Combination of Inflammatory Signaling and an AhR Ligand Leads to Synergistic Induction of IL-6

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Abstract

Research has increasingly shown connections between inflammatory immune responses and carcinogenesis and cancer progression. Evidence points to interleukin 6 (IL-6) differing in its effects by tumor type, in some cancers acting as a positive growth factor, increasing anti-apoptotic signaling, and increasing migration and invasion properties. We are investigating the combinatorial effects of IL-1β and Ah receptor ligands in tumor cell lines and their synergistic induction of IL-6 production. Co-treatment of MCF-7 breast cancer cells and ECC-1 endocervical cancer cells with IL-1β and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for 2 hours was shown via quantitative RT-PCR to result in a synergistic induction of *IL-6* mRNA transcription and a decrease in *CYP1A1* mRNA levels. Quantitative RT-PCR and ELISA assay revealed continuous increases in both mRNA and serum protein levels throughout a 72 hour time course of treatment, with no signs of abating. Additional AhR ligands, including benzo[a]pyrene, similarly show synergistic activity in conjunction with IL-1β, with regard to *IL-6* induction. Although the promoters for *IL-6* and *IL-8* have marked similarities in containing NFκB, C/EBPβ and c-Jun regulatory binding sites, *IL-8* transcription does not follow the pattern of synergistic increase seen with *IL-6*. Therefore, the combination of pro-inflammatory IL-1β and AhR ligands shows a context-specific ability to up-regulate cellular IL-6 output for a sustained period of time. This event could lead to instigation of both autocrine and paracrine loop signaling within the tumor microenvironment. These results would suggest a mechanism that could in part explain the tumor promoting properties of certain AhR ligands (e.g benzo[a]pyrene).

Introduction

The AHR has often been studied through its role in mediating the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), commonly referred to as dioxin. This persistent environmental contaminant bioaccumulates in the food chain and is essentially not metabolized by mammals. TCDD has been shown to be a carcinogen by way of AhR binding, which leads to heterodimerization with ARNT and transcriptional activation of drug metabolizing enzymes, such as CYP1A1 (1). TCDD has also been shown to be a potent tumor promoter in various models, though the precise mechanism(s) of AhR-mediated tumor promotion are largely unknown. The AhR is known to functionally interact with numerous transcription factor pathways, and various reports have demonstrated "cross-talk" between the immunologically relevant estrogen receptor (ER) or NF-κB transcription factors, and the AhR (2). Whether the Ah receptor can modulate expression of key NF-κB target genes such as IL-6 is poorly understood.

IL-6 is a pleiotropic cytokine "classically" involved in the acute phase response and lymphocyte differentiation and proliferation following inflammatory stimuli, as well as being found in various types of non-immune tissues. Additionally, IL-6 expression in tumor cells is known to elicit both pro- and anti-tumorigenic properties. Studies have shown both pro- and anti-proliferative effects of IL-6 on breast cancer cells lines, with the stage of carcinoma and estrogen receptor status playing a role. Supernatants taken from multidrug resistant breast carcinoma cell lines show markedly higher IL-6 levels than those of chemo-sensitive parental cells (3). Numerous clinical studies have shown that cervical cancer leads to elevated IL-6 levels and elevated IL-6 gene expression has been correlated with the presence of invasive cervical carcinomas (4). However, IL-6 expression in breast cancer has been implicated to correlate both positively and negatively with overall prognosis (3). Low IL-6 levels have been observed in certain established breast cancer cell lines, such as MCF-7 cells (5). The estrogen receptor has also been shown to repress IL-6 transcription, thus lending credence to notions of receptor cross-talk and a rationale for the higher IL-6 expression in invasive estrogen receptor negative breast cancer cell lines. For the first time, we show that an AhR ligand can synergize with cytokines to enhance Il-6 production in some tumor cell lines

Figure 1.

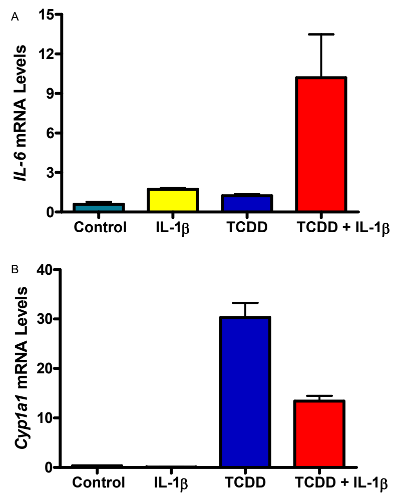


Fig 1. Co-treatment with TCDD and IL-1β results in synergistic *IL-6* transcription, while IL-1β decreases *Cyp1A1* transcription in MCF-7 cancer cells. A & B, MCF-7 cells were plated in 6-well dishes, serum starved for 24 h, and treated for 2 h with DMSO vehicle, 10 ng/ml IL-1β, 1 nM TCDD, or TCDD and IL-1β. *IL-6* and *Cyp1a1* mRNA levels were determined by quantitative real-time PCR.

Figure 2.

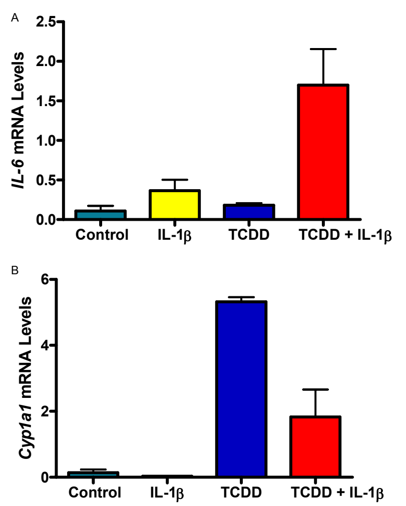


Fig 2. *Cyp1a1* and *IL-6* regulation by combinatorial TCDD and IL-1β treatment is also evident in the ECC-1 endocervical cancer cell line. A & B, ECC-1 cells were plated in 6-well dishes, serum starved for 24 h, and treated for 2 h with DMSO vehicle, 10 ng/ml IL-1β, 1 nM TCDD, or TCDD and IL-1β. *IL-6* and *Cyp1a1* mRNA levels were determined by quantitative real-time PCR.

Figure 3.

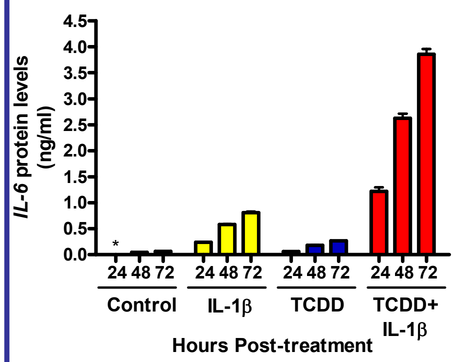


Fig 3. IL-6 protein levels are equivalently raised by combinatorial TCDD and IL-1β treatment for 72 h. MCF-7 cells were plated in 6-well dishes, serum starved for 24 h, and treated with DMSO vehicle, 10 ng/mL IL-1β, 1 nM TCDD, or TCDD and IL-1β for 24, 48, and 72 h. At each time point, media was collected. IL-6 protein levels in media were determined by ELISA

Figure 4.

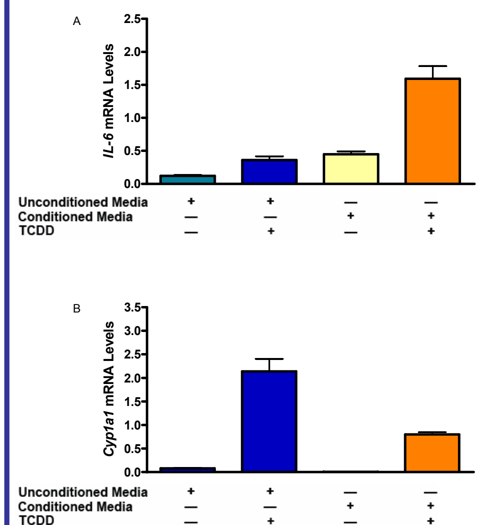


Fig 4. Synergistic induction can be obtained via exposure to differentiated monocyte conditioned media. MCF-7 cells were plated in 6-well dishes and serum-starved for 24 h after which half of the media was replaced with unconditioned or conditioned THP-1 media. Cells were immediately treated with DMSO, 10 ng/ml IL-1β, 1 nM TCDD, or TCDD and IL-1β for 2 h. Levels of *Cyp1a1* and *IL-6* mRNA were determined by quantitative real-time PCR.

Figure 5.

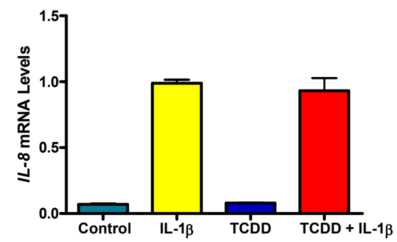
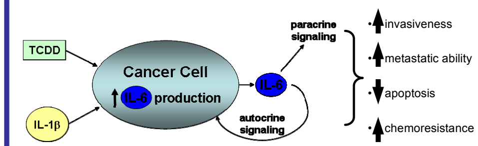


Fig 5. *IL-8* transcription, while being regulated similarly to *IL-6*, is not increased by TCDD and IL-1β co-treatment, thus showing the context-specificity of the synergy. MCF-7 cells were plated in 6-well dishes, serum starved for 24 h, and treated for 2 h with DMSO vehicle, 10 ng/ml IL-1β, 1 nM TCDD, or TCDD and IL-1β. *IL-8* mRNA levels were determined by quantitative real-time PCR.

Conclusions

- Co-treatment of breast cancer and endocervical cancer cell lines with TCDD and IL-1β results in synergistic increases of *IL-6* transcription and lowered *Cyp1A1* transcription
- The synergistic increase in *IL-6* transcription is comparable to an increase in protein secretion, continuing through 72 h
- Conditioned media from activated monocytes is sufficient to result in synergistic *IL-6* induction with TCDD, providing evidence of the possibility of this effect occurring within a tumor microenvironment
- *IL-8*, while regulated similarly to *IL-6*, is not transcriptionally affected by the addition of an AhR ligand

Implication Scheme | Potential Outcomes



Acknowledgements

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