an innovating and consulting company established in 2004. Its focus is to evaluate, assess, and subside effects of human burden 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD) on hazardous human viruses. This field originated from the discovery data on trans-activation with sub-nanomolar TCDD of the HIV-1 in human cells. Today, upregulation of some common viruses by body burden TCDD is considered to manifest its promoter role in several major human cancers. TCDD is also suggested an intracellular trigger increasing the virulence of HIV-1, and influenza A virus.

From a bioscience standpoint, this is a worst-case scenario of chemico-biological interactions, in terms of how the most potent xenobiotic might dangerously interact with invaded viral pathogen by using regular transcription factors of the target human cell. Here, an intracellular Ah receptor for TCDD mediates its effects on replication of the invaded virus by binding to "dioxin response element" (DRE) localized in the promoter region of viral gene.

From a clinical standpoint, the above findings bring to light tremendous medical problems in oncology, organ transplantation and influenza epidemics. Namely, malignization of the major cancers such as cervical cancer and lymphoproliferative disorders is associated with DREs-containing human papillomavirus and Epstein-Barr virus, respectively. Elevated level of hepatitis B virus chronic infection and associated HCC observed in the Arctic natives is due to increased level of background TCDD, and to the HBV possession of multiple DREs. Because the gene encoding the NS1 protein in influenza A virus (including H5N1) contains at least two DREs in its 5' upstream region, it might suggest upregulating effect of body burden TCDD on influenza virus virulence in birds and humans, due to a known ability of NS1 to diminish avian and human interferon-mediated antiviral response.

The RelA NF-kappaB subunit and the aryl hydrocarbon receptor (AhR) cooperate to transactivate the c-myc promoter in mammary cells

Kim DW , Gazourian L , Quadri SA , Romieu-Mourez R , Sherr DH , Sonenshein GE

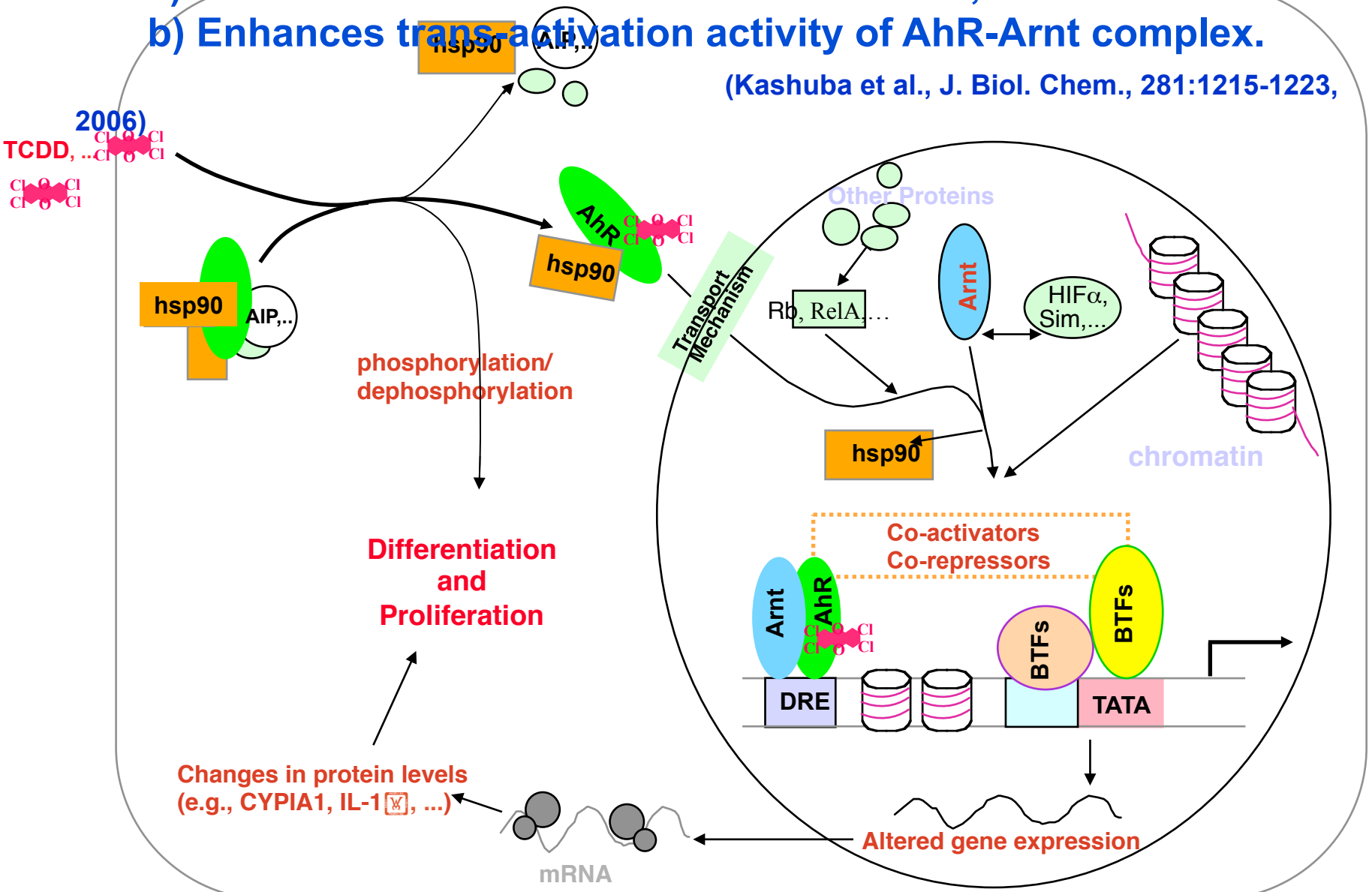
Oncogene **19**:5498-506, 2000

- The RelA NF-kappaB transcription factors regulate many genes involved in control of cellular proliferation, neoplastic transformation, and apoptosis, including the c-myc oncogene.
- Levels of NF-kappaB and aryl hydrocarbon receptor (AhR), which mediates malignant transformation by environmental carcinogens, are highly elevated and appear constitutively active in breast cancer cells.
- A physical and functional association between the RelA subunit of NF-kappaB and AhR was demonstrated resulting in the activation of c-myc gene transcription in breast cancer cells.
- In transient co-transfection, RelA and AhR gene products demonstrated cooperation in transactivation of the c-myc promoter, which was dependent on the NF-kappaB elements, and in induction of endogenous c-Myc protein levels.
- A novel AhR/RelA-containing NF-kappaB element binding complex was identified by electrophoretic mobility shift analysis. Thus, the RelA and AhR proteins functionally cooperate to bind to NF-kappaB elements and induce c-myc gene expression.
- These findings suggest a novel signaling mechanism whereby the Ah receptor can stimulate proliferation and tumorigenesis

Effect of EBV-encoded EBNA-3

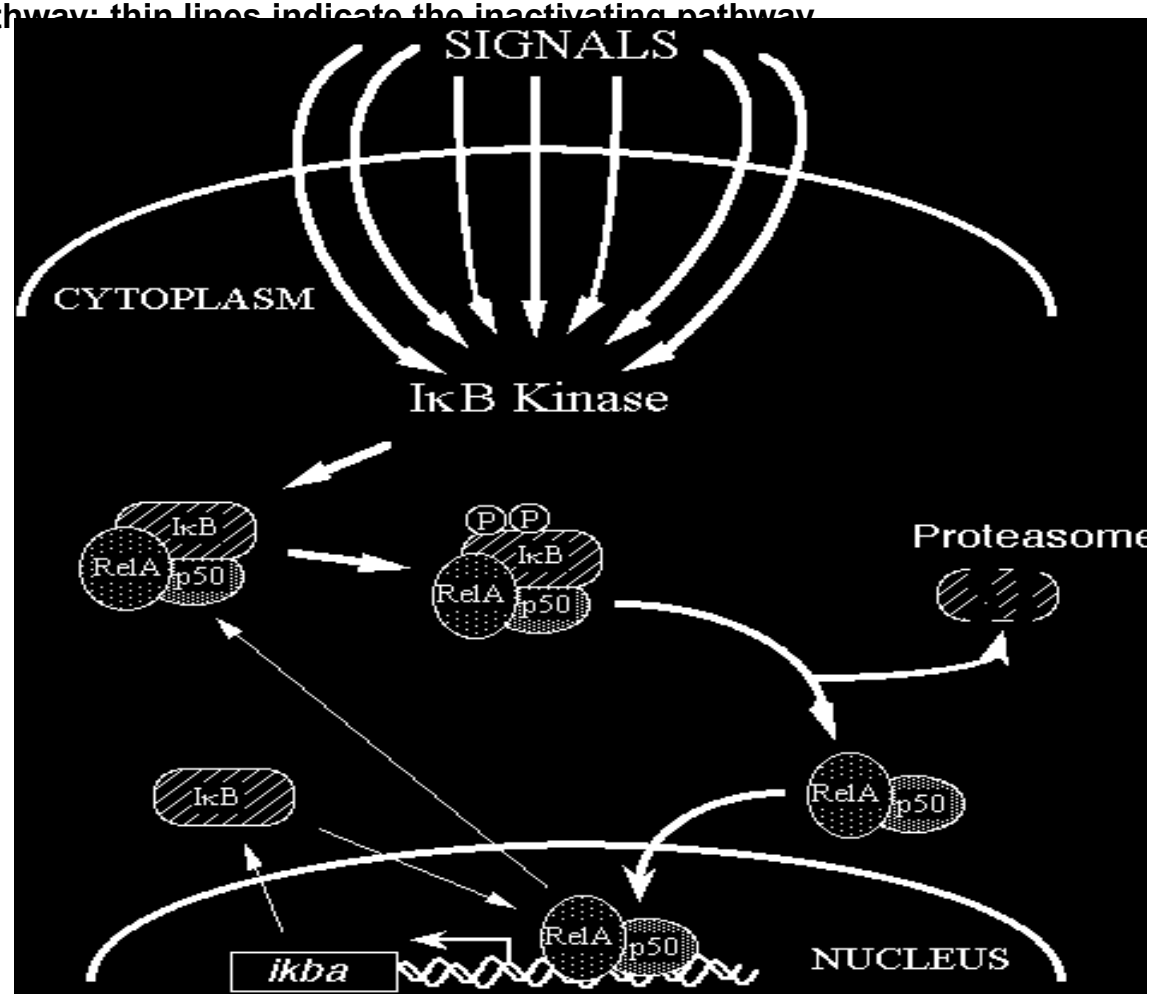
a) Counter-acts effects of XAP2 on the AhR;
b) Enhances trans-activation activity of AhR-Arnt complex.

(Kashuba et al., J. Biol. Chem., 281:1215-1223, 2006)



Rel/NF-kappaB Signal Transduction

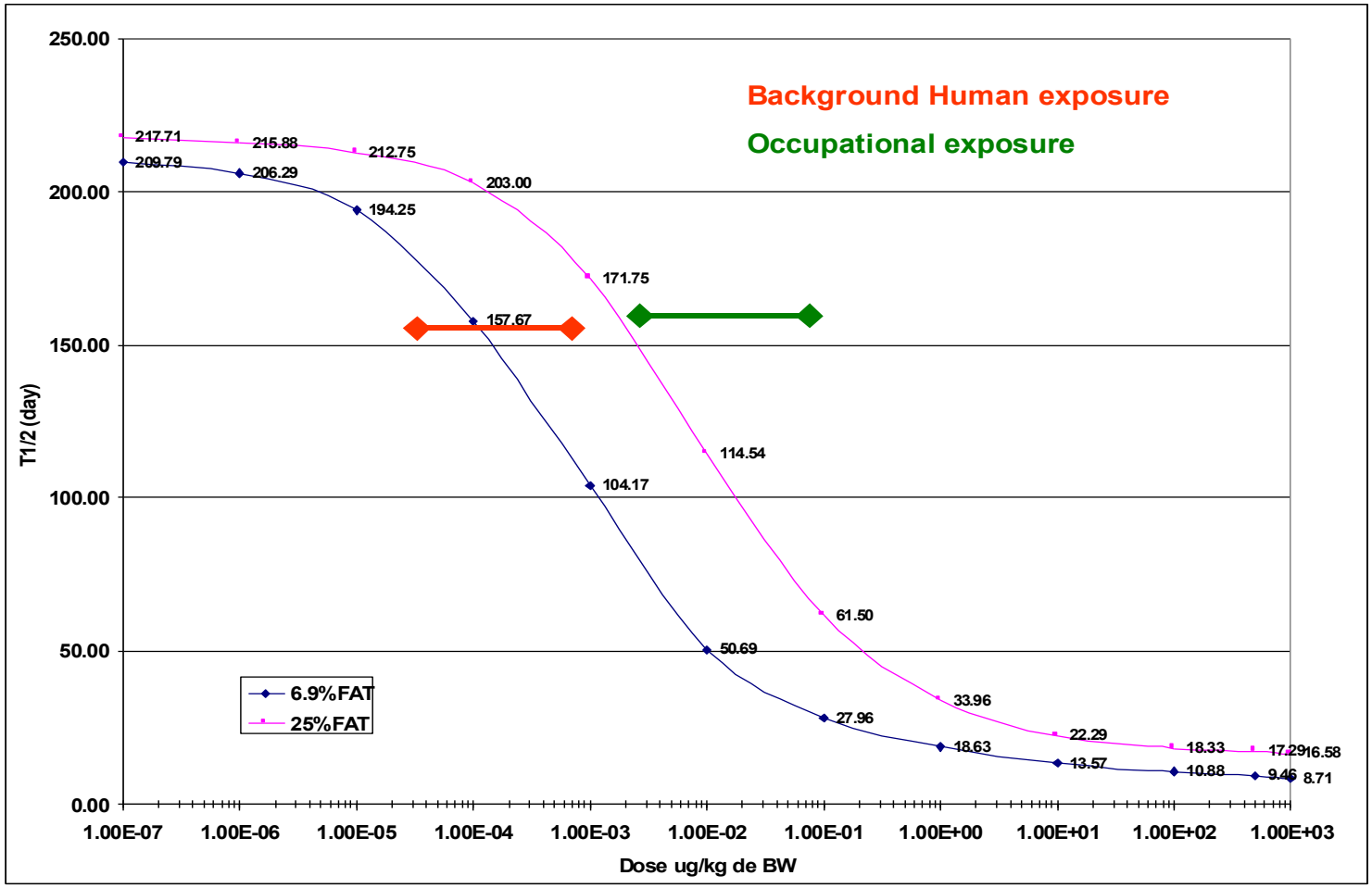
In the classical pathway, various signals converge on activation of the IkB kinase (IKK) complex. IKK then phosphorylates IkB at 2 N-terminal serines, which signals it for ubiquitination and proteolysis. Freed NF-kB (p50-RelA, in this case) enters the nucleus and activates gene expression. One NF-kB target gene encodes IkB. The newly synthesized IkB can enter the nucleus, pull NF-kB off DNA, and export NF-kB back to its resting state in the cytoplasm. Thick lines indicate the activating pathway; thin lines indicate the inactivating pathway.



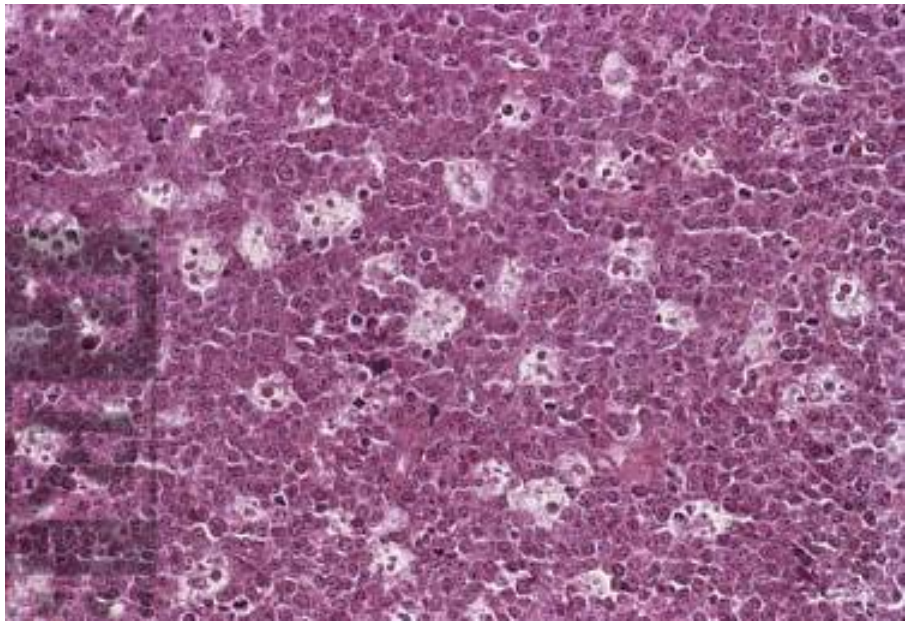

NO
FISHING
POISON

RESEARCH & DEVELOPMENT

Building a scientific foundation for sound environmental decisions



Burkitt's lymphoma cancer



Numerous large, pale macrophages are present, and the small cancerous cells have numerous dark nucleoli within them. This cancer is caused by infection with the Epstein-Barr virus.

Seminar Infection Diseases in Arctic

Hepatitis B Virus Can Be Transcriptionally Up-regulated with Human Body Burden 2,3,7,8-TCDD

Ilya B. Tsyrlov, M.D., Ph.D.

XENOTOX Inc.
Scarsdale, New York, USA
xenotoxit@optonline.net

A constitutively active arylhydrocarbon receptor induces growth inhibition of jurkat T cells through changes in the expression of genes related to apoptosis and cell cycle arrest

Ito T , Tsukumo S , Suzuki N , Motohashi H , Yamamoto M , Fujii-Kuriyama Y , Mimura J , Lin TM , Peterson RE , Tohyama C , Nohara K.

J Biol Chem, 279(24): 25204-10 2004

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is known to suppress T cell-dependent immune reactions through the activation of the arylhydrocarbon receptor (AhR). Our previous findings suggest that TCDD inhibits the activation and subsequent expansion of T cells following antigen stimulation in mice, leading to a decreased level of T cell-derived cytokines involved in antibody production. In the present study, we investigated the effects of activated AhR on T cells by transiently expressing a constitutively active AhR (CA-AhR) mutant in AhR-null Jurkat T cells. In agreement with our previous findings, CA-AhR markedly inhibited the growth of Jurkat T cells. The inhibited cell growth was found to be concomitant with both an increase in the annexin V-positive apoptotic cells and the accumulation of cells in the G(1) phase. The growth inhibition was also shown to be mediated by both xenobiotic response element (XRE)-dependent and -independent mechanisms, because an A78D mutant of the CA-AhR, which lacks the ability of XRE-dependent transcription, partially inhibited the growth of Jurkat T cells. Furthermore, we demonstrated that CA-AhR induces expression changes in genes related to apoptosis and cell cycle arrest. These expression changes were shown to be solely mediated in an XRE-dependent manner, because the A78D mutant of the CA-AhR did not induce them. To summarize, these results suggest that AhR activation causes apoptosis and cell cycle arrest, especially through expression changes in genes related to apoptosis and cell cycle arrest by the XRE-dependent mechanism, leading to the inhibition of T cell growth.

Human viruses associated with infections in the Arctic

(all infect cells and establish latent infections)

Hepatitis B virus

Epstein-Barr virus

Papillomavirus

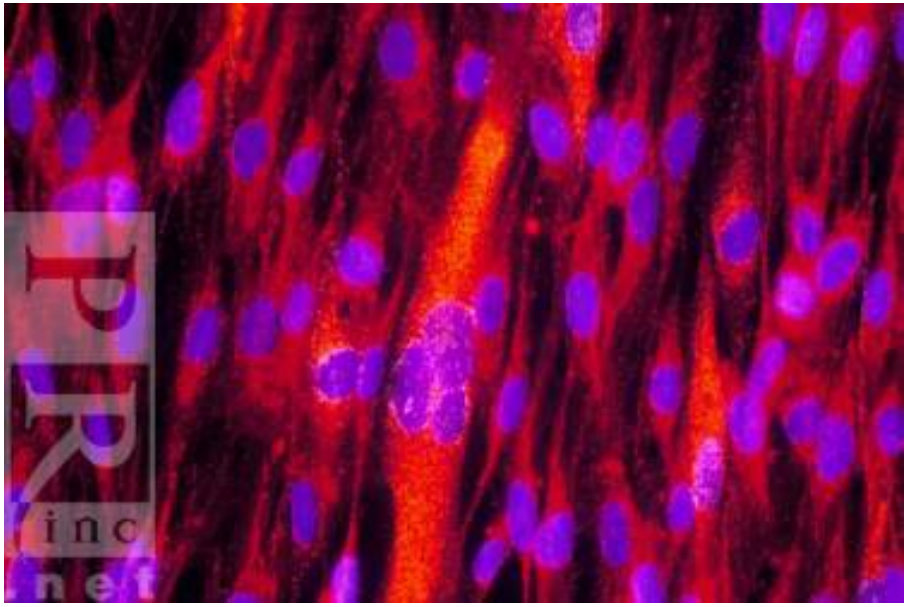
Cytomegalovirus

From: J. Butel, *Carcinogenesis* **21**:405-426, 2000

Properties of accepted and potential* human tumor viruses

	HBV	EBV	HPV	HTLV-1	CMV*
Genome:				ssRNA →	
Nucleic acid	dsDNA	dsDNA	dsDNA	dsDNA	dsDNA
Size, kb/kbp	3.2	172	8	9	240
No. genes	4	~90	8-10	6	7
Cell tropism	Hepatocytes White blood cells	Oropharen-geal epithe-lial cells, B cells	Squamous epithelial cells	T-cells	
Unique biology	Chronic Infection & inflammation	Immortali-zes B cells	Highly tissue specific	Immortali-zes T cells	
Prevalence of Infection	Chronic infection	Common	Common	Common	Common
Transmission	Vertical, pa-renteral, hori-zontal, venereal	Saliva	Venereal, Skin abra-sions	Breast milk, parenteral, venereal	
Human diseases	Hepatitis, Cirrhosis	IM, oral hairy leukoplakia	Skin warts, EV, genital warts, LP	HAM/ TSP	
Human cancers	HCC	BL, NPC, HD, lympho-mas	Cervical, skin, oro-pharynx	ATL	
Transforming genes	HBx?	LMP-1	E6, E7	Tax?	

Cytomegalovirus infected cells



Immunofluorescent light micrograph of human cells infected with cytomegaloviruses. The infected cells are shown by the presence of the virus-specific protein UL37 (orange). Cell nuclei are blue, with mitochondria red.

Table 1.4: General rules of infection and cancer

Infection	Cancer	% Virus-positive	No. of cases worldwide/year	Vaccine developed
HPVs	Cervical cancer	100%	490,000	✓
HBV	Liver cancer	50%	340,000	✓
HCV	Liver cancer	25%	195,000	x
EBV	Burkitt lymphoma	>90%	113,000	x
	Hodgkin lymphoma	>50%		
	Post-transplant lymphoma	>80%		
	Nasopharyngeal carcinoma	100%		
KSHV	Kaposi sarcoma	100%	66,000	x
	Primary effusion lymphoma	100%		
	Multi Castleman's disease	>50%		
HTLV1	Adult T cell leukaemia	100%	3,000	x
H. pylori	Gastric carcinoma	30%	603,000	x
	MALT lymphoma	100%		

From the CDC Third National Report on Human Exposure to Environmental Chemicals, 2005

Background levels of PCBs and dioxins have been associated with impaired neurological developments in newborn and children

- C. Koopman-Esseboom et al., *Dev Med Child Neurol* 39:785, 1997
- J.L. Jacobsen & S.W. Jacobsen, *New Engl J Med* 335:783-789, 1996
- M.P. Longnecker et al., *Env Health Persp* 111:65-70, 2003

Mother's Herpes Virus Infection Associated With Schizophrenia In Her Offspring

Scientists at Johns Hopkins Children's Center and six other research centers have found that mothers who have had a herpes simplex virus type 2 (HSV-2) infection at the time of birth are more likely to give birth to children who develop schizophrenia or other psychotic disorders. HSV-2 is a sexually transmitted disease.

Based on 3,804 stored blood samples and medical records dating as far back as the late 1950s, the correlative study in November of 2001 issue of *Archives of General Psychiatry* is the first to compare direct laboratory evidence of specific maternal infections with the development of psychosis in children. The researchers determined maternal infection by the presence of elevated levels of antibodies to HSV-2.

[The HSV-2 possesses 8 DREs in its gene 5'-flanking region, - *I.T.*]

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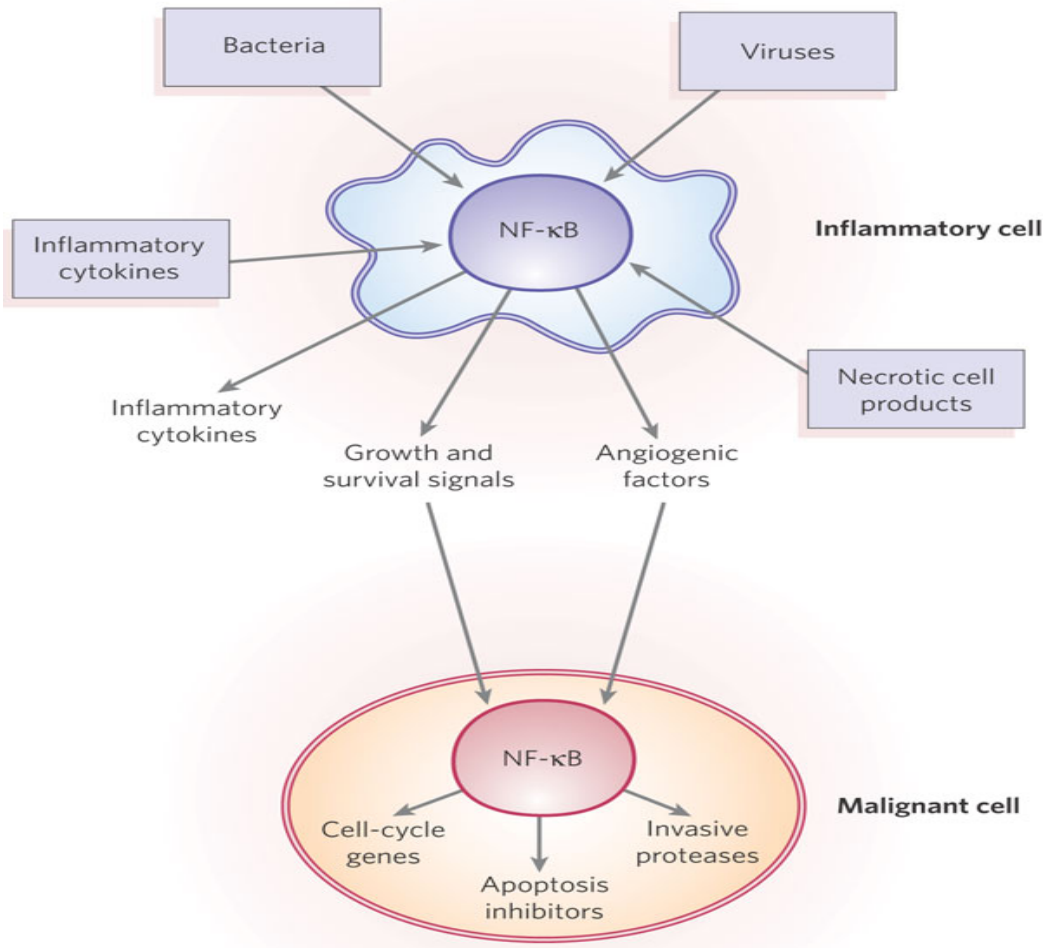
From the following article:

Nuclear Factor-κB in Cancer Development and Progression

(M. Karin, *Nature* **441**:431-436, 2006)



Activation of NF-κB in inflammatory cells in response to infectious agents, inflammatory cytokines and proteins, and danger signals released by necrotic cells lead to the production of secreted factors that enhance the growth and survival of carcinoma cells.

Viral Malignancies in the Era of HAART

- **The introduction of highly active antiretroviral therapy (HAART) has dramatically decreased the incidence of AIDS-related malignancies associated with advanced immunosuppression, such as Kaposi sarcoma[1-3]**

- **At the same time, restoration of immunocompetence in HIV-infected patients with the HAART has no effect on EBV-related non-Hodgkin's lymphomas[1-3], and HPV-related cervical cancer[4].**

- **Malignant disease has been a major cause of death among immunocompetent HIV-infected patients in industrialized nations since the introduction of HAART[3].**

[1] C.S. Rabkin, *Eur J Cancer* **37**:1316-1319, 2001
[2] W.Y. Au et al., *Blood* **104**:243-249, 2004
[3] F. Bonnet et al., *Cancer* **101**:317-324, 2004
[4] Y. Aoki & G. Tosato, *Curr HIV Res* **2**:343-349, 2004