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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Introduction

It is estimated that 15% of all human tumors worldwide are linked to viruses, and environmental factors are suggested to influence viral persistent infection and carcinogenesis[1]. In regard of all cancers, the most potent human pollutant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), has been extensively studied, and currently is listed the "known to be a human carcinogen"[2], based on the data obtained in accidental and industrial cohorts heavily exposed to TCDD. Regarding human cancer risk estimation at or near background level TCDD exposure, opinions are heterogeneous, from those accepting either a non-linear or linear dose-response relationship for TCDD and cancer[3,4], to those challenging the very carcinogenic potency of present low TCDD body burden[5,6]. In addition to that, immunologic effects of background TCDD in a heterogeneous human population are subtle[6], whereas immunossuppresion is traditionally viewed as predetermining condition for both chronic viral infections and malignancies[1].

The above inconsistencies resulted in incomprehension and ignorance of human TCDD possible involvement in viral-associated cancers in immunocompetent people. We have approached this subject, starting from our pioneer findings in the early 1990s[7,8,9], through recent key data related to mechanism of TCDD action[10], and its effective dose limit[11], to the current formulation of a new concept[12].

Results and Discussion

Historically, our concept emerged from a discovered significant trans-activation of the HIV-1 virus in human cells caused by nanomolar TCDD. Measurements of reverse transcriptase activity and viral antigen revealed several fold increase of virus reproduction initially determined with 10 nM TCDD[7,8], and thereafter with 1.0 nM TCDD[9]. It was also shown that 0.08 nM TCDD caused effect on viral reverse transcriptase consisting 62% of that observed with 1.0 nM TCDD (personal communication). Such a marked stimulatory effect of TCDD on HIV-1 reproduction in previously infected lymphoid cells correlated with the data on higher polychlorinated dioxins/furans levels determined in the blood of AIDS patients with opportunistic infections in comparison with HIV-positive patients without any clinical manifestation[13].

Afterwards, our basic data and an assumption that the Ah receptor (AhR)-mediated transcriptional pathway is involved in mechanism of TCDD activation of the HIV-1, all have been confirmed in several laboratories[14,15,16]. However, because of usage TCDD at concentrations 10-15 fold higher than its human background level in the mid-1990, and also a lack of mechanistic knowledge of TCDD action on viruses, all the above remained no more than observations.

A decade later it finally turned out into understanding of the mechanism how body burden TCDD might transcriptionally up-regulate the HIV-1. With already proven data on participation of (overexpressed) AhR in TCDD activation of the virus[8,12,14,16], another obligatory component of the TCDD signaling pathway was revealed in 2002 from the "Species DRE Summary"[10]. Namely, a dioxin response element (DRE) was computationally identified in the 5'-flanking region of the HIV-1 gene, a feature known only for orthologous mammalian genes[17]. DRE includes the substitution intolerant core sequence, GCGTG, and adjacent variable sequences, with a matrix similarity score threshold to rank identified DREs.

Even more intrigued was that several known cancer-associated human viruses were found possessing multiple DREs in viral promoters. According to the above Eukaryotic Promoter Database-derived data[10],

the 5'-flanking regions of the following human viruses contain DREs, the number of which are shown in brackets: Epstein-Barr virus [22]; hepatitis B virus [4]; cytomegalovirus [10]; herpes simplex virus (HSV) type 1 [30], type 2 [8]; papillomavirus (HPV) type 16 [1], type 18 [2]; adenovirus (HAV) types 2 [9], type 5 [5], type 7 [5], type 12 [4]; T-cell leukemia virus type 1 [1].

A significance of this new information acquires from the fact that almost all of the above DRE-containing viruses are designated among those human viruses linked to approximately 15% of human tumors[1,18]. Beneath we present data and suggestions, which apply our concept to particular cancer-associated human viruses possessing multiple DREs.

However, any practical consideration of the concept was impossible until the basic question as to whether human TCDD body burden is potent enough to up-regulate DRE-containing viruses, was resolved in 2002[11]. Namely, a strong up-regulation of cytomegalovirus in host human cells was shown in the presence of 0.3 pM TCDD, i.e., concentration at least twenty times lower than TCDD background level currently determined in general population of this country[3,6]. The involvement of the AhR and AhR nuclear translocator (Arnt) in activation of cytomegalovirus replication was demonstrated, even though an extremely low concentration of TCDD was used in the study[11].

According to "Species DRE Summary" [10], a single DRE is localized in the HIV-1 promoter, whereas 10 DREs are found within powerful cytomegalovirus promoter. If juxtapose these DRE numbers with the above TCDD concentrations causing up-regulation of the HIV-1 and cytomegalovirus, the most susceptible candidates viruses to be augmented with body burden TCDD are those ones possessing at least similar to cytomegalovirus number of promoter DREs.

For instance, it is pretty applicable to the Epstein-Barr virus, which contains 22 DREs in the gene 5'-upstream region, and which is commonly associated with nasopharyngeal carcinoma and human malignant B-lymphomas[1]. There are epidemiological and medical findings showing dioxin-like compounds association with increasing incidence of the lymphomas[19]. Other data demonstrated increased titers of Epstein-Barr viral DNA in the lymphomas observed in immunocompetent individuals[20].

As regards the cytomegalovirus, numerous clinical studies show that this common virus is linked to the malignization of major human tumors, such as breast and colon adenocarcinomas. Thus nuclear acids and the major tegument protein pp65 of cytomegalovirus were detected in 92% of colorectal adenocarcinomas but not in adjacent nonmalignant biopsy samples[21]. Cytomegalovirus infection of Caco-2 cells in vitro resulted in induction of anti-apoptotic Bcl-2 and COX-2, which shift cells to more malignant phenotype contributing to tumor progression[22].

From mechanistic aspects, the data on up-regulation of the HIV-1 by 1.0-10.0 nM TCDD[7-9, 14-16], as well the CMV by 0.3 pM TCDD[11], all show an obligatory involvement of the AhR, a TCDD-activated transcription factor earlier known as mediating expression of genes in the Ah gene battery in mammals[23]. This corresponds to numerous publications on a significant overexpression of the AhR in various cancer cells[24,25]. It was also shown[26] that at low doses a local concentration of TCDD in extrahepatic tissues is determined not only by its partition between lipid and hydrophilic phases, but also by it's binding to the AhR. It might be that an overexpressed AhR binds higher amounts of TCDD. The individual risk assessment of human burden TCDD might also be dependent on human AhR binding affinity to TCDD, which varies ~ 20-fold in human populations[27].

Recent data support the concept, and provide evidence that sub-nanomolar TCDD is fully able to activate, via the AhR transcriptional pathway, the DRE-containing viruses. Thus a mammalian cell-based DRESSA bioassay system is developed for detection of at least 0.5 pM TCDD. The system contains tandem copies of the DREs fused to minimal viral promoter, and subcloned into an expression plasmid upstream of the reporter gene[28].

Our hypothesis that DRE-containing human viruses might be activated with bodily TCDD is not associated with viral theory of cancer origin, it rather relates to an established fact that malignant forms of cancer arise in chronically inflamed tissue. According to the recent IARC report[29], a high frequency of the common

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. In other words, body burden TCDD might be one of those "poorly understood factors that determine the persistence of specific cancer-associated virus in tumor malignancy" [1].

Concurrently, several alternative judgments are rendered on TCDD as human carcinogen, all derived from limited evidence in humans. The International Agency for Research on Cancer (IARC) classified TCDD as a group 1 carcinogen based on an elevation of all cancers combined in several heavily exposed industrial and accidental human cohorts[2]. As for estimation of human cancer risk at low level TCDD exposure, arguments exist over either accepting a threshold dose region for TCDD, or implying any exposure as having a statistical likelihood of causing cancer. The IARC supports a non-linear dose-response relationship for TCDD and cancer, whereas the US EPA characterizes the dose-response function as linear[3]. According to the US EPA, the average level of TCDD found in the general U.S. population is at or near the level that can be linked to adverse health effects, i.e., there little or no "margin of exposure"[4].

An opposite judgment denies any carcinogenic potential for TCDD body burden in humans. Thus Cole et al.[5] challenged the IARC as using Ah receptor-related mechanistic data to interpret cancer risk in humans, and argue with the US EPA over not assessing confounding factors. Hays and Aylward stressed that the falling trends in human TCDD body burden in general population over the three decades lead to its current serum lipid-adjusted level of 2-3 ng/kg (or ppt), which is too low to pose a risk to human health[6].

However, all arguing sides have paid no attention to the feasibility for body burden dioxin of causing cancers in humans by triggering malignancy-associated human pathogens. This quarrel is also at odds with a common view that TCDD-caused immune alterations result in a predisposition to infectious diseases. It is so because though some immunotoxicity of TCDD has been documented in accidental and occupational cohorts, immunologic effects of background TCDD in a heterogeneous human population are subtle, if any[30]. Our mechanistic concept addresses these inconsistencies by postulating that body burden TCDD in immunocompetent individuals might transcriptionally up-regulate the malignancy-associated human viruses, which possess multiple DREs.

As the receptor-mediated signaling pathway constitutes the suggested mechanism of TCDD action on DRE-containing human viruses, this might lead to the development of newly approach to partial inhibition of TCDD-caused augmentation of cancer-associated viruses. Thus antagonists of TCDD binding to the Ah-receptor, and inhibitors blocking binding of activated AhR-Arnt complex at the promoter DRE, might be tested. Clinically proven medicines such as salycilamide, as well as some natural compounds like coplanar bioflavonoids are among potential candidate antagonists.

In summary, human common viruses and body burden TCDD, i.e., two entirely different factors characteristic for the current general populations of industrialized countries, supposedly interfere in certain circumstances thus leading to tumor malignization. From a bioscience standpoint, this is the worst-case scenario of chemico-biological interactions. From a clinical standpoint, new developments in this field might discover preventive tools, which will help solving key problems in virus-linked oncology. The concept might also be utilized for the assessment and regulation of TCDD level, including its body burden, in relation to human viruses associated with major malignancies.

Notes

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