ABSTRACT

MECHANISMS UNDERLYING 2,3,7,8-TETRACHLORODIBENZO-P-DIOXINMEDIATED SUPPRESSION OF B CELL ACTIVATION AND DIFFERENTIATION

By

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Exposure to the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is known to alter B cell function, resulting in marked suppression of the primary immune response. The immunotoxic effects of TCDD involve transcriptional regulation through the aryl hydrocarbon receptor (AHR) but the exact molecular mechanisms are still unknown. To identify novel genes directly modulated by the ligand-activated AHR during B cell differentiation, a genome-wide study was performed in mouse B cells through which Bach2; a direct target of AHR was identified. Bach2 is known to repress expression of Blimp-1, a master regulator of B cell differentiation by binding to Maf elements (MAREs) in the regulatory regions of the gene. Electrophoretic mobility shift assays confirmed the binding of AHR to intron1 of Bach2. TCDD induced expression of Bach2 and decreased expression of Blimp-1 in B cells. Increased binding of Bach2 was observed in presence of TCDD to the intron 5 MARE in the Blimp-1 gene. These studies suggest transcriptional regulation of Bach2 by AHR as one of the mechanisms involved in suppression of B cell differentiation by TCDD.

B cell differentiation can also be affected by the strength of B cell activation, a process initiated upon ligation of the CD40 receptor and by signaling through cytokines IL-2, IL-6 and IL-10. In a previous study, it was shown that TCDD markedly affected B cell activation by decreasing the expression of B cell activation markers CD80, CD86

and CD69. Hence, the second part of this study investigated the mechanisms underlying suppression of human B cell activation by TCDD. BCL-6 was identified as a likely candidate owing to its role as a transcriptional repressor of B cell activation and differentiation. In the presence of TCDD, BCL-6 protein levels were elevated in human B cells in an AHR-dependent manner. A decrease in B cell activation was also evident through the attenuation of surface CD80 and CD69. BCL-6 repressed CD80 in presence of TCDD by binding to the enhancer region of CD80. Moreover, the suppressed activation marker expression was reversed by treatment of cells with a specific BCL-6 inhibitor thus suggesting a role for BCL-6 in decreasing B cell activation in presence of TCDD. Part of the mechanism underlying TCDD-mediated suppression of B cell activation also involves SHP-1, a protein tyrosine phosphatase inhibiting signaling in activated B cells which was identified through the same genome-wide analysis of AHR binding in presence of TCDD. SHP-1 mRNA and protein levels were elevated in presence of TCDD. An increase in SHP-1+ BCL-6+ cells was observed upon TCDDtreatment thereby suggesting cross talk between SHP-1 and BCL-6 pathways. Addition of SHP-1 inhibitor to naïve B cells affected BCL-6 protein levels suggesting possible regulation of BCL-6 by SHP-1 for the first time.

Taken together, the results of this investigation suggest that a) TCDD: AHRmediated inhibition of B cell activation occurs through de-regulation of BCL-6 and SHP1 and that b) the inhibition of B cell differentiation occurs through elevated Bach2 levels in B cells. These studies contribute to the field of TCDD immunotoxicity by presenting novel insights into the mechanisms by which TCDD affects B cell activation and effector function.