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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin-mediated disruption of the CD40 ligand-induced activation of primary human B cells

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ABSTRACT

Suppression of the primary antibody response is particularly sensitive to suppression by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice; however, surprisingly little is known concerning the effects of TCDD on humoral immunity or B cell function in humans. Results from a limited number of previous studies, primarily employing *in vitro* activation models, suggested that human B cell effector function is suppressed by TCDD. The present study sought to extend these findings by investigating, in primary human B cells, the effects of TCDD on several critical stages leading to antibody secretion including activation and plasmacytic differentiation using an *in vitro* CD40 ligand activation model. These studies revealed important differences in the response of human and mouse B cells to TCDD, the most striking being altered expression of plasmacytic differentiation regulators, B lymphocyte-induced maturation protein 1 and paired box protein 5, in mouse but not human B cells. The activation of human B cells was profoundly impaired by TCDD, as evidenced by decreased expression of activation markers CD80, CD86, and CD69. The impaired activation correlated with decreased cell viability, which prevented the progression of human B cells toward plasmacytic differentiation. TCDD treatment also attenuated the early activation of mitogen-activated protein kinases (MAPK) and Akt signaling in human B cells. Collectively, the present study provided experimental evidence for novel mechanisms by which TCDD impairs the effector function of primary human B cells.

Introduction

Immune suppression is among the earliest and most sensitive sequelae of exposure to environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Holsapple et al., 1991). In rodent species, T cell-dependent and -independent antibody responses are particularly sensitive to suppression by TCDD treatment. Although many different leukocyte populations are modulated by TCDD, suppression of humoral immune responses has been demonstrated by direct treatment of purified primary B cells and B-cell lines indicating that TCDD can act directly on B cells to impair their function (Dooley et al., 1990; Dooley and Holsapple, 1988; Sulentic et al., 1998). Using *in vitro* and *in vivo* mouse models the molecular mechanisms by which TCDD impairs B cell function been extensively investigated. Although the identification of specific molecular targets has been challenging, in mouse B cells TCDD suppresses B cell to plasma cell differentiation. Moreover, recently three transcriptional repressors critically involved in regulating B cell differentiation, B lymphocyte maturation protein 1 (Blimp-1), paired box protein 5 (Pax5), and Bach2 were identified as potential targets in mouse B cells subjected to alterations by TCDD. Evidence for direct modulation of either Blimp-1 or Pax5 via an AHR/DRE-mediated mechanism could not be established as the identified DRE-like sites in known regulatory regions of both genes exhibited modest AHR binding affinity (North et al., 2009; Schneider et al., 2008; Yoo et al., 2004). One explanation for the above findings is that the observed impairment of Blimp-1 and Pax5 could be, at least in part, due to alterations in upstream signaling events more proximal to B cell activation (North et al., 2010; Suh et al., 2002). In contrast, TCDD-mediated up regulation of Bach2, a direct transcriptional repressor of Blimp-1 was recently demonstrated (Ochiai et al., 2006). Bach2 was identified in a genome-wide ChIP-on-chip study in which TCDD-inducible AHR binding was demonstrated to a DRE-like site within intron1 of Bach2 (De Abrew et al., 2010). Intron1 has been found to play a vital role in regulating Bach2 transcription (Ochiai et al., 2008). Studies are presently ongoing to further characterize the role of Bach2 in TCDD-mediated suppression of B cell function.

B cell activation is initiated through ligation of surface receptors including the B cell antigen receptor (BCR), CD40, toll-like receptors (TLR) and a number of cytokine receptors. Ligation of these receptors in various combinations drives B cell proliferation followed by differentiation into antibody secreting plasma cells. When activated in a T cell-dependent manner *in vivo*, B cells receive three signals, the primary signal through engagement of BCR by the antigen, the second from activated T cells expressing CD40 ligand (CD40L) which engage B cells

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through CD40 and the third through T cell-derived cytokines which bind cytokine receptors on B cells (McHeyzer-Williams et al., 2000). CD40L, expressed on activated T cells, has been shown to play a critical role in T cell-dependent B cell responses (Bishop and Hostager, 2003). Ligation of constitutively expressed CD40 on B cells by CD40L upregulated on activated T cells initiates B cell signaling cascades involving molecules such as phosphoinositide 3-kinase (PI3K)/Akt and mitogen-activated protein kinases (i.e., c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK) and p38 kinase) (Bishop, 2004; Elgueta et al., 2009; van Kooten and Banchereau, 2000). The activation of these signaling molecules, among which extensive cross-talk is commonly observed, culminate into an "activated" phenotype of B cells characteristically identified by increased expression of surface molecules including CD69, CD80 (B7.1), CD86 (B7.2), MHC II, and intercellular adhesion molecule-1 (ICAM-1) (Bishop and Hostager, 2001). In addition to CD40L, activated T cells also secrete cytokines including interleukin-2 (IL-2), IL-4, IL-5, IL-6, IL-10, and IL-21 that further promote B cell activation, proliferation, and ultimately effector function (i.e., immunoglobulin (Ig) production) by triggering additional downstream signaling pathways that act in a coordinated manner with those activated by CD40 ligation.

CD40L, in combination with T cell cytokines, has been utilized to induce polyclonal activation and differentiation of primary human B cells *in vitro* (Arpin et al., 1995; Rousset et al., 1992). Most recently, a CD40L-dependent IgM response model was established to comparatively assess xenobiotic-mediated effects on human and mouse primary B cells (Lu et al., 2009). Using this model, TCDD was found to suppress the CD40L-induced IgM response in B cells derived from multiple human donors. Interestingly, the magnitude of suppression in those donors that responded to TCDD was comparable to observations made in parallel studies using B cells from C57BL/6 mice (Lu et al., 2010). The above results are consistent with epidemiological studies that reported a potential link between decreased serum Ig and exposure to TCDD or dioxin-like chemicals (Baccarelli et al., 2002; Kim et al., 2003; Lu and Wu, 1985), as well as experimental studies using human tonsillar B cells activated by T cell-independent stimuli, which demonstrated for the first time that TCDD can affect human B cell function (Wood et al., 1993; Wood and Holsapple, 1993). What remains largely unknown is the mechanism by which TCDD alters human and mouse B cells and are the mechanisms similar between the two species. The objective of the present study was to extend the previous investigations by determining the potential effects of TCDD on a series of events involved in human B cell activation and differentiation, two critical stages leading to antibody secretion. In particular, we demonstrate for the first time that TCDD profoundly attenuates the activation of human B cells, as evidenced by decreased expression of surface activation markers CD80, CD86, and CD69. Moreover, we also found the impairment of early signaling events by TCDD, which are critically involved in B cell activation.

Materials and methods

Chemicals and cell culture. TCDD (99.1% pure) was obtained from Accustandard (New Haven, CT) as a solution in dimethyl sulfoxide (DMSO). Isolated human or mouse B cells were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated bovine calf serum (Hyclone, Logan, UT), 100 U/ml of penicillin (Invitrogen), 100 μg/ml of streptomycin (Invitrogen), and 50 μM 2-mercaptoethanol. The stably-transfected mouse fibroblast line expressing human CD40 ligand (CD40L; CD40L-L cell) was a generous gift from Dr. David Sherr (Boston University School of Public Health). CD40L-L cells were maintained in Dulbecco's Modified Eagle Medium (Invitrogen) supplemented with 10% bovine calf serum (HyClone), 100 units/ml of penicillin, 100 μg/ml of streptomycin, 50 μM of 2-mercaptoethanol, and 1 x HT supplement (Invitrogen). In all cases cells were cultured at 37 °C in 5% CO_2.

Human leukocyte packs. Human leukocyte packs collected from anonymous donors were purchased from the Gulf Coast Regional Blood Center (Houston, TX). All donors were screened for human immunodeficiency virus and hepatitis at the blood center.

Mice. Virus-free, female C57BL/6 mice (6 weeks of age) were purchased from Charles River (Portage, MI). Mice were randomized, transferred to plastic cages containing sawdust bedding (five mice per cage), and quarantined for 1 week. Mice were provided food (Purina certified laboratory chow) and water *ad libitum* and were not used for experimentation until their body weight was 17–20 g. Animal holding rooms were kept at 21–24 °C and 40–60% humidity with a 12-h light/dark cycle. The Michigan State University Institutional Animal Care & Use Committee approved all experiments involving the use of animals.

Isolation of human and mouse B cells. Naive human B cells ($CD19^+CD27^-$) were isolated from peripheral blood mononuclear cells (PBMCs) enriched from each leukocyte pack by density gradient centrifugation using Ficoll-Paque Plus (GE Healthcare, Piscataway, NJ). Mouse B cells were isolated from spleens of female C57BL/6 mice, and were made into single-cell suspensions by passage through a 40 μm cell strainer (BD Biosciences, San Jose, CA). Negative selection of human or mouse B cells was conducted using MACS Naive human B cell, or Mouse B Cell Isolation Kits following the manufacturer's protocols (Miltenyi Biotec, Auburn, CA) and as described previously (Lu et al., 2009). In all cases, the purity of isolated B cells was ≥95%.

CD40L-dependent activation. The CD40L-dependent activation model was described previously (Lu et al., 2009). In brief, freshly isolated naive human or mouse B cells (1×10^6 cell/ml) were co-cultured with irradiated CD40L-L cells in the presence of 10 U/ml of recombinant human or mouse IL-2 (Roche Applied Science, Indianapolis, IN), 100 U/ml of IL-6 (human IL-6 from Roche Applied Science, mouse IL-6 from Jena Bioscience, Jena, Germany), and 20 ng/ml of recombinant human or mouse IL-10 (Bender MedSystems, Burlingame, CA) up to 4 days (human) or 3 days (mouse). For experiments that involved later time points, the CD40L stimulation was removed by transferring cells to new culture plates in the absence of CD40L-L cells on Day 4 (human) or Day 3 (mouse), and the cells were further cultured until harvested for analysis. For experiments that assessed the immediate activation/phosphorylation events, human B cells were equilibrated at 37 °C for 2–4 h, and then treated simultaneously with soluble recombinant human CD40L (200 ng/ml; Peprotech, Rocky Hill, NJ) in combination with recombinant human IL-2, IL-6, and IL-10 at concentrations described above for certain time periods. In all cases, cells were treated with TCDD (3, 10 or 30 nM) and/or vehicle (VH, DMSO) prior to stimulation with CD40L and cytokines. The selection of TCDD concentrations used in the present studies was based on prior investigation demonstrating TCDD-mediated suppression of IgM responses in mouse and human primary B cells (Lu et al., 2010).

Real-time PCR. Total RNA was isolated using the RNeasy Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. RNA was reverse-transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The expression of target genes was determined by TaqMan real-time PCR using ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). Relative steady state mRNA levels of the target genes were calculated and normalized to the endogenous reference, 18S ribosomal RNA using the $^{\Delta\Delta}$CT method (Farraj et al., 2004). All primers were purchased from Applied Biosystems: 18S (4319413E), human Blimp-1 (PRDM1) (Hs00153357_m1), mouse Blimp-1 (PRDM1) (Mm00476128_m1), human Pax5 (Hs00277134_m1), and mouse Pax5 (Mm00435501_m1).