TOXICOLOGICAL SCIENCES **120(S1)**, S171–S191 (2011)
doi:10.1093/toxsci/kfq324
Advance Access publication October 15, 2010

The Long Winding Road toward Understanding the Molecular Mechanisms for B-Cell Suppression by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

Courtney E.W. Sulentic* and Norbert E. Kaminski†,‡,[1]

*Department of Pharmacology & Toxicology, Boonshoft School of Medicine, Wright State University, Dayton, Ohio 45435; †Department of Pharmacology and Toxicology, and ‡Center for Integrative Toxicology, Michigan State University, East Lansing, Michigan 48824

[1]To whom correspondence should be addressed at Center for Integrative Toxicology, 315 National Food Safety and Toxicology Building, East Lansing, MI 48824-1317. Fax: (517) 432-3218. E-mail: kamins11@msu.edu.

Received August 25, 2010; accepted October 11, 2010

Suppression of humoral immune responses by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was first reported in the mid-1970s. Since this initial observation, much effort has been devoted by many laboratories toward elucidation of the cellular and molecular mechanisms responsible for the profound impairment of humoral immune responses by TCDD, which is characterized by decreased B cell to plasma cell differentiation and suppression of immunoglobulin production. These efforts have led to a significant body of research demonstrating a direct effect of TCDD on B-cell maturation and function as well as a requisite but as yet undefined role of the aryl hydrocarbon receptor (AhR) in these effects. Likewise, a number of molecular targets putatively involved in mediating B-cell dysfunction by TCDD, and other AhR ligands, have been identified. However, our current understanding has primarily relied on findings from mouse models, and the translation of this knowledge to effects on human B cells and humoral immunity in humans is less clear. Therefore, a current challenge is to determine how TCDD and the AhR affect human B cells. Efforts have been made in this direction but continued progress in developing adequate human models is needed. An in-depth discussion of these advances and limitations in elucidating the cellular and molecular mechanisms putatively involved in the suppression of B-cell function by TCDD as well as the implications on human diseases associated in epidemiological studies with exposure to TCDD and dioxin-like compounds is the primary focus of this review.

Key Words: **2,3,7,8-tetrachlorodibenzo-p-dioxin; B cell; humoral immunity; immune suppression; AhR.**

Halogenated aromatic hydrocarbons such as the polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls are persistent environmental toxicants. The most potent of this class of chemicals is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which is produced as a by-product during a variety of industrial and municipal processes, many of which involve the combustion of organic materials in the presence of chlorine. TCDD has generated much public concern due to several highly publicized incidents of human and wildlife exposure (reviewed by White and Birnbaum, 2009). Additionally, the sensitivity of rodent models to TCDD-induced toxicity has fueled concern regarding the potential health risks to humans. TCDD produces a broad range of biological effects in animal and cellular models including death, a generalized wasting syndrome, lymphoid involution (especially of the thymus), hepatotoxicity, cardiotoxicity, teratogenicity, developmental toxicity, carcinogenesis, neurotoxicity, immunotoxicity, and various biochemical effects (reviewed by Birnbaum and Tuomisto, 2000).

Alterations in immune function, primarily immune suppression, have been observed in virtually every species studied and occurs at doses that do not produce obvious signs of toxicity *in vivo* and at noncytotoxic concentrations *in vitro* (Holsapple *et al.*, 1991b; Kerkvliet, 2002). These alterations include suppression of innate, humoral, and cell-mediated immunity. The immune suppression produced by TCDD is mediated through alterations in the function of various immune cell types, including B cells, which is the focus of this review (Holsapple *et al.*, 1991a; Kerkvliet, 2002). B cells are essential to antibody-mediated clearance of pathogens (i.e., antigens) as they are the only immune cells that express immunoglobulin (Ig) and that secrete antibodies (i.e., soluble Ig). The effects of TCDD on humoral immunity have been extensively studied, especially the effects on primary antibody responses, which are highly sensitive to suppression by TCDD. The biological processes that regulate humoral immune responses are complex and, depending on the antigenic stimulus, can involve cell types other than B cells, which contribute in an accessory manner through cell-cell interactions with B cells and via the production of

© The Author 2010. Published by Oxford University Press on behalf of the Society of Toxicology. All rights reserved.
For permissions, please email: journals.permissions@oup.com

soluble regulatory mediators. These accessory cells and mediators direct B cells through the stages of activation, proliferation, and ultimately lead to differentiation into plasma cell effectors.

Since the mid-1970's, when suppression of humoral immune responses by TCDD was first reported, much effort has been devoted by many laboratories to identify the cellular and molecular mechanisms responsible for the profound impairment that is observed on humoral immune responses and is characterized by suppression of IgM and IgG secretion and decreased B-cell differentiation into Ig-secreting cells (i.e., antibody-forming cells [AFCs] or plasma cells). The objective of this review is to discuss, within the context of an extensive peer-reviewed literature, the cellular and molecular mechanisms putatively involved in the suppression of B-cell function by TCDD as well as its implications on human diseases associated in epidemiological studies with exposure to TCDD and dioxin-like compounds.

EVIDENCE FOR DIRECT EFFECTS OF TCDD ON B-CELL FUNCTION

Some of the earliest reports documenting marked suppression of humoral immune responses after TCDD treatment of laboratory animals, principally mice, occurred during the 1970's and early 1980's (Clark *et al*., 1981; Holsapple *et al*., 1986a; Vecchi *et al*., 1980a,b, 1983; Vos *et al*., 1973). Initially, much effort was devoted toward identifying the specific leukocyte subtype(s) directly targeted by TCDD, which were responsible for suppression of the IgM response. Studies employing defined antigens requiring differential cellular cooperativity to induce an IgM response were used in identifying which cell types were responsible for the suppression of humoral immunity by TCDD. Treatment of mice with TCDD and *in vivo* sensitization with T cell–dependent antigens (e.g., sheep erythrocytes [sRBC] or ovalbumin), T cell–independent antigens (e.g., dinitrophenyl [DNP]-Ficoll or trinitrophenyl [TNP]-lipopolysaccharide [LPS]), or polyclonal B-cell activators (e.g., LPS or anti-Ig), all showed comparable suppression of *in vivo* IgM AFC responses (Dooley and Holsapple, 1988; Tucker *et al*., 1986). Likewise, direct addition of TCDD to naive spleen cell cultures followed by *in vitro* sensitization with either sRBC or LPS similarly showed marked and concentration-dependent suppression of the IgM response (Dooley and Holsapple, 1988; Tucker *et al*., 1986). Extension of these experiments employing splenocytes isolated from TCDD-treated mice and *ex vivo* sensitization affirmed suppression of the IgM response with the magnitude of suppression being strikingly similar regardless of the B-cell activator, sRBC, DNP-Ficoll, or LPS, and the inherent accessory cell requirements (Dooley and Holsapple, 1988; Tucker *et al*., 1986). Collectively, at least two important conclusions could be drawn from these studies. First, they demonstrated the direct interactions of the chemical with leukocytes because direct addition of TCDD to naive splenocyte culture recapitulated the suppression of primary humoral responses observed *in vivo*; hence, ruling out the involvement of an unknown non–leukocyte-derived immunoregulatory factor as a possible contributory mechanism. Second, these results strongly suggested that in the case of TCDD-mediated IgM suppression, the primarily effect was on B-cell function and not via disruption of accessory cell function because the magnitude of suppression of the IgM response was comparable regardless of the antigenic stimulus used to drive B cells to IgM AFC.

Importantly, because the above studies utilized heterogeneous splenocyte preparations, they did not rule out the possibility that suppression of the IgM response by TCDD was due, at least in part, to "by-stander" effects by leukocytes other than B cells. This point is especially critical in light of the numerous regulatory factors produced by leukocytes as well as the cell-cell interactions that occur between different immunocompetent cell types that can promote or dampen immune responses. To address these and other caveats associated with experiments utilizing heterogeneous leukocyte preparations, cell fractionation studies were conducted in which splenocytes were isolated from either TCDD- or vehicle-treated mice and fractionated into adherent populations (macrophages) and nonadherent populations (B and T cells) followed by *in vitro* reconstitution in various combinations and antigen sensitization. These types of experiments initially ruled out the involvement of adherent cells, principally cells of the monocytic lineages (i.e., macrophages and dendritic cells), as being functionally altered by TCDD at least in the context of participating in antibody responses and instead associated the nonadherent cellular fraction, primarily B and T cells as the affected cellular population(s) (Dooley and Holsapple, 1988). The cell fractionation experiments were further extended to examine B and T cells separately and showed that only those experimental groups reconstituted with B cells from TCDD-treated mice exhibited suppression of the IgM response (Dooley and Holsapple, 1988). In addition, purified splenic B cells from TCDD-treated mice exhibited marked suppression of the IgM response when activated *in vitro* with LPS (Dooley and Holsapple, 1988). These studies further supported the concept that B cells are the principal cell type targeted by TCDD in the suppression of IgM responses.

A number of laboratories also examined the direct effects of TCDD *in vitro* on the IgM response using purified splenic B cells isolated from naive mice. In studies by Luster *et al*., direct addition of TCDD to purified splenic B cells that were activated with either TNP-LPS or with anti-Ig plus B-cell growth factors and T-cell replacement factor strongly suppressed the IgM AFC response (Luster *et al*., 1988). In this same study, no suppression of the LPS-induced IgM AFC response by direct addition of TCDD at concentrations as high as 100nM was observed, which was in contrast to similar studies by Holsapple *et al*. (1986a), who showed an IC_{50} concentration of 15nM TCDD for suppression of the LPS-induced IgM AFC response. The primary difference between these two studies was that Luster *et al*. (1988) used purified

B cells which were activated with LPS derived from *Escherichia coli*, whereas Holsapple *et al.* (1986a) used unfractionated splenocytes and LPS derived from *Salmonella typhosa*. Interestingly, Morris *et al.* (1993) purified splenic B cells into high-density B cells, which they termed as being predominantly "resting B cells" in G_0, and low-density B cells, which they termed as being an "activated" population of B cells in G_1 of the cell cycle. These experiments showed that the low-density B cells when stimulated with LPS (*S. typhosa*) were sensitive to suppression by TCDD, as measured by secreted IgM, further suggesting a direct effect on B cells. In these same studies, the high-density B-cell population was found to be refractory to the effects of TCDD (Morris *et al.*, 1993) leading the authors to speculate that B cells were most sensitive to suppression by TCDD when in G_1 of the cell cycle. More recent studies with B-cell lines, such as the murine line, CH12.LX, have confirmed that suppression of the LPS-induced IgM response is through the direct action of TCDD on B cells (Sulentic *et al.*, 2000). Specifically, CH12.LX cells can be induced by LPS to secrete increased levels of IgM in the absence of accessory cells or additional growth factors, and direct addition of TCDD to cultured CH12.LX cells produces a marked suppression of IgM secretion.

Several studies have also investigated whether B cells exhibit greater sensitivity as they progress toward fully differentiated plasma cells. For example, *in vitro* time of addition studies uncovered the temporal effects of TCDD by identifying a critical period of time post-antigen sensitization during which TCDD must be present to suppress antibody responses. In one study, the greatest magnitude of suppression of the anti-sRBC IgM response was achieved if TCDD was added to spleen cell cultures either 60 min prior to antigen sensitization or at the time of antigen sensitization. Addition of TCDD on day 1 post-antigen sensitization resulted in a significant decrease in the magnitude of the TCDD-mediated suppression and addition of TCDD on days 2 through 5 post-antigen sensitization produced no significant suppression of the IgM AFC response (Tucker *et al.*, 1986). Unfortunately, time points between antigen sensitization and day 1 post-sensitization were not included in this study. In a second study using spleen cell cultures activated with LPS, suppression of the IgM AFC response could only be produced if TCDD was added to splenocytes prior to 3 h post-activation (Holsapple *et al.*, 1986a). Taken together, these data suggest that TCDD treatment interferes with an early B-cell activation event, impeding B-cell differentiation into plasma cells capable of productive IgM secretion. However, it is also noteworthy that from studies with a variety of well-characterized immunotoxicants, lymphocytes often exhibit a trend of becoming increasingly refractory to suppression of their respective effector functions as they progress toward terminal differentiation. Nevertheless, the narrow window of sensitivity for suppression by TCDD of the IgM AFC response in activated B cells strongly suggests that TCDD must alter critical events during B-cell activation.

Evidence supporting the B cell as a direct target of TCDD-mediated suppression of the antibody response

- Defined antigens requiring differential cellular cooperativity to induce an IgM response supported the B cell as a direct target of TCDD.
- Analysis of fractionated splenic leukocyte populations from TCDD-treated mice identified the B cell as the cell type principally affected.
- TCDD-mediated suppression of IgM responses has been demonstrated in purified splenic B cells.
- Suppression of the IgM response by TCDD has been demonstrated in AhR-expressing B-cell lines in the absence of other accessory cell types.

ROLE OF THE AhR IN B-CELL DYSFUNCTION

Most biological effects by TCDD are thought to involve the aryl hydrocarbon receptor (AhR). The AhR and its dimerization partner AhR nuclear translocator (ARNT) regulate transcription by binding dioxin response elements (DRE) or xenobiotic response elements in regulatory regions of dioxin-sensitive genes (for review, see Okey, 2007; Swanson and Bradfield, 1993). The most well-studied AhR-regulated genes are metabolic enzymes whose upregulation by xenobiotics, such as planar aromatic hydrocarbons, which bind and activate the AhR, led to the initial theory that the AhR signaling pathway evolved to mediate clearance of xenobiotics through metabolism. However, the wide variety of toxic responses induced by TCDD, the most potent AhR ligand that is also resistant to metabolism, and the development of AhR-null mice has provided considerable evidence for a role of the AhR signaling pathway in physiological processes (Birnbaum and Tuomisto, 2000; Fernandez-Salguero *et al.*, 1995, 1997; McMillan and Bradfield, 2007; Mimura *et al.*, 1997; Schmidt *et al.*, 1996). Nevertheless, the exact mechanisms by which the AhR mediates B-cell dysfunction remain poorly understood. Several studies initially suggested a crucial role for the AhR in the suppression of B-cell differentiation and antibody secretion by TCDD (Davis and Safe, 1988; Kerkvliet *et al.*, 1990; Luster *et al.*, 1988; Tucker *et al.*, 1986; Vecchi *et al.*, 1983). These studies utilized a mixed cell population and either congenic mouse strains that expressed either a low (AhR^d)- or high

Evidence supporting a requisite role for the AhR in TCDD-mediated suppression of antibody responses

- Mice possessing the gene that encodes one of the Ahr^b-alleles of the AhR (e.g., C57Bl/6) are more sensitive to suppression of humoral immune responses by TCDD than mice expressing the Ahr^d-allele of the AhR (DBA/2).
- Mice congenic at the *Ahr* locus demonstrated that expression of the Ahr^{b-1} allele resulted in greater sensitivity to suppression of anti-sRBC IgM AFC response by TCDD than expression of the Ahr^d allele.
- TCDD treatment does not alter IgM responses in mice null for the AhR.
- The murine CH12.LX B-cell line, which expresses high levels of AhR, exhibits remarkable sensitivity to TCDD-mediated suppression of the IgM response; whereas the murine BCL-1 cell line, which does not express the AhR, is insensitive to TCDD-mediated antibody suppression.
- TCDD-induced changes in LSK HSC and B-cell subpopulations is dependent on the AhR.

(AhR^b)- affinity form of the AhR or structure-activity relationship studies with polychlorinated dibenzofuran congeners and therefore could not determine if the AhR was directly or indirectly mediating the effects of TCDD on B-cell function. Later studies identified functional AhR and ARNT in mouse splenocytes (Williams *et al*., 1996) and primary mouse B cells (Marcus *et al*., 1998) confirming the presence of a functional AhR signaling pathway in B cells. Additional support for an AhR-mediated mechanism in the effects of TCDD on antibody secretion was obtained from an *in vitro* B-cell model consisting of two surface Ig^+ (mature) B-cell lines, the CH12.LX and the BCL-1, which differ in their expression of the AhR and in their sensitivity to TCDD. Both cell lines can be induced by LPS to secrete enhanced levels of antibody. Similar to primary B cells (Crawford *et al*., 1997; Marcus *et al*., 1998), TCDD treatment of LPS-stimulated CH12.LX cells resulted in a sensitive and marked suppression of both IgM secretion (Sulentic *et al*., 1998) and the number of IgM-secreting cells (Crawford *et al*., 2003). In contrast, LPS-induced IgM secretion from the AhR-deficient BCL-1 cells was refractory to TCDD (Sulentic *et al*., 1998). Later studies using AhR-null mice demonstrated a similar and essential role for the AhR in TCDD-induced suppression of the IgM AFC response (Vorderstrasse *et al*., 2001). In subsequent sections of this review, the putative role of the AhR and its ability to modulate B-cell function will be discussed in greater detail.

EFFECTS OF TCDD ON B-CELL MATURATION

Although investigated by only a few laboratories, there is evidence supporting TCDD-mediated alterations on different stages of the B-cell maturation process including the pluripotent hematopoietic stem cells (HSC) of the bone marrow. The HSC-enriched $Lin^-/Sca\text{-}1^+/cKit^+$ (LSK) cell population was elevated by 24 h after *in vivo* exposure of mice to TCDD and remained elevated through day 31 post-TCDD treatment (Murante and Gasiewicz, 2000). Consistent with these findings, Sakai *et al.* (2003) demonstrated an increase in the HSC-enriched $CD34^-$ LSK cell population following *in vivo* exposure of mice to TCDD and extended these results by demonstrating that the long-term reconstitution activity of these cells was impaired by TCDD. These effects appear to be dependent on the AhR because TCDD had no effect on these endpoints in AhR-null mice (Sakai *et al*., 2003). Recent studies demonstrated a similar increase in LSK that was AhR dependent. The increase in LSK was likely due to increased cell proliferation and an increased number of LSK cells in S phase, which exhibited diminished short-term (6 weeks) and long-term (40 weeks) reconstitution activity (Singh *et al*., 2009b). As relates to B-cell maturation, the number of functional colony-forming unit (CFU)-preB progenitors decreased over time (days 5 through 9 post-TCDD treatment), whereas CFU-granulocyte, CFU-macrophage, and CFU-granulocyte-macrophage increased. The authors concluded that TCDD increases multipotent progenitors (i.e., LSK) with reduced ability to differentiate into the lymphoid lineage (Singh *et al*., 2009b). Additionally, the effects on LSK proliferation appears to be limited to immature HSC (i.e., LSK) because TCDD did not directly affect BrdU incorporation in lineage-specific cell populations and may reflect a decrease or loss in sensitivity as maturity increases (Singh *et al*., 2009b). Notably, AhR and ARNT messenger RNA (mRNA) and protein expression was also evaluated in bone marrow subsets ($Lin^-/Sca\text{-}1^+$, $Lin^-/Sca\text{-}1^-$, LSK, and $B220^+$) and demonstrated AhR and ARNT expression in all of the subsets including the $B220^+$ B-cell subset. In contrast, Yamaguchi *et al*. (1997) did not detect the AhR in bone marrow $B220^+$ B cells or in an early pre-B-cell line, but as in the above study, functional AhR was detected in bone marrow stromal cell lines (Lavin *et al*., 1998; Yamaguchi *et al*., 1997) and as mentioned earlier in splenic B cells (Marcus *et al*., 1998). Taken together, these results support an AhR-dependent effect by TCDD on early progenitor cell function. In-depth reviews on the influence of the AhR in progenitor cell function and response to xenobiotics have been recently published (Hirabayashi and Inoue, 2009; Singh *et al*., 2009a).

Clearly, an effect on early LSK progenitor cells may be a major contributor to the decrease in the $B220^+$ subset in bone marrow following TCDD treatment (Thurmond and Gasiewicz, 2000; Thurmond *et al*., 2000). Analysis of B-cell maturation subsets demonstrated a decreased percentage of pro/pre-B cells within the total $B220^+$ population of bone marrow cells and a decrease in the absolute number of pro/pre-B and immature B cells at days 6, 9, and 10 post-TCDD treatment, all of which returned to control levels by 31 days post-treatment (Thurmond and Gasiewicz, 2000; Thurmond *et al*., 2000). This effect correlated well with the reduced mRNA levels for terminal deoxynucleotidyl transferase (TdT) and recombinase activation gene-1 (RAG-1) in lymphocyte stem cells (Fine *et al*., 1989; Frazier *et al*., 1994). Because TdT and RAG are more highly expressed in pro- and pre-B-cell subsets (Hardy *et al*., 1991), Thurmond *et al*. concluded that the decrease in TdT and RAG-1 was likely an indirect effect due to decreased pro/pre-B-cell numbers and altered LSK differentiation (Thurmond and Gasiewicz, 2000). The effect of TCDD on mature B-cell subpopulations in the bone marrow appears to be somewhat conflicting because the same group demonstrated no effect on mature B-cell numbers in one study (day 10 post-TCDD treatment) (Thurmond *et al*., 2000) and in another study demonstrated a significant but reversible increase of the mature B-cell population by 24 h which was time- and dose-dependent and gradually decreased below the vehicle control by day 9, returning to control levels on day 31 post-TCDD treatment (Thurmond and Gasiewicz, 2000). The reason for this discrepancy is unclear and may relate to differences between days 9 and 10. Interestingly, TCDD treatment induced a temporal increase in B-lymphocyte progenitor ($B220^+$/

CD24-/AA4.1$^+$) cells, which was detected by 24 h, became maximum by day 9 and returned to normal on day 31 post-treatment (Thurmond and Gasiewicz, 2000).

An analysis of B-cell maturation subsets in the context of AhR expression exhibited a significantly higher total B220$^+$ population of cells in bone marrow of AhR-null mice as compared with AhR$^{+/+}$ mice, and this appears to be due to an increase in the number of pro/pre-B cells and immature B cells with no effect on the total number of mature B cells (Thurmond *et al*., 2000). Whereas TCDD decreased the number of pro/pre-B cells and immature B cells, which was shown to be dependent on the AhR. Therefore, the AhR appears to significantly regulate the B-cell maturation process, particularly the pro/pre-B- and immature B-cell subpopulations (Thurmond *et al*., 2000). AhR expression in both nonhematopoietic and hematopoietic compartments appears to be required for both normal levels of and TCDD-mediated effects on B-cell subsets as evidenced by hematopoietic chimeric studies (Thurmond *et al*., 2000). Analysis of splenic B cells in AhR-null mice demonstrated slightly decreased B-cell numbers in 10-week-old mice and a more marked decrease in 7-month-old mice (Fernandez-Salguero *et al*., 1995, 1997). This is in agreement with a proposed developmental arrest of the bone marrow mature B-cell subpopulation (B220^{hi}/IgM$^+$) and impairment of B cell release into the periphery (Thurmond and Gasiewicz, 2000; Thurmond *et al*., 2000). However, a different AhR-null derived mouse model did not demonstrate decreased B220/IgM$^+$ B cells in the spleen (Schmidt *et al*., 1996). Because the above B-cell maturation studies were conducted with the AhR-null mice exhibiting decreased splenic B220$^+$ B cells, it is unclear if both AhR-null mouse models would exhibit similar changes in B-cell subsets compared with AhR$^{+/+}$ mice. To further understand the biological role of the AhR, Andersson *et al.* (2003) generated mice that expressed a constitutively active AhR (CA-AhR). Analysis of the B-cell subpopulations in the bone marrow and spleen from CA-AhR mice demonstrated an increase in the mature B-cell population compared with wild-type AhR$^{+/+}$ mice (Andersson *et al*., 2003). This was consistent with the increase in mature B cells in the bone marrow of wild-type mice treated with TCDD (Thurmond and Gasiewicz, 2000; Thurmond *et al*., 2000). Conversely, there was very little effect on the pro/pre-B-cell subpopulations in CA-AhR mice (Andersson *et al*., 2003) as opposed to the decrease seen in wild-type mice treated with TCDD (Thurmond and Gasiewicz, 2000; Thurmond *et al*., 2000). Probably, the most significant effect of CA-AhR expression was a marked decrease in the CD5$^+$ B1 cells. B1 cells are primarily located in peritoneal and pleural cavities and originate in the fetal liver and omentum instead of the bone marrow where conventional B (B2) cells are derived. B1 cells unlike B2 cells are self-renewing and spontaneously secrete high levels of antibodies (mostly IgM) with limited antigen specificity and are believed to play an important role in innate immunity.

The mechanism by which AhR may modulate an effect on B-cell maturation has been postulated to involve a number of proteins, including NF-κB, paired box protein-5 (Pax-5), and CD19 (Andersson *et al*., 2003; Thurmond and Gasiewicz, 2000; Thurmond *et al*., 2000). All of which are important regulators of B-cell maturation. NF-κB/Rel proteins are significant regulators of B-cell maturation and differentiation processes. Additionally, several studies, recently summarized in two comprehensive reviews (Tian, 2009; Vogel and Matsumura, 2009), have established a complex interaction between the AhR and NF-κB/Rel signaling pathways, which may contribute to the effects of TCDD on B-cell maturation. Pax-5 is essential for B-cell development and may also be a mechanism for the effects of TCDD on B-cell maturation and differentiation (see below discussion). Interestingly, Masten and Shiverick (1995) have identified a DRE core motif within a Pax-5–binding site that is located in the CD19 promoter region. CD19 is a signal transducing protein that is expressed through the early stages of B-cell development but is lost upon B-cell differentiation to a plasma cell (Tedder *et al*., 1994). Masten and Shiverick (1995) have also demonstrated a TCDD-induced suppression of CD19 mRNA expression in a human B-cell line. They proposed that decreased CD19 is a result of the AhR nuclear complex interfering with the binding of Pax-5 to a common DNA-binding site in the CD19 promoter region. Notably, this interaction between Pax-5 and the AhR is limited to the human CD19 promoter and to a moderate affinity Pax-5 site, which may not play a prominent role in CD19 expression (Kozmik *et al*., 1992); however, nucleotide analysis has identified a DRE-like site within the transcription initiation region of both the mouse and human CD19 genes. It remains to be determined whether these DRE sites mediate an effect on CD19 gene expression. Furthermore, AhR agonists including TCDD inhibited basal and LPS-induced interleukin (IL)-6 expression by a bone marrow stromal cell line perhaps in an NF-κB–dependent manner (Jensen *et al*., 2003). IL-6 has many functions including effects specific to progenitor cells and the maintenance of B-cell progenitors (Kishimoto, 2006; Ogawa, 1993). Therefore, a decrease in IL-6 may contribute to the effects of TCDD on the maturation of B-cell subsets. Additionally, a recent study by Dinatale *et al.* (2010) demonstrated AhR binding to DRE-like sites upstream of the IL-6 promoter, supporting an AhR-mediated mechanism; however, unlike the previous study, TCDD and IL-1β cotreatment produced a synergistic induction of IL-6 transcription in MCF-7 cells. The significance of these findings within the context of B-cell maturation remains unclear.

EFFECTS OF TCDD ON B-CELL ACTIVATION

A number of studies have explored the effects of TCDD treatment on cell activation events in conjunction with induction of antibody responses using heterogeneous leukocyte

preparation as well as purified B cells. Kramer *et al.* (1987) showed that *in vitro* TCDD treatment of naive murine splenocytes in the absence of antigen stimulation induced a threefold increase in the background number of IgM AFC, which occurred in the absence of increased cell proliferation and was accompanied by an increase in non–protein kinase C (PKC) associated basal kinase activity. TCDD treatment of these naive splenocytes led to the phosphorylation of a number of unknown low- to mid-ranged molecular weight proteins from 12 to 63 kDa within 30 min, with some increases in phosphorylation occurring as rapidly as 10 min. In a similar study, direct addition of TCDD (10nM) to purified mouse splenic B cells also induced phosphorylation of membrane-associated proteins spanning a molecular weight range from 17 to 105 kDa as measured at 140 min post-TCDD treatment (Clark *et al.*, 1991). Treatment of cells with TCDD in combination with anti-Ig plus B-cell growth factors appeared to further enhance the magnitude of phosphorylation of several of these proteins. As in the Kramer *et al.* (1987) study, TCDD-induced protein phosphorylation was found to occur independently of PKC activation. Moreover, increased phosphorylation was observed of tyrosine containing control peptides added to cell lysates isolated from TCDD-treated cells when compared with cell lysates from untreated cells, suggesting that the TCDD-induced kinase activity was likely due to the activation of one or more tyrosine kinases. The above results in mouse leukocyte preparations are contrasted against similar studies performed in the mouse hepatocyte line, hepa1c1c7, in which TCDD induced a rapid activation of PKC (within 10 min) followed by increased levels of fos and jun family member proteins and activator protein 1 (AP-1) DNA–binding activity (Puga *et al.*, 1992). Importantly, TCDD-mediated PKC activation was preceded by a rapid (within 2 min) and robust increase in intracellular calcium concentrations. By contrast, TCDD treatment of resting mouse splenic B cells only modestly induced the basal level of intracellular calcium as measured at 18 h post-TCDD with no change in intracellular calcium at 60 min (Karras *et al.*, 1996). B cells treated with TCDD (5nM) and activated with anti-Ig, exhibited a modest twofold increase in intracellular calcium compared with anti-Ig plus vehicle. The TCDD-induced phosphorylation of cellular proteins in leukocytes and hepatocytes, especially those occurring within 10 min of TCDD treatment, as well as the rapid increase in intracellular calcium in hepatocytes are important as they are indicative of biological events that occur independently of AhR-mediated nuclear translocation and changes in gene transcription while potentially still involving the AhR. In addition, the differences in the profile of activity between hepatocytes and leukocytes in the signaling events affected by TCDD are indicative of cell type–specific effects.

More recently, the effect of TCDD on the amount of active (i.e., phosphorylated) ERK, AKT, and JNK was assessed simultaneously in individual resting and activated mouse B cells by flow cytometry in a multiparametric analysis (North *et al.*, 2010). Each of these three kinases is critically involved in Toll-like receptor (TLR)–mediated B-cell activation (Bishop *et al.*, 2000; Yi *et al.*, 1996). Specifically, the percentage of pERK, pAKT, and pJNK B cells was monitored at 15, 30, and 60 min post-TLR-4-, TLR-7-, or TLR-9-induced activation (using LPS, R848, or CpG, respectively) in the absence and presence of TCDD. Not unexpectedly, each of the TLR ligands activated each of the aforementioned kinases but with modestly different kinetics. Most importantly, TCDD treatment consistently decreased the percentage of TLR ligand–driven $pERK^{high}$, $pAKT^{high}$, and $pJNK^{high}$ B cells with the most striking effect being on the decreased percentage of $pERK^{high}$, $pAKT^{high}$, and $pJNK^{high}$ triple-positive B cells at 15 min post-R848 activation compared with the vehicle control group. TCDD also markedly suppressed the IgM response induced by each of the TLR ligands. TCDD produced no effect on background kinase activity in nonactivated B cells. Concurrent with the decreased percentage of $pERK^{high}$, $pAKT^{high}$, and $pJNK^{high}$ produced by TCDD treatment, there was also a marked decrease in the percentage of B cells expressing CD80 and CD86, a hallmark of B-cell activation. Therefore, in addition to changes at the level of gene transcription, which have been widely demonstrated to occur though ligand activation of the AhR, TCDD treatment can also induce biochemical changes in cells through mechanisms that are independent of AhR-mediated gene regulation. Moreover, the TCDD-mediated early changes in various aspects of cell signaling are consistent with time of addition studies, suggesting that TCDD perturbs an early B-cell activation event that leads to impairment of plasma cell formation and Ig production.

Lymphocytes in general express relatively low levels of AhR when compared with other cells yet they are one of the most sensitive targets of TCDD. Interestingly, activation of mouse and human B cells (Allan and Sherr, 2005; Crawford *et al.*, 1997; Marcus *et al.*, 1998; Sulentic *et al.*, 1998) and mouse T cells (Lawrence *et al.*, 1996) leads to an upregulation of the AhR. It is tempting to speculate that the upregulation of AhR upon activation contributes to the sensitivity of lymphocytes to TCDD-induced toxicity. Furthermore, in the absence of addition of exogenous AhR ligands, activation of splenocytes induced CYP1A1 expression and AhR-DRE binding (Crawford *et al.*, 1997). These effects on AhR expression and activity in stimulated lymphocytes are not unique to mouse cells but have more recently been demonstrated in human primary B cells following activation with two different B-cell stimuli: (1) bacterial CpG, which activates through TLR9 and resembles B-cell activation during an innate immune response and (2) CD40 ligand, which binds the CD40 receptor expressed on the B-cell surface and models an adaptive immune response in which T-helper cells expressing surface CD40 ligand directly interact with and activate B cells (Allan and Sherr, 2005). These results suggest a physiological role for the AhR during B-cell activation; however, the precise role is unclear

because it appears that B cells from AhR-null mice (Vorderstrasse *et al*., 2001) and AhR-deficient BCL-1 cells (Sulentic *et al*., 1998) can be induced to secrete antibody. Overall, there is surprisingly little known concerning the physiological role of the AhR in cellular processes, including those processes important to B-cell activation.

TCDD-MEDIATED SUPPRESSION OF B-CELL PROLIFERATION AS A PUTATIVE CAUSE OF IMPAIRED DIFFERENTIATION

Historically, suppression of humoral immune responses after TCDD treatment has been assessed primarily either by the Jerne plaque assay or ELIspot, both of which enumerate the number of antibody-secreting cells (AFC response), or by ELISA, which measures the amount of antibody secreted by B cells (antigen specific or total) most often in culture supernatants or serum. Of these approaches, the most widely used has been the Jerne IgM AFC response. Almost without exception, TCDD-mediated suppression of the IgM AFC response has been observed regardless of the antigenic stimulus and whether TCDD treatment was performed *in vivo* or by direct addition of TCDD to B cells in culture. In spite of extensive research, the molecular mechanism responsible for the decreased number of IgM AFC and decrease IgM production remains poorly understood but is indicative that TCDD impairs B cell to plasma cell differentiation.

Resting B cells in response to antigenic stimulation undergo four to six rounds of cell division prior to initiating the differentiation program that leads to plasma cell formation and high-level Ig secretion. One putative mechanism by which TCDD could mediate a decrease in IgM AFCs is through a generalized suppression in B-cell proliferation. Some of the earliest investigations suggested the contrary by demonstrating that TCDD produced modest effects on B-cell proliferation in spite of the strong magnitude of suppression on humoral immune responses. In mice receiving a single 5 μg/kg dose of TCDD, a greater than 80% suppression of the *in vivo* anti-sRBC IgM AFC response was produced with no significant effect on spleen cellularity, leading the authors to conclude that TCDD primarily impairs B-cell differentiation and not proliferation (Tucker *et al*., 1986). Similar results were observed in these same studies using LPS to induce IgM AFC. Likewise, Dooley and Holsapple (1988) showed that five consecutive days of TCDD administration by oral gavage at doses up to 10 μg/kg produced no significant decrease in spleen weight or body weight, suggesting no direct cytotoxic effects on spleen cells. In a related study, mice dosed for 14 consecutive days by oral gavage with 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin and sensitized with sRBC on day 11 demonstrated an 18% decrease in spleen cell number at 2.0 μg/kg while exhibiting a 70% suppression of the IgM AFC response (Holsapple *et al*., 1984). In yet another study, a single 20 μg/kg oral dose of TCDD to mice suppressed germinal center (GC) formation in the spleen and decreased the frequency of high-affinity AFC, which the authors ascribed to decreased B-cell proliferation (Inouye *et al*., 2003). Their conclusion was based primarily on a decrease in the absolute number of splenic B cells recovered at the time of sacrifice; in spite of the fact that the percentage of total B cells to other cell types in the spleen was either similar to or within 5% of the control, being more consistent with the interpretation that TCDD produced a modest overall decrease in all cell types in the spleen.

The effect of TCDD on B-cell proliferation has also been examined *in vitro*. Direct addition of TCDD at concentrations up to 10nM to cultured mouse splenocytes activated with anti-Ig plus B-cell growth factors strongly suppressed the IgM response but not B-cell proliferation even at TCDD concentrations as high as 50nM (Luster *et al*., 1988). Direct addition of TCDD at concentrations up to 33nM to mouse spleen cell cultures activated with LPS also showed no suppression on the lymphoproliferative response (Holsapple *et al*., 1986b). Similarly, activation of naive mouse B cells with CD40L plus IL-2, IL-6, and IL-10 in the presence of TCDD at concentrations up to 30nM produced minimal affects on the number of rounds B cells proliferated, although a slightly lower percentage of cells were observed in the later rounds of proliferation in the TCDD treatment group (Lu *et al*., 2010). Somewhat in contrast, TCDD was reported to suppress LPS-induced proliferation of low-density B cells ("activated" population of B cells in G_1) but not high-density B cells (i.e., "resting B cells" in G_o), which were closely correlated with suppression of IgM secretion (Morris *et al*., 1993). The effects of TCDD on cell division in the CH12.LX cell line have also been examined within the context of LPS-induced IgM secretion. In these experiments, TCDD produced a concentration-dependent suppression of secreted IgM with 92% suppression at 72 h in supernatant IgM at 10nM TCDD and a 37% decrease in cell number, which was correlated with an increase in doubling time from ~16 to 20 h (Crawford *et al*., 2003).

Collectively, these studies show that B-cell proliferation induced by a variety of different stimuli signaling through a number of different receptors including the B-cell antigen receptor, TLRs, and CD40 can be affected by TCDD but that the effects are modest in comparison to the magnitude of IgM suppression. Therefore, although a decrease in B-cell proliferation may be a contributing factor, it cannot account solely for the magnitude of suppression produced by TCDD on humoral immunity.

POSSIBLE MOLECULAR TARGETS INVOLVED IN B-CELL DYSFUNCTION BY TCDD

The AhR signaling pathway culminates in transcriptional regulation through binding of the AhR nuclear complex to DRE sites within regulatory regions of dioxin-sensitive genes. Undeniably, metabolic genes, particularly CYP1A1, have been

the most thoroughly studied. However, it is commonly believed that an upregulation of metabolic genes is not a toxic response *per se* and is not responsible for the various dioxin-induced toxicities observed in various animal models. Research has been and continues to be directed toward identifying genes whose regulation has been altered by TCDD. These transcriptional changes are complex and can be envisioned to result from a combination of events including through direct transcriptional regulation by the AhR, via secondary genes whose regulation is affected by genes directly regulated by the AhR and transcriptional events influenced by "secondary" signaling events initiated by AhR activation but independent of AhR DNA binding. Such a complex scenario is likely responsible for the plethora of toxic effects and diverse genes identified to be transcriptionally regulated after TCDD treatment. Congruently, an assortment of genes, including those encoding cell signaling proteins, cell survival, and apoptotic proteins, cytokines, and transcription factors have been shown to be upregulated or downregulated by TCDD. In addition, DRE-like sites have been identified in the promoter regions of numerous genes some of which have been found to be modulated by AhR activation and some that have yet to be tested (Lai *et al*., 1996; Schneider *et al*., 2009). Recently, a combination of whole-genome microarray-based chromatin immunoprecipitation (ChIP-on-chip) and time course gene expression microarray analysis was performed on the mouse B-cell line CH12.LX following exposure to LPS or LPS and TCDD to identify the primary and downstream transcriptional elements of B-cell differentiation that are altered by the AHR (De Abrew *et al*., 2010). ChIP-on-chip analysis identified 1893 regions with a significant increase in AhR binding with TCDD treatment. Transcription factor binding site analysis on the ChIP-on-chip results showed enrichment in AhR response elements. Other transcription factors showed significant coenrichment with AhR response elements. When ChIP-on-chip regions were compared with gene expression changes at the early time points (8 and 12 h post-TCDD treatment), 78 genes were identified as potential direct targets of the AhR, which are now being further investigated on an individual basis. Because the primary focus of this review is on the impairment by TCDD of B-cell effector function, we will focus on specific proteins involved in B cell to plasma differentiation, including the transcriptional regulation of the Ig heavy chain, Paired Box Protein 5 (Pax5), B lymphocyte-Induced Maturation Protein-1 (Blimp-1), and B-Cell Lymphoma 6 Protein (BCL6).

Ig Heavy Chain Regulation and the 3′IgH Regulatory Region

The primary antibody response is characterized by the differentiation of antigen-activated virgin B cells into IgM-secreting plasma cells. Ig molecules typically are composed of four polypeptide chains, two light chains and two heavy chains, joined into a macromolecule by disulfide bonds. IgM is secreted by plasma cells in a polymeric form in which individual IgM monomers are joined together by a J chain into pentamers or hexamers. A coordinated and increased rate of transcription of genes coding for the light chain, heavy chain, and joining chain is necessary for high-level production of IgM that is characteristic in plasma cells. Of these three Ig chains, to date the regulation of the murine Ig heavy chain (IgH) locus is the most extensively characterized.

In CH12.LX cells, the IgH gene was inhibited by TCDD (Henseler *et al*., 2009; Sulentic *et al*., 2000; Yoo *et al*., 2004). Expression of the light chain (IgL) and the J chain of Ig are likewise suppressed by TCDD (Yoo *et al*., 2004). Studies focused primarily on IgH gene expression and Ig protein secretion demonstrated a structure-activity relationship with polychlorinated dibenzo-*p*-dioxins such that suppression of the aforementioned was closely correlated with AhR-binding affinity. These findings are highly consistent with studies demonstrating an essential role for the AhR in suppression of humoral immune responses. Further analysis of these results demonstrated a general concordance between the IC_{50} values for IgH expression and Ig secretion and the EC_{50} for induction of CYP1A1 expression, which further supports a common AhR-mediated mechanism (Sulentic *et al*., 2000). Because CYP1A1 induction is an established AhR/DRE-mediated event, suppression by TCDD of IgM secretion may be, at least in part, due to an AhR/DRE-mediated effect on IgH gene expression. Consistent with this, DRE-like sites have been identified within the 3′IgH regulatory region (3′IgHRR) and AhR/ARNT binding to these DRE sites has been demonstrated (Sulentic *et al*., 2000).

Regulation of the mouse IgH locus is governed through a complex interaction of several regulatory elements whose activity is B cell specific and dependent on the state of B-cell maturation. The most 5′ regulatory element is the V_H promoter, which lies immediately upstream of each variable region and contributes to B cell specific activity of the IgH locus (Fig. 1). A 40-kb region, downstream of the α constant

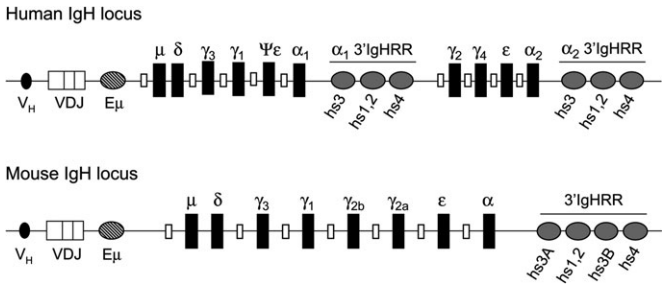

FIG. 1. Schematic of the human versus mouse IgH gene locus. Simplified diagram of a rearranged human and mouse Ig heavy chain (IgH) locus which includes the variable heavy chain promoter (V_H), the intronic or μ enhancer (Eμ), the heavy chain constant regions for the μ-, δ-, γ-, ε-, α-isotypes and subclasses (black rectangles), the germline promoters upstream of each heavy chain constant region (open rectangles), and the enhancers (hs3, hs1,2, and hs4) of the 3′IgH regulatory region (3′IgHRR).

region (Cα), demonstrates enhancer activity that is largely restricted to plasma cells and appears to regulate processes late in B-cell differentiation such as upregulation of IgH gene expression and secretion as well as class switch recombination (Klein *et al*., 1984; Madisen and Groudine, 1994; Saleque *et al*., 1997; Singh and Birshtein, 1993; Wabl and Burrows, 1984). Class switch recombination is a DNA recombination event by which B cells shift from the production of IgM to the expression of IgG, IgE, or IgA isotypes. Within this regulatory region, four separate enhancers, hs3a, hs1,2, hs3b, and hs4, corresponding to DNase1 hypersensitive (hs) sites, were identified and are collectively termed the 3′IgHRR (formerly the 3′α enhancer) (Fig. 1). Using various B-cell lines at differing maturation phases, the activity of the 3′IgHRR enhancers appeared to be dependent on the developmental stage of the B cell with hs4 being activated at the pre-B-cell stage, whereas hs1,2 and hs3 were only active in mature B cells (Chauveau *et al*., 1998; Mills *et al*., 1997; Ong *et al*., 1998; Stevens *et al*., 2000). Measurement of enhancer activity in mature B cells identified hs1,2 as having the greatest activity followed by hs4, and the least active was hs3 (Mills *et al*., 1997); however, the greatest enhancer activity was observed when all four enhancers were linked (Chauveau *et al*., 1998; Stevens *et al*., 2000; Mills *et al*., 1997).

DNA-binding sites for several transcription factors, including NF-κB/Rel (bind κB sites), Pax5 previously termed B cell–specific activator protein (BSAP), Oct, and AP-1, have been identified within the 3′IgHRR and appear to be important regulators of its activity (Kanda *et al*., 2000; Khamlichi *et al*., 2000; Michaelson *et al*., 1996; Saleque *et al*., 1999; Stevens *et al*., 2000). Interestingly, mutational analysis of κB, Pax5, or Oct binding sites within the hs1,2 or hs4 enhancers (when in isolation) demonstrated a negative influence of these transcription factors on hs1,2 basal activity versus a positive influence on hs4 basal activity in mature B cells (Michaelson *et al*., 1996). A similar dichotomy in the effects of TCDD on hs4 versus 3′IgHRR activity was identified and demonstrated a synergistic and sensitive activation of LPS-induced hs4 activation (maximum at 3.0nM TCDD) and a markedly sensitive suppression of LPS-induced 3′IgHRR activity [maximum at 0.3nM or 90 pg TCDD which, depending on the lipid content of individuals, may be within the range of current human body burdens (lipid-adjusted TEQ for PCDD/PCDF was 2–20 ppt for the year 2000)] (Sulentic *et al*., 2004). Studies by Michaelson *et al*. (1996) have identified κB binding as a critical regulator of basal hs4 activity in mature B cells and in plasma cells. This appears to translate to an important effect on the overall 3′IgHRR activity because mutation of the κB motif within the hs4 enhancer led to a significant suppression of 3′IgHRR activity (Kanda *et al*., 2000). Interestingly, the observed dichotomy between NF-κB/Rel regulation of the hs4 versus hs1,2 enhancers in mature B cells is not observed in plasma cells in which κB binding becomes a positive regulator of both the hs4 and the hs1,2 (Michaelson *et al*., 1996). This suggests either a shift in the NF-κB/Rel dimers (homo- or heterdimers of RelA, cRel, RelB, p50, p52) binding to the κB site within the hs1,2 and/or an altered interaction between the NF-κB/Rel proteins and other transcription factors, such as Pax5 (which is downregulated in plasma cells), Oct, AP-1, or perhaps the AhR, which is induced by TCDD to bind DRE-like sites within the hs4 and hs1,2 enhancers (Sulentic *et al*., 2000).

TCDD-induced suppression of Ig may be mediated directly through binding of the AhR to DREs within the regulatory regions of the Ig genes (such as 3′IgHRR) and/or indirectly through regulation of other transcriptional factors known to regulate these genes (Sulentic *et al*., 2004; Yoo *et al*., 2004). For example, Pax5, the NF-κB/Rel proteins, Oct-1, and AP-1 are all modulated by TCDD and are significant regulators of B-cell activation and differentiation and of 3′IgHRR activation (Kanda *et al*., 2000; Michaelson *et al*., 1996; Puga *et al*., 2000; Schneider *et al*., 2008; Suh *et al*., 2002; Sulentic *et al*., 2000; Yoo *et al*., 2004). For NF-κB/Rel proteins, previous reports have identified interactions between the NF-κB/Rel and AhR signaling pathways, which has been postulated as an important mechanism in mediating the toxicities of TCDD (Tian *et al*., 2002; Vogel *et al*., 2007a, 2007b). Consequently, the overall effect of TCDD on the 3′IgHRR may involve an interaction between these regulatory proteins within and between the enhancers of the 3′IgHRR.

Regulatory Mechanisms Controlling B-Cell Differentiation into Plasma Cells

Terminally differentiated antibody-secreting plasma cells are the end stage of the humoral immune response. Based on studies in mouse and to a lesser extent human B cells, three transcription factors which sequentially influence each others expression, BCL6, Blimp-1 (the gene product of *prdm-1*), and Pax5, have emerged as crucial regulators of the B-cell differentiation program (Fig. 2).

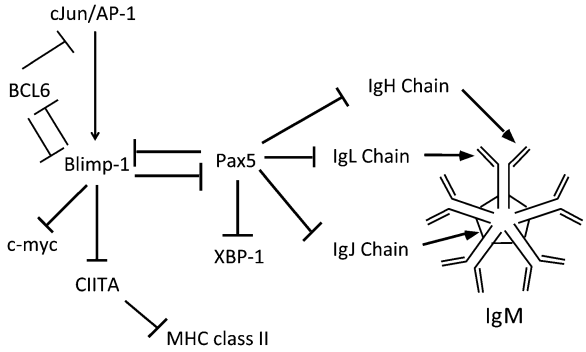

FIG. 2. Schematic representation of the regulators controlling B cell to plasma cell differentiation. Positive regulation is depicted with arrows and negative regulation is depicted by brackets.

Paired Box Protein 5

Pax5 has emerged as a key regulator not only of B-cell development but also of B-cell differentiation and of the IgM response via transcriptional repression of *IgH, Igκ*, and the *J chain*. Pax5 is a highly expressed transcription factor throughout B-cell development, including in pre-B cells, immature B, and mature B cells. Targeted disruption of the Pax5 gene resulted in a blockade in B-cell development at the pro-B-cell stage establishing its essential role in B-cell development (Nutt *et al*., 1999; Urbanek *et al*., 1994). When B cells begin to differentiate into plasma cells, Pax5 is progressively decreased (Barberis *et al*., 1990; Rinkenberger *et al*., 1996) concordant with one of its principal functions, repression of *IgH, Igκ*, and *J chain*. Repression of IgH is mediated by Pax5 through its binding to Pax5 DNA–recognition motifs present in the hs1,2 and hs4 domains within the 3′IgHRR (Neurath *et al*., 1995, 1994). More recently, it has been demonstrated that repression of hs1,2, enhancer activity by Pax5 also involves the recruitment of a member of the Groucho family corepressors, Grg4 (Linderson *et al*., 2004). Such a mechanism of corecruitment of additional DNA-binding proteins can explain how Pax5 can repress certain genes (e.g., *IgH, Igκ, and J chain*) while simultaneously positively regulating others (e.g., CD19). Pax5 repression of Igκ expression, although less well understood, appears to be mediated by disruption of Igκ 3′ enhancer activity by suppressing the transactivating capability of the transcription factor, PU.1, through direct protein:protein interactions (Maitra and Atchison, 2000). Likewise, repression of the J chain by Pax5 is through DNA binding to a negative regulatory element located on base pairs −113 to −97 within the J chain promoter and thus blocking the ability of PU.1 and B-MEF-2 to bind their respective response elements (Wallin *et al*., 1999). In addition, Pax5 also strongly represses the expression of a fourth gene that is essential for IgM secretion, XBP-1, which it does by binding to and inhibiting *Xbp-1* promoter activity (Reimold *et al*., 1996). XBP-1 is a basic-region leucine zipper protein in the ATF/CREB family of transcription factors and has been linked to triggering the assembly of the secretory apparatus necessary for IgM secretion by plasma cells (Calfon *et al*., 2002). XBP-1 is one of only a few proteins known to be essential for Ig secretion, as demonstrated in mouse lymphoid chimeras deficient in XBP-1, which possessed a normal number of activated B cells that proliferated, secreted normal amounts of cytokines, and formed GCs but expressed very little Ig and were devoid of plasma cells (Reimold *et al*., 2001). Conversely, in Pax5-deficient cells, XPB-1 and Blimp-1 expression were found to be upregulated and IgM secretion was significantly elevated, a profile of activity concordant with differentiated B cells. These characteristics of differentiated B cells were all reversed when Pax5 expression was restored (Nera *et al*., 2006). These findings are important not only because they demonstrate the crucial role Pax5 plays in B-cell differentiation but also suggest reciprocal repression between Pax5 and Blimp-1. More recently *Blimp-1* repression by Pax5 has been confirmed (Mora-Lopez *et al*., 2007). Collectively, the above studies implicate Pax5 as a potent repressor of *IgH*, *Igκ*, *J chain*, *XBP-1*, and *Blimp-1* but alone insufficient to activate the terminal differentiation program in B cells as evidenced by functional deletion (cre/loxP) of *Pax5* in mature B cells (Horcher *et al*., 2001).

B lymphocyte–Induced Maturation Protein-1 (Blimp-1)

Blimp-1, termed by some as the B cell "master regulator," is a 96-kDa transcriptional repressor whose activity is conferred by binding a 12-bp consensus site (Keller and Maniatis, 1991). Blimp-1 is a critical regulator of plasmacytic differentiation and is highly expressed in plasma cells (Kallies *et al*., 2004; Shapiro-Shelef and Calame, 2005; Shapiro-Shelef *et al*., 2003). Ectopic expression of Blimp-1 is in itself sufficient to drive BCL-1 cells and splenic B cells to differentiation into IgM-secreting plasma cells (Angelin-Duclos *et al*., 1999; Piskurich *et al*., 2000; Schliephake and Schimpl, 1996; Turner *et al*., 1994). Although not fully understood how a single transcriptional repressor can regulate B-cell differentiation, the effect of Blimp-1 on four known targets that are also critically involved in B-cell differentiation provide important insights. First, Blimp-1 strongly represses *Pax5* transcription (Lin *et al*., 2002), which in turn releases *IgH*, *Igκ*, *J chain*, and *XBP-1* from Pax5-mediated repression (Fig. 2). Second, Blimp-1 represses *c-myc* transcription (Lin *et al*., 1997), which in turn stops the cell cycle and proliferation (Eilers, 1999). Third, Blimp-1 represses BCL6, which also leads to cessation of cell cycle and proliferation. It is noteworthy that as in the case of Pax5 and Blimp-1, BCL6 and Blimp-1 also reciprocally repress each other. Hence, in the absence of appropriate signaling events initiated during B-cell activation, Blimp-1 is dually repressed by BCL6 and Pax5 to keep B cells from aberrantly initiating the B-cell differentiation program. Fourth, Blimp-1 represses class II, major histocompatibility complex transactivator (CIITA) (Piskurich *et al*., 2000), which in turn leads to the downregulation of MHC class II expression on plasma cells (Silacci *et al*., 1994).

B-Cell Lymphoma 6 Protein (BCL6)

BCL6 is a zinc finger transcription factor which functions primarily as a sequence-specific transcriptional repressor. Despite its importance in B-cell differentiation, BCL6 is best known for its role in non-Hodgkin's lymphoma, a group of heterogeneous cancers most commonly derived from GC B cells and in which BCL6 is frequently expressed as a result of chromosomal translocations (Ye *et al*., 1997). BCL6 protein is tightly regulated, being predominately expressed in mature B cells with highest expression in GC B cells; however, BCL6

is not found in pre-B cells or plasma cells. The primary role of BCL6 is to promote GC differentiation and to block plasmacytic differentiation. This notion is supported by a number of related observations in addition to its high level of expression in GC B cells. For example, mice deficient in BCL6 display normal B cell, T cell, and lymphoid organ development but fail to develop GCs (Dent *et al*., 1997; Ye *et al*., 1997). Likewise, it has been shown that nascent pre-GC-B cells upregulate BCL6 protein, migrate to the follicular area, and initiate GC formation (Fukuda *et al*., 1997). Conversely, downregulation of BCL6 in B cells is through degradation by ubiquitin/proteasome pathway and is associated with induction of Ras/MAPK activity (Niu *et al*., 1998). More recently, studies aimed at understanding BCL6–mediated blockade of plasmacytic differentiation demonstrated that BCL6 represses the transcription of *Blimp-1* via a novel regulatory pathway that targets the *Blimp-1* promoter. Specifically, the zinc finger domain of BCL6 can bind Jun proteins, c-Jun, JunB, and JunD but not fos family members (Vasanwala *et al*., 2002). By doing so, BCL6 sequesters crucial Jun proteins involved in the formation of AP-1 complexes to inhibit transactivation through AP-1 sites located at −1813 and −1647 bp in the human *Blimp-1* promoter (Vasanwala *et al*., 2002). Analogous AP-1 sites have been identified within the mouse *Blimp-1* promoter.

Effects of TCDD on the B-Cell Differentiation Program

As discussed above, the discovery of functionally relevant DREs within the regulatory domains of the 3′IgHRR (Sulentic *et al*., 2000) led to the identification of one of the first genes directly targeted by the ligand-activated AhR and contributing to TCDD-mediated impairment of B-cell function. Likewise, it provided, at least in part, a potential mechanism explaining the significant decreases in IgH mRNA levels observed in activated B cells treated with TCDD. However, further examination also revealed that in addition to decreased IgH mRNA levels, *in vitro* TCDD treatment of LPS-activated splenic B cells (Schneider *et al*., 2008) as well as CH12.LX cells also produced a comparable decrease in mRNA levels of *Igκ* and *J chain*. Based on the critical role Pax5 plays in coordinately repressing *IgH*, *Igκ*, and *J chain*, the effects of TCDD on Pax5 regulation were further examined in the CH12.LX cell line. As would be predicted, LPS activation of CH12.LX cells induced a time-dependent downregulation in *Pax5* mRNA levels over a 72-h time period, which was closely correlated with downregulation of Pax5 protein levels and DNA-binding activity to a Pax5 consensus site (Yoo *et al*., 2004). Conversely, in the presence of TCDD, downregulation of *Pax5* mRNA levels, protein, and DNA-binding activity was impaired and was in turn closely correlated in a time-dependent manner with suppression of LPS-induced IgM secretion. Moreover, the impaired downregulation of *Pax5* mRNA levels was observed in multiple splice variants of *Pax5* (Schneider *et al*., 2008). In addition, in the presence of TCDD, LPS-activated CH12.LX cells also showed a decreased level of the active 54 kDa form of XBP-1 protein when compared with LPS-activated controls further confirming the functional consequences of the aberrantly elevated Pax5 levels. Evaluation of *Xbp-1* mRNA levels likewise demonstrated time-dependent induction over 72 h post-LPS activation in CH12.LX cells and mouse splenocytes, which was suppressed in the presence of TCDD (Schneider *et al*., 2008). Finally, confirmatory experiments using ectopic expression of Pax5 in CH12.LX cells showed suppression of LPS-induced IgM secretion, which mimicked that observed with TCDD treatment (Schneider *et al*., 2008). Taken together, TCDD-mediated suppression of *IgH*, *Igκ*, and *J chain* as well as *XBP-1* are all consistent with the inability of B cells to appropriately downregulate Pax5.

Although transcriptional regulation of *Pax5* has only been partially elucidated, recent studies have investigated putative mechanisms by which TCDD treatment might lead to abnormally high levels of Pax5 in activated B cells, hence blocking B cell to plasma cell differentiation. Motif analysis of the *Pax5* promoter identified 10 putative DRE-like sites within the first −3500-bp region of the transcriptional start site. Unfortunately, experiments assessing whether TCDD treatment induces *Pax5* transcription through one or more of these putative DRE-like sites, using a *Pax5* promoter reporter for transient transfection assays in CH12.LX cells were not informative due to the expulsion of the reporter plasmid by 24 h (Schneider *et al*., 2009). Importantly, similar experiments transfecting the *Pax5* promoter reporter into Hepa 1c1c7 cells and NIH3T3 cells, which are more amendable to transfection, showed no induction of reporter activity above background after TCDD treatment, suggesting that the activated AhR likely does not directly induce *Pax5* transcription. Additional studies in CH12.LX cells examining Blimp-1 activity showed that LPS activation of CH12.LX cells induced Blimp-1 DNA binding in a time-dependent manner to the Blimp-1 response element within the *Pax5* promoter. By contrast, a significant decrease in Blimp-1 DNA–binding activity was observed in LPS-activated CH12.LX cells concomitantly treated with TCDD when compared with time-matched controls. The most pronounced effects on Blimp-1 being observed at 24 and 48 h post-LPS activation (Schneider *et al*., 2009). In addition, *Blimp-1* mRNA levels were closely correlated with Blimp-1 DNA–binding activity thus showing strong induction in response to LPS activation in the absence of TCDD and marked impairment in the presence of TCDD. A detailed analysis of the first −2000 bp of the *Blimp-1* promoter identified two putative DRE sites located within the first −350 bp of the transcriptional start site. Electrophoretic mobility shift assays demonstrated very low TCDD-inducible DNA-binding activity to both of these DRE-like sites (Schneider *et al*., 2009). Taken together, these studies suggest that TCDD does not impair *Blimp-1* transcription via direct binding of the AhR to either of the putative DRE sites identified within the *Blimp-1* promoter.

Transcriptional upregulation of *Blimp-1* during B-cell differentiation is controlled in part by AP-1 binding to multiple TRE sites within its promoter (Vasanwala *et al*., 2002). Concordant with TCDD suppressing Blimp-1 induction, impairment of AP-1 DNA–binding activity by TCDD in activated B cells was first reported in LPS-activated CH12.LX cells and was correlated with a decrease in c-jun expression (Suh *et al*., 2002). A more recent evaluation of three separate TRE within the −2000 bp of the *Blimp-1* promoter using nuclear extracts from CH12.LX cells showed induction of AP-1 binding in response to LPS activation, which was markedly impaired in a time- and concentration-dependent manner in the presence of TCDD treatment (Schneider *et al*., 2009). In addition, the TCDD-mediated inhibition in AP-1–binding activity was sustained up to 72 h post-LPS activation. Interestingly, TCDD treatment in nonactivated B cells produced a modest induction of AP-1 binding to several of the TRE within the *Blimp-1* promoter, which is consistent with a prior report by Puga *et al*. who showed TCDD-mediated induction of AP-1 binding in cultured mouse hepatoma cells and postulated a mechanism involving AhR-dependent p38-MAPK activation (Puga *et al*., 2000; Weiss *et al*., 2005). Presently, the molecular mechanism responsible for modulation of AP-1 activity in B cells by TCDD treatment is intriguing but difficult to explain in the context of AhR activation. It is also noteworthy as discussed in an earlier section that altered levels of kinase activity after TCDD treatment have been demonstrated, specifically on pERK, pAKT, and pJNK as rapidly as 15 min post-B-cell activation (North *et al*., 2010). These early events that almost certainly occur independent of AhR-mediated transcriptional changes do not rule out the involvement of the AhR at the level of protein-protein interaction and may represent critical events that lead to impaired AP-1 function, Blimp-1 upregulation, and altered B-cell differentiation. In addition, in a recent study in which a combination of whole-genome microarray-based ChIP-on-chip and time course gene expression microarray analysis was performed on CH12.LX cells following exposure to LPS or LPS and TCDD, Bach2, a transcriptional repressor of *Blimp-1* was identified (De Abrew *et al*., 2010). AhR-mediated upregulation of Bach2 may represent another, as well as important, contributing event in the molecular mechanisms by which TCDD impairs B-cell differentiation.

North *et al*. (2009) recently investigated the effects of TCDD on B-cell differentiation after induction of a primary immune response *in vivo*. In these studies, mice were administered a single dose of TCDD by oral gavage and then sensitized with LPS 4 days later. These studies showed a dose-dependent suppression of the IgM AFC responses as well as a corresponding decrease in $CD138^+$ B cells (i.e., plasma cells) with TCDD treatment. Analysis by real-time PCR of splenic mRNA on sequential days up to day 3 post-LPS sensitization revealed that TCDD dose dependently suppressed LPS-induced *IgH*, *Igκ*, *J chain*, *XBP-1*, and *Blimp-1*. In addition, as assessed by flow cytometry, TCDD also impaired the upregulation of Blimp-1 at the protein level on $CD19^+$ B cells. These findings are important because they confirmed *in vivo* for the first time observations previously made *in vitro* in B-cell lines as well as primary B cells showing altered B-cell differentiation by TCDD including deregulation of Blimp-1 and XBP-1.

Recently, stochastic modeling of B-cell differentiation and its suppression by TCDD as well as a systems computational biology modeling approach has been used to further characterize the interactions between BCL6, Blimp-1, and Pax5 during B-cell differentiation (Bhattacharya *et al*., 2010; Zhang *et al*., 2010). Computational modeling in this area has focused in part on the interactions between BCL6, Blimp-1, and Pax5 because these three key regulators form two coupled mutually repressive feedback loops at the Blimp-1 node predicting that they can function as a bistable biological switch directing B cells to differentiate into plasma cells. The interest on this putative bistable switch is that in biological systems, it is becoming increasingly clear that coupled feedback loops, as formed by BCL6, Blimp-1, and Pax5, increase the robustness of the bistable properties of the signal transduction circuitry as well as the biological behavior that ensues. Hence, in B cells, there is increasing evidence that this bistable switch dictates whether the B cell remains as a B cell or whether it initiates terminal differentiation into a plasma cell. Using a bifurcation analysis, the model proposes that TCDD suppresses plasmacytic differentiation by raising the threshold dose of antigen necessary to trigger the bistable switch. The most significant implication from this computation model is the prediction that suppression of humoral responses by TCDD is due to a decrease in the overall proportion of B cells that receive sufficient antigenic stimulation to trigger the bistable switch to initiate B cell to plasma cell differentiation (Bhattacharya *et al*., 2010). The predicted phenomenon is highly consistent with past observations that suppression of primary humoral immune responses by TCDD is accompanied by a decrease in the total number of IgM plasma cells.

EFFECTS OF TCDD ON HUMAN B CELLS

A significant data gap is studies investigating the effects of TCDD and dioxin-like compounds on human primary B cells. This data gap is due to several factors. First, inducing human primary B cells *in vitro* to become antibody-secreting cells historically has been technically challenging. Human B cells do not respond to LPS as they lack TLR-4. Some success of activating human B cells to become antibody secretors has been achieved with pokeweed mitogen, although antibody responses tend to be relatively modest. Other activation stimuli include the super-antigen, toxic shock syndrome toxin-1 (TSST-1), CpG, and CD40 ligand. Notably, all are polyclonal B-cell activators because inducing primary antibody responses using specific antigens is limited by small numbers of B-cell

precursors possessing appropriate antigenic specificity to any given antigen. Second, a routine source of human B cells has also been an obstacle. Sources include human spleens from accident victims as well as tonsils from patients undergoing tonsillectomy. The former is not a reliable routine source and the later has drawn concern as tonsils are typically not removed from healthy patients. The most noninvasive source of human B cells is peripheral blood; however, B cells only constitute ~5% of circulating leukocytes. Recently, due to enhanced cell isolation/purification technologies, peripheral blood has become a reliable and readily available source of human B cells. Another major deterrent of immunotoxicology studies of human primary B cells has been the inherent donor-to-donor variability.

In spite of the above challenges, several studies have investigated the direct effects of TCDD on human primary B cells. In one study, human tonsillar lymphocytes were stimulated *in vitro* with pokeweed mitogen, in the absence and presence of TCDD followed by measurements of total IgM, IgG, and IgA by reverse plaque assay. TCDD was found to have no effect on either the pokeweed mitogen-induced Ig responses or [^{3}H]-thymidine incorporation as a measure of B-cell proliferation (Wood *et al.*, 1992). Studies were performed in parallel using mouse spleen cells, which demonstrated strong suppression of the anti-sRBC IgM AFC response but no suppression of either pokeweed mitogen-induced IgM response or proliferation. By contrast, in a second study by the same laboratory, purified human tonsillar B cells activated with TSST-1 exhibited TCDD-mediated suppression of the IgM response in the absence of effects on proliferation (i.e., [^{3}H]-thymidine incorporation) (Wood and Holsapple, 1993). Among the four donors assayed in this study, variability in sensitivity between donors was observed with IC_{50} values for suppression of the IgM response being < 0.3, < 0.3–5.0 and ~25nM. No suppression of TSST-1–induced B-cell proliferation was observed. Recently, a study was conducted in which the direct effects of TCDD were evaluated in purified naive ($CD19^+CD27^-$) peripheral blood B cells from 12 donors. Human naive B cells were used specifically because this population of B cells most closely reflects the population of B cells found in the mouse spleen, which is typically greater than 95% $CD19^+CD27^-$ (Lu *et al.*, 2009). The study objectives were twofold: (1) to assess the induction of known AhR-responsive genes in primary human B cells as a measure of early biological responses to TCDD and (2) to evaluate the direct effect of TCDD on CD40 ligand–induced IgM secretion by human primary B cells (Lu *et al.*, 2010). These studies showed that AhR-responsive genes (i.e., *CYP1A1, CYP1B1, AHR* repressor, and TCDD-inducible *poly [ADP-ribose] polymerase*) in human B cells exhibited slower kinetics and reduced magnitude of induction by TCDD when compared with mouse B cells, which was consistent with the generally observed 10-fold lower sensitivity of human AhR to TCDD binding and *CYP1A1* induction (reviewed by Harper *et al.*, 2002 and Denison *et al.*, 2002). Nevertheless, these studies confirmed activation by TCDD of the AhR signaling cascade in human primary B cells. In addition, these studies identified two general phenotypes among the 12 donors with the majority of donors (i.e., 9 of 12 donors) exhibiting similar sensitivity to suppression by TCDD of the IgM response as mouse B cells from C57Bl/6 mice with significant suppression at 10 and 30nM TCDD. These donors were termed "responders." Assuming the 10-fold lower TCDD-binding affinity to the human AhR, when compared with TCDD-responsive mouse strains, the comparable sensitivity of human and mouse B cells to TCDD suppression of the IgM response suggest that in these donors, there are important mechanistic differences in how TCDD impairs this B-cell function that is independent of differences in AhR-binding affinity for TCDD. Importantly, no effect by TCDD was observed on B-cell proliferation as assessed by carboxyfluorescein succinimidyl ester (CSFE) staining, which was consistent with prior studies in human B cells (Wood and Holsapple, 1993; Wood *et al.*, 1992). In contrast, a minority of donors (3 of 12 donors) showed no suppression of the IgM response by TCDD even at high concentrations (50–100nM) and therefore were termed "non-responders." Interestingly, sequence analysis of exons of the human *AHR* for three "responsive" and three "nonresponsive" donors showed that two of the three nonresponsive donors possessed previously characterized polymorphisms within the exons of the *AHR*: one has 132 T>C in codon 44 of exon 2 encoding part of the basic-helix-loop-helix domain and the other has 1661 G>A in condon 554 of exon 10 encoding the transactivation domain (Harper *et al.*, 2002). The polymorphism identified at condon 554 G>A was previously shown to cause failure to induce *CYP1A1* induction *in vitro* when combined with two other *AHR* polymorphisms (Wong *et al.*, 2001). By contrast, no polymorphisms were identified within the exons of the *AHR* from all three responsive donors. Although it is tempting to speculate that the absence of sensitivity in nonresponsive donors was due, at least in part, to polymorphisms within the *AHR*, the findings at this point are at best only suggestive. Taken together, although there have only been a limited number of published investigations employing human primary B cells, the existing data demonstrate that TCDD is capable of suppressing the primary antibody response in human B cells. Moreover, the relative sensitivity of human B cells to suppression by TCDD appears to be comparable to that observed in responsive mouse strains, such as the C57Bl/6 and B6C3F1. Equally intriguing is the observation that in the limited study of 12 donors, 3 donors were found to be unresponsive to suppression of the IgM response even at considerably high TCDD concentrations of 50–100nM, suggesting the existence within the general population of sensitive and less sensitive subpopulations to suppression of humoral immune responses by TCDD. There is considerable evidence for species and strain differences in sensitivity to TCDD and other AhR

ligands, which may be related to differences in the *AHR* gene (e.g., Ahr^b versus Ahr^d mouse alleles; species differences in ligand selectivity) or differences in another component of the AhR or dioxin response pathway (e.g., AhR affinity for TCDD is similar in the dioxin-resistant hamster vs. the highly dioxin-sensitive guinea pig). These topics are covered in-depth in several fairly recent reviews (Denison *et al*., 2002; Flaveny and Perdew, 2009; Hahn, 2002; Harper *et al*., 2002).

Potential impact of TCDD on human health

- Reduced vaccine titers, increased incidence of chicken pox, and increased incidence of otitis media within a Dutch children cohort.
- Decreased plasma IgG levels correlated with TCDD plasma levels in Seveso, Italy, residents living in a high TCDD exposure zone.
- Increased IgE production from B cells isolated from patients with allergic rhinitis, atopic eczema/dermatitis syndrome or bronchial asthma and activated *in vitro*.
- Sufficient evidence and limited/suggestive evidence for a positive association between non-Hodgkin's lymphoma and multiple myeloma, respectively, and exposure to dioxin-like compounds.
- Greater incidence of non-Hodgkin's lymphoma in residents living in close proximity to a solid waste incinerator, an area possessing the highest concentrations of TCDD, in Besançon, France.

IMPLICATION OF TCDD-MEDIATED B-CELL TOXICITY ON HUMAN HEALTH

As already discussed, there have been a limited number of studies investigating the effects of TCDD specifically on human primary B-cell function, with several having demonstrated suppression of antibody responses under *in vitro* conditions. However, presently, the long-term consequence of TCDD-mediated B-cell immunotoxicity on human health, especially that which is supported by epidemiologic findings, is less unclear. A longitudinal study of a cohort of Dutch children suggested an association between prenatal exposure to TCDD and dioxin-like compounds and changes in humoral immune status. Specifically, at 42 months of age, children within this cohort demonstrated an association between reduced vaccine titers, increased incidence of chicken pox, and increased incidence of otitis media with higher TEQ (ten Tusscher *et al*., 2003). In another study, 20 years after the industrial accident in Seveso, Italy, 62 individuals from the most highly exposed zone were compared for immunologic effects of dioxin with 58 individuals in a surrounding noncontaminated area. Interestingly, circulating plasma IgG levels were markedly decreased with increasing TCDD plasma levels (Baccarelli *et al*., 2002). In fact, the changes in circulating IgG associated with TCDD plasma levels were highly significant after adjustment for age, smoking, and consumption of domestic livestock and poultry. This epidemiologic evidence taken together with the limited number of *in vitro* studies further supports that humoral immune responses in humans can be suppressed by TCDD exposure. Less clear is what level of exposure to TCDD and dioxin-like compounds is required to impair humoral immunity and whether these levels are attainable through environmental exposure. Furthermore, an interesting study by Kimata *et al*. demonstrated that TCDD (100 pM–10nM) increased the production of IgE (no effect on IgG1, IgG2, IgG3, IgG4, IgM, IgA1, or IgA2) from B cells isolated from patients with allergic rhinitis, atopic eczema/dermatitis syndrome, or bronchial asthma and activated *in vitro* with anti-CD40 plus IL-4 (Kimata, 2003). In this study, TCDD had no effect on antibody secretion in similarly activated B cells isolated from healthy control patients. In contrast, rat and mouse studies evaluating the effects of TCDD on allergic reactions demonstrated a decrease in IgE titers (Fujimaki *et al*., 2002; Luebke *et al*., 2001).

B-cell leukemias are a second category of B-cell sequelea for which an epidemiological association with TCDD exposure has been identified. In 1993, a National Academy of Sciences (NAS) Committee on Vietnam Veterans and Agent Orange concluded that there is sufficient evidence for a positive association between non-Hodgkin's lymphoma and exposure to the herbicides, 2,4-D, 2,4,5-T, and its contaminant TCDD (Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides, 1994). As a follow-up in 1996, the NAS concluded that there is also limited/suggestive evidence between exposure to the herbicides, 2,4-D, 2,4,5-T, and its contaminant TCDD and multiple myeloma. In addition, several epidemiological studies have established an association between occupational or accidental exposure to chemicals contaminated with dioxins and non-Hodgkin's lymphoma (Becher *et al*., 1996; Hooiveld *et al*., 1998; Kogevinas *et al*., 1997, 1995; Saracci *et al*., 1991). Less clear is the impact of exposure to low environmental levels of TCDD and dioxin-like compounds on human health. Although exposure through the consumption of high-fat containing foods is typically considered the primary source of human exposure to TCDD, municipal incineration represents a significant source for the generation of dioxin-like compounds. Interestingly, a case-controlled study was conducted after the identification of a cluster of non-Hodgkin's lymphoma patients living in close proximity to a solid waste incinerator in Besançon, France. Among individuals living in the area with the highest concentrations of dioxin, there was a 2.3 times greater incidence of non-Hodgkin's lymphoma than those living in surrounding areas with lower dioxin concentrations (Floret *et al*., 2003).

The molecular mechanisms responsible for suppression of TCDD-mediated antibody responses and non-Hodgkin's lymphomas, although presently unknown, may be mechanistically related. Studies in murine models have unequivocally demonstrated that TCDD treatment impairs B cell to plasma cell differentiation, which is closely correlated with strong

attenuation of Blimp-1 upregulation. Blimp-1 is essential for plasma cell formation and high-level IgM secretion. Characteristic of B-cell lymphomas is a blockage in B-cell differentiation and uncontrolled proliferation. Importantly, the Blimp-1 locus lies on chromosome 6q21-q22.1, a region frequently deleted in B-cell lymphomas, suggesting that it may harbor a tumor suppressor gene. Notably, diffuse large B-cell lymphoma (DLBCL) represents the most frequent type of B-cell non-Hodgkin's lymphoma in the adult, accounting for 40% of all diagnoses (Abramson and Shipp, 2005). In a recent study, most non–GC-type DLBCL cases ($n = 20/26$, 77%) lacked Blimp-1 protein expression (Pasqualucci *et al.*, 2006). In addition, this study also found that a significant number of patients of activated B cell–like DLBCL, also a subclass of non-Hodgkin's lymphomas possess an inactive Blimp-1 gene. These studies strongly suggest that altered regulation of Blimp-1 is a critical event in certain types of non-Hodgkin's lymphomas. In the case of TCDD exposure, the deregulation of Blimp-1 may occur through alterations in cell signaling which controls Blimp-1 expression and/or function rather than through direct alterations to the gene, such as deletions to the Blimp-1 locus described above. This notion is further supported by studies of B-cell differentiation in murine models which have demonstrated that Blimp-1 and BCL6 reciprocally repress each others expression (see Fig. 2), hence deregulation of Blimp-1 also leads to altered regulation of BCL6. As already discussed, the primary role of BCL6 is to block B cell to plasma cell differentiation. BCL6 protein is tightly regulated and its deregulation is believed to be one of the major contributing factors in non-Hodgkin's lymphoma (Bastard *et al.*, 1994; Otsuki *et al.*, 1995).

An additional mechanism may include altered 3′IgHRR activity. Burkitt's lymphoma, another type of B-cell non-Hodgkin's lymphoma, has been characterized by a chromosomal translocation between the *c-myc* locus (chromosome 8) and the Ig loci on chromosome 2, 14, or 22. The most common is the chromosomal 8–14 translocation, which links the *c-myc* proto-oncogene to the *IgH* gene and to the 3′IgHRR (Kanda *et al.*, 2000). Studies demonstrated that the κB site within the hs4 enhancer is required for the transcriptional activation of the translocated *c-myc* gene and that NF-κB/Rel proteins regulate *c-myc* expression in human Burkitt's lymphoma cell lines (Kanda *et al.*, 2000). Additionally, in a transgenic mouse, the 3′IgHRR was demonstrated to deregulate *c-myc* expression *in vivo*. These mice displayed an increased c-Myc expression selective to B cells, higher rates of cell proliferation and cell cycle progression, and the development of B-cell lymphomas histologically similar to Burkitt's lymphoma (Wang and Boxer, 2005). These studies support a significant role of the 3′IgHRR in *c-myc* deregulation and the development of Burkitt's lymphoma.

In determining the actual risk of altered 3′IgHRR activity by TCDD and dioxin-like compounds and the development of Burkitt's lymphoma in humans, an important consideration would be whether the mouse and human 3′IgHRR are regulated similarly. Comparisons between the mouse and human *IgH* locus and the 3′IgHRR have identified structural differences including (1) a large duplication at the 3′ end of the human *IgH* locus, which consists of several constant region genes and the 3′IgHRR (Fig. 1); (2) a 3′IgHRR (hs3, hs1,2, and hs4 homologous to mouse hs3a, hs1,2, and hs4) located 3′ of $C\alpha_1$ and $C\alpha_2$ with the two hs1,2 enhancers inverted with respect to each other; and (3) an overall similarity between the mouse and human core 3′IgHRR enhancer sequences (~500 bp) of ~65% (Chen and Birshtein, 1997; Mills *et al.*, 1997; Pinaud *et al.*, 1997). However, short segments of high similarity were identified in the vicinity of each 3′IgHRR enhancer (hs3a, hs1,2, and hs4), some of which corresponded to known transcription factor binding sites and to distinctive structural features (Sepulveda *et al.*, 2005). The human hs1,2 enhancer and to a lesser extent the hs4 enhancer have also been shown in a human mature B-cell line to activate the germline promoters for the α_1, α_2, and γ_3 heavy chains with the greatest activation occurring when all of the human 3′IgHRR enhancers are linked (Hu *et al.*, 2000). These results validate a role of the 3′IgHRR in human Ig expression and class switch recombination as demonstrated in mice.

Additionally, similar transcription factors such as NF-κB, AP-1, Oct, and Sp-1 are thought to regulate the human hs3, hs1,2, and hs4 enhancers (Denizot *et al.*, 2001; Guglielmi *et al.*, 2004). Closer examination of transcription factor binding sites has identified differences in that each of the two human hs1,2 core regions contain one of the two Pax5 sites found in the mouse hs1,2 and also lack one of the κB and μE5 sites (Mills *et al.*, 1997). Moreover, when fragments of the human hs1,2 located immediately outside the minimal core region were included in reporter assays, significantly less reporter activity was observed, suggesting the possibility of additional negative regulatory sequences. Similarly, the human hs4 core lacks the Pax5 site and has one versus two octomer sites (Mills *et al.*, 1997; Sepulveda *et al.*, 2004). With further analysis, protein binding to either the κB or the octomer site of the hs4 enhancer appeared to be critical to the activity of both the human and the mouse hs4 in mature cells (Kanda *et al.*, 2000; Michaelson *et al.*, 1996; Mills *et al.*, 1997).

Because TCDD has been shown to alter NFκB, Oct, and AP-1 binding and to suppress 3′IgHRR activity in mouse cellular models, the human 3′IgHRR may be a sensitive target for regulation by dioxins. Indeed, the overlapping DRE- and κB-binding motifs are highly conserved between the mouse and human hs4 enhancer with 10 of 11 identical nucleotides for the DRE sequences and 9 of 10 identical nucleotides for the κB sequences (Guglielmi *et al.*, 2004). Additionally, the DRE in the hs1,2 enhancer was also fairly well conserved with 8 of 11 nucleotides identical, including a consensus core DRE

(GCGTG) (Denizot *et al.*, 2001). Therefore, the human AhR/ARNT may also bind to the DRE within the human hs1,2 and hs4 enhancer. Additionally, the close proximity of the κB- and DRE-binding motifs in the human enhancers may implicate an interaction between the AhR and NF-κB/Rel as suggested in the CH12.LX cells. Therefore, the human 3′IgHRR may also be sensitive target of TCDD and dioxin-like compounds. Independent of the 3′IgHRR, other studies with mammary cancer cell lines have demonstrated a TCDD-induced co-operative interaction between the AhR and the NF-κB/Rel subunit RelA that resulted in transactivation of a c-myc promoter (Kim *et al.*, 2000). Therefore, multiple mechanisms may play a role in the risk of developing non-Hodgkin's lymphomas following exposure to TCDD and other dioxin-like compounds.

In summary, there is a significant body of research demonstrating a direct effect of TCDD on B-cell maturation and function as well as various lines of evidence supporting an as yet undefined role of the AhR in these effects. Additionally, considerable progress has been made in identifying molecular targets that may be responsible in mediating the effects of TCDD and other AhR ligands on B-cell dysfunction. The immerging picture is, not surprisingly, complex and one involving several molecular pathways including the AhR. The actual interactions between these pathways are unclear and appear to involve interactions at various levels of regulation (i.e., transcriptional, protein-protein, negative feedback loops, indirect and direct effects, etc.). Our current understanding has primarily relied on mouse models, and the translation to effects on human B cells and humoral immunity is less clear. Therefore, the current challenge is to determine how TCDD and the AhR affect human B cells and humoral immunity and to confirm or refute that similar molecular pathways are affected in human B cells as in mouse B cells. As discussed in this review, efforts have been made in this direction but continued progress in developing adequate human models is needed. Notably, there are examples of differences between the human and mouse (e.g., DRE and Pax5 regulation of CD19 and the Ig and 3′IgHRR sequence differences) that may complicate the translation of mouse studies to human. Likewise, it is noteworthy that much of the current understanding surrounding the molecular biology of B-cell maturation, differentiation, and Ig gene regulation comes from studies performed in the mouse and remains to be confirmed in human B cells. Although the above discussion has focused primarily on the immunotoxic effects of TCDD, the ever-increasing evidence pointing toward a physiological role for the AhR in biological processes supports continued efforts in elucidating AhR function. Advancement in this area could have significant clinical implications and facilitate insight into disease processes and potential therapeutic development.

Summary of key findings for the effects of TCDD on B cells

- B cells are directly targeted by TCDD.
- B-cell activation induces marked upregulation of AhR expression.
- AhR expression in B cells is absolutely required for suppression of IgM production by TCDD.
- B cells are affected by TCDD at multiple stages of maturation and differentiation.
- TCDD affects immediate early events (i.e., transcriptionally independent) as well as later events (i.e., transcriptionally dependent) in B cells.
- TCDD exerts modest effects on B-cell proliferation.
- A subset of genes have been identified in B cells that are transcriptionally altered by the TCDD-activated AhR.
- TCDD impairs B-cell differentiation and IgM regulation.
- Suppression of IgM secretion has been demonstrated in mouse and human B cells; however, it is yet unclear whether the molecular mechanisms for B-cell suppression are identical in both species.

FUNDING

National Institutes of Health (R01 ES002520, P42 ES004911) to N.E.K.; National Institutes of Health (R01ES014676) and Colgate-Palmolive Grants for Alternative Research to C.E.W.S.

ACKNOWLEDGMENTS

We thank Mrs Kimberly Hambleton for administrative assistance in preparing and submitting the manuscript.

REFERENCES

Abramson, J. S., and Shipp, M. A. (2005). Advances in the biology and therapy of diffuse large B-cell lymphoma: moving toward a molecularly targeted approach. *Blood* **106,** 1164–1174.

Allan, L. L., and Sherr, D. H. (2005). Constitutive activation and environmental chemical induction of the aryl hydrocarbon receptor/transcription factor in activated human B lymphocytes. *Mol. Pharmacol.* **67,** 1740–1750.

Andersson, P., Ridderstad, A., McGuire, J., Pettersson, S., Poellinger, L., and Hanberg, A. (2003). A constitutively active aryl hydrocarbon receptor causes loss of peritoneal B1 cells. *Biochem. Biophys. Res. Commun.* **302,** 336–341.

Angelin-Duclos, C., Cattoretti, G., Chang, D. H., Lin, K. I., Lin, Y., Yu, J., and Calame, K. (1999). Role of B-lymphocyte-induced maturation protein-1 in terminal differentiation of B cells and other cell lineages. *Cold Spring Harb. Symp. Quant. Biol.* **64,** 61–70.

Baccarelli, A., Mocarelli, P., Patterson, D. G., Jr., Bonzini, M., Pesatori, A. C., Caporaso, N., and Landi, M. T. (2002). Immunologic effects of dioxin: new results from Seveso and comparison with other studies. *Environ. Health. Perspect.* **110,** 1169–1173.

Barberis, A., Widenhorn, K., Vitelli, L., and Busslinger, M. (1990). A novel B-cell lineage-specific transcription factor present at early but not late stages of differentiation. *Genes Dev.* **4,** 849–859.

Bastard, C., Deweindt, C., Kerckaert, J. P., Lenormand, B., Rossi, A., Pezzella, F., Fruchart, C., Duval, C., Monconduit, M., and Tilly, H. (1994). LAZ3 rearrangements in non-Hodgkin's lymphoma: correlation with

histology, immunophenotype, karyotype, and clinical outcome in 217 patients. *Blood* **83,** 2423–2427.

Becher, H., Flesch-Janys, D., Kauppinen, T., Kogevinas, M., Steindorf, K., Manz, A., and Wahrendorf, J. (1996). Cancer mortality in German male workers exposed to phenoxy herbicides and dioxins. *Cancer Causes Control* **7,** 312–321.

Bhattacharya, S., Conolly, R. B., Kaminski, N. E., Thomas, R. S., Andersen, M. E., and Zhang, Q. (2010). A bistable switch underlying B-cell differentiation and its disruption by the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol. Sci.* **115,** 51–65.

Birnbaum, L. S., and Tuomisto, J. (2000). Non-carcinogenic effects of TCDD in animals. *Food Addit. Contam.* **17,** 275–288.

Bishop, G. A., Hsing, Y., Hostager, B. S., Jalukar, S. V., Ramirez, L. M., and Tomai, M. A. (2000). Molecular mechanisms of B lymphocyte activation by the immune response modifier R-848. *J. Immunol.* **165,** 5552–5557.

Calfon, M., Zeng, H., Urano, F., Till, J. H., Hubbard, S. R., Harding, H. P., Clark, S. G., and Ron, D. (2002). IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* **415,** 92–96.

Chauveau, C., Pinaud, E., and Cogne, M. (1998). Synergies between regulatory elements of the immunoglobulin heavy chain locus and its palindromic 3′ locus control region. *Eur. J. Immunol.* **28,** 3048–3056.

Chen, C., and Birshtein, B. K. (1997). Virtually identical enhancers containing a segment of homology to murine 3'IgH-E(hs1,2) lie downstream of human Ig C alpha 1 and C alpha 2 genes. *J. Immunol.* **159,** 1310–1318.

Clark, D. A., Gauldie, J., Szewczuk, M. R., and Sweeney, G. (1981). Enhanced suppressor cell activity as a mechanism of immunosuppression by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Proc. Soc. Exp. Biol. Med.* **168,** 290–299.

Clark, G. C., Blank, J. A., Germolec, D. R., and Luster, M. I. (1991). 2,3,7,8-Tetrachlorodibenzo-p-dioxin stimulation of tyrosine phosphorylation in B lymphocytes: potential role in immunosuppression. *Mol. Pharmacol.* **39,** 495–501.

Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides, I. O. M. (1994). Cancer. In *Veterans and Agent Orange: Health Effects of Herbicides Used in Vietnam*, pp. 433–590. The National Academies Press, Washington, DC.

Crawford, R. B., Holsapple, M. P., and Kaminski, N. E. (1997). Leukocyte activation induces aryl hydrocarbon receptor up-regulation, DNA binding, and increased Cyp1a1 expression in the absence of exogenous ligand. *Mol. Pharmacol.* **52,** 921–927.

Crawford, R. B., Sulentic, C. E., Yoo, B. S., and Kaminski, N. E. (2003). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) alters the regulation and posttranslational modification of p27kip1 in lipopolysaccharide-activated B cells. *Toxicol. Sci.* **75,** 333–342.

Davis, D., and Safe, S. (1988). Immunosuppressive activities of polychlorinated dibenzofuran congeners: quantitative structure-activity relationships and interactive effects. *Toxicol. Appl. Pharmacol.* **94,** 141–149.

De Abrew, K. N., Kaminski, N. E., and Thomas, R. S. (2010). An integrated genomic analysis of aryl hydrocarbon receptor-mediated inhibition of B-cell differentiation. *Toxicol. Sci.* **118,** 454–469.

Denison, M. S., Pandini, A., Nagy, S. R., Baldwin, E. P., and Bonati, L. (2002). Ligand binding and activation of the Ah receptor. *Chem. Biol. Interact.* **141,** 3–24.

Denizot, Y., Pinaud, E., Aupetit, C., Le Morvan, C., Magnoux, E., Aldigier, J. C., and Cogne, M. (2001). Polymorphism of the human alpha1 immunoglobulin gene 3′ enhancer hs1,2 and its relation to gene expression. *Immunology* **103,** 35–40.

Dent, A. L., Shaffer, A. L., Yu, X., Allman, D., and Staudt, L. M. (1997). Control *of* inflammation, cytokine expression, and germinal center formation by BCL-6. *Science* **276,** 589–592.

Dinatale, B. C., Schroeder, J. C., Francey, L. J., Kusnadi, A., and Perdew, G. H. (2010). Mechanistic insights into the events that lead to synergistic induction of IL6 transcription upon activation of the Ah receptor and inflammatory signaling. *J. Biol. Chem.* **285,** 24388–24397.

Dooley, R. K., and Holsapple, M. P. (1988). Elucidation of cellular targets responsible for tetrachlorodibenzo-p-dioxin (TCDD)-induced suppression of antibody responses: I. The role of the B lymphocyte. *Immunopharmacology* **16,** 167–180.

Eilers, M. (1999). Control of cell proliferation by Myc family genes. *Mol. Cells* **9,** 1–6.

Fernandez-Salguero, P., Pineau, T., Hilbert, D. M., McPhail, T., Lee, S. S., Kimura, S., Nebert, D. W., Rudikoff, S., Ward, J. M., and Gonzalez, F. J. (1995). Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. *Science* **268,** 722–726.

Fernandez-Salguero, P. M., Ward, J. M., Sundberg, J. P., and Gonzalez, F. J. (1997). Lesions of aryl-hydrocarbon receptor-deficient mice. *Vet. pathol.* **34,** 605–614.

Fine, J. S., Gasiewicz, T. A., and Silverstone, A. E. (1989). Lymphocyte stem cell alterations following perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Mol. Pharmacol.* **35,** 18–25.

Flaveny, C. A., and Perdew, G. H. (2009). Transgenic humanized AHR mouse reveals differences between human and mouse AHR ligand selectivity. *Mol. Cell. Pharmacol.* **1,** 119–123.

Floret, N., Mauny, F., Challier, B., Arveux, P., Cahn, J. Y., and Viel, J. F. (2003). Dioxin emissions from a solid waste incinerator and risk of non-Hodgkin lymphoma. *Epidemiology* **14,** 392–398.

Frazier, D. E., Jr., Silverstone, A. E., Soults, J. A., and Gasiewicz, T. A. (1994). The thymus does not mediate 2,3,7,8-tetrachlorodibenzo-p-dioxin-elicited alterations in bone marrow lymphocyte stem cells. *Toxicol. Appl. Pharmacol.* **124,** 242–247.

Fujimaki, H., Nohara, K., Kobayashi, T., Suzuki, K., Eguchi-Kasai, K., Tsukumo, S., Kijima, M., and Tohyama, C. (2002). Effect of a single oral dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin on immune function in male NC/Nga mice. *Toxicol. Sci.* **66,** 117–124.

Fukuda, T., Yoshida, T., Okada, S., Hatano, M., Miki, T., Ishibashi, K., Okabe, S., Koseki, H., Hirosawa, S., Taniguchi, M., Miyasaka, N., and Tokuhisa, T. (1997). Disruption of the Bcl6 gene results in an impaired germinal center formation. *J. Exp. Med.* **186,** 439–448.

Guglielmi, L., Truffinet, V., Magnoux, E., Cogne, M., and Denizot, Y. (2004). The polymorphism of the locus control region lying downstream the human IgH locus is restricted to hs1,2 but not to hs3 and hs4 enhancers. *Immunol. Lett.* **94,** 77–81.

Hahn, M. E. (2002). Aryl hydrocarbon receptors: diversity and evolution. *Chem. Biol. Interact.* **141,** 131–160.

Hardy, R. R., Carmack, C. E., Shinton, S. A., Kemp, J. D., and Hayakawa, K. (1991). Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow. *J. Exp. Med.* **173,** 1213–1225.

Harper, P. A., Wong, J. Y., Lam, M. S., and Okey, A. B. (2002). Polymorphisms in the human AH receptor. *Chem. Biol. Interact.* **141,** 161–187.

Henseler, R. A., Romer, E. J., and Sulentic, C. E. (2009). Diverse chemicals including aryl hydrocarbon receptor ligands modulate transcriptional activity of the 3′immunoglobulin heavy chain regulatory region. *Toxicology* **261,** 9–18.

Hirabayashi, Y., and Inoue, T. (2009). Aryl hydrocarbon receptor biology and xenobiotic responses in hematopoietic progenitor cells. *Biochem. Pharmacol.* **77,** 521–535.

Holsapple, M. P., Dooley, R. K., McNerney, P. J., and McCay, J. A. (1986a). Direct suppression of antibody responses by chlorinated dibenzodioxins in cultured spleen cells from (C57BL/6 x C3H)F1 and DBA/2 mice. *Immunopharmacology* **12,** 175–186.

Holsapple, M. P., McCay, J. A., and Barnes, D. W. (1986b). Immunosuppression without liver induction by subchronic exposure to 2,7-dichlorodibenzo-p-dioxin in adult female B6C3F1 mice. *Toxicol. Appl. Pharmacol.* **83,** 445–455.

Holsapple, M. P., McNerney, P. J., Barnes, D. W., and White, K. L., Jr. (1984). Suppression of humoral antibody production by exposure to 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin. *J. Pharmacol. Exp. Ther.* **231,** 518–526.

Holsapple, M. P., Morris, D. L., Wood, S. C., and Snyder, N. K. (1991a). 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced changes in immunocompetence: possible mechanisms. *Annu. Rev. Pharmacol. Toxicol.* **31,** 73–100.

Holsapple, M. P., Snyder, N. K., Wood, S. C., and Morris, D. L. (1991b). A review of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced changes in immunocompetence: 1991 update. *Toxicology* **69,** 219–255.

Hooiveld, M., Heederik, D. J., Kogevinas, M., Boffetta, P., Needham, L. L., Patterson, D. G., Jr., and Bueno-de-Mesquita, H. B. (1998). Second follow-up of a Dutch cohort occupationally exposed to phenoxy herbicides, chlorophenols, and contaminants. *Am. J. Epidemiol.* **147,** 891–901.

Horcher, M., Souabni, A., and Busslinger, M. (2001). Pax5/BSAP maintains the identity of B cells in late B lymphopoiesis. *Immunity* **14,** 779–790.

Hu, Y., Pan, Q., Pardali, E., Mills, F. C., Bernstein, R. M., Max, E. E., Sideras, P., and Hammarstrom, L. (2000). Regulation of germline promoters by the two human Ig heavy chain 3′ alpha enhancers. *J. Immunol.* **164,** 6380–6386.

Inouye, K., Ito, T., Fujimaki, H., Takahashi, Y., Takemori, T., Pan, X., Tohyama, C., and Nohara, K. (2003). Suppressive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the high-affinity antibody response in C57BL/6 mice. *Toxicol. Sci.* **74,** 315–324.

Jensen, B. A., Leeman, R. J., Schlezinger, J. J., and Sherr, D. H. (2003). Aryl hydrocarbon receptor (AhR) agonists suppress interleukin-6 expression by bone marrow stromal cells: an immunotoxicology study. *Environ. Health.* **2,** 16.

Kallies, A., Hasbold, J., Tarlinton, D. M., Dietrich, W., Corcoran, L. M., Hodgkin, P. D., and Nutt, S. L. (2004). Plasma cell ontogeny defined by quantitative changes in blimp-1 expression. *J. Exp. Med.* **200,** 967–977.

Kanda, K., Hu, H. M., Zhang, L., Grandchamps, J., and Boxer, L. M. (2000). NF-kappa B activity is required for the deregulation of c-myc expression by the immunoglobulin heavy chain enhancer. *J. Biol. Chem.* **275,** 32338–32346.

Karras, J. G., Morris, D. L., Matulka, R. A., Kramer, C. M., and Holsapple, M. P. (1996). 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) elevates basal B-cell intracellular calcium concentration and suppresses surface Ig- but not CD40-induced antibody secretion. *Toxicol. Appl. Pharmacol.* **137,** 275–284.

Keller, A. D., and Maniatis, T. (1991). Identification and characterization of a novel repressor of beta-interferon gene expression. *Genes Dev.* **5,** 868–879.

Kerkvliet, N. I. (2002). Recent advances in understanding the mechanisms of TCDD immunotoxicity. *Int. Immunopharmacol.* **2,** 277–291.

Kerkvliet, N. I., Steppan, L. B., Brauner, J. A., Deyo, J. A., Henderson, M. C., Tomar, R. S., and Buhler, D. R. (1990). Influence of the Ah locus on the humoral immunotoxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin: evidence for Ah-receptor-dependent and Ah-receptor-independent mechanisms of immunosuppression. *Toxicol. Appl. Pharmacol.* **105,** 26–36.

Khamlichi, A. A., Pinaud, E., Decourt, C., Chauveau, C., and Cogne, M. (2000). The 3′ IgH regulatory region: a complex structure in a search for a function. *Adv. Immunol.* **75,** 317–345.

Kim, D. W., Gazourian, L., Quadri, S. A., Romieu-Mourez, R., Sherr, D. H., and Sonenshein, G. E. (2000). The RelA NF-kappaB subunit and the aryl hydrocarbon receptor (AhR) cooperate to transactivate the c-myc promoter in mammary cells. *Oncogene* **19,** 5498–5506.

Kimata, H. (2003). 2,3,7,8-tetrachlorodibenzo-p-dioxin selectively enhances spontaneous IgE production in B cells from atopic patients. *Int. J. Hyg. Environ. Health* **206,** 601–604.

Kishimoto, T. (2006). Interleukin-6: discovery of a pleiotropic cytokine. *Arthritis Res. Ther.* **8**(Suppl. 2), S2.

Klein, S., Sablitzky, F., and Radbruch, A. (1984). Deletion of the IgH enhancer does not reduce immunoglobulin heavy chain production of a hybridoma IgD class switch variant. *Embo. J.* **3,** 2473–2476.

Kogevinas, M., Becher, H., Benn, T., Bertazzi, P. A., Boffetta, P., Bueno-de-Mesquita, H. B., Coggon, D., Colin, D., Flesch-Janys, D., Fingerhut, M., Green, L., Kauppinen, T., Littorin, M., Lynge, E., Mathews, J. D., Neuberger, M., Pearce, N., and Saracci, R. (1997). Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. An expanded and updated international cohort study. *Am. J. Epidemiol.* **145,** 1061–1075.

Kogevinas, M., Kauppinen, T., Winkelmann, R., Becher, H., Bertazzi, P. A., Bueno-de-Mesquita, H. B., Coggon, D., Green, L., Johnson, E., Littorin, M., *et al.* (1995). Soft tissue sarcoma and non-Hodgkin's lymphoma in workers exposed to phenoxy herbicides, chlorophenols, and dioxins: two nested case-control studies. *Epidemiology* **6,** 396–402.

Kozmik, Z., Wang, S., Dorfler, P., Adams, B., and Busslinger, M. (1992). The promoter of the CD19 gene is a target for the B-cell-specific transcription factor BSAP. *Mol. Cell Biol.* **12,** 2662–2672.

Kramer, C. M., Johnson, K. W., Dooley, R. K., and Holsapple, M. P. (1987). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) enhances antibody production and protein kinase activity in murine B cells. *Biochem. Biophys. Res. Commun.* **145,** 25–33.

Lai, Z. W., Pineau, T., and Esser, C. (1996). Identification of dioxin-responsive elements (DREs) in the 5′ regions of putative dioxin-inducible genes. *Chem. Biol. Interact.* **100,** 97–112.

Lavin, A. L., Hahn, D. J., and Gasiewicz, T. A. (1998). Expression of functional aromatic hydrocarbon receptor and aromatic hydrocarbon nuclear translocator proteins in murine bone marrow stromal cells. Arch. *Biochem. Biophys.* **352,** 9–18.

Lawrence, B. P., Leid, M., and Kerkvliet, N. I. (1996). Distribution and behavior of the Ah receptor in murine T lymphocytes. *Toxicol. Appl. Pharmacol.* **138,** 275–284.

Lin, K. I., Angelin-Duclos, C., Kuo, T. C., and Calame, K. (2002). Blimp-1-dependent repression of Pax-5 is required for differentiation of B cells to immunoglobulin M-secreting plasma cells. *Mol. Cell. Biol.* **22,** 4771–4780.

Lin, Y., Wong, K., and Calame, K. (1997). Repression of c-myc transcription by Blimp-1, an inducer of terminal B cell differentiation. *Science* **276,** 596–599.

Linderson, Y., Eberhard, D., Malin, S., Johansson, A., Busslinger, M., and Pettersson, S. (2004). Corecruitment of the Grg4 repressor by PU.1 is critical for Pax5-mediated repression of B-cell-specific genes. *EMBO Rep.* **5,** 291–296.

Lu, H., Crawford, R. B., North, C. M., Kaplan, B. L., and Kaminski, N. E. (2009). Establishment of an immunoglobulin m antibody-forming cell response model for characterizing immunotoxicity in primary human B cells. *Toxicol. Sci.* **112,** 363–373.

Lu, H., Crawford, R. B., Suarez-Martinez, J. E., Kaplan, B. L., and Kaminski, N. E. (2010). Induction of the aryl hydrocarbon receptor-responsive genes and modulation of the immunoglobulin M response by 2, 3, 7, 8–tetrachlorodibenzo-p-dioxin in primary human B cells. *Toxicol. Sci.* **118,** 86–97.

Luebke, R. W., Copeland, C. B., Daniels, M., Lambert, A. L., and Gilmour, M. I. (2001). Suppression of allergic immune responses to house dust mite (HDM) in rats exposed to 2,3,7,8-TCDD. *Toxicol. Sci.* **62,** 71–79.

Luster, M. I., Germolec, D. R., Clark, G., Wiegand, G., and Rosenthal, G. J. (1988). Selective effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin and corticosteroid on in vitro lymphocyte maturation. *J. Immunol.* **140,** 928–935.

Madisen, L., and Groudine, M. (1994). Identification of a locus control region in the immunoglobulin heavy-chain locus that deregulates c-myc expression in plasmacytoma and Burkitt's lymphoma cells. *Genes Dev.* **8,** 2212–2226.

Maitra, S., and Atchison, M. (2000). BSAP can repress enhancer activity by targeting PU.1 function. *Mol. Cell. Biol.* **20,** 1911–1922.

Marcus, R. S., Holsapple, M. P., and Kaminski, N. E. (1998). Lipopolysaccharide activation of murine splenocytes and splenic B cells increased the expression of aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator. *J. Pharmacol. Exp. Ther.* **287,** 1113–1118.

Masten, S. A., and Shiverick, K. T. (1995). The Ah receptor recognizes DNA binding sites for the B cell transcription factor, BSAP: a possible mechanism for dioxin-mediated alteration of CD19 gene expression in human B lymphocytes. *Biochem. Biophys. Res. Commun.* **212,** 27–34.

McMillan, B. J., and Bradfield, C. A. (2007). The aryl hydrocarbon receptor sans xenobiotics: endogenous function in genetic model systems. *Mol. Pharmacol.* **72,** 487–498.

Michaelson, J. S., Singh, M., Snapper, C. M., Sha, W. C., Baltimore, D., and Birshtein, B. K. (1996). Regulation of 3′ IgH enhancers by a common set of factors, including kappa B-binding proteins. *J. Immunol.* **156,** 2828–2839.

Mills, F. C., Harindranath, N., Mitchell, M., and Max, E. E. (1997). Enhancer complexes located downstream of both human immunoglobulin Calpha genes. *J. Exp. Med.* **186,** 845–858.

Mimura, J., Yamashita, K., Nakamura, K., Morita, M., Takagi, T. N., Nakao, K., Ema, M., Sogawa, K., Yasuda, M., Katsuki, M., and Fujii-Kuriyama, Y. (1997). Loss of teratogenic response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. *Genes Cells* **2,** 645–654.

Mora-Lopez, F., Reales, E., Brieva, J. A., and Campos-Caro, A. (2007). Human BSAP and BLIMP1 conform an autoregulatory feedback loop. *Blood* **110,** 3150–3157.

Morris, D. L., Karras, J. G., and Holsapple, M. P. (1993). Direct effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on responses to lipopolysaccharide (LPS) by isolated murine B-cells. *Immunopharmacology* **26,** 105–112.

Murante, F. G., and Gasiewicz, T. A. (2000). Hemopoietic progenitor cells are sensitive targets of 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57BL/6J mice. *Toxicol. Sci.* **54,** 374–383.

Nera, K. P., Kohonen, P., Narvi, E., Peippo, A., Mustonen, L., Terho, P., Koskela, K., Buerstedde, J. M., and Lassila, O. (2006). Loss of Pax5 promotes plasma cell differentiation. *Immunity* **24,** 283–293.

Neurath, M. F., Max, E. E., and Strober, W. (1995). Pax5 (BSAP) regulates the murine immunoglobulin 3′ alpha enhancer by suppressing binding of NF-alpha P, a protein that controls heavy chain transcription. *Proc. Natl. Acad. Sci. U.S.A.* **92,** 5336–5340.

Neurath, M. F., Strober, W., and Wakatsuki, Y. (1994). The murine Ig 3′ alpha enhancer is a target site with repressor function for the B cell lineage-specific transcription factor BSAP (NF-HB, S alpha-BP). J. *Immunol.* **153,** 730–742.

Niu, H., Ye, B. H., and Dalla-Favera, R. (1998). Antigen receptor signaling induces MAP kinase-mediated phosphorylation and degradation of the BCL-6 transcription factor. *Genes Dev.* **12,** 1953–1961.

North, C. M., Crawford, R. B., Lu, H., and Kaminski, N. E. (2009). Simultaneous in vivo time course and dose response evaluation for TCDD-induced impairment of the LPS-stimulated primary IgM response. *Toxicol. Sci.* **112,** 123–132.

North, C. M., Crawford, R. B., Lu, H., and Kaminski, N. E. (2010). 2,3,7,8-tetrachlorodibenzo-p-dioxin-mediated suppression of toll-like receptor stimulated B-lymphocyte activation and initiation of plasmacytic differentiation. *Toxicol. Sci.* **116,** 99–112.

Nutt, S. L., Heavey, B., Rolink, A. G., and Busslinger, M. (1999). Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. *Nature* **401,** 556–562.

Ogawa, M. (1993). Differentiation and proliferation of hematopoietic stem cells. *Blood* **81,** 2844–2853.

Okey, A. B. (2007). An aryl hydrocarbon receptor odyssey to the shores of toxicology: the Deichmann Lecture, International Congress of Toxicology-XI. *Toxicol. Sci.* **98,** 5–38.

Ong, J., Stevens, S., Roeder, R. G., and Eckhardt, L. A. (1998). 3' IgH enhancer elements shift synergistic interactions during B cell development. *J. Immunol.* **160,** 4896–4903.

Otsuki, T., Yano, T., Clark, H. M., Bastard, C., Kerckaert, J. P., Jaffe, E. S., and Raffeld, M. (1995). Analysis of LAZ3 (BCL-6) status in B-cell non-Hodgkin's lymphomas: results of rearrangement and gene expression studies and a mutational analysis of coding region sequences. *Blood* **85,** 2877–2884.

Pasqualucci, L., Compagno, M., Houldsworth, J., Monti, S., Grunn, A., Nandula, S. V., Aster, J. C., Murty, V. V., Shipp, M. A., and Dalla-Favera, R. (2006). Inactivation of the PRDM1/BLIMP1 gene in diffuse large B cell lymphoma. *J. Exp. Med.* **203,** 311–317.

Pinaud, E., Aupetit, C., Chauveau, C., and Cogne, M. (1997). Identification of a homolog of the C alpha 3′/hs3 enhancer and of an allelic variant of the 3′IgH/hs1,2 enhancer downstream of the human immunoglobulin alpha 1 gene. *Eur. J. Immunol.* **27,** 2981–2985.

Piskurich, J. F., Lin, K. I., Lin, Y., Wang, Y., Ting, J. P., and Calame, K. (2000). BLIMP-I mediates extinction of major histocompatibility class II transactivator expression in plasma cells. *Nat. Immunol.* **1,** 526–532.

Puga, A., Barnes, S. J., Chang, C., Zhu, H., Nephew, K. P., Khan, S. A., and Shertzer, H. G. (2000). Activation of transcription factors activator protein-1 and nuclear factor-kappaB by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Biochem. Pharmacol.* **59,** 997–1005.

Puga, A., Nebert, D. W., and Carrier, F. (1992). Dioxin induces expression of c-fos and c-jun proto-oncogenes and a large increase in transcription factor AP-1. *DNA Cell Biol.* **11,** 269–281.

Reimold, A. M., Iwakoshi, N. N., Manis, J., Vallabhajosyula, P., Szomolanyi-Tsuda, E., Gravallese, E. M., Friend, D., Grusby, M. J., Alt, F., and Glimcher, L. H. (2001). Plasma cell differentiation requires the transcription factor XBP-1. *Nature* **412,** 300–307.

Reimold, A. M., Ponath, P. D., Li, Y. S., Hardy, R. R., David, C. S., Strominger, J. L., and Glimcher, L. H. (1996). Transcription factor B cell lineage-specific activator protein regulates the gene for human X-box binding protein 1. *J. Exp. Med.* **183,** 393–401.

Rinkenberger, J. L., Wallin, J. J., Johnson, K. W., and Koshland, M. E. (1996). An interleukin-2 signal relieves BSAP (Pax5)-mediated repression of the immunoglobulin J chain gene. *Immunity* **5,** 377–386.

Sakai, R., Kajiume, T., Inoue, H., Kanno, R., Miyazaki, M., Ninomiya, Y., and Kanno, M. (2003). TCDD treatment eliminates the long-term reconstitution activity of hematopoietic stem cells. *Toxicol. Sci.* **72,** 84–91.

Saleque, S., Singh, M., and Birshtein, B. K. (1999). Ig heavy chain expression and class switching in vitro from an allele lacking the 3' enhancers DNase I-hypersensitive hs3A and hs1,2. *J. Immunol.* **162,** 2791–2803.

Saleque, S., Singh, M., Little, R. D., Giannini, S. L., Michaelson, J. S., and Birshtein, B. K. (1997). Dyad symmetry within the mouse 3' IgH regulatory region includes two virtually identical enhancers (C alpha3'E and hs3). *J. Immunol.* **158,** 4780–4787.

Saracci, R., Kogevinas, M., Bertazzi, P. A., Bueno de Mesquita, B. H., Coggon, D., Green, L. M., Kauppinen, T., L'Abbe, K. A., Littorin, M., Lynge, E., *et al.* (1991). Cancer mortality in workers exposed to chlorophenoxy herbicides and chlorophenols. *Lancet* **338,** 1027–1032.

Schliephake, D. E., and Schimpl, A. (1996). Blimp-1 overcomes the block in IgM secretion in lipopolysaccharide/anti-mu F(ab')2-co-stimulated B lymphocytes. *Eur. J. Immunol.* **26,** 268–271.

Schmidt, J. V., Su, G. H., Reddy, J. K., Simon, M. C., and Bradfield, C. A. (1996). Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. *Proc. Natl. Acad. Sci. U.S.A.* **93,** 6731–6736.

Schneider, D., Manzan, M. A., Crawford, R. B., Chen, W., and Kaminski, N. E. (2008). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-mediated impairment of B cell differentiation involves dysregulation of paired box 5 (Pax5) isoform, pax5a. *J. Pharmacol. Exp. Ther.* **326,** 463–474.

Schneider, D., Manzan, M. A., Yoo, B. S., Crawford, R. B., and Kaminski, N. (2009). Involvement of Blimp-1 and AP-1 dysregulation in the 2,3,7,8-Tetrachlorodibenzo-p-dioxin-mediated suppression of the IgM response by B cells. *Toxicol. Sci.* **108,** 377–388.

Sepulveda, M. A., Emelyanov, A. V., and Birshtein, B. K. (2004). NF-kappa B and Oct-2 synergize to activate the human 3′ Igh hs4 enhancer in B cells. *J. Immunol.* **172,** 1054–1064.

Sepulveda, M. A., Garrett, F. E., Price-Whelan, A., and Birshtein, B. K. (2005). Comparative analysis of human and mouse 3′ Igh regulatory regions identifies distinctive structural features. *Mol. Immunol.* **42,** 605–615.

Shapiro-Shelef, M., and Calame, K. (2005). Regulation of plasma-cell development. *Nat. Rev. Immunol.* **5,** 230–242.

Shapiro-Shelef, M., Lin, K. I., McHeyzer-Williams, L. J., Liao, J., McHeyzer-Williams, M. G., and Calame, K. (2003). Blimp-1 is required for the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells. *Immunity* **19,** 607–620.

Silacci, P., Mottet, A., Steimle, V., Reith, W., and Mach, B. (1994). Developmental extinction of major histocompatibility complex class II gene expression in plasmocytes is mediated by silencing of the transactivator gene CIITA. *J. Exp. Med.* **180,** 1329–1336.

Singh, K. P., Casado, F. L., Opanashuk, L. A., and Gasiewicz, T. A. (2009a). The aryl hydrocarbon receptor has a normal function in the regulation of hematopoietic and other stem/progenitor cell populations. *Biochem. Pharmacol.* **77,** 577–587.

Singh, K. P., Wyman, A., Casado, F. L., Garrett, R. W., and Gasiewicz, T. A. (2009b). Treatment of mice with the Ah receptor agonist and human carcinogen dioxin results in altered numbers and function of hematopoietic stem cells. *Carcinogenesis* **30,** 11–19.

Singh, M., and Birshtein, B. K. (1993). NF-HB (BSAP) is a repressor of the murine immunoglobulin heavy-chain 3′ alpha enhancer at early stages of B-cell differentiation. *Mol. Cell. Biol.* **13,** 3611–3622.

Stevens, S., Ong, J., Kim, U., Eckhardt, L. A., and Roeder, R. G. (2000). Role of OCA-B in 3′-IgH enhancer function. *J. Immunol.* **164,** 5306–5312.

Suh, J., Jeon, Y. J., Kim, H. M., Kang, J. S., Kaminski, N. E., and Yang, K. H. (2002). Aryl hydrocarbon receptor-dependent inhibition of AP-1 activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin in activated B cells. *Toxicol. Appl. Pharmacol.* **181,** 116–123.

Sulentic, C. E., Holsapple, M. P., and Kaminski, N. E. (1998). Aryl hydrocarbon receptor-dependent suppression by 2,3,7, 8-tetrachlorodibenzo-p-dioxin of IgM secretion in activated B cells. *Mol. Pharmacol.* **53,** 623–629.

Sulentic, C. E., Holsapple, M. P., and Kaminski, N. E. (2000). Putative link between transcriptional regulation of IgM expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin and the aryl hydrocarbon receptor/dioxin-responsive enhancer signaling pathway. *J. Pharmacol. Exp. Ther.* **295,** 705–716.

Sulentic, C. E., Zhang, W., Na, Y. J., and Kaminski, N. E. (2004). 2,3,7,8-tetrachlorodibenzo-p-dioxin, an exogenous modulator of the 3'alpha immunoglobulin heavy chain enhancer in the CH12.LX mouse cell line. *J. Pharmacol. Exp. Ther.* **309,** 71–78.

Swanson, H. I., and Bradfield, C. A. (1993). The AH-receptor: genetics, structure and function. *Pharmacogenetics* **3,** 213–230.

Tedder, T. F., Zhou, L. J., and Engel, P. (1994). The CD19/CD21 signal transduction complex of B lymphocytes. *Immunol. Today* **15,** 437–442.

ten Tusscher, G. W., Steerenberg, P. A., van Loveren, H., Vos, J. G., von dem Borne, A. E., Westra, M., van der Slikke, J. W., Olie, K., Pluim, H. J., and Koppe, J. G. (2003). Persistent hematologic and immunologic disturbances in 8-year-old Dutch children associated with perinatal dioxin exposure. *Environ. Health Perspect.* **111,** 1519–1523.

Thurmond, T. S., and Gasiewicz, T. A. (2000). A single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin produces a time- and dose-dependent alteration in the murine bone marrow B-lymphocyte maturation profile. *Toxicol. Sci.* **58,** 88–95.

Thurmond, T. S., Staples, J. E., Silverstone, A. E., and Gasiewicz, T. A. (2000). The aryl hydrocarbon receptor has a role in the in vivo maturation of murine bone marrow B lymphocytes and their response to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol. Appl. Pharmacol.* **165,** 227–236.

Tian, Y. (2009). Ah receptor and NF-kappaB interplay on the stage of epigenome. *Biochem Pharmacol* **77,** 670–680.

Tian, Y., Rabson, A. B., and Gallo, M. A. (2002). Ah receptor and NF-kappaB interactions: mechanisms and physiological implications. *Chem. Biol. Interact.* **141,** 97–115.

Tucker, A. N., Vore, S. J., and Luster, M. I. (1986). Suppression of B cell differentiation by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Mol. Pharmacol.* **29,** 372–377.

Turner, C. A., Jr., Mack, D. H., and Davis, M. M. (1994). Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells. *Cell* **77,** 297–306.

Urbanek, P., Wang, Z. Q., Fetka, I., Wagner, E. F., and Busslinger, M. (1994). Complete block of early B cell differentiation and altered patterning of the posterior midbrain in mice lacking Pax5/BSAP. *Cell* **79,** 901–912.

Vasanwala, F. H., Kusam, S., Toney, L. M., and Dent, A. L. (2002). Repression of AP-1 function: a mechanism for the regulation of Blimp-1 expression and B lymphocyte differentiation by the B cell lymphoma-6 protooncogene. *J. Immunol.* **169,** 1922–1929.

Vecchi, A., Mantovani, A., Sironi, M., Luini, W., Cairo, M., and Garattini, S. (1980a). Effect of acute exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin on humoral antibody production in mice. *Chem. Biol. Interact.* **30,** 337–342.

Vecchi, A., Mantovani, A., Sironi, M., Luini, W., Spreafico, F., and Garattini, S. (1980b). The effect of acute administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on humoral antibody production and cell-mediated activities in mice. *Arch. Toxicol. Suppl.* **4,** 163–165.

Vecchi, A., Sironi, M., Canegrati, M. A., Recchia, M., and Garattini, S. (1983). Immunosuppressive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in strains of mice with different susceptibility to induction of aryl hydrocarbon hydroxylase. *Toxicol. Appl. Pharmacol.* **68,** 434–441.

Vogel, C. F., and Matsumura, F. (2009). A new cross-talk between the aryl hydrocarbon receptor and RelB, a member of the NF-kappaB family. *Biochem. Pharmacol.* **77,** 734–745.

Vogel, C. F., Sciullo, E., Li, W., Wong, P., Lazennec, G., and Matsumura, F. (2007a). RelB, a New Partner of Aryl Hydrocarbon Receptor-Mediated Transcription. *Mol. Endocrinol.* **21,** 2941–2955.

Vogel, C. F., Sciullo, E., and Matsumura, F. (2007b). Involvement of RelB in aryl hydrocarbon receptor-mediated induction of chemokines. *Biochem. Biophys. Res. Commun.* **363,** 722–726.

Vorderstrasse, B. A., Steppan, L. B., Silverstone, A. E., and Kerkvliet, N. I. (2001). Aryl hydrocarbon receptor-deficient mice generate normal immune responses to model antigens and are resistant to TCDD-induced immune suppression. *Toxicol. Appl. Pharmacol.* **171,** 157–164.

Vos, J. G., Moore, J. A., and Zinkl, J. G. (1973). Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the immune system of laboratory animals. *Environ. Health. Perspect.* **5,** 149–162.

Wabl, M. R., and Burrows, P. D. (1984). Expression of immunoglobulin heavy chain at a high level in the absence of a proposed immunoglobulin enhancer element in cis. *Proc. Natl. Acad. Sci. U.S.A.* **81,** 2452–2455.

Wallin, J. J., Rinkenberger, J. L., Rao, S., Gackstetter, E. R., Koshland, M. E., and Zwollo, P. (1999). B cell-specific activator protein prevents two activator factors from binding to the immunoglobulin J chain promoter until the antigen-driven stages of B cell development. *J. Biol. Chem.* **274,** 15959–15965.

Wang, J., and Boxer, L. M. (2005). Regulatory elements in the immunoglobulin heavy chain gene 3'-enhancers induce c-myc deregulation and lymphomagenesis in murine B cells. *J. Biol. Chem.* **280,** 12766–12773.

Weiss, C., Faust, D., Durk, H., Kolluri, S. K., Pelzer, A., Schneider, S., Dietrich, C., Oesch, F., and Gottlicher, M. (2005). TCDD induces c-jun expression via a novel Ah (dioxin) receptor-mediated p38-MAPK-dependent pathway. *Oncogene* **24,** 4975–4983.

White, S. S., and Birnbaum, L. S. (2009). An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev.* **27,** 197–211.

Williams, C. E., Crawford, R. B., Holsapple, M. P., and Kaminski, N. E. (1996). Identification of functional aryl hydrocarbon receptor and aryl hydrocarbon receptor nuclear translocator in murine splenocytes. *Biochem. Pharmacol.* **52,** 771–780.

Wong, J. M., Harper, P. A., Meyer, U. A., Bock, K. W., Morike, K., Lagueux, J., Ayotte, P., Tyndale, R. F., Sellers, E. M., Manchester, D. K., *et al*. (2001). Ethnic variability in the allelic distribution of human aryl hydrocarbon receptor codon 554 and assessment of variant receptor function in vitro. *Pharmacogenetics* **11,** 85–94.

Wood, S. C., and Holsapple, M. P. (1993). Direct suppression of superantigen-induced IgM secretion in human lymphocytes by 2,3,7,8-TCDD. *Toxicol. Appl. Pharmacol.* **122,** 308–313.

Wood, S. C., Karras, J. G., and Holsapple, M. P. (1992). Integration of the human lymphocyte into immunotoxicological investigations. *Fundam. Appl. Toxicol.* **18,** 450–459.

Yamaguchi, K., Near, R. I., Matulka, R. A., Shneider, A., Toselli, P., Trombino, A. F., and Sherr, D. H. (1997). Activation of the aryl hydrocarbon receptor/transcription factor and bone marrow stromal cell-dependent preB cell apoptosis. *J. Immunol.* **158,** 2165–2173.

Ye, B. H., Cattoretti, G., Shen, Q., Zhang, J., Hawe, N., de Waard, R., Leung, C., Nouri-Shirazi, M., Orazi, A., Chaganti, R. S., *et al*. (1997). The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation. *Nat. Genet.* **16,** 161–170.

Yi, A. K., Chace, J. H., Cowdery, J. S., and Krieg, A. M. (1996). IFN-gamma promotes IL-6 and IgM secretion in response to CpG motifs in bacterial DNA and oligodeoxynucleotides. *J. Immunol.* **156,** 558–564.

Yoo, B. S., Boverhof, D. R., Shnaider, D., Crawford, R. B., Zacharewski, T. R., and Kaminski, N. E. (2004). 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) alters the regulation of Pax5 in lipopolysaccharide-activated B cells. *Toxicol. Sci.* **77,** 272–279.

Zhang, Q., Bhattacharya, S., Kline, D. E., Crawford, R. B., Conolly, R. B., Thomas, R. S., Kaminski, N. E., and Andersen, M. E. (2010). Stochastic modeling of B lymphocyte terminal differentiation and its suppression by dioxin. *BMC Syst. Biol.* **4,** 40.