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Review Paper

Effects of Persistent Organic Pollutants on the Immune System: The Case of Dioxins

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ABSTRACT

Persistent organic pollutants can be traced in air, water, soil and biota in industrialized and non industrialized regions. Although the production of these chemicals has been banned since 1980's when their toxicity was proven, their use, trade and disposal as well as persistence due to previous use, continues to contaminate the environment and threaten human health. Recent studies on the immunological consequences of dioxin contamination and exposure indicate that these compounds and specifically 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin activates the aryl hydrocarbon receptor on lymphocytes. This activation results in an array of effects on T, B and APC cells, biological mediators of the immune response and thereby results in suppression or remodelling of the immune response. This review attempts to shed light on the recent research developments in this field and to provide insight into the vast and long term health consequences of persistent organic pollutants.

Keywords: *Persistent organic pollutants,Dioxins,Immune response, Aryl hydrocarbon receptor, Pollution*

INTRODUCTION

The production of thousands of chemicals has contributed to industrial and economic development in many parts of the world. This trend however has been associated with the release of new chemicals and possibly toxic substances into the environment and food chain and adversely effecting human health in many instances. Notably, the increased incidence of asthma in urban and industrial areas has been attributed to airborne chemicals and pollutants (Ebtekar and Moien, 2001). Polycyclic aromatic hydrocarbons have been shown to affect immune competence in several studies (Laupeze et al., 2002).

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Among these chemicals some were later recognized to remain intact and active for very long periods of time in the environment and thus easily transported to other regions. Termed Persistent Organic Pollutants (POPs), were known to adversely affect human health and biodiversity. According to current statistics from 1929 to 1989, 1.5 million tons of POPs were produced globally. Industrial activity, ferrous and nonferrous metal facilities and foundries emit Polychlorinated Biphenyls (PCBs) which are one of the most important types of POPs. Chlorinated pesticides are also less biodegradable and pose serious health and environmental risks. Polychlorinated biphenyls are compounds with 2-6 carbon rings in which 1-6 hydrogens are replaced by chlorine. To date 150 types of PCBs have been recognized. Dioxins are an important

category known to have direct immune system effects.

POPs may enter the host through the food cycle or inhaled air; they pass mucosal barriers and interact with the immune system. The toxicity of PCBs was recognized in 1980, and it became clear that PCBs accumulate in the food chain particularly in aquatic food. Trace amounts of dioxins can be measured in the peripheral blood of most residents in industrialized and urban areas, while 50 years ago it was not traceable. Realizing the serious health risks and particularly the persistence of these compounds, the global community took action to develop a legal international treaty to prevent the production, use and trade and proper management of these substances. This international campaign led to the creation of the Stockholm Convention on Persistent Organic Pollutants in 2000. The Islamic Republic of Iran has signed the treaty and the final stages of ratification are followed in the Parliament.

The Iranian Department of Environment has recently initiated an international project with the Convention for enabling the country to fulfil its commitments to the Convention. The Convention has entered into force recently and therefore there is much hope to create better environmental conditions with regard to POPs (Ebtekar, 2000).

Among the environmental factors linked to the development of autoimmunity are heavy metals such as mercury, chlorinated pesticides containing chlordecone methoxychlore, crystalline silica dust and solvents such as trichloroethylene. Mice exposed to occupationally relevant doses of the contaminant trichloroethylene in their drinking water developed lupus like syndromes and autoimmune hepatitis in association with the activation of IFN-gamma producing CD4+T cells. Studies on one of its major metabolites trichloroacetaledhyde (TCAA) also shows that TCAA promotes T cell activation via stimulation of the mitogen activated protein kinase pathway in association with Schiff base formation on T cell surface proteins. By demonstrating that TCAA can stimulate T cell function directly these results may explain how the environment toxicant promotes T cell activation and related autoimmunity in vivo (Gilbert et al., 2004).

Molecular mechanisms of dioxins Dioxins as a class of POPs that can be found in both contaminated air and water are relatively difficult to detect (Shimomura et al., 2001) and interact specifically with the immune system.

Studies indicate that dioxin acts specifically on cells by ligand activated induction of transcription through the cyotosolic Aryl Hydrocarbon Receptor (AHR). The Ah receptor exists on cells including lymphocytes, in the thymus, lung and liver. Further studies have elucidated the molecular mechanisms after binding of dioxins with AHR. This complex faces conformational changes and translocates to the nucleus to bind to specific regulatory sequences and induce transcription through NF-kb (Baccarelli et al., 2002; Smialowicz, 2002).

2, 3, 7, 8 tetrachlorodibenzo-p dioxin (TCDD) has been recognized to be a potent antagonist of the aryl hydrocarbon receptor (AHR).

Activation of the aryl hydrocarbon receptor (AHR), a basic helix-loop-helix transcription factor in lymphocytes by TCDD has been shown to cause thymic atrophy in every species studied. Studies indicated that AHR was only activated in thymocytes to cause thymic atrophy. Therefore, intrathymic progenitor cells are direct targets of TCDD in the thymus and TCDD cause thymic atrophy by reducing entrance to the cell cycle in these populations (Laiosa et al., 2003).

Moreover, dioxins have been demonstrated to induce a 40% increase in double positive cells in murine fetal thymus cells and over expression of CD44 and MHC class I on thymocytes. A higher rate of positive selection has also been reported due to the effect of dioxins. This event could increase the incidence of autoimmunity in the host (Kerkvliet et al., 2002). The influence of TCDD on haematopoietic stem cells which possess the ability to reconstitute long term multilineage haematopoiesis was studied in another

work. This work indicated that TCDD treated stem cells almost lost long term reconstitution activity (Sakai et al., 2003). Rats exposed to TCDD developed signs of immunosuppression, with reduced thymus weight, suppressed T cell responses and reduced NK as well as Ab specific responses (Smialowicz, 2002).

Effects on T cells and cytokine production T cells upon activation undergo apoptosis, a process termed *activation induced cell death* (AICD). Research indicates that the immunotoxic effects of TCDD in activated peripheral T cells may stem from increased AICD mediated through Fas-Fas ligand interactions (Camacho et al., 2001). One study has shown that TCDD induces Fas-dependent activation induced cell death in superantigen- primed T cells (Camacho et al., 2002). In other studies it was shown that TCDD induced suppression of CD4+ T cells involves, in part increased cell death that may be mediated by Fas/FasL interaction (Dearstyne and Kerkevliet, 2002) .

Exposure to TCDD clearly impairs T cell dependent immune responses. (Shepard et al., 2000). In a recent study on T cell receptor transgenic mice it was shown that TCDD suppressed the antibody response while in wild type mice only the expansion and differentiation of CD8+ cells was suppressed (Mitchell and Lawrence, 2003A). Also TCDD can induce the Il-2 gene through the AhR (Vorderstrasse and Kerkvliet, 2001). Other studies showed that the murine Il-2 promoter contains distal regulatory elements responsive to the AhR receptor, these motifs after binding to the AhR are sufficient to transactivate luciferase expression (Jeon and Esser, 2000).

In one study the effect of TCDD on T cell derived cytokine production in mice was observed. TCDD caused an initial increase in IL-2 but this increase was suppressed by day 4, also the production of Th2 cell derived cytokines IL-4, IL-5 and IL-6 were significantly suppressed as compared with control mice (Ito et al, 2002, Fujimaki et al., 2002). Therefore impaired suppression of antigen specific antibody production following exposure may be related to the impaired cytokine response. (Ito et al, 2002; Nohara et al., 2002)

Effects on B cells and humoral immunity Research also has demonstated that TCDD alters B cell differentiation as evidenced by a marked decrease in IgM secretion and the number of antibody forming cells. Findings also suggest that cyclin dependent kinase inhibitors may be an important intracellular target in TCDD mediated inhibition of B-cell differentiation. Other studies demonstrate that TCDD exposure inhibits the generation of high affinity antibody forming cells and high affinity antibody production during the primary humoral immune response and suggest that these alterations were caused by the suppression of antigen responding B-cell proliferation induced by TCDD during GC formation (Inouye et al., 2003).

TCDD also directly effects spontaneous IgE production in B cells from atopic patients. TCDD failed to induce other immunoglobulin types and in non atopic patients TCDD could not induce increased IgE production. These results suggest that TCDD may aggravate allergic diseases by enhancing IgE mediated allergic responses (Kimata, 2003). Nuclear factor kappa-beta and AP-1 both play an important role in B-cell activation, differentiation and immunoglobulin gene expression. Studies show that LPS induced DNA binding and transcriptional activity of AP-1 was markedly inhibited by TCDD, however the chemical had no effect on the activity of NK-kb. This study indicates that The TCDD induced inhibition of IgM expression by B cells may be mediated at least in part through a down regulation of AP-1 activity in an Ah-R dependent manner (Sulentic et al., 2000; Suh et al., 2002). According to one study TCDD exposure suppressed rather than enhanced the development of allergic immune responses as well as the expression of immune mediated lung disease (Luebke et al., 2001).

Effects on APCs TCDD has been demonstrated to induce activation changes in splenic dendritic cells in the absence of antigen challenge. It

however did not affect the ability of splenic dendritic cells to internalize latex beads administered in vivo. This study concludes that TCDD does not suppress the ability of DC to process and present antigen but may enhance their ability to provide activation signals to T cells.This might alter the survival of the T cells, the DC or both and may contribute to the dysregulation of the immune response (Vorderstrasse et al., 2003). TCDD has also been shown to affect the number and function of murine splenic dendritic cells and their expression of accessory molecules. These effects were dose dependant and persisted for two weeks after exposure. DC from TCDD treated mice
Expressed higher levels of several accessory molecules including ICAM-1, CD24, B7-2 and CD40 while the expression of LFA-1 was significantly reduced (Vorderstrasse and Kerkvliet, 2001).

Response to infection In mice treated with TCDD and infected by influenza A virus, researchers observed a significant increase in mortality as compared with infected mice not contaminated with TCDD. The study indicated that decreased antibody production and hyper inflammation may contribute to the increased mortality of mice (Vorderstrasse et al., 2003).
Exposure to TCDD suppressed the clonal expansion of influenza virus specific CD8+ T cells resulting in a three to five fold reduction in the number of cytotoxic T lymphocytes (CTL) in the lymph node as compared to control mice. Studies to identify possible mechanisms for the diminished CTL response failed to show evidence for increased apoptosis in virus specific CD8+ T cells from TCDD exposed mice. In this study however treatment with TCDD reduced the number of proliferating virus specific CD8+ T cells by as much as 70% on day 7 post infection. Restimulation could not completely restore proliferation or IFN gamma production by CD8+ cells suggesting that exposure to TCDD drives antigen specific CD8+ cells into a state of unresponsiveness similar to anergy (Mitchell and Lawrence, 2003B).
Other studies indicate that TCDD increased IFN gamma and TNF alpha gene expression thereby enhancing the production of proinflammatory cytokines. (Ho-Jun et al., 2002).
In one research mice exposed to TCDD and infected with influenza virus did not have an increased pulmonary virus burden suggesting either that TCDD treatment alters the host response to infection, creating a cellular environment that is less supportive for viral growth, or that exposure to TCDD directly affects influenza virus leading to impaired virus replication within lung epithelial cells (Lawrence et al., 2000).
In another study on lactational exposure to TCDD in *Listeria* infection in newborn mice, the exposure had little effect on the weight of immune organs but it enhanced the production of tumor necrosis factor alpha (TNF-alpha) and interferon gamma in the serum after *Listeria* infection.The clearance of *Listeria monocytogenes* from the spleen was impaired in the newborn (Sugita-Konishi et al., 2003).
The effect of TCDD on myocarditic coxsackievirus B3 infection in mice was studied focusing on inflammatory lesion and mortality. TCDD seemed to have limiting effect on viral replication and the development of the inflamematory lesion in the myocardium; however mortality was increased by TCDD in this infection model (Funseth et al., 2002).

Response to allogenic tumours Studies performed on mice challenged with P815 tumour cells and exposed to TCDD indicated an increased percentage of CD11b+ Mac-1 + (Gr1 +) cells in the spleens of these mice. These CD11b+ Gr-1 + myeloid suppressor cells MSC have been described as that which prevents cytotoxic T lymphocyte (CTL) development in a variety of disease states. This study also shows that TCDD exposure alters the host response to allogeneic tumour graft resulting in enhanced myelopoiesis perhaps as a compensatory response to the suppressed T cell mediated immune response in the face of an increasing P815 tumour burden.

Furthermore, within the context of the P815 response, TCCD appears to alter the functional capabilities of mature neutrophils, by enhancing their oxidative burst capacity but reducing the tumouricidal response (Choi et al., 2003)

Studies on CTL precursors show that TCDD induces an early defect in CTLp activation that is not due to insufficient numbers or deletion of CD8+ cells and may implicate a novel mechanism by which ligands of the Ah R disrupt CTL precursor activation (Prell et al., 2000).

Genetic studies Genetic studies have indicated that dioxins may induce certain genes including oncogenes, cytochrome p450, plasmino- gen activator inhibitor 2, Il-1beta and certain cytokines. PCBs have been known to induce genes which affect cell decisions on proliferation and differentiation therefore possibly disrupting cell funtions and homeostasis in a broader sense. Expression of NF-kb, c-jun and p27 (kip1) genes was increased by TCDD treatment according to one study (Crawford et al., 2003). In addition, some genes like insulin like growth factor binding protein-6 and IL-5R alpha subunit genes were upregulated while the other genes like CD14 were down regulated (Park et al., 2001).

Human studies The residents of Seveso Italy were exposed in 1976, due to an accident to high levels of TCDD. Recent studies on the immunoglobulin and complement plasma levels in a random sample of the most highly exposed and in the surrounding contaminated area showed that plasma IgG levels decreased with increasing TCDD plasma concentration. Also the increased incidence of lymphatic tumours in the area of the accident requires more investigation (Baccarelli et al., 2002).

Recent advances and future prospects Many studies have been conducted to elucidate the underlying biochemical mechanisms by which dioxins disrupt immunological functions and much research has been done focussing on the effects of TCDDi . Some studies point to a new paradigm for the mechanism of suppression of immune functions by TCDD emphasising on inappropriate activation of cells, leading to anergy or death and the consequent premature termination of the immune response. This paradigm has also been characterized by enhanced activation of B cells, DC and CD4+ T cells by TCDD and earlier disappearance of the two latter populations from the peripheral lymphoid organs (Kerkvliet, 2002). The exact mechanism involved and the signalling pathways need to be studied and identified. Considering the continued use of many chemicals including persistent organic pollutants in most parts of the world and also the increased risks of contamination, studies to understand and identify the possible risks and effects on human health should continue. The long term consequences and serious adverse effects of these chemicals point to the importance of environmental regulation and monitoring to control their use and release into the environment at local as well as international levels.

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