CURRENT HUMAN BODY BURDEN OF DIOXINS MIGHT UPREGULATE DRE-CONTAINING CYTOMEGALOVIRUS LINKED TO INFLAMMATION AND MALIGNANCY PATHWAYS

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Abstract

The low picomolar TCDD concentration has been shown to cause overreplication of cancer-associated viruses in infected human cells, exemplified here in cytomegalovirus-infected human macrophage cell line. Presumably, TCDD at body burden level might augment replication of viruses by triggering the TCDD-AhR-Arnt transcription complex binding to DREs revealed in promoter regions of cytomegalovirus and other human common viruses. Natural and synthetic antagonists of the Ah-receptor are suggested as universal tool for prevention and treatment of progressive inflammation and malignization driven by DRE-containing human viruses.

Introduction

While previous studies had failed to link characteristics of infectious diseases to human cancer, researchers nowadays have better knowledge of the role common viruses play in the etiology of particular cancers, including some major malignancies. With using immunohistochemistry, *in situ* hybridization, microarray displays, and PCR, the oncogenic potential of viral gene products has been demonstrated. A high frequency of the 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[1].

However, the factors that determine the persistence of specific cancer-associated virus, and mechanisms of their involvement cellular malignancy are still poorly understood. The data obtained from experiments with human macrophage cell line allow us to suggest that 2,3,7,8-tetrachlodibenzo-p-dioxin (TCDD) at low picomolar concentrations might trigger transcriptional activation of human cytomegalovirus (hCMV).

It is known that human general population has been accumulating the most potent pollutants, TCDD and TCDD-like compounds, mainly due to consuming contaminated food. After being swallowed, just small amount of TCDDD pass through intestines into the blood. Nevertheless, its body burden monitoring is routinely conducted using blood samples. Decreasing trends in TCDD body burden over three decades in the Unites States culminated in its current serum level of about 2-3 ng/kg, lipid[2]. Such body burden is commonly assumed too low to cause direct adverse effects on TCDD human health[3]. However, a scenario of possible triggering effect of bodily TCDD on human common viruses that contain numerous dioxin response elements (DRE) within viral 5' upstream regulatory region[4], was completely overlooked.

Meanwhile, up-regulating effect of a nanomolar TCDD on human viruses had been postulated owing to trans-activation of HIV-1 reverse transcriptase and accumulated viral protein in infected human cells[5,6]. Thereafter, our data on HIV-1 have been confirmed in other laboratories[7-9]. Lately, it was recognized and demonstrated that TCDD at sub-picomolar concentrations augment replication of hCMV in infected human fibroblast cell line[10]. Here, we tested TCDD effects on hCMV in human macrophage cell line, as among the various cell types shown infected by HCMV *in vivo*, macrophages play a particular pathogenic role[11].

Materials and Methods

The human non-adherent monocytic cell line THP-1 (obtained from ATCC), passages 20-40, was used. Cells were passaged three times a week in RPMI 1640, supplemented with 10% heat-inactivated fetal serum and penicillin (100 U/ml) and streptomycin (100 μg/ml). THP-1 cells were differentiated into macrophages by exposure to 15 nM PMA for 18 h, then cells were centrifugated, counted, and viability determined. Cells were seeded into 24-well plates at $5x10^5$ cell/well/ml.

After reaching confluency, THP-1 cells were inoculated with stock hCMV at a moi of 0.1, and then treated at 37°C in 5%CO_2/95% air with 1.0 ml DMEM medium containing either the above supplements (control) or TCDD (0.3, 3.0, 30.0 or 300 pM) for 120 hrs. Production of infectious hCMV was determined using a plaque assay[12]. Dot blot hybridization for detection of CMV DNA was conducted as described in[13]. The amplified PCR products from *UL54* DNA polymerase gene region were used as the hybridization probes.

Results and Discussion

Replication of hCMV in THP-1 cell cultures after treatment with TCDD was analyzed in four separate series. TCDD at any concentration used has no toxic effects upon cell-free CMV. We demonstrated a significant (4.8-fold) increase in viral production at 5 days after treatment of infected cells with 3.0 pM TCDD (0.001 pg TCDD/ml). The level of production of the virus in THP-1 cells treated with 0.3 pM TCDD was also significantly higher than that in control cells, but comprised only 43% of the level of hCMV production observed with 3.0 pM TCDD. Augmenting effects on hCMV production of TCDD at neither 30.0 pM (5.0-fold) nor 0.3 nM (5.3-fold) significantly differed from those of 3.0 pM TCDD.

We determined a 7.0-fold increase of hCMV DNA replication caused by 3.0 pM TCDD, as compared to viral DNA replication level in the THP-1 cells non-treated with TCDD. At the same time, effect of TCDD at 0.3 pM on the level of hCMV DNA replication in THP-1 cells did not differ significantly from the level in control cells.

Preliminary results were obtained in three separate series that flavonoid quercetin, analgetic drug salicylamide, brassica vegetables' indole-3-carbinol, and tricyclic furocoumarin 8-methoxypsoralen (used at concentration of 10μM) all reduced to various extents activation effects of 3.0 pM TCDD on hCMV production and viral DNA replication in THP-1 cells (data not shown). The above compounds are known as aryl hydrocarbon receptor (AhR) antagonists thus confirming earlier data that upregulation by TCDD of HIV-1in infected target cells is regulated by the AhR[6,8,10], a ubiquitous TCDD-activated transcription factor, known to mediate expression of genes in the *Ah* gene battery in mammals. Assuming that body burden TCDD might augment human persistent viruses, like hCMV, one would raise the question of what determine the individual risk of chronic viral inflammation and cellular malignization development? From mechanistic standpoint, upregulation of the hCMV with a picomolar TCDD should necessarily include the AhR as a factor contributing to the risk estimation.

This supposition corresponds well with previous findings that AhR is distinctly overexpressed exclusively in some cancer cells, including colon adenocarcinoma[14]. Also, the individual cancer risk assessment, in relation to human body burden of TCDD, might be dependent on the AhR affinity to TCDD, which varies ~ 15-fold in human populations[15].

However, only a high-affinity AhR interaction with TCDD with the formation of activated TCDD:AhR complex would not lead to transcriptional regulation of the target genes, unless a binding of TCDD:AhR:Arnt complex to DRE sites within promoter region is to occur[16]. With regard to viral genes, a gap in our knowledge of TCDD action was filled by pioneer information from Tim Zacharewski lab on DREs detected within promoters of several human viruses[4]. According to the data, while a single DRE was determined in the HIV-1 promoter, ten DREs were found within a powerful hCMV promoter. As the minimum effective concentration of TCDD causing transactivation of the HIV-1 was determined no lower than 1.0 nM, so the concentration-dependent effects of TCDD on reactivation of latently infected hCMV became a matter of interest.

It has been shown earlier that TCDD at concentration of 0.3 pM significantly increased the replication of hCMV in human fibroblast cell line[10]. In this study, human monocyte/macrophage cell line THP-1 was used. By utilizing a plaque assay for measurement of infectious hCMV replication, and dot blot hybridization for detection of hCMV DNA, we found that the lowest effective concentration of TCDD was at least ten times greater than the corresponding concentration used with fibroblast cell line. Thus TCDD at 3.0 pM increased viral production (virus titer) 4.8 times, and viral UL54 DNA level 7.0 times, compared to the control samples.

Macrophages were chosen because these cells are believed to play an important role in hCMV dissemination and latency[17]. Macrophages are targets for hCMV infection in different tissues. It is becoming increasingly clear that hCMV benefits from inducing a strong host inflammatory response[18]. Viral proteins representing all stages of permissive hCMV infection were detected in macrophages, suggesting that these cells support the complete viral replication cycle.

New findings suggest that macrophages are infected by hCMV in vivo, and they play an important role in the hematogenous spread of hCMV into solid organs, due to endothelial-cell tropism of both macrophages and hCMV[19]. The malignant endothelial tissue is exemplified here by colon cancer, the second-leading cause of cancer-related deaths in the U.S. Nucleic acids and the major tegument protein pp65 of hCMV were detected in 92% of colorectal adenocarcinomas, but not in adjacent nonmalignant biopsy samples[20].

The hCMV is a herpes virus that infects and is carried by 70-100% of the world's population, and may persist in an immunocompetent host. The possible role of this virus was highlighted in the development of various diseases, in particular inflammatory diseases, autoimmune diseases and, more recently, with certain forms of cancers. Researchers are focused on determining whether hCMV plays a causative role in these diseases or is merely an epiphenomenon of inflammation. Inflammation plays a central role in the pathogenesis of hCMV. By influencing the regulation of various cellular processes including the cell cycle, apoptosis and migration as well as tumor invasiveness and angiogenesis, hCMV may also participate in cancer development[21].

Also, hCMV is able to reactivate other oncogenic viruses, via virus superinfection (as with Epstein-Barr virus), or thru a specific transactivator, like TNF-α (with HIV-1). As for modulatory effects of hCMV on tumor cell biology, there are many data regarding this subject. Thus hCMV infection of Caco-2 cells *in vitro* resulted in induction of anti-apoptotic Bcl-2 and COX-2, which shifts cells to more malignant phenotype contributing to tumor progression[22].

Historically, our data on TCDD activation of the HIV-1, and an assumption about AhR-mediated transcriptional pathway involvement[5,6,23], all have been confirmed and further developed[7-9]. However, as effective TCDD concentrations were found 10-100 times higher than its human body burden level in the mid-1990, and because the lack of mechanistic knowledge regarding TCDD mechanism of action on viruses, all the above remained no more than observations. A decade later it finally turned out into mechanistic concept underlying transcriptional up-regulation of human viruses by TCDD. With already proven data on participation of the AhR in TCDD activation of the virus[6,8,9], second critical component of the TCDD-activated signaling pathway was revealed from the “Species DRE Summary”. Namely, DRE sites were computationally identified in the 5’-flanking region of viral genes, a feature known only for orthologous mammalian genes[4]. DRE includes the substitution intolerant core sequence, GCGTG, and adjacent variable sequences, with a matrix similarity score threshold to rank identified DREs.

However, no practical consideration has been given to the concept, until basic question whether human TCDD body burden might upregulate DRE-containing viruses, was finally addressed[10]. Namely, a strong augmentation of hCMV replication in human fibroblast cells was shown in the presence of 0.3 pM TCDD, i.e., concentration twenty times lower than TCDD background level currently determined in general population of this country[2,3]. The involvement of the AhR and Arnt in activation of hCMV replication was also demonstrated although extremely low concentration of TCDD was used in the study[10].

More intrigued was that several known cancer-associated human viruses were found possessing multiple DREs in viral promoters. According to[4], 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]; herpes simplex virus type 1 [30], type 2 [8]; papillomavirus type 16 [1], type 18 [2]; adenovirus 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 human viruses linked to approximately 15% of human tumors[24,25].

Finally, we postulate that DRE-containing cancer-associated human viruses are newly target genes regulated by TCDD-activated AhR transcriptional complex. Specific effects of TCDD on replication of each of the above DRE-containing cancer-linked human viruses remain to be demonstrated. If proven, new intervention tool for the treatment of progressive malignization associated with persistent virus infection in cancer cells might be suggested. Particularly, strong competitive antagonists of the AhR, such as synthetic drugs like salicylamide, and naturally derived compounds like bioflavonoids[26], were successfully tested in preliminary analyses conducted during this study.

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