

HUMAN BURDEN 2,3,7,8-TCDD MAY AUGMENT COMMON VIRUSES ASSOCIATED WITH CANCER MALIGNIZATION

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Elevated levels of dioxin associated with the herbicide Agent Orange is still routinely found in blood samples from persons living in areas sprayed with Agent Orange between 1962 and 1970. By Vietnam estimations, more than a million of its people were exposed to the spraying with Agent Orange contaminated by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The effects of TCDD on human health can be studied in Vietnam because of the unique situation where both current and older dioxin contamination exists for a potentially large population of exposed men, women, and children over several generations. It was hypothesized that a major route of current is from the movement of dioxin from soil into river sediment, then into fish, and from fish consumption into people (Schechter et al., 2001).

According to the Institute of Medicine, sufficient evidence is shown for an association between Agent Orange and the following cancers: Soft tissue sarcoma, Non-Hodgkin lymphoma (NHL), Hodgkin disease, Chronic lymphocytic leukemia (CLL). Almost all of these cancers are strictly or tentatively associated with common human viruses, such as Epstein-Barr virus (EBV), Herpes simplex viruses (HSV), or cytomegalovirus (CMV). For other viruses, like Hepatitis B virus (HBV), there are conflicting reports on its role in the development of malignant hepatocellular carcinoma in Vietnam (Ngaon & Yoshimura, 2001).

In general, human cancers associated with papillomavirus (HPV), HBV, EBV, and human T cell leukemia-lymphoma virus (TCLLV) infections are responsible for approximately 15 percent of the worldwide cancer incidence. Cancer of the cervix and hepatocellular carcinoma (in Africa) account for about 80 percent of virus-linked cancers. Because experimental and epidemiologic data imply the key role for viruses, particularly in cervical

and liver cancer, viruses must be thought of as the second most important risk factor for cancer development in humans, exceeded only by tobacco consumption.

The RNA and DNA tumor viruses have made fundamental contributions to two major areas of cancer research. Viruses were vital, first, to the discovery and analysis of cellular growth control pathways and the synthesis of current concepts of cancer biology and, second, to the recognition of the etiology of some human cancers. DNA tumor viruses encode oncogenes of viral origin that are essential for viral replication and cell transformation; viral oncoproteins complex with cellular proteins to stimulate cell cycle progression and led to the discovery of tumor suppressors. Viral systems support the concept that cancer development occurs by the accumulation of multiple cooperating events. The infectious nature of viruses distinguishes them from all other cancer-causing factors; tumor viruses establish long-term persistent infections in humans, with cancer an accidental side effect of viral replication strategies. Viruses are usually not complete carcinogens, and the known human cancer viruses display different roles in transformation. Many years may pass between initial infection and tumor appearance and most infected individuals do not develop cancer. Variable factors that influence viral carcinogenesis are suggested, including possible synergy between viruses and environmental cofactors (Butel, 2000).

Here, we present a newly concept, which historically came into existence in the 1990s when we discovered TCDD ability to significantly trans-activate the HIV-1 virus in the target human cells (Pokrovsky & Tsyrllov, 1991; Tsyrllov & Pokrovsky, 1993). Inasmuch as our discovery has been confirmed in several labs in the US and Japan, it took about ten more years before it turned out into revelation of a health threatening ability of human body burden TCDD to transcriptionally up-regulate cancer-associated human viruses.

The understanding of the mechanisms of TCDD action on human viruses emerged from the “Species DRE Summary”, which appeared in 2002 on the website of Michigan State University. It showed that 5’-flanking regions of genes of several cancer-associated human common viruses possess multiple “dioxin-response elements” (DREs), a feature earlier known only for mammalian genes.

The key question still remained, namely is human body burden TCDD potent enough to up-regulate common viruses? This obstacle to the concept was cleared after it was

demonstrated that human CMV was strongly up-regulated in human cells with only 0.3 pM TCDD, i.e., concentration at least twenty times lower than dioxin background level currently determined in general population of this country (Murayama et al., 2002).

According to “Species DRE Summary”, a single DRE is localized in the HIV-1 promoter, while 10 DREs are found within powerful CMV promoter. If juxtapose these with the above TCDD concentrations causing up-regulation of the HIV-1 and CMV, the most susceptible candidates viruses to be augmented with body burden TCDD are those viruses possessing at least similar to CMV amount of promoter DREs. For instance, it is fully applicable to the abovementioned EBV, which contains 22 DREs in the gene 5’ upstream region, and which is commonly associated with human malignant human B-lymphomas. There are several epidemiological and medical findings showing TCDD as a factor associated with increasing incidence of the lymphomas, and demonstrating increased titers of EBV DNA in the lymphomas observed even in immunocompetent patients.

As regards the CMV, numerous clinical studies show that this common virus is linked to the malignization of such major human tumors as breast and colon adenocarcinomas. Thus nuclear acids and the major tegument protein pp65 of CMV were detected in 92% of colorectal adenocarcinomas but not in adjacent nonmalignant biopsy samples (Harkins et al., 2002). CMV 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 (Cinatl et al., 2004).

In addition to the above viruses, there are several other cancer-associated human common viruses also containing multiple DREs in the 5’ flanking region of their gene, namely human papillomavirus (HPV), type 18 (2 DREs); hepatitis B virus (4 DREs); adenovirus (HAV), types 5 and 7 (5 DREs), and 12 (4 DREs); herpes simplex virus (HSV), type 1 (30 DREs), and type 2 (8 DREs).

From the mechanistic point, all the data published on up-regulation of the HIV-1 by 1.0-10.0 nM TCDD, as well the CMV by 0.3 pM TCDD, show an involvement of the aryl hydrocarbon receptor (AhR), a TCDD-activated transcription factor earlier known as mediating expression of genes in the Ah gene battery in mammals. This

corresponds very well to numerous publications on a significant overexpression of the AhR in various cancer cells. It was shown (Diliberto et al., 2001) that at low doses a local dose of TCDD in extrahepatic tissues is determined not only by its partition between lipid and hydrophilic phases, but also by its binding to the AhR. It might be proposed 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.

The IARC classification of TCDD as a group 1 carcinogen (IARC 1997) has stirred some controversy. Some authors ignored the original IARC focus on high-exposure subcohorts, ignored the positive exposure-response analyses, and raised the issue of possible confounding by smoking and other chemical carcinogens without any serious consideration of whether such possible confounding is likely, or whether it could account for the observed elevation of all-cancer mortality in those with higher TCDD exposure. In our view, the epidemiologic and toxicologic evidence since the IARC (1997) classification of TCDD as a human carcinogen has strengthened the case for IARC's decision. Furthermore, the dose-response assessments for TCDD and cancer indicate that TCDD exposure levels close to those in the general population may be carcinogenic and argue for caution in setting the upper ranges of long-term permissible exposure to dioxins.

This totally relates to our concept on body burden TCDD's ability to augment malignancy-associated human viruses. According to the IARC biennial report of 2003, 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 still "poorly understood factors that determine the persistence of specific cancer-associated virus in tumor malignancy".

It is important that a mammalian cell-based bioassay system is already developed that is enabled detection of 0.5 pM TCDD. The system is called the fast-track DRESSA (dioxin-responsive-element-based sensing via secreted alkaline phosphatase), where tandem copies of the DREs fused to minimal viral promoter are subcloned into an expression plasmid upstream of the

reporter gene (Kasai et al., 2005). Thus is the most vivid practical demonstration that TCDD at doses lower than its current human body burden is able to stimulate viral expression plasmid containing DREs in its 5'-upstream region. Moreover, it was shown that TCDD action is mediated thru the AhR transcriptional pathway, as the AhR antagonists blocked the above stimulation, and therefore were used for elimination of nonspecific, false-positive responses.

As the receptor concept is fully applicable to TCDD action on human common viruses, the above might lead to the development of a new tool for inhibition (complete or partial) of TCDD-caused up-regulation of cancer-associated viruses. I am talking here about already known antagonists of TCDD binding at the Ah-receptor, and inhibitors blocking binding of activated AhR-Arnt complex at the DRE. These antagonists/inhibitors include pharmaceutically used medicines such as salycilamide, as well as some natural compounds like coplanar bioflavonoids in green tea, etc.

Summarizing the above, the common viruses and body burden TCDD, i.e., two entirely different endogenous factors characteristic for the current general population, supposedly interfere in certain circumstances (like currently in Vietnam) 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 and organ transplantation. These consist the essence of the XENOTOX, Inc., an innovating and consulting company, which focuses on the assessment and regulation of the effect of TCDD, at or near its body burden, on human common viruses linked to specific malignancies.