4. IMMUNOTOXICITY

4.1. INTRODUCTION

Concern over the potential toxic effects of chemicals on the immune system arises from the critical role of the immune system in maintaining health. It is well recognized that suppressed immunological function can result in increased incidence and severity of infectious diseases as well as some types of cancer. Conversely, inappropriate enhancement of immune function or the generation of misdirected immune responses can precipitate or exacerbate development of allergic and autoimmune diseases. Thus, both suppression and enhancement of immune function are considered to represent potential immunotoxic effects of chemicals.

The immune system consists of a complex network of cells and soluble mediators that interact in a highly regulated manner to generate immune responses of appropriate magnitude and duration. Consequently, comprehensive evaluation of immunotoxicity must include specific assessments of multiple functional parameters on a kinetic basis. In addition, because an immune response develops in a time-dependent manner relative to antigen exposure, the immunotoxicity of a chemical can be profoundly influenced by the timing of chemical exposure relative to antigen challenge. Consideration of these levels of complexity involved in immunotoxicology assessment is critical for interpreting the effects of chemical exposure on immune function (Kerkvliet, 1994).

Extensive evidence has accumulated to demonstrate that the immune system is a target for toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and structurally related polyhalogenated aromatic hydrocarbons (PHAHs), including the polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and polybrominated biphenyls (PBBs). This evidence was derived primarily from numerous studies in various animal species, mainly rodents, but also guinea pigs, rabbits, monkeys, marmosets, and cattle. Epidemiological studies also provide some evidence that PHAHs alter immune parameters in humans. In animals, relatively high doses of PHAHs produce lymphoid tissue depletion, except in the thymus, where lower doses cause cellular depletion. Alterations in specific immune effector functions and increased susceptibility to infectious disease have been identified at doses of TCDD well below those that cause significant lymphoid tissue depletion. Both cell-mediated and humoral immune responses are suppressed following TCDD exposure, which suggests that multiple cellular targets within the immune system are altered by TCDD. Evidence also suggests that the immune system is indirectly targeted by TCDD-induced changes in nonlymphoid tissues. In addition, in parallel with increased understanding of the cellular and molecular mechanisms involved in immunity, studies on TCDD are beginning to establish

biochemical and molecular mechanisms of TCDD immunotoxicity. These advances are highlighted in this document.

There is an enormous literature based on descriptive studies of the immunotoxic effects of TCDD and related PHAHs in laboratory animals. Unfortunately, widely differing experimental designs, exposure protocols, and immunologic assays used, have made it difficult to define a "TCDD-induced immunotoxic syndrome" in a single species, let alone across species. Before 1994, only one report directly compared the effects of TCDD on the immune system of rats, mice, and guinea pigs, and even then, different immunologic parameters were assessed, and different antigens were used in the different species (Vos et al., 1973). In that study, the delayed-type hypersensitivity (DTH) response to tuberculin was evaluated in guinea pigs and rats for assessment of cell-mediated immunity, and the graft versus host (GVH) response was measured in mice. A decreased DTH response to tuberculin was observed in guinea pigs following 8 weekly doses of 40 ng/kg TCDD (total dose, 320 ng/kg), whereas in rats the DTH response to tuberculin was unaffected by 6 weekly doses of 5 µg/kg TCDD (total dose, 30,000 ng/kg). The GVH response in mice was suppressed by 4 weekly doses of 5 µg/kg TCDD (total dose, 20,000 ng/kg). The greater sensitivity of guinea pigs compared with rats and mice to the immunosuppressive effects of TCDD is consistent with their greater sensitivity to other toxic effects of TCDD (McConnell et al., 1978; Poland and Knutson, 1982).

Although the results of Vos et al. (1973) appear to suggest that cell-mediated immunity in mice is more sensitive to TCDD than in rats, no studies have directly compared cell-mediated immunity in rats and mice using the same antigens and endpoints. However, a 1994 study made a direct comparison of the effects of TCDD on humoral immunity in rats and mice (Smialowicz et al., 1994). In this study, the primary plaque-forming cell (PFC) response to sheep red blood cells (SRBCs) was suppressed in B6C3F$_1$ mice (ED$_{50}$ of 0.68 µg/kg TCDD), but the anti-SRBC response was either unaffected or enhanced in Long Evans and Fischer 344 rats, respectively, at a dose as high as 30 µg/kg TCDD. This response of rats was corroborated by Fan et al. (1996), who observed that TCDD at 20 and 40 µg/kg did not alter serum IgM levels to SRBC, whereas IgG levels were enhanced. In contrast, the primary PFC and serum antibody response to the T cell–independent (TI) antigen trinitrophenyl lipopolysaccharide (TNP-LPS) was reported to be suppressed in both mice and rats following exposure to TCDD at 10 and 30 µg/kg, respectively (Smialowicz et al., 1996).

In a study in mice (Clark et al., 1981), the DTH response to oxazolone was suppressed by 4 weekly doses of 4 µg/kg TCDD (total dose, 16,000 ng/kg), whereas the DTH response to SRBC was unaffected by a 10-fold higher dose of TCDD, which illustrates that DTH responses to different antigens are not equally sensitive to TCDD-induced suppression, even in the same species. When PCB and PBB studies are considered, variable effects on DTH and other immune

reactions are also apparent (Vos and van Driel-Grootenhuis, 1972; Thomas and Hinsdill, 1978; Fraker, 1980; Luster et al., 1980a). Because the exact basis for the interstudy variability is not known, it would serve no useful purpose in terms of risk assessment to catalog all of the reported effects of TCDD and other PHAHs on the immune system. Several comprehensive reviews have been published on the immunotoxic effects of PHAHs in general (Kerkvliet, 1984; Vos and Luster, 1989; Kerkvliet and Burleson, 1994; Holsapple, 1995) and TCDD in particular (Holsapple et al., 1991a, b). The reader is also referred to the previous EPA TCDD risk assessment document (Sonawane et al., 1988) for another perspective on TCDD immunotoxicity. The present document does not address this extensive literature, but rather emphasizes more recent developments in the field of PHAH immunotoxicity that may assist in the risk assessment process. Gaps in our knowledge that require further research are also identified.

4.2. ROLE OF THE AH LOCUS IN PHAH IMMUNOTOXICITY

One of the most important advances in the study of PHAH toxicity in recent years has been the elucidation of a genetic basis for sensitivity to the toxicity of these chemicals, which may ultimately provide a logical explanation for many of the controversial data in the literature on PHAH toxicity in different species and in different tissues of the same species. In this regard, many biochemical and toxic effects of PHAHs appear to be mediated via binding to an intracellular protein known as the aryl hydrocarbon (Ah) or TCDD receptor in a process similar to steroid hormone receptor-mediated responses (Poland and Knutson, 1982; Cuthill et al., 1988). Ah receptor (AhR) activation follows stereospecific ligand binding; interaction of the receptor-ligand complex with dioxin-response elements (DREs) in the genome induces transcription of the structural genes encoding mRNA for CYP1A1 enzyme activity (i.e., cytochrome P4501A1) as well as expression of additional unidentified genes, the products of which are hypothesized to mediate PHAH toxicity (Whitlock, 1990). Differences in toxic potency between various PHAH congeners generally correlate with differences in AhR-binding affinities. The most toxic PHAH congeners are approximate stereoisomers of 2,3,7,8-TCDD and are halogen substituted in at least three of the four lateral positions in the aromatic ring system.

In mice, allelic variation at the Ah locus has been described (Poland et al., 1987; Poland and Glover, 1990). The different alleles code for AhRs that differ in their ability to bind TCDD and thus help to explain the different sensitivities of various inbred mouse strains to TCDD toxicity. Ah^{bb}-C57Bl/6 (B6) mice represent the prototype "responsive" strain and are the most sensitive to TCDD toxicity, whereas Ah^{dd}-DBA/2 (D2) mice represent the prototypic "nonresponsive" strain and require higher doses of TCDD to produce the same toxic effect. Congenic Ah^{dd} mice on a B6 background have been derived that differ from conventional B6 mice primarily at the Ah locus. The spectrum of biochemical and toxic responses to TCDD

exposure was similar in both strains, but the doses needed to bring about the responses were significantly higher in congenic mice homozygous for the Ah^d allele compared with mice carrying two Ah^b alleles (Birnbaum et al., 1990; Kerkvliet et al., 1990a).

Two lines of evidence have been used to investigate AhR dependence of acute immunotoxicity of TCDD and related PHAHs: (1) comparative studies using PCDD, PCDF, and PCB congeners that differ in their binding affinity for the AhR and (2) studies using mice of different genetic background known to differ at the Ah locus.

A comparison of the relative potency of PHAHs that differ in their binding affinity for the AhR is presented in Tables 4-1 and 4-2. These data are based on single-dose exposure (oral or intraperitoneal) of B6 mice to the various PHAHs for suppression of the anti-SRBC response and the cytotoxic T lymphocyte (CTL) response, respectively. As shown in Table 4-1, the potency of TCDD to suppress the primary antibody response to SRBCs has been reported by several laboratories, with remarkable agreement in the ID$_{50}$ value of 0.7 µg/kg in B6 mice. The ID$_{50}$ of B6C3F$_1$ mice has been reported to be lower (<0.1 µg/kg) (Narasimhan et al., 1994), similar (<1 µg/kg) House et al., 1990; Smialowicz et al., 1994), or slightly higher (1.2 µg/kg) (Holsapple et al., 1986a) in comparison with B6 mice. It should be noted that 4 weekly i.p. doses of 10 but not 1 or 0.1 µg/kg TCDD significantly suppressed the anti-SRBC response in B6 mice (Clark et al., 1981). This sub-chronic dosing protocol (Clark et al., 1981) does not readily explain this decreased potency because Vecchi et al. (l983) reported that 5 weekly doses of 2 µg/kg or 8 weekly doses of 0.5 µg/kg TCDD significantly suppressed the anti-SRBC response. The basis for the discrepancies between the data of Clark et al. (1981) and other laboratories regarding the potency of TCDD to suppress the anti-SRBC response is unknown.

In contrast to the reproducible data on TCDD, relative potency for other PHAH congeners shown in Table 4-1 is difficult to evaluate because few congeners have been examined in more than one study. In the few cases where the same congener has been evaluated independently, discrepancies in the data exist. For example, both Davis and Safe (1990) and Silkworth et al. (1984) evaluated the potency of the 2,3,4,5,3′,4′-HxCB congener (PCB 156) in the anti-SRBC response. The ID$_{50}$s from these two data sets differ by almost two orders of magnitude (0.7 mg/kg vs. 31 mg/kg, respectively). When the same congener was compared with TCDD for suppression of the CTL response, the ID$_{50}$ was 70 mg/kg (Table 4-2). Although the basis for these discrepancies between laboratories and immune function endpoints is unknown, it is apparent that the immunotoxicity database should be expanded so that these differences can be resolved.

Vecchi et al. (1983) were the first to report that antibody response to SRBCs was differentially suppressed by TCDD in B6 mice compared with D2 mice, such that D2 mice required a dose approximately 10 times higher to produce the same degree of suppression.

Immunosuppression in F1 and backcross mice supported the role of the Ah locus in expression of TCDD immunotoxicity. 2,3,7,8-TCDF was significantly less potent than TCDD and showed a similar differential immunosuppressive effect in B6 and D2 mice. At the same time, Silkworth and Grabstein (1982) reported a B6 versus D2 strain-dependent difference in sensitivity to suppression of the anti-SRBC response by 3,4,3′,4′-tetrachlorobiphenyl (PCB 77), a ligand for the AhR. In comparison, the 2,5,2′,5′-tetrachlorobiphenyl (PCB 52) isomer, which lacks affinity for the AhR, was not immunosuppressive in either B6 or D2 mice. Structure-activity relationships were extended by Kerkvliet et al. (1985) in studies that compared the immunosuppressive potency of chlorinated dioxin and furan isomers that contaminate technical-grade pentachlorophenol. The 1,2,3,6,7,8-hexachlorinated dibenzo-p-dioxin (HxCDD), 1,2,3,4,6,7,8-heptachlorinated dibenzo-p-dioxin (HpCDD), and 1,2,3,4,6,7,8-heptachlorinated dibenzofuran (HpCDF) isomers, which bind the receptor, were all significantly immunosuppressive. The dose of each isomer that produced 50% suppression of the anti-SRBC response (ID_{50}) was 7.1, 85, and 208 µg/kg for HxCDD, HpCDD, and HpCDF, respectively (Figure 4-1). The ID_{50} for TCDD was 0.65 µg/kg based on the data of Vecchi et al. (1980). More extensive structure-dependent immunosuppressive activities of technical grade PCB mixtures (Davis and Safe, 1990), PCB congeners (Davis and Safe, 1989), and PCDF congeners (Davis and Safe, 1988) have also been reported. Results of these studies using different PHAH congeners are summarized in Table 4-1.

The role of the AhR in suppression of the anti-SRBC response was verified in studies using B6 mice congenic at the Ah locus (Kerkvliet et al., 1990a). As expected, congenic Ah^{dd}-B6 mice were significantly less sensitive to TCDD-induced immune suppression as compared with wild-type Ah^{bb}-B6 mice. Unexpectedly, however, the dose response in congenic Ah^{dd}-B6 mice appeared to be bimodal, with a portion of the response sensitive to suppression by low doses of TCDD. Because of the bimodal response, the data did not permit extrapolation of an ID_{50} dose in the congenic mice. The results were interpreted to suggest potential non-AhR-mediated immunosuppressive effects. It should be noted, however, that studies by Silkworth et al. (1993) using rederived congenic Ah^{dd}-B6 mice did not corroborate a bimodal dose response. Their data, which indicated an ID_{50} of 7.5 µg/kg in congenic Ah^{dd}-B6 mice compared with an ID_{50} of 0.54 µg/kg in Ah^{bb}-B6 mice, are consistent with an AhR-dependent mechanism of immune suppression.

AhR dependency of PHAH immunotoxicity has also been demonstrated in mice using other immunologic responses. For example, Kerkvliet et al. (1990a) reported that the ID_{50} for suppression of the antibody response to TNP-LPS in Ah^{bb}-B6 mice was 7.0 µg/kg compared with a significantly higher ID_{50} of 30 µg/kg in congenic Ah^{dd}-B6 mice. Because the antibody response to TNP-LPS shows little requirement for macrophages or T helper cells (Jelinek and Lipsky,

1987), these results suggest an AhR-dependent B cell response. In terms of cytotoxic T cells, Clark et al. (1983) were first to report data suggesting that TCDD and PCB isomers suppressed in vitro CTL responses of B6 and D2 mice through an AhR-dependent mechanism. Subsequently, Kerkvliet et al. (1990b) reported that B6 mice congenic at the Ah locus showed Ah-dependent sensitivity to suppression of the CTL response following exposure to either TCDD or 3,4,5,3′,4′,5′-hexachlorobiphenyl (HxCB) (PCB 169). Furthermore, the potency of TCDD and of three HxCB congeners to suppress the CTL response of mice directly correlated with their relative binding affinities for the AhR (Table 4-2, from Kerkvliet et al., 1990b). The ID_{50} of TCDD for suppression of the CTL response in B6 mice was 7.0 μg/kg.

It should be noted that the dose of TCDD required to suppress the CTL response reported by Kerkvliet et al. (l990b) is significantly greater than that reported by Clark et al. (1981), who reported CTL suppression following 4 weekly doses of 0.1 μg/kg TCDD. Clark et al. (1983) also reported that doses of TCDD as low as 4 ng/kg to B6 mice suppressed in vitro generation of CTL and that the suppression was Ah dependent. The potency of TCDD described in Clark's studies has not been corroborated by other laboratories (Holsapple et al., 1991b; Hanson and Smialowicz, 1994). For example, the in vivo and in vitro CTL response in B6 mice was not affected at doses ranging from 0.01 to 3.0 μg/kg given at weekly intervals for 4 weeks (Hanson and Smialowicz, 1994).

If immunotoxicity of TCDD and structurally related PHAHs depends on AhR-mediated mechanisms, then co-exposure to subsaturating levels of more than one Ah agonist should produce additive effects. An additive interaction has been demonstrated in mice coexposed to 1,2,3,6,7,8-HxCDD and 1,2,3,4,6,7,8-HpCDD, two relatively strong AhR ligands (Kerkvliet et al., 1985). On the other hand, coexposure of mice to an immunotoxic dose of TCDD and a subimmunotoxic dose of different commercial Aroclors or certain PCB congeners resulted in partial antagonism of TCDD suppression of the anti-SRBC response (Bannister et al., 1987; Davis and Safe 1988, 1989). An apparently similar antagonism was observed following coexposure to 2,3,7,8-TCDF (10 μg/kg) and TCDD (1.2 μg/kg) (Rizzardini et al., 1983). The mechanism for this antagonism has not been fully elucidated, but the effects are consistent with competition for binding at the AhR because the weaker agonist was administered in excess compared with TCDD. In other studies, Silkworth et al. (1988, 1993) have shown that immunotoxicity of TCDD can be modified by coexposure to other PHAHs present as cocontaminants of actual environmental samples from Love Canal, New York. Smialowicz et al. (1997) examined the anti-SRBC response in mice cotreated with TCDD and 2, 2', 4, 4', 5, 5'-hexachlorobiphenyl (PCB153). PCB153 alone enhanced this response such that when given with an immunosuppressive dose of TCDD (1 μg/kg) the anti-SRBC response was not suppressed.

These results indicate that PCB153 acts as a functional rather than an AhR or dispositional antagonist of TCDD-induced immunosuppression.

Recent work by Sulentic et al. (1998) provides evidence for the critical role of the AhR in TCDD-induced suppression of IgM secretion. B cell lines, which differ in their expression of the AhR (i.e., CH12.LX cells express AhR and BCL-1 cells do not), were used in this study. TCDD treatment resulted in marked induction of AhR expression as well as suppression of LPS-induced IgM secretion in CH12.LX but not BCL-1 cells. Furthermore, TCDD induced CYP1AI induction in CH12.LX but not BCL-1 cells. These results implicate the AhR as a critical factor in TCDD-induced inhibition of IgM secretion.

The data indicate that suppression of the antibody response to T cell–dependent and –independent antigens and the CTL response by PHAHs are primarily AhR-dependent. However, the underlying mechanism(s) for these effects remain to be elucidated. It should also be emphasized that the data supporting an AhR dependency have been obtained from studies in inbred mice using an acute or subacute exposure regimen. Except for thymic atrophy, structure-immunotoxicity relationships in other species, including rats, have not been established, and inbred strains of other species with defined Ah genotype are not currently available. Nevertheless, it is important to note that thymic cortical atrophy does not occur in AhR deficient (AhR$^{-/-}$) mice despite having received a 10-fold higher TCDD dose than mice expressing a functional AhR (AhR$^{+/-}$), which experience significant reduction in the size and cellularity of thymic cortical areas (Fernandez-Salguero et al., 1996).

Results from other studies suggest that non-Ah-dependent effects may also occur. For example, mice exposed for 14 days to 2,7-dichlorodibenzo-p-dioxin (2,7-DCDD), a dioxin congener with very weak affinity for the AhR, had suppressed antibody responses to SRBC (Holsapple et al., 1986b). This suppression was observed in the absence of any change in thymus weight or in AHH activity. More recently, Morris et al. (1992) reported that sensitivity of D2 mice to TCDD-induced suppression of the anti-SRBC response increased significantly when TCDD was administered daily over 2 weeks rather than as an acute single dose. Unfortunately, in these studies, the lowest dose of TCDD produced near-maximum suppression of the anti-SRBC response of B6C3F$_1$ mice in the acute exposure model, precluding detection of any similar increase in sensitivity of the B6C3F$_1$ mice to chronic dosing. In contrast to these findings, Vecchi et al. (1983) reported that multiple exposures to TCDD (2 µg/kg for 5 weeks or 0.5 µg/kg for 8 weeks) did not increase the sensitivity of D2 mice to suppression of the anti-SRBC response. Thus, the basis for any change in potency resulting from multiple treatment or chronic exposure to TCDD and the role of AhR-mediated events in the phenomenon remain to be elucidated.

Some in vitro studies also suggest that suppression of the in vitro antibody response may occur independent of the AhR. Tucker et al. (1986) and Holsapple et al. (1986a) reported that direct addition of TCDD in vitro suppressed the antibody response to SRBCs. However, based on the response of cells from congenic mice as well as a limited structure-activity study, the data of Tucker et al. (1986) supported an AhR-dependent suppression, whereas the data of Holsapple et al. (1986a) did not. In the latter study, the magnitude of suppression was comparable using cells from responsive B6C3F$_1$ or congenic heterozygous (Ah^{bd}-B6) mice compared with nonresponsive D2 or homozygous Ah^{dd}-B6 mice. In addition, Holsapple et al. (1986a) reported that 2,7-DCDD, which lacks affinity for the AhR, was equipotent with TCDD in suppressing the in vitro response.

Davis and Safe (1991) directly compared the in vitro structure-immunotoxicity relationships for a series of PHAH congeners that show >14,900-fold difference in in vivo immunotoxic potency. Results of these studies indicated that all of the congeners were equipotent in vitro and produced a similar concentration-dependent suppression of the in vitro anti-SRBC response using cells from either B6 or D2 mice. Coexposure to the AhR antagonist α-napthoflavone antagonized the immunosuppression induced by either TCDD or 1,3,6,8-TCDF (a weak AhR agonist). Collectively, these results suggested a mechanism of suppression in vitro that was independent of the AhR.

Morris et al. (1991) demonstrated that suppression of the in vitro antibody response to SRBC by TCDD was critically dependent on the type and concentration of the serum used in the in vitro culture. When splenocytes were cultured in the presence of normal mouse serum (NMS), the profile of activity was dependent on the genotype (i.e., Ah^{bb} or Ah^{dd}) of the lymphocytes rather than the source of NMS (Morris et al., 1994). These results are important, because they may help to explain the variable effects observed by different laboratories performing in vitro TCDD-antibody response assays.

The majority of evidence indicates that immunotoxicity of PHAHs is AhR mediated. This evidence comes primarily from the in vivo mouse data described above. Recent studies also indicate that murine T lymphocytes and leukocytes express the Ah receptor (Lawrence et al., 1996; Williams et al., 1996). A complete understanding of the role of the AhR in murine leukocytes and PHAH-mediated immunotoxicity, however, requires further study. Although other data suggest that non-AhR mechanisms may have some role in TCDD-induced immunotoxicity, further studies are required to provide definitive evidence for this mechanism. Studies employing novel approaches -such as the development and use of variant lymphoma cells that express variable levels of the AhR (e.g., hepatoma cells [Miller et al., 1983; Karenlampi et al., 1988]), the use of AhR knockout mice (Fernandez-Salguero et al., 1995; Gonzalez et al.,

1995), or the use of pure binding antagonists (Lu et al., 1995, 1996), would help to provide such evidence.

4.3. SENSITIVE TARGETS FOR PHAH IMMUNOTOXICITY

Despite considerable investigation, the cells that are most sensitive to alteration by PHAH exposure leading to suppressed immune function have not been unequivocally identified. The in vivo immunotoxicity of TCDD, expressed in terms of suppression of the anti-SRBC response of B6 or B6C3F$_1$ mice, is highly reproducible between laboratories. Because the magnitude of the anti-SRBC response depends on the concerted interactions of antigen-presenting cells (APCs), regulatory T cells (helper and suppressor), and B cells, this response has been used most widely to evaluate target cell sensitivity to PHAHs. In addition, the CTL response has served as a model for evaluating PHAH-induced suppression of T cell function. These immune responses can be modulated by nonimmunological factors, including hormonal and nutritional variables, and PHAHs are known to affect endocrine and metabolic functions. These indirect effects will be apparent only in in vivo studies, whereas direct effects on APC and lymphocyte functions would be evident following in vitro exposure to PHAHs.

One potentially important indirect mechanism is through effects on the endocrine system, because the activity of several endocrine hormones (e.g., glucocorticoids, sex steroids, thyroxine, growth hormone, and prolactin) that regulate immune responses have been shown to be altered by TCDD and other PHAHs (see Chapter 3, Acute, Subchronic and Chronic Toxicity and Chapter 5, Developmental and Reproductive Toxicity). Consequently, studies have been performed to examine the possible role of PHAH-induced indirect effects on the immune systems of rodents.

Kerkvliet et al. (1990b) reported that exposure of mice to 3,4,5,3′,4′,5′-HxCB (PCB169)followed by injection of P815 allogeneic tumor cells induced a dose-dependent elevation of serum corticosterone concentrations that correlated with the dose-dependent suppression of the anti-P815 CTL response. However, because adrenalectomy or treatment with the glucocorticoid receptor antagonist RU38486 failed to protect mice from the immunosuppressive effect of PCB169 (DeKrey et al., 1993), a role for the elevated CS in the suppression of the CTL response seems unlikely. Adrenalectomy and hypophysectomy also failed to prevent TCDD-induced thymic atrophy in rats (van Logten et al., 1980).

In other studies using the P815 allogeneic tumor model, Kerkvliet and Baecher-Steppan (1988a) reported that male mice were more sensitive than female mice to suppression of the CTL response by PCB169. Castration of male mice partially ameliorated the immunosuppressive effects of HxCB (DeKrey et al., 1993), suggesting a role for testosterone in suppression of this response. Male mice were found to be more sensitive than female mice to PCB169-induced

reductions in serum prolactin levels (DeKrey et al., 1994). However, it was concluded that PCB169-induced hypoprolactinemia was not responsible for CTL suppression in mice, because bromocryptine, which severely suppressed serum prolactin levels, failed to alter CTL activity.

Pazdernik and Rozman (1985) suggested that thyroid hormones may play a role in TCDD immunotoxicity based on the finding that radiothyroidectomy prevented the suppression of the anti-SRBC response in rats treated with TCDD. However, because thyroidectomy alone suppressed immune function, the significance of the findings requires further study. Taken together, the current data do not provide convincing evidence supporting a role for hormones in PHAH-induced indirect mechanisms of immunosuppression.

Kerkvliet and Brauner (1987) compared the sensitivity of antibody responses to antigens that differ in their requirements for APC and T cells as an in vivo approach to evaluate the cellular targets of 1,2,3,4,6,7,8-HpCDD humoral immunotoxicity. The TI antigens, DNP-Ficoll and TNP-LPS, were used in these studies. These TI antigens differ from each other in their requirement for APC (higher for DNP-Ficoll) and their sensitivity to regulatory (amplifier and suppressor) T cell influence (DNP-Ficoll is sensitive, TNP-LPS is not) (Braley-Mullen, 1982). Obviously, all antibody responses require B cell differentiation into antibody-secreting plasma cells. Although HpCDD produced dose-dependent suppression of the antibody response to all three antigens, sensitivity to suppression directly correlated with the sensitivity of the response to T cell regulation. The ID_{50}s were 53, 127, and 516 µg/kg for SRBC, DNP-Ficoll, and TNP-LPS, respectively. These results were interpreted as follows: If one assumes that B cell function is targeted in the TNP-LPS response, then regulatory T cells and/or APC may represent the more sensitive target in the SRBC and DNP-Ficoll responses. The difference in sensitivity between the SRBC and DNP-Ficoll responses suggests that the T helper cell may be a particularly sensitive target. The differential sensitivity of the antibody responses to TNP-LPS versus SRBC has been corroborated in TCDD-treated mice (House et al., 1990; Kerkvliet et al., 1990a). Thus, the in vivo sensitivity of the antibody response to SRBC, described in these studies, appears to depend on the T cell and/or APC components of the response rather than the B cell, unless the B cells that respond to SRBC are different from the B cells that respond to TNP-LPS. Currently, evidence for such a difference is lacking.

These in vivo results differ from the ex vivo data of Dooley and Holsapple (1988). Using in vitro immunization with SRBC, DNP-Ficoll or LPS of separated and reconstituted splenic T cells, B cells, and adherent cells from vehicle- and TCDD-treated mice, they reported that B cells from TCDD-treated mice were functionally compromised in in vitro antibody responses but T cells and macrophages were not. These ex vivo results corroborated an earlier study in which TCDD suppressed the in vitro antibody response to these same antigens in a dose-related manner and at comparable concentrations (Holsapple et al., 1986a). Taken together, these ex vivo data

suggested that the B cell is the primary target for the direct effects of TCDD, because the antigens employed differ in their dependence on T cells and accessory cells (Holsapple, 1995).

The basis for the different responses to TI antigens in these studies has not been established. However, it has been suggested for the ex vivo work that the effects of TCDD on T cells may be indirectly induced following antigen exposure such that removal of the cells from the TCDD environment of the host prior to antigen challenge would preclude detection of T cell dysfunction (Kerkvliet and Burleson, 1994).   This interpretation is supported by the findings of Tomar and Kerkvliet (1991) that spleen cells taken from TCDD-treated mice were not compromised in their ability to reconstitute the antibody response of lethally irradiated mice, and the reported lack of direct effects of TCDD and other PHAHs on T cells in vitro (Clark et al., 1981; Kerkvliet and Baecher-Steppan, 1988a, b).

Although the direct effects of TCDD on T cells in vitro have not been demonstrated, it is clear that functional T cell responses generated in vivo are compromised following in vivo exposure. Nude mice that are congenitally T cell deficient were significantly less sensitive to HpCDD-induced immunotoxicity when compared with their T cell–competent littermates (Kerkvliet and Brauner, 1987). Likewise, exposure to TCDD or PCB169 suppressed the development of CTL activity following alloantigen challenge (Kerkvliet et al., 1990b). The influence of TCDD exposure on regulatory T cell functions has been addressed in several studies. Clark et al. (1981) first proposed that T suppressor cells were induced by TCDD in the thymus that were responsible for the suppressed CTL response. However, increased suppressor cell activity in peripheral lymphoid tissue was not observed in mice exposed to TCDD (Dooley et al., 1990) or PCB169 (Kerkvliet and Baecher-Steppan, 1988b). In terms of T helper cell activity, Tomar and Kerkvliet (1991) reported that a dose of 5 µg/kg TCDD suppressed the in vivo generation of carrier-specific T helper cells. Lundberg et al. (1990) reported that thymocytes from B6 mice treated with TCDD (50 µg/kg) were less capable of providing help for an in vitro anti-SRBC response. However, Clark et al. (1983) reported in ex vivo studies that T cells from TCDD-treated mice produced normal levels of interleukin-2 (IL-2). A study using the P815 tumor allograft model suggests that TCDD alters early CD4^{+} T cell activation events, which lead to premature termination of cytokine production by CD8^{+} T cells, suppression of CTL activity, and suppression of alloantibγody production by B cells (Kerkvliet et al., 1996). Tumor necrosis factor (TNF), IL-2, and interferon γ (IFNγ) were suppressed in P815-injected mice exposed to TCDD. In contrast, TNF and IL-2 were not affected and IFNγ was reduced in anti-CD3 injected mice exposed to TCDD (Prell et al., 1995). These results suggest that the effects of TCDD on cytokine production may be determined by the inducing stimulus.

The influence of TCDD exposure on B cell function has been addressed primarily in in vitro studies, but direct effects of PHAHs on macrophages and T cells in vitro have not been

described. The issue of TCDD effects on B cells is difficult to address in vivo given that most B cell responses (except perhaps anti-LPS responses) depend on interactions with T cells and macrophages. In vitro studies have described the direct effects of TCDD on activation and differentiation of purified B cells (Holsapple et al., 1986a; Luster et al., 1988; Morris et al., 1991; Karras and Holsapple, 1994a, b; Karras et al., 1995). These studies suggest that TCDD inhibits terminal differentiation of B cells via alteration of an early activation event (Luster et al., 1988; Karras and Holsapple, 1994b). Increased phosphorylation and tyrosine kinase activity in TCDD-treated B cells may underlie this B cell dysfunction (Kramer et al., 1987; Clark et al., 1991a). Also, Karras and Holsapple (1994a) suggest that the antiproliferative effect of TCDD on B cells is due to inhibition of calcium-dependent activation; this inhibition results in suppression of B cell surface Ig-induced antibody production (Karras et al., 1996).

Macrophage functions have also been examined following TCDD exposure and generally found to be resistant to suppression by TCDD when assessed ex vivo. Macrophage-mediated phagocytosis, macrophage-mediated tumor cell cytolysis or cytostasis, oxidative reactions of neutrophils and macrophages, and spontaneous natural killer (NK) cell activity were not suppressed following TCDD exposure, with doses as high as 30 μg/kg failing to suppress NK and macrophage functions (Vos et al., 1978; Mantovani et al., 1980). More recent studies, however, indicate that virus-augmented pulmonary NK activity, but not spontaneous pulmonary NK activity, is suppressed by TCDD (Yang et al., 1994). Phorbol ester–activated antitumor cytolytic and cytostatic activity of neutrophils is also selectively inhibited by TCDD (Ackermann et al., 1989).

On the other hand, it is interesting to note that the pathology associated with TCDD toxicity often includes neutrophilia and an inflammatory response in liver and skin characterized by activated macrophage and neutrophil accumulation (Vos et al., 1973; Weissberg and Zinkl, 1973; Vos et al., 1974; Puhvel and Sakamoto, 1988; Herbert et al., 1990). Although these observations may reflect a normal inflammatory response to tissue injury, some experimental evidence suggests that inflammatory cells may be activated by TCDD exposure. For example, Alsharif et al. (1994) reported that TCDD increased superoxide anion production in rat peritoneal macrophages. In addition, it has been shown that TCDD exposure results in an enhanced inflammatory response following SRBC challenge (Kerkvliet and Oughton, 1993). This effect of TCDD was characterized by a twofold to fourfold increase in the number of neutrophils and macrophages locally infiltrating the intraperitoneal site of SRBC injection. However, the kinetics of the cellular influx were not altered by TCDD. Likewise, the expression of macrophage activation markers (I-A and F4/80) and the antigen-presenting function of the peritoneal exudate cells were unaltered by TCDD. Using specific inhibitors of the proinflammatory cytokines TNF and IL-1 (Moos et al., 1994) found that the TCDD-induced

hyperinflammatory response was mediated by TNF . However, although exogenous TNF suppressed the antibody response to SRBC, the increased inflammatory response and suppression of the response to SRBC were not apparently linked, because coincident treatment with the soluble TNF receptor rhuTNFR:Fc, which blocks TNF activity, further suppressed rather than normalized the immunosuppression by TCDD alone (Moos and Kerkvliet, 1995). Thus, the relationship, if any, between the inflammatory and immune effects of TCDD remains to be elucidated.

Evidence also suggests that the long-recognized hypersusceptibility of TCDD- and PCB-treated animals to endotoxin (LPS) (Thomas and Hinsdill, 1978, 1979; Vos et al., 1978; Loose et al., 1979) may be related to an increased production of proinflammatory factors. TNF production may be responsible for endotoxin hypersensitivity in TCDD-treated mice and that the Ah locus mediates this response (Clark et al., 1991b; Taylor et al., 1992). The ability of methylprednisolone to reverse the mortality associated with TCDD/LPS treatment is also consistent with an inflammatory response (Rosenthal et al., 1989). Similarly, increased inflammatory mediator production may underlie the enhanced rat paw edema response to carrageenan and dextran in TCDD-treated rats (Theobald et al., 1983; Katz et al., 1984). Whereas serum complement activity has been reported to be suppressed in dioxin-treated mice (White et al., 1986), enhanced activity was reported at the lowest exposure level when 1,2,3,6,7,8-HxCDD was tested. Work by Sutter et al. (1991) indicates that IL-1β gene expression, as well as plasminogen activator inhibitor-2, in keratinocytes is elevated by TCDD. On the other hand, House et al. (1990) reported that inflammatory macrophages obtained from TCDD-treated mice produced control levels of IL-1 when examined ex vivo. Thus, the effect of TCDD on inflammatory mediator production may be a "priming effect" and require coexposure to antigen or LPS. The influence of TCDD on inflammatory mediator production and action is an important area for further study.

Since the rapid influx of phagocytic cells to the site of pathogen invasion is an important factor in host resistance to infection, the ability of TCDD to augment the production of inflammatory chemoattractive mediators would imply that TCDD exposure could result in enhanced host resistance. However, because TCDD exposure is, at the same time, immunosuppressive, which results in decreased specific immune responses generated by T and B lymphocytes, the overall impact of TCDD exposure on disease susceptibility will likely vary depending on the nature of the pathogen and the major mode of host response to the specific infectious agent. Such effects may in fact help explain the disparate effects of TCDD in different host resistance models that are described below.

Taken together, it is obvious that multiple targets exist for PHAH-induced immunosuppression, including both T and B lymphocytes. Lymphocyte precursor cells as well

as lymphoid-associated tissue such as thymus epithelium and bone marrow stromal elements are also affected by dioxins (discussed later in the chapter). The class or subclass of T or B lymphocytes that is the proximate target for PHAH-induced immunosuppression remains to be determined, as do the mechanisms by which immunosuppression by these chemicals is achieved.

4.4. INFLUENCE OF TCDD ON HOST RESISTANCE TO DISEASE

The ability of an animal to resist and/or control viral, bacterial, parasitic, and neoplastic diseases is determined by both nonspecific and specific immunological functions. Decreased functional activity in any immunological compartment may result in increased susceptibility to infectious and neoplastic diseases. Animal host resistance models that mimic human disease are available and have been used to assess the effect of TCDD on altered host resistance.

TCDD exposure increases susceptibility to challenge with the gram-negative bacterium *Salmonella*. TCDD was given per os at 0.5 to 20 µg/kg once a week for 4 weeks to male 4-week-old C57Bl/6Jfh (J67) mice and challenged 2 days after the fourth dose (when mice were 8 weeks old) with either *Salmonella bern* or *Herpesvirus suis* (also known as pseudorabies virus). Results with *S. bern* indicated increased mortality at 1 µg TCDD/kg (total dose of 4 µg/kg) and reduced time to death after bacterial challenge with 5 µg TCDD/kg (total dose of 20 µg/kg). In contrast, the same doses of TCDD did not alter the time to death or the incidence of mortality following *Herpesvirus suis* infection (Thigpen et al., 1975). A TCDD feeding study by Hinsdill et al. (1980) also demonstrated increased susceptibility of 7-week-old Swiss Webster outbred female mice to *S. typhimurium* var. *copenhagen*. Mice were fed control feed or feed containing 10, 50, or 100 ppb TCDD for 8 weeks, after which they were injected intravenously with $10^{3.5}$ *S. typhimurium* var. *copenhagen*. Results indicated that 50 and 100 ppb TCDD increased mortality from *Salmonella* and shortened the time to death, whereas 10 ppb caused an increased bacteremia.

Vos et al. (1978) reported that TCDD resulted in increased sensitivity to endotoxin (*E. coli* O 127:B 8 lipopolysaccharide) and suggested that the increased susceptibility to *Salmonella* caused by TCDD might be caused by the endotoxin of this gram-negative bacterium. Vos et al. demonstrated reduced resistance to endotoxin with a single oral dose of 100 µg TCDD/kg using 3- to 4-week-old outbred female mice and challenged with endotoxin 5 days later. Vos et al. also reported enhanced mortality from intravenous injection of endotoxin 2 days after the final oral dose of TCDD (1.5, 5, 15, or 50 µg/kg, once a week for 4 weeks) in 3- to 4-week-old male outbred Swiss mice. These studies indicate a reduced resistance to endotoxin after single or multiple doses of TCDD. Thomas and Hinsdill (1979), using *S. typhimurium* lipopolysaccharide, demonstrated a reduced resistance to endotoxin in the offspring of female Swiss Webster mice fed TCDD prior to mating, during gestation, and between parturition and weaning. Rosenthal et

al. (1989) used female B6C3F$_1$, DBA/2, as well as congenic mice to demonstrate that acute doses of 50, 100, or 200 µg/kg TCDD per os increased endotoxin-induced mortality in B6C3F$_1$ mice, which was associated with hepatotoxicity and decreased clearance of the endotoxin. D2 and Ah^{dd} congenic mice were relatively resistant to this effect, implicating AhR-dependent mechanisms in endotoxin hypersensitivity.

White et al. (1986) reported that *Streptococcus pneumoniae*, a gram-positive bacterium that does not contain endotoxin, caused increased mortality in 5- to 6-week-old female B6C3F$_1$ mice after subchronic oral administration of TCDD (1 µg/kg for 14 days) and challenged with *S. pneumoniae* intraperitoneally 1 day after the last treatment. The 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HCDD) isomer also resulted in a dose-dependent increase in susceptibility to *S. pneumoniae*.

Enhanced susceptibility to viral disease has also been reported after TCDD administration. Clark et al. (1983) injected TCDD intraperitoneally once a week for 4 weeks and challenged mice 7 to 22 days later with *Herpes simplex* type II strain 33 virus. Mice receiving TCDD at 0.04, 0.4, or 4.0 µg/kg weekly (total dose of 160, 1,600, and 16,000 ng/kg) all had significantly enhanced mortality to Herpesvirus type II infection. House et al. (1990) also reported an enhanced susceptibility to viral infection following low-level singledose TCDD administration intraperitoneally. B6C3F$_1$ female mice, 6 to 8 weeks of age, were challenged with influenza A/Taiwan/1/64 (H2N2) virus 7 to 10 days following TCDD. TCDD administration at 10, 1.0, or 0.1 µg/kg decreased resistance to virus. The lowest observable effect level was 0.1 µg/kg, making this one of the most sensitive endpoints for TCDD immunotoxicity. However, the only immune parameter suppressed by TCDD, which is related to host resistance to this virus, was the serum hemagglutination antibody titers to influenza. Unfortunately, only mice dosed at 10 µg/kg TCDD were evaluated for these virus-specific antibodies. No alterations in macrophage function, NK cell activity, or interferon levels were observed in TCDD-exposed mice (House et al., 1990).

Recently, the sensitivity of TCDD-exposed mice to influenza virus challenge was corroborated by Burleson et al. (1996), who observed enhanced mortality to influenza A/Hong Kong/8/68 (H3N2) virus following a single exposure to 0.1, 0.05, or 0.01 µg/kg TCDD. Increased mortality at 0.01 µg/kg TCDD was the lowest observable effect level and as such represents the most sensitive adverse effect level yet reported for TCDD (Burleson et al., 1996). However, the role of TCDD in altering immune-mediated mechanisms important in resistance to this virus remains to be elucidated. Furthermore, because increased mortality was not correlated with increased virus titers in the lungs of mice, and TCDD did not alter the expected virus-enhanced increase in lung:body weight ratio nor the virus-induced decrease in thymus weight, other nonimmune virus-mediated physiological changes may be involved in this increased

mortality. Further studies are warranted to confirm this very low level, TCDD-induced effect and to delineate the underlying mechanisms responsible for this altered host resistance.

Rats exposed to TCDD daily for 14 days at a total dose of 10 µg/kg had significantly augmented rat-adapted influenza virus replication in the lungs (Yang et al., 1994). The increased virus replication was correlated with a significant suppression of virus-augmented but not spontaneous pulmonary NK cell activity, suggesting that reduced NK activity may be at least in part related to enhanced susceptibility of rats to influenza virus (Yang et al., 1994).

TCDD exposure has also been shown to result in more severe infections from parasites. Tucker et al. (1986) studied the effects of TCDD administration on *Plasmodium yoelii* 17 XNL, a nonlethal strain of malaria, in 6- to 8-week-old B6C3F$_1$ female mice. A single dose of TCDD at 5 µg/kg or 10 µg/kg per os resulted in increased susceptibility to *P. yoelii*. The peak parasitemia was greater and of longer duration in TCDD-treated animals than in controls, the difference being significant at 5 µg/kg on day 10 and at 10 µg/kg on days 12 and 14. A single dose of TCDD at 10 or 30 µg/kg intraperitoneally 7 days prior to infection of B6C3F$_1$ mice with *Trichinella spiralis* resulted in delayed onset of adult parasite elimination, and at 1.0 µg/kg TCDD suppressed the proliferative response of splenocyte and mesenteric lymph node cells stimulated with *T. spiralis* antigen (Luebke et al., 1994). Tissue levels of TCDD were also higher in infected versus noninfected mice in this study. In a separate study, TCDD at 1, 10, or 30 µg/kg administered intraperitoneally 7 days prior to infection of Fischer 344 rats with *T. spiralis* did not affect adult parasite elimination or the numbers of encysted larvae in muscle. Furthermore, proliferative responses of lymphocytes from rats dosed at 30 µg/kg and stimulated with parasite antigen were enhanced in contrast to the suppression observed in B6C3F$_1$ mice (Luebke et al., 1995). The different responses observed in mice versus rats for this host resistance model are similar to those reported by Smialowicz et al. (1994) for the antibody response to SRBCs, in that the latter response was suppressed in mice and enhanced in rats. In a recent study, aged (76-week-old) rats exposed to TCDD and infected with *T. spiralis*, were less able to limit the burden of encysted larvae, compared to young (10-week-old) rats (Luebke et al. 1999). These results suggest that TCDD may exacerbate the age-related decreased resistance to this parasite.

Luster et al. (1980a) demonstrated enhanced growth of transplanted tumors in mice treated with TCDD at doses of 1.0 or 5.0 µg/kg in B6C3F$_1$ mice. Dams were given TCDD by gavage at day 14 of gestation and again on days 1, 7, and 14 following birth; host resistance studies were performed 6 to 8 weeks after weaning. This exposure protocol resulted in an increased incidence of PYB6 tumors in pups from dams receiving repeated doses of 1.0, but not 5.0, µg TCDD/kg.

Although it is clear that TCDD adversely affects numerous host resistance models detailed above, the effects of TCDD on susceptibility to *Listeria monocytogenes* infections are ambiguous. The disparate results may reflect different study designs, including dose, route, single versus multiple administrations, mouse strain, age, or sex. However, it is clear that TCDD, under certain conditions, results in increased susceptibility to *Listeria*. Hinsdill et al. (1980) reported the increased susceptibility of 7-week-old Swiss Webster outbred female mice to *Listeria*. Mice were fed control feed or feed containing 10 or 50 ppb TCDD for 8 weeks, after which they were injected intravenously with 10^5 *Listeria*. Results indicated that the 50 ppb diet increased bacteremia and mortality. Luster et al. (1980b) used doses of 1.0 or 5.0 µg TCDD/kg in B6C3F$_1$ mice. Dams were given TCDD by gavage at day 14 of gestation and again on days 1, 7, and 14 following birth, and host resistance studies were performed 6 to 8 weeks after weaning. This exposure protocol resulted in an increased susceptibility to *Listeria* in pups from dams receiving repeated doses of 5.0 µg TCDD/kg. However, Vos et al. (1978) reported that oral administration of 50 µg TCDD/kg once a week for 4 weeks to 3- to 4-week-old male Swiss mice followed by intravenous challenge 4 days after the last dose with *Listeria* had no effect on nonspecific phagocytosis and killing of *Listeria*. House et al. (1990) used B6C3F$_1$ female mice, 6 to 8 weeks of age and challenged intravenously with *Listeria* 7 to 10 days following a single dose of TCDD at 10, 1.0, and 0.1 µg/kg. TCDD did not enhance mortality from *Listeria*.

In summary, results from host resistance studies provide evidence that under certain conditions exposure to TCDD results in increased morbidity and mortality for bacterial, viral, parasitic, and neoplastic disease. These effects are observed at relatively low doses and likely result from TCDD-induced suppression of immunological function. However, the specific immunological functions targeted by TCDD in each of the host resistance models remain to be fully defined. Possible nonimmunological mechanisms, which may also contribute to altered host resistance by PHAHs, should also be investigated. Future work should also address the role of the AhR in host resistance.

4.5. ROLE OF THE THYMUS IN PHAH IMMUNOTOXICITY

Thymic involution is one of the hallmarks of exposure to TCDD and related PHAHs in all species examined. In mice, thymic involution occurs by an AhR-dependent mechanism. Poland and Knutson (1982) demonstrated that C57Bl/6 mice were 10-fold more sensitive to TCDD-induced thymic atrophy than DBA/2 mice. Because the thymus has a critical role in the ontogeny of T lymphocytes, thymic involution is often referred to as an immunotoxic effect. However, although an intact thymus is crucial to the developing immune system during the prenatal and early postnatal period of rodents as well as during the prenatal period of humans, the physiological role played by the thymus in adult life has not been established. In animal models,

adult thymectomy has little effect on the quantity or quality of T lymphocytes, which have already matured and populated the secondary lymphoid organs (Benjamini and Leskowitz, 1991). Likewise, in humans, childhood and adult thymectomy produces no clearly identifiable adverse consequences in terms of altered immune function, although some might argue that such studies have not been done. On the basis of this knowledge, it is not surprising that a direct relationship between the effects of TCDD on the thymus and immune suppression has not been established in studies using adult animals. In fact, adult thymectomy prior to PHAH exposure did not modify TCDD- or HpCDD-induced suppression of the anti-SRBC response (Tucker et al., 1986; Kerkvliet and Brauner, 1987). Furthermore, suppression of immune responses occurs at dose levels of PHAH significantly lower than those required to induce thymic atrophy (Vos et al., 1978; Silkworth and Antrim, 1985; Holsapple et al., 1986b; Tucker et al., 1986; Kerkvliet and Brauner, 1990). Thus, thymic involution does not represent a surrogate marker for TCDD immunotoxicity in adult animals. On the other hand, it is possible that chronic exposure to TCDD resulting in chronic thymic atrophy may produce more delayed, subtle effects on immune function not yet identified (Clarke and MacLennan, 1986).

In contrast to adult animals, congenital thymic aplasia or neonatal thymectomy results in severe reduction in the number and function of T lymphocytes and produces a potentially lethal wasting disease (Benjamini and Leskowitz, 1991). Similarly, there is evidence from studies that rodents exposed to TCDD or PCBs during the prenatal or neonatal period are more sensitive to immune suppression compared with rodents exposed as adults and that the prenatal effects are more selective for cell-mediated immunity (Vos and Moore, 1974; Faith and Moore, 1977; Luster et al., 1980b). Perinatal exposure of mice and rats to TCDD alters thymocyte differentiation and maturation in vivo (Holladay et al., 1991; Blaylock et al., 1992; Gehrs and Smialowicz, 1997, 1999; Gehrs et al., 1997).

TCDD has also been shown to alter thymocyte maturation in vitro by inducing terminal differentiation of thymic epithelial cells (Greenlee et al., 1985; Cook et al., 1987). Addition of TCDD to fetal thymic organ cultures (FTOCs) also alters thymocyte maturation (Dencker et al., 1985; d'Argy et al., 1989). 3,3',4,4'-Tetrachlorobiphenyl (PCB77) has also been found to alter the normal developmental pathways of fetal thymocytes in vitro (Esser and Welzel, 1993; Kremer et al., 1994). In mouse FTOCs the addition of TCDD or PCB77 resulted in an overall reduction in thymocytes but a significant increase in mature $CD4^-CD8^+$ thymocytes which were $CD3^+,\alpha\beta TCR^+,CD69^+,HSA^-,IL\text{-}2R^-$ (Esser and Welzel, 1993; Kremer et al., 1995). These mature $CD8^+$ thymocytes were functionally competent, in that they responded to stimulation by Con A or anti-CD3 and possessed cytotoxic activity when cultured with H-2 allogeneic spleen cells (Lai, et al., 1995). Data from experiments using FTOC recultivation techniques, in which intact thymic lobes and thymocyte-depleted (i.e., stroma) lobes were employed, suggested that TCDD drives

thymocytes into differentiation faster than the precursor pool can be replenished by self-renewal (Kremer et al., 1994). Because TCDD affected thymic stroma but did not directly affect thymocytes, the findings of Kremer et al. (1994) suggest that the nonlymphoid compartment (i.e. stroma) rather than the thymocytes themselves may be the target of these PHAHs.

Work by Silverstone et al. (1994a) indicates that TCDD-induced thymic atrophy can be mediated, at least in part, by damage to prethymic T cell precursor stem cells in both bone marrow and liver. Perinatal exposure of mice to TCDD resulted in alteration in the lymphocyte stem cell population, as indicated by a significant reduction in the lymphocyte stem cell–specific enzyme terminal deoxynucleotidyl transferase (TdT) in fetal liver and neonatal bone marrow lymphoid cells (Fine et al., 1989). In contrast, thymic TdT synthesis was relatively unaffected on a per cell basis by perinatal TCDD exposure. TCDD also inhibited the ability of pre-T stem cells (prothymocytes), from both fetal livers and neonatal bone marrow, to repopulate the thymus of irradiated syngeneic mice (Fine et al., 1990). TCDD-induced thymic atrophy did not involve apoptotic mechanisms in thymocytes affected by the bcl-2 proto-oncogene, and this atrophy appears to be mediated through the AhR and not through effects on the estrogen receptor (Silverstone et al., 1994a, b; Frazier et al., 1994).

Thymic alterations in adult mice exposed to TCDD appear to depend on AhR activation in hemopoietic cells. Using chimeric mice with TCDD-responsive (AhR^{+/+}) stromal components and TCDD-unresponsive (AhR^{-/-}) hematopoietic components, or the reverse, Staples et al. (1998a) demonstrated that the hemopoietic compartment is the target of TCDD-induced thymic atrophy and thymic phenotype alterations. The mechanisms underlying these alterations are not clear, although TCDD-induced apoptosis of thymocytes, via Fas-Fas ligand interactions, has been reported (Kamath et al., 1997, 1999). However, other evidence indicates that apoptosis is not a key mechanism of TCDD-induced thymic atrophy (Staples et al. 1998b)

Taken together, the data indicate that PHAHs induce alterations of T cell maturation in the thymus. These are mediated in part by effects on accessory cells and on extra-thymic precursor cells. In addition, TCDD also influences B cell development in the bursa of chick embryos (Nikolaidis et al., 1990), as well as lymphocyte stem cells in the fetal liver and bone marrow of mice (Fine et al., 1989, 1990). Such alterations may have an important role in the observed suppression of immune function by PHAHs in perinatally exposed animals.

4.6. IMMUNOTOXICITY FOLLOWING PRENATAL/NEONATAL EXPOSURE TO PHAHS

The reported increase in susceptibility of very young animals to PHAH immunotoxicity necessitates close examination of the available literature on prenatal or neonatal immunotoxic effects. Several studies have examined immune function in mice, rats, and guinea pigs following

exposure to TCDD or PCB during fetal development (Vos et al., 1973; Vos and Moore, 1974; Thomas and Hinsdill, 1979; Luster et al., 1980b).

Results of work in which exposure of the progeny occurred via placental transfer and lactation are summarized in Table 4-3. Study 1 presents results of two studies, one using B6 (Vos and Moore, 1974) and the other B6C3F$_1$ mice (Luster et al., 1980a); study 2 presents results of outbred Swiss mice (Thomas and Hinsdill,1979); and study 3 presents results from two studies of rats (Vos and Moore, 1974; Faith and Moore, 1977). The most sensitive indicator of TCDD immunotoxicity in these studies was an increase in the growth of transplanted tumor cells in the offspring of B6C3F$_1$ mice (Ah responsive strain) treated with 1 µg/kg TCDD at 4 weekly intervals. (Total TCDD dose to dam was 4 µg/kg; dose to offspring was not determined.) The offspring of Swiss mice fed a diet containing 1 ppb TCDD for 7 weeks showed enhanced mortality following endotoxin challenge, while the plaque-forming cell response to SRBCs and delayed hypersensitivity response were suppressed in offspring of mice fed 5.0-ppb TCDD diets. (Estimated daily dose to 20 g dam consuming 5 g of 5-ppb TCDD diet is equivalent to 1.25 µg/kg TCDD/day.) Rats appeared to be relatively more resistant to the immunotoxic effects of prenatal or neonatal exposure to TCDD than mice based on the finding that 5, but not 1, µg/kg TCDD given four times at weekly intervals produced immunotoxicity in the offspring. Immunotoxic end points that were unaffected by the highest exposure levels in these studies included blastogenesis induced by LPS and serum antibody titers to bovine gamma globulin (BGG). However, a significant finding of this study was that suppression of the DTH response to oxazalone was relatively long lived in that suppression of this response was evident in 145-day-old rats. Taken together, these studies suggest that cell-mediated rather than humoral immunity is more sensitive to perinatal TCDD exposure.

Two later studies examined immune function in offspring of female mice, in the first study exposed to TCDD (Holladay et al., 1991) and in the second study to PCBs (Kanechlor 500) (Takagi et al., 1987), with the offspring cross-fostered to unexposed lactating mice at birth. Thus, exposure was limited to placental exposure. B6 mice exposed to 3.0 µg/kg TCDD on gestational days 6 to 14 gave birth to offspring that had significant thymic atrophy and hypoplasia measured on gestational day 18 or on day 6 postnatally. The thymic effects were no longer apparent by day 14. At 7 to 8 weeks postnatally, mitogen responses and antibody plaque-forming cell response to SRBCs were unaltered, but the CTL response was significantly suppressed compared with controls (Holladay et al., 1991). These results suggest a selectivity of prenatal TCDD on the CTL and not the T helper cells involved in the antibody response to SRBCs. In contrast to these results, Takagi et al. (1987) exposed female C3H mice per os to 50 mg/kg Kanechlor 500 twice per week for 4 weeks, at which time steady-state tissue levels were noted. The offspring derived from mating to unexposed males had an unaltered antibody response to the

T-independent antigen DNP-dextran. On the other hand, carrier-primed T helper cell activity assessed by adoptive transfer was significantly suppressed by PCB exposure when assessed 4 and 7 weeks after birth but fully recovered by 11 weeks. Together, these studies confirm prior studies to indicate that T cell function is selectively altered by PHAH when exposure is prenatal. Although both T helper cells and CTL show altered function, T helper cell activity may recover faster than CTL function.

Fine et al. (1990) reported on TCDD levels in mouse offspring following maternal treatment with TCDD (10 µg/kg) on gestational day 14. The fetal liver had the highest concentration on gestational day 18 (235 fg/mg), which declined slightly by postnatal day 6 to around 100 fg/mg. Concentration of TCDD in the thymus on gestational day 18 was 140 fg/mg, which declined to 20 fg/mg on day 6 after birth. (These thymic TCDD concentrations are equivalent to 60 to 425 pM, assuming 1 kg of tissue is equivalent to 1 L of water.) TCDD concentrations in the spleen remained constant at about 40 fg/mg during the same timeframe, whereas bone marrow concentrations were very low (about 3 fg/mg). These concentrations of TCDD were associated with thymic atrophy (Fine et al., 1989) and significant reduction in the ability of prothymocytes in liver and bone marrow to repopulate an irradiated thymus (Fine et al., 1990).

Recent studies in rats indicate that perinatal exposure to TCDD results in relatively long-lived and persistent suppression of immune responses. Lactating female Leeds rats were exposed to TCDD in their feed, starting on postnatal day 1 through 18, at 0.1, 0.5, or 2.5 µg TCDD/kg, with an estimated total TCDD consumption of 0.2, 1.0, or 5.0 µg/kg/body weight, respectively (Badesha et al., 1995). Both T cell–dependent (i.e., SRBC) and T cell–independent (i.e., DNP-Ficoll, TNP-LPS, and LPS) antibody responses were suppressed in a dose-related manner in 130-day-old offspring.

In another group of studies, a single oral exposure of pregnant Fischer 344 dams on GD-14 to 0.1, 0.3, 1.0, or 3.0 µg TCDD/kg resulted in suppression of the DTH response to BSA (Gehrs et al., 1997; Gehrs and Smialowicz, 1999). Depressed DTH responses persisted for up to 19 months in the male offspring of dams exposed to 3.0 µg TCDD/kg. While both male and female offspring exposed to TCDD on GD-14 displayed reduced DTH responses, the DTH response of males appears to be more affected than that of females. A cross-fostering study indicated that both gestational and lactation exposure of the offspring are required for suppression of the DTH response following gestational day 14 exposure of the dams (Gehrs et al., 1997). A single low dose (0.1 µg/kg) of TCDD given to dams on gestational day 14 resulted in suppression of the DTH response that persists in the offspring for up to age 14 months (Gehrs and Smialowicz, 1999). These results suggest that perinatal exposure to TCDD may lead to a permanent defect in the immune defenses associated with the DTH response, which are critical

for protection against certain intracellular bacterial and parasitic infections as well as certain viruses. If so, then the fetus and neonate may be more at risk for TCDD-induced immune perturbation, which may manifest, early or later in life, as susceptibility to certain infectious diseases.

4.7. IMMUNOTOXICITY OF PHAHS IN NONHUMAN PRIMATES

Studies using nonhuman primates as surrogate models for humans have been conducted to assess PHAH immunotoxicity. Immunological effects were described in rhesus monkeys and their offspring chronically exposed to TCDD at levels of 5 or 25 ppt for 4 years (Hong et al., 1989). In the mothers, the total number of T cells increased in monkeys fed 25 ppt TCDD, with a selective increase in $CD8^+$ cells and a decrease in $CD4^+$ cells. However, no significant effect on T cell function was established when assessed as proliferation response to mitogens, alloantigens, or xenoantigens. NK cell activity and production of antibodies to tetanus immunization were normal. In the offspring of TCDD-exposed dams examined 4 years after exposure, a significantly increased antibody response to tetanus toxoid (TT) immunization was observed that correlated with TCDD tissue levels. Body burden of TCDD in the offspring ranged from a low of 290 ppt to a high of 1,400 ppt. Interestingly, there was no strict correlation between exposure levels and resulting body burden. Immunoenhancement of the antibody response to TT in the offspring of the monkeys exposed to TCDD is in contrast to immunosuppression observed in rodents exposed perinatally. However, other studies in nonhuman primates cited below indicate that humoral immunity is suppressed in nonhuman primates by other PHAHs.

In other TCDD studies, a single injection of TCDD in marmosets (*Callithrix jacchus*) resulted in a delayed decrease in the percentage of $CD4^+$ T cells and $CD20^+$ B cells in the blood and an increase in the percentage of $CD8^+$ cells (Neubert et al., 1990). The total number of T cells was not significantly altered by TCDD exposure. The $CD4^+$ subset most affected was the $CDw29^+$ "helper-inducer" or "memory" subset, with significant effects observed after a TCDD dose of 10 ng/kg. The no-observed-effect level for this effect was 3 ng/kg TCDD. Concomitant with suppression of the CDw29 subset in TCDD treated animals, the percentage of $CD4^+CD45RA^+$ cells increased. This subset has been classified as "suppressor-inducer" or "naive" cells. The changes in the T cell subsets were intensified following in vitro culture of the cells with mitogen (Neubert et al., 1991).

Interestingly, however, another study from the same laboratory reported that chronic exposure of young marmosets to very low levels of TCDD (0.3 ng/kg/week for 24 weeks) produced the opposite effect on the $CD4^+CDw29^+$ subset, resulting in a significant increase in this population (Neubert et al., 1992). Concomitantly, the $CD4^+CD45RA^+$ subset decreased.

Upon transfer of the animals to a higher dose of TCDD (1.5 ng/kg/week) for 3 weeks, the enhancement effect was reversed and suppression of the CD4$^+$CDw29$^+$ subset was observed, with maximum suppression after 6 weeks of exposure to the higher dose. In addition, the CD8$^+$CD56$^+$ T cytotoxic T cell subset was transiently increased but normalized even though TCDD dosing continued. After discontinuation of dosing, the reduction in the percentage and absolute number of CD4$^+$CDw29$^+$ cells persisted for 5 weeks, reaching normal range 7 weeks later. These results led the authors to conclude that extrapolation of the results obtained at higher doses to very low exposures is not justified with respect to the effects induced by TCDD on the immune system of marmosets (Neubert et al., 1992).

Neubert et al. (1995b) performed a study to determine if a functional deficiency could be detected in marmosets that displayed altered peripheral blood T and B subsets following exposure to TCDD (Neubert et al., 1990). No reduction was observed in the in vitro lymphoproliferative response to TT by peripheral blood lymphocytes from marmosets vaccinated with tetanus and exposed to a single dose of 100 ng/kg TCDD at the time of the second booster vaccination (Neubert et al., 1995b). These results are interesting because no association was established between this functional endpoint and the earlier observed TCDD-induced peripheral blood lymphocyte subset changes in mature marmosets given a single injection of TCDD (Neubert et al., 1990).

Immunomodulatory effects of chronic low-level PCB exposure in monkeys have also been investigated. In early studies, Thomas and Hinsdill (1978) reported that rhesus monkeys fed diets containing 2.5 or 5 mg/kg of Aroclor 1248 had significantly suppressed antibody responses to SRBCs but not to TT. These monkeys also had chloracne, alopecia, and facial edema. Similarly, exposure of cynomolgus monkeys to Aroclor 1254 (100 or 400 µg/kg/day) for 3 months suppressed antibody responses to SRBCs but not TT (Truelove et al., 1982). Suppressive effects on anti-SRBC responses were more severe in cynomolgus monkeys when the PCB mixture contained PCDFs (Hori et al., 1982). Tryphonas et al. (1989; 1991a, b) reported results of studies in rhesus monkeys exposed chronically to Aroclor 1254 (5 to 80 µg/kg/day) for 23 or 55 months. These exposures resulted in steady-state blood PCB levels that ranged from a mean low of 0.01 ± 0.001 ppm in the 5 µg/kg group to a mean high of 0.11 ± 0.01 ppm in the 80 µg/kg group. The only consistently altered immune parameter was the primary and anamnestic antibody responses to SRBCs, which were suppressed in a dose-dependent manner. In contrast, the antibody response to pneumococcus vaccine antigen measured at 55 months of exposure was not significantly altered. At 23 months, the percentage of T helper cells in the blood was significantly decreased in the 80 µg/kg group, and the percentage and absolute number of T suppressor cells were increased; however, these effects were not apparent at 55 months of exposure (Tryphonas et al., 1991b). Lymphoproliferative responses to PHA and Con A were not

significantly altered at 23 months but were dose- dependently suppressed at 55 months. Proliferation to alloantigens was not significantly altered. Likewise, serum immunoglobulin and hydrocortisone levels did not differ between treatment groups. After 55 months, chemiluminescent response (time to peak) of monocytes from PCB-exposed monkeys was slower than that from controls. Also noted at 55 months were a significant elevation in serum hemolytic complement levels, a dose-related increase in NK cell activity, and a dose-related increase in thymosin alpha-1 levels but not thymosin beta-4 levels (Tryphonas et al., 1991a). Effects on interferon levels were inconsistent, and TNF production was not altered.

The studies in nonhuman primates are important from the standpoint that the antibody response to SRBCs emerges as the only immunological parameter consistently suppressed by PHAH in several different animal species. Notable exceptions are the reports that TCDD does not suppress antibody response to SRBCs in rats (Smialowicz et al., 1994; Fan et al., 1996). At the present time, it is not clear why the antibody response to SRBCs is most consistently altered by PHAH exposure in different species. Sensitivity of the anti-SRBC response does not appear to be caused solely by T cell dependency of the response because antibody responses to other T-dependent antigens (e.g., TT, BGG) are not suppressed and may be enhanced following PHAH exposure. It is possible that the particulate nature of the SRBC antigens is an important factor even though a mechanistic basis for this is not readily apparent. The sensitivity of the technique used to quantify the antibody response may also contribute to apparent increased sensitivity of the SRBC model, which is most often measured as the PFC response rather than serum antibody titers which are usually more variable. Nonetheless, the finding that the SRBC response is also suppressed in nonhuman primates exposed to PCBs lends support to the use of the anti-SRBC database for risk assessment of PHAHs.

4.8. IMMUNOTOXICITY OF PHAHS IN HUMANS: IN VIVO EXPOSURE

Immunotoxicity of TCDD and related PHAHs in humans has been the subject of several studies derived from accidental, occupational, or environmental exposure to PCDDs, PCDFs, and/or PCBs(see Chapter 7, Human Effects). Perhaps the most important human case studies involve two incidents in which large numbers of individuals were exposed to PCB-contaminated rice oil containing PCDFs and other PHAHs. The first occurred in Japan in 1968 and the second in Taiwan in 1979, and the resulting symptoms associated with exposure were called "Yusho" and "Yu-Cheng" which mean "oil disease" in Japanese and Chinese, respectively (Tsukamoto, 1969; Chang et al., 1980a). The most common clinical symptoms included acne-form eruptions and follicular accentuation, pigmentation of the skin and nails, swelling of the eyelids and increased discharge, nausea, headaches, and numbness of the limbs (Chang et al., 1980b).

Clinical studies revealed decreased serum concentration of γ-globulin and decreased DTH responses in Yu-Cheng patients (Chang et al., 1980b).

Patients also presented with increased frequency of various kinds of infection, especially of the respiratory tract and skin (Shigematsu et al., 1978; Nakanishi et al., 1985; Lu and Wu, 1985). Immunologic effects, in studies that compared a group of 30 Yu-Cheng patients and 23 normal controls, included decreased serum IgA and IgM, but not IgG, and decreased percentage of total T cells (E rosettes), active T cells (active E rosettes), and T helper (Tμ) cells in peripheral blood (Chang et al., 1981). Monocytes and polymorphonuclear cells from these exposed patients also had reduced numbers of Fc receptors compared with controls (Chang et al., 1982a). In a subsequent study, the DTH response to streptokinase and streptodornase in 30 exposed patients was significantly reduced compared with 50 controls (Chang et al., 1982b). The percentage of anergic patients increased, and the degree of induration decreased with increased PCB concentration in the blood. In contrast, lymphoproliferative responses of peripheral blood lymphocytes (PBLs) to PHA, pokeweed mitogen (PWM), and tuberculin PPD, but not Con A, were significantly augmented in PCB-exposed patients (Lu and Wu, 1985). PCB concentrations in the blood ranged from 3 to 1,156 ppb, with a mean of 89 ± 6.9 ppb. The oil was contaminated at PCB concentrations of 4.8 to 204.9 ppm, with a mean of 52 ± 39 ppm (Ko et al., 1981a, b). Followup studies 3 years later indicated that the DTH to PPD was reduced, that the percentage of total T cells had recovered but the percentage of helper T cells was reduced and of suppressor T cells increased, and that lymphoproliferative responses were enhanced in exposed patients compared with controls (Wu et al., 1984; Lu and Wu, 1985).

The patterns of immune function alterations in the Yu-Cheng patients described above were relatively consistent between the original and 4-year followup studies. These observations would suggest a rather robust and persistent alteration in the immune parameters examined. Unfortunately, it is not clear how many, if any, of the same patients were tested in each of these different studies to allow a direct assessment of the persistence of these effects.

Mothers of Yu-Cheng children reported increased incidence of pneumonia and bronchitis in their children during the first 6 months of life (Rogan et al. 1988). In a followup study, school-age children prenatally exposed to PCBs and PCDFs (n=103), were compared with nonexposed control children (n=96) for middle-ear disease (Chao et al., 1997). The exposed children had a higher prevalence of otitis media compared with their matched controls. Furthermore, exposed children with ear disease had higher serum levels of 2,3,4,7,8-pentachloro- and 1,2,3,4,7,8-hexachloro-dibenzofurans than did children with no middle-ear disease. In the summer of 1995, 105 Yu-Cheng children and 101 control children were given physical exams (Yu et al., 1998). The frequency of influenza, but not otitis media, asthma, or enteronitis attacks, during the 6 months prior to examination, as reported by the parents of the Yu-Cheng children,

was higher than for the controls. Blood samples were obtained from 29 Yu-Cheng and 22 control children for evaluation of total serum IgM, IgG, and IgA levels, as well as percentages of circulating T cells, B cells, and NK cells. There were no differences between Yu-Cheng and control children for any of these immune parameters (Yu et al., 1998).

Tests of altered immune function were also described in Michigan dairy farmers exposed to PBBs through contaminated dairy products and meat in 1973 (Bekesi et al., 1979). As in the PCB-exposed patients, the percentage and absolute numbers of T cells in peripheral blood of PBB-exposed farmers were significantly reduced compared with a control group. However, in contrast to PCB-exposed individuals (Lu and Wu, 1985), lymphoproliferation responses to PHA, PWM, and allogeneic leukocytes were significantly decreased in PBB-exposed persons. Also in contrast to PCB exposure, skin testing using standard recall antigens indicated that PBB-exposed farmers had significantly increased responses, particularly to candida and Varidase. Tissue levels of PBBs in the subjects were not determined in this study.

In contrast, another study of Michigan residents exposed to PBBs revealed no significant differences in lymphocyte number or function compared with nonexposed individuals (Landrigan et al., 1979; Silva et al., 1979). The exposed cohort in this study was drawn from individuals who lived on PBB-quarantined farms, who received food directly from such farms, or who worked or were related to workers engaged in PBB manufacture. No differences in the total leukocyte counts, the absolute numbers of T or B peripheral blood lymphocytes, or the in vitro responses to PHA, Con A, and PWM were observed between this high-exposure group (mean serum PBB level of 787 ppb [range of 188 to 2560], n=32) and a low- exposure group (mean serum PBB level of 2.8 ppb [range of <1 to 11], n=51).

Several studies have also examined the effects of occupational or environmental exposure to TCDD in human populations. Webb et al. (1989) reported the findings from immunologic assessment of 41 persons from Missouri with documented adipose tissue levels of TCDD resulting from occupational, recreational, or residential exposure. Of the participants, 16 had tissue TCDD levels less than 20 ppt, 13 had levels between 20 and 60 ppt, and 12 had levels greater than 60 ppt. The highest level was 750 ppt. Data were analyzed by multiple regression based on adipose tissue level and the clinical dependent variable. Increased TCDD levels were correlated with an increased percentage and total number of $OKT8^+$ ($CD8^+$) cells and increased percentages of $OKT11^+$ (i.e., $CD2^+$) and $OKT3^+$ (i.e., $CD3^+$) T lymphocytes. However, the percentage and total number of $OKT4^+$ cells were not altered. Lymphoproliferative responses to Con A, PHA, PWM, or TT were unaltered, as was the cytotoxic T cell response. Serum IgA, but not IgG, was increased. No adverse clinical disease was associated with TCDD levels in these subjects. Only 2 of the 41 subjects reported a history of chloracne.

The above findings differ from those reported for the Quail Run Mobile Home Park resident cohort study using 152 exposed and 151 unexposed individuals (tissue levels unknown) in which decreased T cell percentages and suppressed cell-mediated immunity were reported (Hoffman et al., 1986). The exposed group was reported to have decreased percentages of PBL OKT3$^+$, OKT4$^+$, and OKT11$^+$ T cells. Using seven standardized recall antigens (i.e., tetanus, diphtheria, *Streptococcus*, tuberculin, *Candida*, *Proteus*, and *Trichophyton*), the researchers observed a significant decrease in the DTH response of TCDD-exposed individuals compared with unexposed controls. The exposed group had an increased frequency of anergy (11.8% vs. 1.1%) and relative anergy (35.3% vs. 11.8%) compared with unexposed controls. However, it is important to note that there were significant technical problems with the interpretation of the skin test responses in this study. Nearly 50% of the skin test data were not used because two of the four skin test readers were inexperienced. Subsequent retesting of these anergic subjects, however, failed to confirm the DTH anergy (Evans et al., 1988). The only T cell measures outside the normal range, which were determined from the initial Quail Run study of Hoffman et al. (1986), were the percentage of OKT4$^+$ cells and the OKT4$^+$/OKT8$^+$ ratios, which were lower compared with unexposed controls (Evans et al., 1988). On the other hand, when serum from some of these individuals was tested for levels of the thymic peptide, thymosin alpha-1 (Thy$_{a-1}$), the entire frequency distribution for the TCDD-exposed group shifted toward lower Thy$_{a-1}$ levels (Stehr-Green et al., 1989). The significant difference between the TCDD-exposed persons and controls remained after controlling for age, sex, and socioeconomic status, with a trend of decreasing Thy$_{a-1}$ levels with increasing number of years of residence in the TCDD-contaminated residential area. Thy$_{a-1}$ levels were not correlated with changes in other immune system parameters or with any increased incidence of clinically diagnosed immune suppression. The decrease in Thy$_{a-1}$ levels in humans contrasts with the increase in Thy$_{a-1}$ seen in PCB-treated monkeys (Tryphonas et al., 1991b). Thy$_{a-1}$, a product of thymic epithelial cells, has a role in modulating the differentiation of prothymocytes to mature thymocytes (Low and Goldstein, 1984).

In July 1976 an explosion in a chemical plant producing trichlorophenol herbicides near Seveso, Italy resulted in the release of TCDD estimated at 1.7 kg (Pocchiari et al., 1979). Several epidemiological evaluations were performed on exposed individuals after the accident. Pocchiari et al. (1979) summarized results of periodic evaluations of immune status of a group of 45 children (21 of whom had chloracne) age 3 to 7 years exposed to TCDD in Zone A, which was the most heavily contaminated area. No abnormalities were reported for the following parameters: serum immunoglobulin concentrations, levels of circulating complement, lymphoproliferative responses to T and B cell mitogens or alloantigens in the MLR, or PBL T and B cell populations. Interestingly, in the summary of a study conducted 6 years after the

explosion, a different cohort of TCDD-exposed children was reported to have exhibited a significant increase in complement protein levels, which reportedly correlated with incidence of chloracne as well as increased numbers of peripheral blood lymphocytes and increased lymphoproliferative responses (Tognoni and Bonaccorsi, 1982; Mocarelli et al., 1986, 1991). However, no specific health problems were correlated with dioxin exposure in these children. Unfortunately, none the studies cited above present any quantitative data. Specific information about the methods and results are not presented. A cursory description of the tests employed and the results obtained are the only information provided for the immune function evaluations. This is extremely unfortunate, because a critical evaluation of these studies and their conclusions cannot be made.

A more thorough study examined possible associations between occupational exposure to the herbicide Agent Orange and its dioxin contaminate and adverse health experienced by Air Force personnel who served in Operation Ranch Hand units in Vietnam from 1962 to 1971 (Roegner et al., 1991) and is summarized by Wolfe et al. (1992). Immunological tests were carried out on a random sample of approximately 40% of the 1,670 participants for whom dioxin measurements in serum were made. Statistical models were used to evaluate associations between test parameters and serum dioxin levels using estimated initial and current serum dioxin levels. The statistical models were implemented using minimal assumptions (only Ranch Hands with current dioxin levels above 10 ppt) and maximal assumptions (all Ranch Hands with current dioxin levels above 5 ppt). The immunological tests included the following: DTH response to the recall antigens *Candida*, mumps, *Trichophyton*, and Staphage-lysate; PBL subsets CD2 (total T cells), CD20 (B cells), CD4 (helper/inducer T cells), CD8 (suppressor/cytotoxic T cells), CD14 (APC monocytes), CD25 (IL-2 receptor positive activated T cells), HLR-DR (B cells, activated T cells and monocytes that present antigen to $CD4^+$ T cells), CD4/CD8 ratio, and TLC (total lymphocytes in circulation); total serum IgM, IgG, and IgA levels; lymphoproliferative response to PHA; NKCI and NKCA activity, which measure NK cell lytic activity with and without IL-2 treatment, respectively; and mixed leukocyte culture (MLC) response (Roegner et al., 1991). This immunological assessment did not find any clinically significant alterations associated with current or initial levels of serum dioxin. A significant association between initial dioxin level and increased IgA levels was found; however, IgM and IgG levels did not indicate the presence of any dioxin-related effects. There was no association between the DTH response and serum dioxin levels. Despite several significant findings for some of the other immunological parameters examined, these data were deemed to be either internally inconsistent or not in a direction expected in an impaired immune system (Roegner et al., 1991; Wolfe et al., 1992).

Results of a followup examination of Air Force personnel involved in Operation Ranch Hand are presented in a report by Grubbs et al. (1995). Many of the immunologic parameters in

the earlier study were examined in this study, with the following exceptions. The PBL subset CD3 (pan T cells) replaced CD2, and CD5 (T cells and B cells) and CD16+CD56 (NK cells) were added to the test screen. The PBL PHA response, NKCI and NKCA activity assays, and MLC response were not performed. However, an autoantibody lupus panel, including tests for antinuclear antibodies; thyroid microsomal antibody; mouse stomach and kidney (MSK) smooth muscle, mitochondrial, and parietal antibodies; and rheumatoid factor, was included in this study. A marginal positive association was found between IgA levels and initial serum dioxin (Grubbs et al., 1995), as reported in the earlier study (Roegner et al., 1991). Also, an inverse relationship was found with dioxin exposure and the presence of autoantibodies to MSK smooth muscle, rheumatoid factor, and the lupus panel summary index. Although Grubbs et al. (1995) recommended that these findings be investigated and clarified in further followups, they concluded that no clinically significant indicators that reflected a consistent relationship between serum dioxin and immune function deficiencies were found in this study.

The most recent followup examination of Air Force personnel involved in Operation Ranch Hand occurred in 1992, and the results and analyses of immune parameters and dioxin body burden were reported by Michalek et al. (1999). A total of 2,233 veterans (Ranch Hand, n=952; Comparison, n=1,281) participated in this physical exam. The immunologic parameters examined in this study were the same as those in the previous followup study (Grubbs et al., 1995). Analysis of the results of the 1992 followup exam revealed only three statistically significant differences. First, an increase was observed in absolute CD20$^+$ (B cells) count in the Background group, whereas the mean absolute count of CD16$^+$CD56$^+$CD3$^+$ (T) cells was decreased in the High category. Second, three dioxin-exposed veteran categories (Background, Low, and High) had increased positive thyroid microsomal autoantibody tests; however, only the Low category was significant. Third, there was no significant association between dioxin-exposed veterans and total serum immunoglobulin levels, or in the presence of other autoantibodies. As with the earlier Ranch Hand studies, the authors concluded that there was no consistent relationship between dioxin exposure category and immune system alteration in Ranch Hand veterans (Michalek et al., 1999).

Other human studies involving smaller cohorts, in which exposure to PHAHs was documented, report alterations in certain immunological parameters. Eighteen workers (8 of whom had chloracne) were evaluated for immunological abnormalities 17 years after exposure to TCDD (no tissue levels were reported) in an industrial accident in a plant manufacturing the herbicide 2,4,5 trichlorophenoxyacetic acid (Jennings et al., 1988). Immunological parameters evaluated were the following: serum IgM, IgG, and IgA levels; antinuclear antibodies; immune complexes; T lymphocyte subsets and NK cells; and lymphoproliferative response to PHA. Antinuclear antibodies and immune complexes were detected more frequently in the peripheral

blood of workers exposed to dioxin. No differences were observed in the total number of T or B lymphocytes or in the T4/T8 ratio; however, NK cells identified by the surface marker Leu-7 were higher in the dioxin-exposed workers (Jennings et al, 1988). The significance of these findings is unknown, because only a small cohort was studied and the autoantibody results were opposite to those reported by Grubbs et al. (1995). Also, there are no animal data that indicate increased NK cells associated with TCDD exposure. The NK results also differ from results of a more recent human study by Svensson et al. (1994), who reported that men with high consumption of PHAH-contaminated Baltic Sea fatty fish (n=23) had lower proportions and numbers of PBL NK cells (i.e., CD56$^+$ cells) compared with men with virtually no fish consumption (n=20). NK cells were negatively associated with blood levels of several persistent organochlorine compounds and with estimated intake of fish. Fish consumption, however, was not associated with any alterations in other cell subsets, plasma immunoglobulin levels, or liver enzyme activities. It is important to point out that no attempt was made to determine if the decreased numbers of NK cells were correlated with reduced NK function in this study or with increased NK activity in the study described by Jennings et al. (1988).

A retrospective cohort morbidity study of 158 men accidentally exposed in 1953 to TCDD during the production of trichlorophenol was reported by Zober et al. (1994), in which 73 men had back calculated TCDD values of >1,000 ppt and 85 had values of <1,000 ppt. Increased frequency of upper respiratory tract infections was observed in individuals who had severe chloracne. However, it was indicated that differences in the utilization rates of medical care by the exposed and referent groups could have biased the findings. A clinical study of the 138 men exposed to TCDD in the accident described above attempted to determine if any TCDD dose relationships existed within the exposed population for a number of clinical and immunological parameters (Ott et al., 1994). Increases in serum IgA and IgG and increases in complement C3 and C4 were seen with high current and back-calculated TCDD concentrations and with high current TCDD concentrations, respectively. Marginal decreases in the percentage of lymphocytes, NK cells, T cells, T helper cells, and T suppressor cells were also observed (Ott et al., 1994). However, mean values for all of these parameters were comparable to those of internal referent groups of 42 to 196 individuals (number of referents differed among the parameters examined) who were not part of this clinical study but who participated in routine occupational medical examinations during the same period (Ott et al., 1994).

In another retrospective study, T helper cell function was evaluated in 11 workers, 45 to 63 years of age, who were exposed to high levels of TCDD and other PCDDs between 1966 and 1976 in production and maintenance operations at a German chemical factory producing 2,4,5-trichlorophenol (Tonn et al., 1996). TCDD body burdens were still high (43 to 874 pg/g blood or a mean of 330, which is 10 times higher than in the average German population) 20 years after

exposure. No differences were detected between exposed and control groups for surface marker distribution (e.g., CD3, CD4, CD8, CD19, CD45RO, CD45RA, CD56 or CD57) or mitogen-induced lymphoproliferation responses (e.g., PHA or PWM). However, the exposed group showed reduced lymphoproliferation in response to human lymphocyte antigen-allogeneic lymphocytes (e.g., MLR) and IL-2 stimulation. It was concluded that TCDD has a long-term immunosuppressive effect on T helper cells manifested by reduced function of these cells rather than by reduction in the cell numbers in peripheral blood (Tonn et al., 1996).

Neubert et al. (1993) attempted to determine if workers with moderately increased body burdens of TCDD and related PHAHs (25 to 140 ppt TCDD or 104 to 522 ppt International-Toxicity Equivalencies [I-TE] in blood lipids) displayed altered patterns of PBL subsets similar to those observed in TCDD-exposed marmosets (Neubert et al., 1990, 1991, 1992). A slight trend in increased percentage of $CD4^+CD45RO^+$ helper-inducer T cells was observed. However, Neubert et al. (1993) concluded that these alterations were of no medical relevance. Furthermore, the data did not support an assumption that moderately increased body burdens of PCDDs/PCDFs in adults result in decreased cellular components of the human immune system. Because adult humans, as well as adult marmosets, are less susceptible to PCDD/PCDF-associated alterations in PBL subsets than are adolescent marmosets, Neubert et al. (1993) suggested that exposure to PHAHs during early development may be the important factor influencing altered lymphocyte subsets.

In a more recent study, Neubert et al. (1995a) evaluated the in vitro lymphoproliferative responses to Con A, PHA, PWM, anti-CD3, and TT of PBL from the same workers described above. No decrease in the lymphoproliferative capacity of lymphocytes from these workers with moderately increased PCDD/PCDF body burdens was observed compared with lymphocyte responses of individuals with PCDD/PCDF body burdens within the reference range (1 to 3 ppt TCDD or 9 to 29 ppt I-TE in blood lipids) for any of these lymphocyte stimulators.

A retrospective examination of possible associations between altered immune parameters and occupational exposure to TCDD of chemical plant workers involved in the manufacture of 2,4,5-trichlorophenate and its derivatives between 1951 and 1972 was reported by Halperin et al. (1998). A total of 259 workers and 243 unexposed referents were included in this study. The workers had had substantial exposure to TCDD as indicated by a lipid adjusted mean serum TCDD concentration of 229 ppt, whereas the controls had a mean TCDD concentration of 6 ppt. Peripheral blood leukocytes and lymphocytes were enumerated, and lymphocytes were evaluated by flow cytometry for a variety of populations including T cells, B cells, and NK cells. In vitro assays for peripheral blood NK cell activity and lymphoproliferative activity of cultured lymphocytes in the presence of the mitogens PHA, ConA, or PWM, or the antigens mumps, Candida, or TT were performed. Total serum concentrations of IgM, IgG, and IgA and

complement factor C3 were also determined. Of the various TCDD categories (based on workers' serum TCDD concentration) except the lowest, there were increased odds of having lower CD26 (activated T cells) counts. In addition, there was a decreased spontaneous proliferation of cultured lymphocytes (i.e., no mitogen or antigen present in the culture); however, lymphocytes from workers in the high TCDD category had increased proliferation in the presence of ConA and PWM. No other immune parameters were affected. It was concluded that the results were unlikely to be of clinical significance (Halperin et al., 1998).

The immunologic effects of pre- and postnatal background exposure to PCBs/dioxins of Dutch infants from birth to 18 months of age were presented in a study by Weisglas-Kuperus et al. (1995). Prenatal PCB exposure was estimated from the sum of PCB congeners 118, 138, 153, and 180 in maternal blood (i.e., 2.25 ± 0.98 µg/L, n=206) and the TEQ level in milk based on 17 PCDDs/PCDFs and 8 dioxin-like PCB congeners (i.e., 66.6 ± 24.4 pg/g fat, n=80). Postnatal exposure was calculated as a product of the total TEQ level in milk multiplied by the weeks of breast-feeding. No relationship was found between pre- and postnatal PCB/dioxin exposure and respiratory tract symptoms (i.e., number of periods with rhinitis, bronchitis, tonsillitis, and otitis) or humoral antibody production at 18 months to vaccination against mumps, measles and rubella at 14 months. Higher prenatal exposure was associated with alterations in T cell subsets, in which increased number of $TCR\gamma\delta^+$ T cells correlated with a total higher TEQ level at birth. Further, higher prenatal exposure was also associated with increased total numbers of T cells, $CD8^+$ cells and $TCR\gamma\delta^+$ T cells at 18 months of age; correlating with higher TEQ levels. A higher TEQ level was also associated with a decreased number of monocytes and granulocytes at 3 months. Despite these statistical associations of cell types with TEQ levels, all values were found to be within normal range. It was indicated that the subtle changes in the number of blood leukocytes do not necessarily mirror alterations in the cell composition of lymphoid and nonlymphoid organs nor do they reflect functional defects (Weisglas-Kuperus et al., 1995).

In a followup study, Weisglas-Kuperus et al. (2000) examined whether changes associated with prenatal exposure to PCBs persist into later childhood. At 42 months of age, antibody levels to measles and mumps were negatively correlated with cord and maternal PCB levels, respectively. A higher prevalence of recurrent middle ear infections and chicken pox, as well as a lower incidence of allergic reactions, were associated with current PCB body burdens. Also, there were significant positive correlations between prenatal PCB exposure and the numbers of peripheral blood lymphocytes and $CD3^+CD8^+$(cytotoxic), $CD4^+CD45RO^+$ (memory), $TcR\alpha\beta^+$, and $CD3^+HLA-DR^+$ (activated) T cells. These values, however, were within the normal range for children at this age. The authors concluded that perinatal background exposure to PCBs and dioxins might be associated with increased susceptibility to infectious diseases.

In summary, no clear pattern of altered immune parameters following exposure to TCDD or related PHAHs has emerged from studies in humans. The basis for the lack of consistent, exposure-related effects is unknown and may depend on several factors. These include the generic difficulties in assessing subclinical immunomodulation using assays with very broad ranges of normal responses, which reduce the sensitivity to detect small changes in a heterogeneous human population. Furthermore, the choice of immune parameters used to evaluate humans exposed to TCDD and related PHAHs has been based to a greater extent on what is clinically feasible than on what has been shown to be sensitive in animal studies. Thus, lack of consistent or significant immunotoxic effects in humans resulting from TCDD exposure may be as much a function of the assays used as of the immune status of the cohort.

An interesting observation, relative to the human data, is that T cells and T cell functions appear to be more frequently affected than other immune cells or functions when PHAH exposure related effects have been reported. This may simply reflect the fact that T cell assays predominate over other assays employed in these studies. Nevertheless, it is interesting that several studies report slight reductions in CD4$^+$ T helper cells (Chang et al., 1981; Wu and Lu, 1984; Lu and Wu, 1985; Hoffman et al., 1986; Evans et al., 1988). Reductions in CD4$^+$ cells and CD4$^+$ subsets have also been reported in non-human primates exposed to PHAHs (Hong et al., 1989; Neubert et al., 1990, 1991, 1992; Tryphonas et al., 1991b). These reductions may not translate into significant immune effects. However, the fact that these cells play a pivotal role in regulating immune responses and that their reduction may presage immunosuppression suggests that these observations are worth further investigation.

4.9. IMMUNOTOXICITY OF PHAHS IN HUMANS: IN VITRO EXPOSURE

Several laboratories have examined the direct effects of TCDD on human lymphocytes in vitro. Neubert et al. (1991) reported decreased PBL subpopulations from humans and nonhuman primates cultured in the presence of TCDD. CD4$^+$CD29$^+$ helper-inducer/memory T cells and CD20$^+$ B cells were dose-dependently decreased in PWM-stimulated cultures of human PBLs at concentrations as low as 10^{-12} to 10^{-14} M TCDD. However, an attempt to corroborate these findings failed to detect any suppression in human PBL subpopulations, including CD4$^+$CD29$^+$ helper-inducer/memory T cells and CD19$^+$ B cells, at TCDD concentrations ranging from 10^{-7} to 10^{-14} M (Lang et al., 1994). Furthermore, PWM- or anti-CD3-stimulated lymphocyte proliferation was not altered by TCDD at concentrations of 10^{-7} to 10^{-11} M (Lang et al., 1994).

Similar negative results were obtained by Wood et al. (1992), who reported that exposure of human tonsillar lymphocytes (HTLs) to concentrations of 3×10^{-8} to 3×10^{-10} M TCDD did not affect either PWM-induced proliferation or IgM antibody production. In contrast, 3×10^{-8} to 3×10^{-10} M TCDD suppressed toxic shock syndrome toxin (TSST-1)–induced IgM secretion of

HTL B cells (Wood and Holsapple, 1993). The sensitivity of the HTL B cells to TCDD suppression of TSST-1–induced IgM secretion, however, was found to be highly variable among the different donors. In a separate study, concentrations of 3×10^{-8} to 3×10^{-10} M TCDD suppressed the background proliferation and IgM secretion of low-density HTL B cells (predominately activated cells), but not high-density HTL B cells (predominately resting cells) (Wood et al., 1993). TCDD also suppressed LPS plus T cell replacement factor–stimulated proliferation and IgG secretion of low-density, but not high-density, HTL B cells. These results suggest that TCDD may have a direct effect on HTL low-density B cells and that this lymphocyte subpopulation may be a sensitive target for TCDD.

More recently, Masten and Shiverick (1995) investigated the effect of TCDD on expression of the CD19 gene in the IM-9 human B lymphocyte cell line in an attempt to determine a possible mechanism for TCDD-induced inhibition of human and murine B lymphocyte Ig production. CD19 is a B cell surface signal transducing protein that is expressed from early stages of B cell development, but is lost when B cells differentiate into antibody-producing plasma cells (Tedder et al., 1994). TCDD treatment of IM-9 cells decreased the steady state levels of CD19 mRNA by 67%. A DNA-binding complex in IM-9 nuclear extracts was identified that, based on several criteria, appeared to be the AhR. Furthermore, the AhR complex recognized a DNA binding site for B cell lineage–specific activator protein (BSAP) in the promotor region of the CD19 gene. This BSAP binding site is similar to the consensus AhR DNA binding site. Based on these results, Matsen and Shiverick (1995) suggest that the decrease in CD19 gene expression following TCDD exposure may result from the AhR interfering with BSAP-stimulated CD19 transcription. Further work, however, is required to support this hypothesis. Nevertheless, it is interesting that this work supports the animal in vitro work of Holsapple and coworkers, which indicates that the B cell is a primary cellular target for the direct effects of TCDD (Holsapple, 1995). It also goes beyond the in vitro animal work in that it provides further evidence that the AhR is involved in PHAH-induced immunotoxicity.

4.10. SUMMARY

Cumulative evidence from a number of studies indicates that the immune system of various animal species is a target for toxicity of TCDD and structurally related PHAHs, including other PCDDs, and PCDFs and PCBs. Both cell-mediated and humoral immune responses are suppressed following TCDD exposure, suggesting that multiple cellular targets within the immune system are altered by TCDD. Evidence also suggests that the immune system is indirectly targeted by TCDD-induced changes in nonlymphoid tissues. TCDD exposure of experimental animals results in decreased host resistance following challenge with certain

infectious agents, which likely result from TCDD-induced suppression of immunological functions.

The primary antibody response to the T cell–dependent antigen SRBCs is the most sensitive immunological response that is consistently suppressed in mice exposed to TCDD and related PHAHs. The degree of immunosuppression is related to the potency of the dioxin-like PHAH congeners. There is remarkable agreement among several different laboratories for the potency of a single acute dose of TCDD (suppression at a dose as low as 0.1 µg TCDD/kg with an average ID_{50} value of approximately 0.7 µg TCDD/kg) to suppress this response in Ah-responsive mice. Results of studies that have compared the effects of acute exposure to individual PCDD, PCDF, and PCB congeners, which differ in their binding affinity for the AhR, on this response have provided critical evidence that certain dioxin-like congeners are also immunosuppressive. The degree of immunosuppression has been found to be related to potency of the dioxin-like congeners. Antibody responses to T cell–independent antigens, such as TNP-LPS, and the CTL response are also suppressed by a single acute exposure to TCDD, albeit at higher doses than those that suppress the SRBC response. A limited number of studies reveal that dioxin-like congeners also suppress these responses, with the degree of suppression related to the congeners' AhR binding affinity. Although a thorough and systematic evaluation of the immunotoxicity of TCDD-like congeners in different species and for different immunological endpoints has not been performed, it can be inferred from the available data that dioxin-like congeners are immunosuppressive.

In addition to the TCDD-like congener results, studies using strains of mice that differ in the expression of the AhR have provided critical evidence to support a role for Ah-mediated immune suppression following PHAH exposure. Recent in vitro work also supports a role for Ah-mediated immune suppression. Other in vivo and in vitro data, however, suggest that non-Ah-mediated mechanisms may also function in PHAH-induced immunotoxicity. However, more definitive evidence remains to be developed to support this latter view.

Although in mice the immunosuppressive potency of individual PHAHs is related to their structural similarity to TCDD, this pattern of suppression is observed only after exposure to an individual PHAH. The immunotoxicity of TCDD and related congeners can be modified by co-exposure to PHAHs in simple binary or more complex mixtures resulting in additive or antagonistic interactions. Dose-response data are needed on acute, subchronic, and chronic exposure to the individual PHAHs in a mixture and on the mixture itself in order to fully evaluate potential synergistic, additive, or antagonistic effects of environmentally relevant PHAH mixtures.

Perinatal exposure of experimental animals to TCDD results in suppression of primarily T cell immune functions, with evidence of suppression persisting into adulthood. The effects on

T cell functions appear to be related to the fact that perinatal TCDD exposure of mice alters thymic precursor stem cells in the fetal liver and bone marrow, and thymocyte differentiation in the thymus. These studies suggest that perinatal development is a critical and sensitive period for TCDD-induced immunotoxicity. Efforts should be made to determine the consequences of perinatal exposure to TCDD congeners and PHAH mixtures on immune system integrity.

Animal host resistance models that mimic human disease have been used to assess the effects of TCDD on altered host susceptibility. TCDD exposure increases susceptibility to challenge with bacteria, viruses, parasites, and tumors. Mortality is increased in TCDD-exposed mice challenged with certain bacteria. Increased parasitemia occurs in TCDD-exposed mice and rats challenged with parasitic infections. Low doses of TCDD also alter resistance to virus infections in rodents. Increased susceptibility to infectious agents is an important benchmark of immunosuppression; however, the role of TCDD in altering immune-mediated mechanisms important in murine resistance to infectious agents remains to be elucidated. Also, since little is known about the effects of dioxin-like congeners on host resistance and nothing is known about the relation between the AhR and host resistance, more research is recommended in this area.

Studies in nonhuman primates exposed acutely, subchronically, or chronically to PHAHs have revealed variable alterations in lymphocyte subpopulations, primarily T lymphocyte subsets. On the other hand, in three separate studies in which monkeys were exposed subchronically or chronically to PCBs, the antibody response to SRBC was consistently found to be suppressed. These results in nonhuman primates are important because they corroborate the extensive database of PHAH-induced suppression of the antibody response to SRBC in mice and thereby provide credible evidence for immunosuppression by PHAHs across species. In addition, these data indicate that the primary antibody response to this T cell–dependent antigen is the most consistent and sensitive indicator of PHAH-induced immunosuppression.

The available database derived from well-controlled animal studies on PHAH immunotoxicity can be used for the establishment of no-adverse-effect levels. Because the antibody response to SRBCs has been shown to be dose-dependently suppressed by TCDD and related PHAHs, this database is best suited for the development of dose-response modeling.

Accidental or occupational exposure of humans to TCDD and related PHAHs variably affects a number of immunological parameters. Unfortunately, evaluation of immune system integrity in humans exposed to PHAHs has provided data that are inconsistent across studies. The broad range of "normal" responses in humans due to the large variability inherent in a heterogenous population, the limited number and sensitivity of tests performed, and the poor exposure characterization of the cohorts in these studies compromise any conclusions about the ability of a given study to detect immune alterations. Consequently, there are insufficient clinical data from these studies to fully assess human sensitivity to PHAH exposure. Nevertheless, the

database of the results of the extensive animal work is sufficient to indicate that immune effects could occur in the human population from exposure to PHAHs at some dose level.

It is interesting that a common thread in several human studies is the observed reduction in $CD4^+$ T helper cells, albeit generally within the "normal" range, in cohorts exposed to PHAHs. These reductions may not translate into clinical effects, but it is important to note that such cells have an important role in regulating immune responses and that their reduction in clinical diseases is associated with immunosuppression. Another important consideration is that a primary antibody response following immunization was not evaluated in any of the human studies. In that this immune parameter has been revealed to be the most sensitive in animal studies, this parameter should be included in future studies of human populations exposed to PHAHs. It is also recommended that research continue on delineating the mechanism(s) underlying PHAH-induced immunotoxicity.

Table 4-1. Acute single dose ID_{50}s for polychlorinated dioxins, furans, and biphenyls based on suppression of the PFC response to SRBCs in Ah-responsive B6 mice

Congener	ID_{50}	Reference
2,3,7,8-TCDD	0.74 µg/kg	Kerkvliet and Brauner, 1990
"	0.65 µg/kg	Vecchi et al., 1980
"	0.77 µg/kg	Davis and Safe, 1988
"	0.60 µg/kg	Kerkvliet et al., 1990a
1,2,3,6,7,8-HxCDD	7.1 µg/kg	Kerkvliet et al., 1985
1,2,3,4,6,7,8-HpCDD	85.0 µg/kg	Kerkvliet et al., 1985
OCDD	>500 µg/kg	Kerkvliet et al., 1985
2,3,4,7,8-PCDF	1.0 µg/kg	Davis and Safe, 1988
2,3,7,8-TCDF	4.3 µg/kg	Davis and Safe, 1988
1,2,3,4,6,7,8-HpCDF	208 µg/kg	Kerkvliet et al., 1985
1,2,3,7,9-PenCDF	239 µg/kg	Davis and Safe, 1988
1,3,6,8-TCDF	11 mg/kg	Davis and Safe, 1988
3,4,3′,4′-TCB	28 mg/kg[a]	Silkworth and Grabstein, 1982
2,3,4,5,3′,4′-HxCB	0.7 mg/kg	Davis and Safe, 1990
2,3,4,5,3′,4′-HxCB	31 mg/kg[a]	Silkworth et al., 1984
2,4,3′,4′,5′,6′-HxCB	43 mg/kg	Davis and Safe, 1990
2,3,4,3′,5′-PenCB	65 mg/kg	Ibid.
2,3,4,5,3′,5′-HxCB	72 mg/kg	Ibid.
2,4,2′,4′-TCB	>100 mg/kg	Silkworth et al., 1984
2,4,5,2′,4′,6′-HxCB	>360 mg/kg	Davis and Safe, 1990
2,4,6,2′,4′,6′-HxCB	>360 mg/kg	Ibid.
2,4,5,2′,4′,5′-HxCB	>360 mg/kg	Biegel et al., 1989
Aroclor 1260	104 mg/kg	Davis and Safe, 1989
Aroclor 1254	118 mg/kg	Ibid.
Aroclor 1254	207 mg/kg	Lubet et al., 1986
Aroclor 1248	190 mg/kg	Davis and Safe, 1989
Aroclor 1242	391 mg/kg	Ibid.
Aroclor 1016	408 mg/kg	Ibid.
Aroclor 1232	464 mg/kg	Ibid.

[a]Interpolated from two data points.

Table 4-2. ID_{50}s for sppression of alloantigen (P8l5)-specific CTL response in C57Bl/6 mice

Congener	ID_{50}	Reference
TCDD	7 µg/kg	Kerkvliet et al., 1990b
3,4,5,3′,4′,5′-HxCB	7 mg/kg	Ibid.
2,3,4,5,3′,4′-HxCB	70 mg/kg[a]	Ibid.
2,4,5,2′,4′,5′-HxCB	>300 mg/kg	Ibid.

[a] Interpolated from two data points.

Table 4-3. Immunotoxic effects of TCDD in the offspring following prenatal/neonatal exposure to TCDD

Protocol[a]	Endpoints	Effect	LOAEL[b]
Study 1			
Pregnant B6 or	PYB6 tumor incidence	Increased	1 µg/kg × 4
B6C3F$_1$ mice given 1, 2, 5,	Allograft rejection time	Increased	2 µg/kg × 4
or 15 µg/kg TCDD orally	Body, thymus, spleen wt	Decreased	5 µg/kg × 4
on day -7, 0, +7, +14	Bone marrow cellularity	Decreased	5 µg/kg × 4
relative to parturition	T cell blastogenesis	Decreased	5 µg/kg × 4
on day 0	*Listeria monocytogenes*-induced mortality	Decreased	5 µg/kg × 4
	Bone marrow colony formation (CFU-S)	Decreased	5 µg/kg × 4
	LPS blastogenesis	—	>15 µg/kg × 4
	Anti-SRBC serum titers	—	>15 µg/kg × 4
Study 2			
Pregnant Swiss mice	Endotoxin mortality	Increased	1.0 ppb diet
fed diets containing	Thymus weight	Decreased	2.5 ppb diet
1.0, 2.5, or 5.0 ppb	PFC response to SRBC	Decreased	5.0 ppb diet
TCDD for 7 weeks prenatally	DTH response	Decreased	5.0 ppb diet
and postnatally	Anti-SRBC serum titers	—	>5.0 ppb diet
	T and B cell blastogenesis	—	>5.0 ppb diet
	Listeria-induced mortality	—	>5.0 ppb diet
Study 3			
Pregnant Fischer 344	Allograft rejection time	Increased	5 µg/kg × 4
rats given 1 or 5 µg/kg	T cell blastogenesis	Decreased	5 µg/kg × 4
TCDD orally on day -3, 0,	DTH response	Decreased	5 µg/kg × 4
+7, and +14 relative to	*Listeria*-induced mortality	Decreased	5 µg/kg × 4
parturition on day 0	Body and thymus weight	Decreased	5 µg/kg × 4
	Anti-BGG serum titers	—	>5 µg/kg × 4

[a]Study 1: Vos and Moore, (1974), Luster et al. (1980a); Study 2: Thomas and Hinsdill (1979); Study 3: Vos and Moore, (1974) , Faith and Moore (1977).
[b]Abbreviations used: LOAEL - lowest observable adverse effect level; BGG - bovine gamma globulin; LPS - lipopolysaccharide; PHA - phytohemagglutinin; Con A - Concanavalin A; SRBC - sheep red blood cell; DTH - delayed-type hypersensitivity; PFC - plaque-forming cell; CFU-S - colony-forming units-spleen.
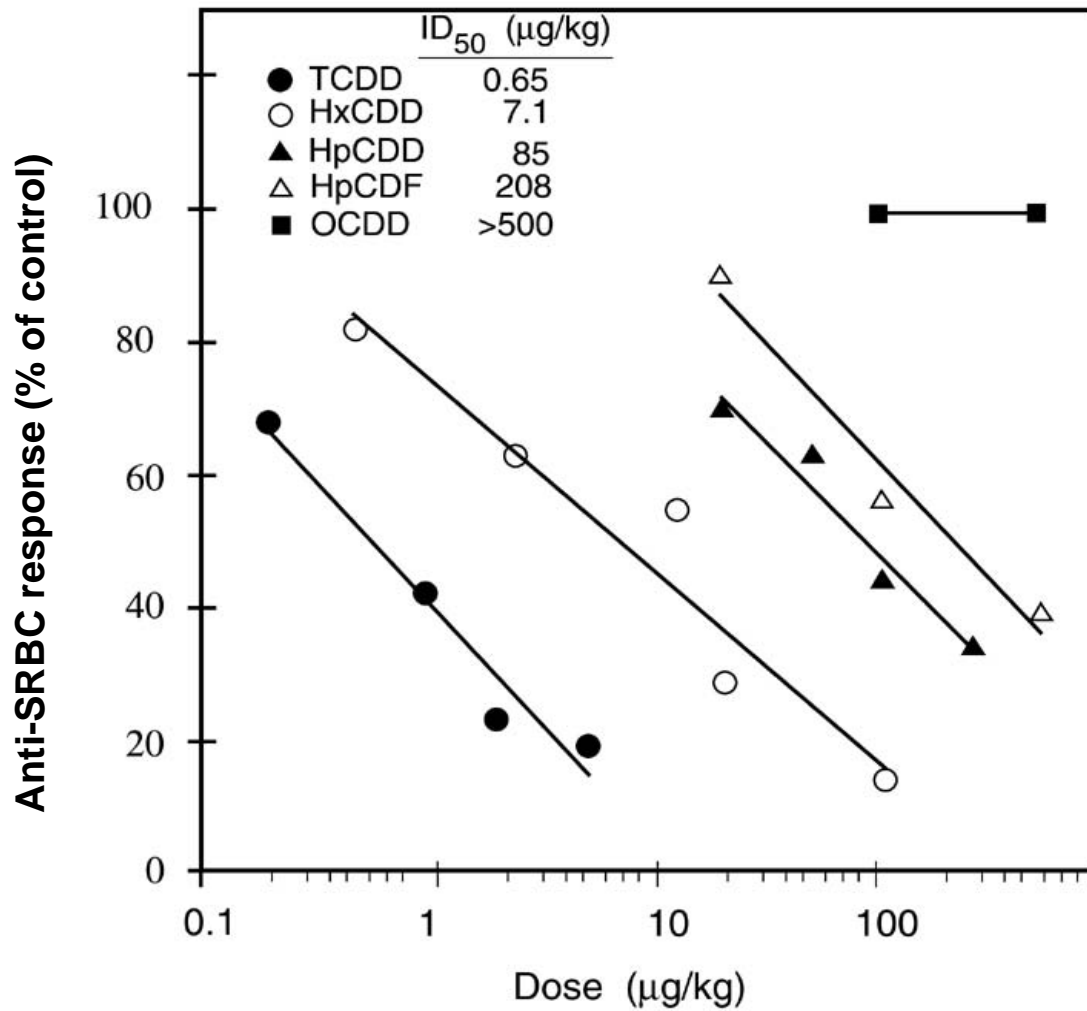
Figure 4-1. Structure-dependent immunotoxicity of some polychlorinated dioxin and furan isomers. Immunotoxicity assessed by suppression of the splenic antibody response to SRBCs (modified from Kerkvliet et al., 1985).

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