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2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN-INDUCED CHANGES IN IMMUNOCOMPETENCE: Possible Mechanisms

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INTRODUCTION

Few environmental contaminants have produced the human health concerns associated with exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, more popularly known as "dioxin" or "TCDD", and referred to hereafter as 2,3,7,8-TCDD. It is the most biologically potent of the halogenated aromatic hydrocarbons (HAH), a chemical family that includes the polychlorinated and polybrominated biphenyls (PCB and PBB, respectively) and the polychlorinated dibenzofurans (PCDF), in addition to the polychlorinated dibenzo-*p*-dioxins (PCDD). 2,3,7,8-TCDD has been called the most toxic chemical ever created. Ironically, 2,3,7,8-TCDD is a true contaminant, formed primarily as a byproduct in the manufacture of products from chlorinated phenols or during the combustion of chlorinated materials, and has no known human benefit. As environmental contaminants, HAH have been most closely associated with the herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and Agent Orange (a 1 :1 mixture of the butyl esters of 2,4-D and 2,4,5-T). Other sources include pentachlorophenol,

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hexachlorophene, pulp and paper manufacturing (chlorine bleaching), automobile exhaust (leaded gasoline), and the combustion of municipal and industrial wastes, chlorinated benzenes, and chlorinated biphenyls. The persistence of HAH in the environment and the potent toxicity of 2,3,7,8-TCDD in certain laboratory animal species have raised concerns of potential health hazards associated with human exposure.[1]

The toxic potential of 2,3,7,8-TCDD has been extensively studied and is characterized by wide variation in effect and potency between species (1–3). Exposure to PCDD has been associated with a generalized wasting syndrome, lymphoid involution (especially of the thymus), pancytopenia, immunosuppression, hepatomegaly and hepatotoxicity, chloracne and hyperkeratosis, gastric lesions, urinary tract hyperplasia, edema, tumor promotion, teratogenicity and embryotoxicity, and decreased spermatogenesis. Decreased immunocompetence, including functional deficits seen at doses that do not produce organ toxicity, is among the earliest and most sensitive manifestations of 2,3,7,8-TCDD exposure, and is almost universally observed among the animal species in which it has been evaluated.

Immunotoxicity studies with 2,3,7,8-TCDD were initially undertaken to further characterize the findings of general toxicity studies showing that exposure produced lymphoid involution, especially of the thymus (4–6). Since the thymus is the central organ for maturation of T-lymphocytes, the initial studies concentrated on T-cell-mediated immunity (CMI). Among the different species tested, suppression of CMI was shown to be dependent upon the age at which the animal was exposed. Exposures in younger animals produced a more extensive and longer-lasting suppression, with in utero exposure during organogenesis required to induce maximum effects in some

[1]Abbreviations: Ab, antibody; AHH, arylhydrocarbon hydroxylase; *Ah*-R, receptor controlled by the *Ah* locus; putative dioxin receptor; CMI, cell-mediated immunity; Con A, Concanavalin A, a T-cell mitogen; CTL, cytotoxic T-lymphocyte; 2,7-DCDD, 2,7-dichlorodibenzo-*p*-dioxin: DRE, dioxin regulatory element; EGF, epidermal growth factor; EROD, ethoxyresorufin-O-deethylase; HAH, halogenated aromatic hydrocarbons; HI, humoral immunity; 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin; 3,4,5,3',4',5'-HxCB, 3,4,5,3',4',5'-hexachlorinated biphenyl; 2,3,4,5,2',4'-HxCB, 2,3,4,5,2',4'-hexachlorinated biphenyl; 2,4,5,2',4',5'-HxCB, 2,4,5,2',4',5'-hexachlorinated biphenyl; 1,2,3,6,7,8-HxCDD, 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin; LPS, lipopolysaccharide; a B-cell mitogen & polyclonal activator; octa-CDD, octachlorodibenzo-*p*-dioxin; PBB, polybrominated biphenyls; PBL, peripheral blood lymphocyte; PCB, polychlorinated biphenyls; PCDD, polychlorinated dibenzodioxins; 1,2,3,7,9-PCDF, 1,2,3,7,9-pentachlorodibenzofuran; 2,3,4,7,8-PCDF, 2,3,4,7,8-pentachlorodibenzofuran; PFC, plaque-forming cell; PHA, phytohemagluttinin; a T-cell mitogen; PI, phosphoinositide; PKC, protein kinase C; PMA, phorbol 12-myristate, 13-acetate; PRL, prolactin; PWM, pokeweed mitogen; primarily a B-cell mitogen; SAR, structure-activity relationships; SRBC, sheep erythrocytes; a T-dependent antigen; 2,3,7,8-TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; 1,3,6,8-TCDF, 1,3,6,8-tetrachlorodibenzofuran; 2,3,7,8-TCDF, 2,3,7,8-tetrachlorodibenzofuran; TRF, T-cell replacing factor.

species. Consistent with these findings, it appears that 2,3,7,8-TCDD induces the terminal differentiation of thymic epithelial cells, preventing thymocyte maturation. In contrast, humoral immune (HI) responses, which are mediated by the production of antibodies (Ab), were largely unaffected in perinatally exposed animals. However, it was demonstrated that 2,3,7,8-TCDD exposure in adult animals produces a profound suppression of the antibody response, which was subsequently shown to be the primary result of a direct effect on the B-lymphocyte. Although the functional deficit has not been identified, studies suggest that the differentiative processes of these cells are affected. Thus, the data suggest that 2,3,7,8-TCDD either indirectly (in the case of CMI) or directly (in the case of HI) affects the maturational or differentiative capabilities or processes of immunocompetent cells, and that such cells are sensitive to the effects of this xenobiotic during periods of activity associated with such processes. This interpretation is consistent with findings in other cell systems in which 2,3,7,8-TCDD has been reported to produce an enhanced or accelerated differentiation to end-stage development. Despite the significant phenomenological characterization of 2,3,7,8-TCDD-induced immunotoxicity, an understanding of the underlying mechanism(s) remains to be elucidated.

Studies into structure-activity relationships (SAR) utilizing enzyme induction as an endpoint, while not a toxic response per se, have led to the most accepted and best supported model of actions associated with exposure to HAH (1, 3). In this model, which has many similarities to the classical steroid receptor model, the HAH congener binds to a cytosolic receptor that is a principal gene product of the *Ah* locus. The bound ligand-receptor complex is then translocated to the nucleus where binding to specific "dioxin regulatory element" (DRE) sites precipitates a pleiotropic gene induction culminating in a measurable increase in mRNA specific for the various enzymes and gene products regulated at that site (7–9). Differences in potency among different HAH congeners are explained by structure-activity differences in affinity for the *Ah* locus receptor (*Ah*-R). A strong correlation has been shown between receptor binding affinity and enzyme induction for all HAH families. Differences in potency and the spectrum of toxic effects between species have been putatively explained by differences in gene activation and either the presence, distribution, or functional capacity of the *Ah*-R.

Although this model is well established for HAH-mediated enzyme induction, and either a parallel SAR or a correlation with *Ah*-R functionality in responsive and non-responsive strains or species has been reported for certain toxic endpoints, the association is not conclusive. No causal relationship has been established between specific genes activated by *Ah*-R binding and the induction of toxic symptoms following 2,3,7,8-TCDD exposure. Additionally, a number of reports have been published that present conflicting toxicity

data that are not readily explained by *Ah*-R-based mechanisms. The observed immunotoxicity, particularly the suppression of HI, has been reported by different laboratories to be both dependent upon, and to be independent of, cellular mechanisms regulated through the *Ah* gene locus and its associated receptor (*Ah*-R). At the very least, the available data suggest that other effector mechanisms may be involved in the immunotoxicity of these chemicals. The focus of this review is to present what is currently understood about the effect of 2,3,7,8-TCDD exposure on immunocompetence, with particular emphasis on possible mechanisms for these effects, including the possibility of dual actions with both *Ah*-dependent and *Ah*-independent processes. Because 2,3,7,8-TCDD is considered to be the prototype HAH and is the most extensively studied chemical in this class, this review focuses primarily on the toxic effects associated with exposure to this chemical. However, results of studies with other HAH have been included as warranted.

A review on the effects of 2,3,7,8-TCDD on immunocompetence is timely from two major perspectives. First, from the standpoint of immunotoxicology, few xenobiotics have been as thoroughly studied as 2,3,7,8-TCDD. More than 20 different laboratories have reported on some aspect of this effect. The extensive characterization of the immunocompetent cellular targets for 2,3,7,8-TCDD are unparalleled by any other xenobiotic with suspected immunotoxic potential. Second, from the standpoint of the mechanism(s) for the actions by 2,3,7,8-TCDD and related HAH, the results from immunotoxicological studies are some of the more convincing evidence for dual actions with both *Ah*-dependent and *Ah*-independent mechanisms. This possibility is important because a model based on dual actions could account for the nature of the complex profile of toxicity associated with 2,3,7,8-TCDD and HAH and has not been critically evaluated in any previous review.

CELLULAR BASIS FOR TCDD-INDUCED IMMUNOTOXICITY

Because immunocompetence is based on a cooperative interaction between several cell types, many investigators have tried to determine if exposure to 2,3,7,8-TCDD produced a differential sensitivity, thereby specifically targeting a given cell type, as a first step toward defining the mechanism. Perhaps the best-characterized alteration in CMI induced by 2,3,7,8-TCDD exposure, and possibly the most sensitive, is the specific reduction in the generation of cytolytic T-lymphocytes (CTLs). Clark et al reported that 2,3,7,8-TCDD at doses ranging from 0.004 to 40 μg/kg could significantly affect the generation of CTLs in C57B1/6 mice, without causing any significant alteration in cellularity of the spleen or lymph nodes, or a suppression of either the delayed hypersensitivity response ($\geq$4 μg/kg) or the Ab response to sheep erythro-

cytes (SRBC; 40 μg/kg) (10). It was further shown by limiting dilution analysis that 2,3,7,8-TCDD did not deplete the number of CTL precursors but induced a population of suppressor cells that prevented the generation of functional CTLs in vitro. T-helper cell function, as assessed by the ability to secrete IL-2, appeared normal (11). Using different strains of mice (C57B1/6 and DBA/2) and bone marrow chimeras, Clark and coworkers further demonstrated that the effects of 2,3,7,8-TCDD and related PCDD on CTL generation segregate with the *Ah* gene locus and the sensitivity to induction of ethoxyresorufin-O-deethylase (EROD) activity (11, 12). The studies carried out in Clark's laboratory were the first to suggest that the basis for the actions of 2,3,7,8-TCDD on immunocompetence was a specific effect on one cell type, i.e. that the suppression of the CTL response was due to induction of T-suppressor cells.

Studies by Holsapple et al and by Tucker et al suggested that exposure to 2,3,7,8-TCDD caused a selective inhibition of B-lymphocyte differentiation into antibody producing cells (13, 14). This conclusion was based on the finding that the Ab response to both SRBC (T-dependent antigen) and lipopolysaccharide (LPS; T-independent) were suppressed at doses that caused no alteration in mitogen-induced proliferation. Time-of-addition studies demonstrated that 2,3,7,8-TCDD must be present early in culture, within the first 24 hours after stimulation with SRBC (14), and within the first three hours after stimulation with LPS (13), to suppress the plaque-forming cell (PFC) response, measured on day 5 or day 2, respectively.

Utilizing separation/reconstitution techniques, Dooley & Holsapple (15) showed conclusively that the B-lymphocyte is the primary target for suppression of the T-dependent Ab response in the B6C3F1 mouse strain. Their results demonstrated that the suppression of three different Ab responses (LPS, DNP-Ficoll, and SRBCs), which varied in their requirements for cellular cooperativity, were the result of a specific effect on the B-cell, and not to effects on either the adherent macrophage population or the T-cell. The lack of effect by 2,3,7,8-TCDD on macrophage antigen-presenting cells was recently confirmed in a preliminary study by Kerkvliet et al (16). More recent work by Dooley and coworkers (17) confirmed that the effects of 2,3,7,8-TCDD on the T-dependent humoral immune response were not due to an alteration in helper T-cell function. These results were based both on titration experiments with T-cells from 2,3,7,8-TCDD-treated mice and on the lack of effect on IL-2 release following mitogen stimulation. The absence of effects on the helper T-cell are in agreement with earlier findings (11, 18). However, the report by Dooley et al (17) differs somewhat from the earlier report by Clark's group (11) by demonstrating that the effect of 2,3,7,8-TCDD on humoral responsiveness, specifically the effects on the B-cell, were not mediated by the activation of a suppressor T-cell. These results further

support the interpretation for a separation in the mechanisms of the suppression of these two cellular targets of dioxin toxicity. While several laboratories have ruled out that T-helper cells are targeted by 2,3,7,8-TCDD, there is at least one report for a defect in T-cell regulation. An earlier study by Kerkvliet et al (19) demonstrated that 1,2,3,4,6,7,8-HpCDD could suppress the antibody response to both T-dependent (SRBC) and T-independent (TNP-LPS and DNP-ficoll) antigens, but produced a significantly greater suppression of the response to SRBC. In addition, nu/nu (i.e. athymic) mice were shown to be significantly more resistant to the immunosuppressive effects of HpCDD on the Ab response to DNP-ficoll (at doses of $\leq$ 100 μg/kg) as compared with their nu/+ littermates, an observation further suggesting that this compound produces a defect at the level of regulatory T-cells. Additional support for an effect on B-cells comes from the results of Luster et al who used an in vitro model of B-cell activation to assess the direct effects of 2,3,7,8-TCDD on the proliferation and differentiation of isolated B-lymphocytes (20). In this report, 2,3,7,8-TCDD caused the specific suppression of IgM secretion in B-cells that were activated by anti-Ig and T-cell replacing factor (TRF); but had no effect on proliferation, again suggesting a selective effect on B-cell differentiation. This conclusion was further supported by the finding that neither the expression of Ia nor the proliferative marker, 7D4, was affected, whereas the expression of the plasma cell specific marker, PC.2, was significantly reduced. Therefore, several investigators have concluded that 2,3,7,8-TCDD caused an alteration in early cellular programming of B-cells, prior to or during antigen stimulation, which results in a specific alteration in their capacity to differentiate into Ab-producing cells (13, 14, 20).

POSSIBLE MECHANISMS OF TCDD-INDUCED IMMUNOTOXICITY

Ever since the original reports by Nebert et al (21) and by Poland et al (22), which described a specific binding site for 2,3,7,8-TCDD and related HAH, scientists have implicated a role for this putative "dioxin receptor" in all actions associated with this chemical class. As described above, this receptor is associated with the *Ah* locus, has been referred to as the *Ah*-R throughout this review, and has many similarities to the model for the mechanism of action associated with classical steroid receptors. However, as discussed in several reviews (2, 3), the *Ah*-R is distinct from any known steroid system and the endogenous ligand for the *Ah*-R is presently unknown. The effect produced by 2,3,7,8-TCDD and related HAH that has been most clearly demonstrated to be associated with the *Ah*-R has been the induction of P-450 enzymes as elegantly reviewed by Whitlock (8, 9). A detailed discussion of the relationship between the *Ah*-R and enzyme induction is beyond the scope

of this review. However, because one of the main points we raise is for dual actions by 2,3,7,8-TCDD on immunocompetence with both *Ah*-R-dependent and *Ah*-R-independent mechanisms, it is important to emphasize that other actions of 2,3,7,8-TCDD, including some types of enzyme induction, have already been reported to be mediated through processes that do not involve the *Ah*-R.

Nebert has recently outlined a series of enzymes and proteins that are increased in the presence of polycyclic hydrocarbons and 2,3,7,8-TCDD (7). He states that although many of the actions associated with 2,3,7,8-TCDD exposure correlate with the presence of the high affinity *Ah*-R and/or induction of the CYP1A1 and CYP1A2 enzymes, there are exceptions to the rule. In the rat, hepatic CYP2A1 (having testosterone 7-alpha-hydroxylase activity) gene expression is increased in the presence of 3-MC or TCDD through a mechanism independent of the *Ah*-R (23). Moreover, of the many enzymes and proteins induced by 2,3,7,8-TCDD in a number of different tissues, only a small number have been conclusively shown to correlate with arylhydrocarbon hydroxylase (AHH) induction. As indicated by Nebert (7), additional research is necessary to determine how many of these additional genes are regulated by dioxin-responsive elements (DRE) and whether any of these genes can be directly correlated with toxicity. Recent work presented by Pohjanvirta et al (24), comparing a TCDD-susceptible rat strain and a TCDD-resistant rat strain, found that the sensitivity of these two strains to 2,3,7,8-TCDD did not correlate with either *Ah*-R levels or induction of CYP1A1 enzyme activity in the liver. The two strains exhibited a 300-fold difference in acute i.p. LD_{50} levels for 2,3,7,8-TCDD (25). These results also deviate from the current view of *Ah*-R-dependent toxicity and are strong evidence that other, non-*Ah*-R-dependent mechanisms may be involved in toxic responses to dioxin.

In addition to results indicating that not all actions by TCDD are mediated through the *Ah*-R, recent results challenge the dogma associated with classical steroid receptors—i.e. that all actions associated with steroids are mediated through the translocation of a ligand-receptor complex to the nucleus that ultimately results in gene transcription. Recent evidence suggests that steroids can act at a second site, the cellular membrane, to alter receptor-mediated infuxes of modulatory ions such as Ca^{++} and Cl^{-}. In a recent article, Touchette (26) discusses the role of steroids in the modulation of various ion channels to facilitate ion transport across the membrane. These effects occur within seconds of steroid addition and can not easily be explained by binding to cytosolic steroid receptors and subsequent gene transcription. In addition, these new studies show that a variety of steroids, including progesterone, estrogen, testosterone, and their metabolites, have membrane receptors. Thus, the interaction between 2,3,7,8-TCDD and hormone systems (as dis-

cussed below), and the actions of the various hormones themselves, may be related to actions elicited at the membrane. This possibility may partially explain toxicities by TCDD and hormones that do not segregate with the *Ah*-R and subsequent gene transcription via the *Ah* gene locus.

Therefore, all actions by TCDD may not be mediated through the *Ah*-R, and all actions by steroids may not be mediated through events involving gene transcription. One final point related to the possible involvement of the *Ah*-R and a steroidlike mechanism of action in TCDD-induced toxicity needs to be emphasized. To date, no one has conclusively demonstrated that gene transcription must precede the development of any toxic effect. Two of the best models for dioxin-induced toxicity have been developed and characterized by Greenlee and coworkers and involve thymic epithelial cells (27) and keratinocyte cell lines (28), which are in vitro correlates for thymic atrophy and hyperkeratosis, respectively. In both cases, they have described results indicating that exposure to 2,3,7,8-TCDD caused a change in the control of cell differentiation, and that this change was a "push" toward terminal differentiation. With the latter cell type, they have speculated that the mechanism for this change was a 2,3,7,8-TCDD-induced decrease in the expression of EGF receptors. As discussed below, several investigators have suggested that the decreased expression of EGF receptors may be due to a change in their state of phosphorylation, presumably by a 2,3,7,8-TCDD-induced activation of a kinase.

Some preliminary evidence supports a similar profile of activity in regards to the effects of TCDD on lymphocyte function. Sharma & Gehring (29), working with the outbred CD-1 mouse strain, demonstrated that animals exposed to low levels of TCDD (0.01–10.0 μg/kg) once a week for eight weeks caused a significant increase in spontaneous lymphocyte transformation in the spleens of animals at both two and four weeks following initial dosing. At eight weeks, this response was reduced to near control levels and was suggestive of a change in lymphocyte activation by 2,3,7,8-TCDD that was both dose- and time-dependent. This effect was directly correlated with mitogen responsiveness of splenic lymphocytes to both PWM and PHA, where stimulation indices for both mitogens were reduced at both two and four weeks, but recovered to control levels at eight weeks posttreatment. The reductions in stimulation indices were noted to result from the increases seen in the levels of spontaneous lymphocyte transformation in the absence of mitogen challenge. In addition, increases in serum immunoglobulin levels were noted at both two and four weeks at doses of 0.01–1.0 μg/kg, but were reduced at 10 μg/kg, suggesting an effect on lymphocyte transformation and immunoglobulin production that is both biphasic and dose-related. Similar effects were observed by Clark et al (10) in which increases in background PFC formation were detected at doses of 2,3,7,8-TCDD from 0.04 to 40

μg/kg. Also in agreement with the work by Sharma & Gehring was the finding that the increases were most noted at the lower dose level (0.04 μg/kg), and likewise occurred at levels of dioxin that did not cause a notable induction of the liver. As in the previous report, suppression of the Ab response occurred at higher doses of 2,3,7,8-TCDD (40 μg/kg) in which induction of liver enzymes and effects on the thymus were prominent. That a similar profile of activity could be produced by direct exposure of cultured lymphocytes to 2,3,7,8-TCDD was reported by Kramer et al (30). Their results indicated that exposure to 30 nM 2,3,7,8-TCDD caused an increase in the background (i.e. no antigen) PFC response that peaked on day 3 of culture. In this same study, Kramer and coworkers demonstrated that 2,3,7,8-TCDD caused an increase in basal kinase activity in isolated B-cells and speculated that the biochemical effects (i.e. increased kinase activity) and functional changes (i.e. increased background Ab production) were causally related. The effects of 2,3,7,8-TCDD on phosphorylation are discussed in greater detail below. Interestingly, several epidemiological studies have suggested that exposure to 2,3,7,8-TCDD and related HAH can cause an activation of, or enhanced activity in, human lymphocytes. In exposed children from Seveso, Italy, peripheral blood lymphocyte counts and mitogen responses increased (31). Nonstatistical increases in mitogen responsiveness were also reported for individuals exposed to the HAH in Times Beach, Missouri (32). Hoffman and coworkers studied a related population from the Quail Run trailer park and reported elevations in mitogen responses to PHA, Con A and PWM (33). Finally, Lipson (34) reported an enhanced release of IgG in lymphocyte cultures taken from blood specimens of PBB-exposed Michigan farmers. While the full interpretation of these effects is forthcoming, the results from both animal and epidemiological studies collectively suggest that exposure to HAH can cause an aberrant activation of lymphocytes.

Role of the Ah-*Receptor*

Investigations into the role of the *Ah*-R in dioxin-induced toxicity have generally represented the driving force in mechanistic studies to date. The objective of this section is to critically evaluate the evidence both for and against a role by the *Ah*-R in 2,3,7,8-TCDD-induced immunotoxicity. This section is divided into three parts. The first part discusses the evidence for the presence of the *Ah*-R in lymphocytes, as indicated by the induction of enzymes controlled by the *Ah* locus, especially AHH. The second part presents the direct evidence for the presence of the *Ah*-R on lymphocytes, as indicated by binding studies in lymphoid tissue or isolated lymphocytes. In the third part, more indirect approaches to the presence of the *Ah*-R, including SAR and genetic variations, are discussed.

AHH ACTIVITY IN LYMPHOCYTES As reviewed by Safe (3), and more recently by Whitlock (9), the induction of AHH and associated enzymes is not simply a function of the presence of the *Ah*-R. The complexity of this relationship, which is particularly relevant to the material discussed below, is demonstrated by studies using the rat thymus. As reported by several groups (35–37), rat thymus has an *Ah*-R concentration equal to that of liver, but has less than 1/1000th the activity of AHH that is observed in liver. Moreover, Okey and coworkers (38) had previously characterized AHH inducibility in several rodent and primate cultured cell lines and concluded that the presence of the *Ah*-R was necessary, but not sufficient, for AHH induction. Nonetheless, the ability of 2,3,7,8-TCDD and related compounds to cause induction of AHH in a given cell type or organ system is often presented as indirect evidence for the presence and activity of the *Ah*-R within that cell or organ. Many studies have demonstrated that AHH can be induced in immunocompetent cells. The AHH activity of human peripheral blood lymphocytes (PBL) was increased twofold by benz(a)anthracene (39), while AHH in human peripheral blood monocytes was increased 4- to 30-fold with benzo(a)pyrene (40). In agreement with the earlier studies, Kouri et al (41) showed that AHH activity in PBL was increased by between 1.7- and 2.9-fold by 2,3,7,8-TCDD or 3-methylcholanthrene (3-MC). Comparable induction by 2,3,7,8-TCDD occurred at a concentration 40 to 60 times less than 3-MC. The paper by Whitlock and coworkers (39) was also important because it indicated that the state of lymphocyte activation could affect the inducibility of human lymphocytes. Stimulation with PHA or PWM produced a greater than twofold increase in AHH activity (i.e. comparable to that observed in lymphocytes treated with benz(a)anthracene). The combination of benz(a)anthracene and PHA produced an almost sevenfold increase, while the combination of benz(a)anthracene and PWM produced a greater than tenfold increase. This observation was confirmed and extended in two subsequent studies (42, 43). While the full implication of this observation is not readily apparent, it is important to emphasize that an increase in mitogen responsiveness has been reported in humans exposed to various HAH. Taken together, these results suggest a possible relationship between AHH inducibility and the state of activation of the lymphocyte. Support for this speculation comes from Alfred & Wojdani (44), who indicated that AHH induction by treatment with benz(a)anthracene or 3-MC was measurable only in mitogen-activated mouse splenocytes and showed a nonlinear relation to blastogenesis.

The paper by Alfred & Wojdani (44) is also important as evidence for a primary role by the *Ah*-R in AHH induction in lymphocytes. They demonstrated that benzanthracene and 3-MC produced greater blastogenesis in splenocytes from C57BL/6 mice and C3H mice (i.e. *Ah*-responsive) than in DBA/2 mice (i.e. *Ah*-nonresponsive). As expected, these agents only caused

an induction of AHH in the "responsive" mouse strains. Additional support for the involvement of the *Ah*-R in the enzyme inducibility of mouse splenocytes comes from Blank et al (45). Although these researchers failed to show any dose-relatedness in the induction of EROD activity by 2,3,7,8-TCDD (i.e. from 1 to 50 nM), they did demonstrate that the induction by dioxin was reversed by alpha-naphthoflavone, a suspected antagonist of the *Ah*-R.

Ah-RECEPTOR IN LYMPHOCYTES As described above, several groups (35–37) have demonstrated that the thymus has a high concentration of *Ah*-R. Moreover, as described by Poland & Glover (46), dioxin-induced thymic atrophy segregated with the *Ah*-R. While it is tempting to use these results collectively as indirect evidence that thymocytes possess *Ah*-R, which contribute to the dioxin-induced changes in the thymus, such an interpretation is not consistent with results reported by Greenlee et al (27). These results indicated that the primary cellular targets responsible for thymic atrophy were thymic epithelial cells and not thymocytes, which were shown to lack sensitivity to the direct effects of 2,3,7,8-TCDD. This observation was consistent with earlier work by Nagarkatti et al (12), which used bone marrow chimeras to demonstrate that the susceptibility of the 2,3,7,8-TCDD-induced suppression of the CTL response was determined by the genotype of the host and not the genotype of the grafted lymphomyeloid cells. Taken together, these results suggest that if there is a role in the suppression of CMI that is regulated by the *Ah*-R, then this role is not at the level of the T-cell. Interestingly, Greenlee and coworkers (27) included binding studies and determined that specific binding by 2,3,7,8-TCDD occurs in the cytosol of thymocytes, but at a lower level than that observed in thymic epithelial cells (25 fmol/mg cytosol protein compared to 88 fmol/mg cytosol protein). These results are important because they indicate that different cell types from the same organ system can express markedly different levels of the *Ah*-R. Greenlee et al also determined that there were 29 fmol/mg protein of the *Ah*-R in the cytosol of whole thymus.

Several laboratories have also determined that there is specific binding by 2,3,7,8-TCDD in the cytosol of spleen (37, 47). While these results do indicate that the *Ah*-R is present in the spleen, they do not allow any speculation as to a possible role for this receptor in 2,3,7,8-TCDD-induced suppression of HI. Results from Roberts and coworkers (48) were argued to support a role by the *Ah*-R in dioxin-induced immunotoxicity. This interpretation was based primarily on the differences between the amount of specific binding in the cytosol of spleen from responsive C57BL/6 mice (18 fmol/mg protein) and nonresponsive DBA/2 mice (none detected), and between Hartley guinea pigs (15 fmol/mg protein), a dioxin-sensitive species, and Golden Syrian hamsters (none detected), a dioxin-resistant species. Interestingly,

Roberts et al (48) also showed that a nonhuman primate, the adult Rhesus monkey, had the greatest amount of binding activity in the spleen (36 fmol/mg protein) when compared to the other species.

Unlike the results from Greenlee and coworkers with the thymus as described above, we are not aware that anyone has determined the relative distribution of the *Ah*-R in the various cell types that make up the spleen. Such an investigation would seem warranted because of the debate over whether a given immunocompetent cell type is especially sensitive to the effects of 2,3,7,8-TCDD and therefore represents a "primary" cell target. In addition, because of the apparent relationship between AHH inducibility and the state of activation of the lymphocyte as discussed above, it is important that the levels of the *Ah*-R be compared in isolated lymphocyte populations which are confirmed to be resting or activated, and/or determined during the various stages of lymphocyte activation and differentiation.

Despite numerous studies implying a role for the *Ah*-R in dioxin-induced immunotoxicity, as discussed below, surprisingly very few studies to date have attempted to directly measure the presence of the *Ah*-R in a purified population of lymphoid cells, and *none* has been reported in any animal model. While Vos & Luster (49) have claimed in a recent review that the *Ah*-R has been identified in murine lymphocytes, the study upon which this claim was based was by Blank et al (45), which is discussed above. In this study, the levels of the *Ah*-R were determined in the cytosol of whole spleen and not on an enriched population of lymphocytes. Moreover, the level of the binding protein that they determined in the cytosol of spleen from B6C3F1 mice, 7 fmol/mg protein, was quite low, especially when compared to the values for spleen reported by other groups. In one study using cytosol from human PBL (50), it was reported that the concentration of the TCDD-binding protein varied greatly, was frequently absent, and was measured at 42 fmol/mg protein as its highest concentration. Similar results were obtained in a more recent study, which also used human PBL (51). The levels of the *Ah*-R in the later study varied from 0 to 50 fmol/mg cytosolic protein. Although this trend may simply reflect that humans are a highly variable population, a component of the variability observed in human peripheral blood lymphocytes may be due to the fact that the levels of the *Ah*-R have been determined in cells in various states of activation. In summary, the *Ah*-R appears to be present in human lymphocytes. In general, the amount of *Ah*-R in human tissue is subject to much greater variability when compared to various animal models. However, in agreement with animal models, it is still unresolved if this protein has a primary or secondary role in dioxin-induced toxicity in humans.

APPROACHES TO THE ROLE OF THE *AH*-R IN IMMUNOTOXICITY The most extensively used approaches to determining a role of the *Ah*-R in 2,3,7,8-

induced immunotoxicity have been indirect. These approaches have capitalized either on the fact that there is a definite SAR associated with HAH binding to the receptor, or on the fact that the expression of the receptor is controlled by the *Ah* locus and that various mouse strains differ in their expression of the receptor. Poland & Glover (2, 22) used both approaches to demonstrate that HAH-induced thymic atrophy segregated with the *Ah*-R. Several groups have also used these approaches to investigate a role for the *Ah*-R in the effects of 2,3,7,8-TCDD and related HAH on either CMI or HI.

The most recent work on the role of the *Ah*-R in the HAH-induced suppression of CMI has come from the laboratory of Kerkvliet and coworkers (52), who used an in vivo model of the CTL response to characterize the acute effects of several PCB and of 2,3,7,8-TCDD. Their results indicated that 3,4,5,3',4',5'-HxCB and 2,3,4,5,3',4'-HxCB, but not 2,4,5,2'4'5'-HxCB, could suppress the CTL and were in agreement with a role by the *Ah*-R. As reported earlier by Silkworth & Grabstein (53), only those PCB congeners that could obtain a planar configuration can bind to the *Ah*-R, and only those congeners were immunosuppressive. Kerkvliet and coworkers also used congenic mice to demonstrate that both 3,4,5,3',4',5'-HxCB and 2,3,7,8-TCDD were markedly more active in Ah^{bb} C57BL/6 mice than in Ah^{dd} C57BL/6 mice (52). The latter observation is also consistent with a role by the *Ah*-R and with previous results presented by Clark and coworkers (11, 12). Therefore, the available evidence does support a role of the *Ah*-R in the suppression of CMI. However, as discussed above, the basis for this role seems to be an effect on thymic epithelial cells and not on T-cells. Such an interpretation is consistent with the recent results of Kerkvliet et al (52), which indicated that the suppression of the CTL response by 3,4,5,3',4',5'-HxCB and 2,3,7,8-TCDD is apparent only at doses that also caused a significant atrophy of the thymus.

In contrast with what has been reported for T-cells, several groups have reported that 2,3,7,8-TCDD can have direct effects on B-cells (13, 14, 20). Therefore, HI may be a better model system to characterize the mechanism of dioxin-induced immunosuppression and, in fact, the Ab response has been more extensively studied than any parameter reflecting CMI. The results represent the strongest evidence to date that *Ah*-R-independent mechanisms may be involved and are, therefore, the most controversial. Studies to assess humoral immunotoxicity have been conducted with both in vivo and in vitro exposure to 2,3,7,8-TCDD and related compounds.

The first study to investigate the role of the *Ah*-R in the dioxin-induced suppression of the Ab response was by Vecchi and coworkers (54). They used acute exposures to 2,3,7,8-TCDD to demonstrate strain differences in the susceptibility of the Ab response to SRBC. In the C57BL/6 mouse, 1.2 μg/kg was immunosuppressive, whereas it took 6 μg/kg in the DBA/2 to demonstrate a comparable suppression. Vecchi and coworkers also reported that the

sensitivity of DBA/2 mice was not affected if 2,3,7,8-TCDD was administered over a longer period of time, i.e. once a week for 5 or 8 weeks. More recently, additional support for a role by the *Ah*-R was demonstrated by an in vivo SAR study. Davis & Safe (55) used acute exposures to 2,3,7,8-TCDD, 2,3,4,7,8-PCDF, 2,3,7,8-TCDF, 1,2,3,7,9-PCDF, and 1,3,6,8-TCDF to demonstrate a good correlation between *Ah*-R binding and suppression of the Ab response.

In contrast to these studies is the work that suggests a possible non-*Ah*-dependent component to dioxin-induced immunosuppression. Holsapple and coworkers showed that 2,7-DCDD, a dioxin congener with very weak affinity for the *Ah*-R, suppressed the Ab response to SRBC when administered at 1 or 10 μg/kg/day for 14 days (56). There was no change in thymic weight or in a variety of parameters reflecting the potential of this congener to cause enzyme induction, including AHH activity. In contrast, exposure to 2,3,7,8-TCDD caused both a marginal, but significant, reduction in thymic weight and a marked elevation in AHH activity, in addition to a suppression of the Ab response. In the same study, they also determined that subchronic exposure to octa-CDD was completely devoid of any activity. Interestingly, the paper by Holsapple & coworkers (56) also demonstrated that the magnitude of the suppression by 2,3,7,8-TCDD was comparable whether the dioxin was administered at 1.0 μg/kg/day over 5 or 14 days; but that there was a marked difference in the activity of 2,7-DCDD, where 14 daily exposures of 1.0 μg/kg produced a significant 56% suppression, while 5 daily exposures of the same dose produced a nonsignificant reduction of 20%. These results suggested that there may be an important temporal relationship to the immunosuppression by 2,7,DCDD. Further studies are necessary to evaluate this relationship by characterizing the subchronic effects of other HAH with minimal affinity for the *Ah*-R in "responsive" mice, or by determining the effects of subchronic exposure to 2,3,7,8-TCDD in "nonresponsive" mice. Because of the complexity of the pharmacokinetic model associated with 2,3,7,8-TCDD (57), it is difficult to compare studies based on 14 daily exposures, as described by Holsapple and coworkers, with studies based on administering the compounds once a week for several weeks, as described by Vecchi and coworkers. Interestingly, the study by Holsapple et al (56) is not the only study based on in vivo exposure to 2,3,7,8-TCDD to suggest that there may be *Ah*-independent mechanisms involved in immunosuppression. A recent preliminary study by Kerkvliet et al (58) indicated a biphasic dose-response curve in Ah^d C57BL/6 congenic mice, which, they argued, was indicative of an *Ah* locus-independent aspect.

The earliest study to demonstrate that any HAH could directly affect the function of cultured lymphocytes was by Holsapple et al (59), in which they demonstrated that 1,2,3,6,7,8-HxCDD could suppress several models of the

in vitro Ab response. Two subsequent papers reported a similar action by 2,3,7,8-TCDD (13, 14). In the first (13), 2,3,7,8-TCDD was shown to produce a suppression of three models of the in vitro Ab response (i.e. LPS, DNP-ficoll and SRBC), which was dose-related, parallel, and comparable in magnitude. This paper (13) also presented the first evidence to refute the hypothesis that the mechanism of immunotoxic action by the dioxins may not be solely mediated through the *Ah*-R. Using the Ab response to LPS, they demonstrated that 2,3,7,8-TCDD produced a comparable suppression of splenocytes from B6C3F1 (Ah^b) and DBA/2 (Ah^d) mice and from heterozygous congenic (Ah^{bd}) and homozygous congenic (Ah^{dd}) mice. Moreover, they demonstrated that 2,3,7,8-TCDD and 2,7-DCDD produced a comparable suppression of the Ab response. Similar results were observed using the Ab response to SRBC (Holsapple et al, unpublished observations). The suppression of the in vitro Ab response by 2,7-DCDD was in agreement with the suppression by this congener following subchronic exposure in the whole animal, as discussed above. Although controversial for some time (13), these results have recently been confirmed and extended by the work of Davis & Safe (60), which demonstrated that the PCDF congeners, 2,3,4,7,8-PCDF, 1,2,3,7,9-PCDF, 1,3,6,8-TCDF, and 2,3,7,8-TCDF, produced a similar profile of suppression of the in vitro Ab response to SRBC as that produced by 2,3,7,8-TCDD. Earlier studies by Tucker et al (14) and by Luster et al (20) presented results which were argued as support for a role by the *Ah*-R in the suppression of the in vitro Ab response. However, their results were based on limited dose-response curves and a limited number of HAH congeners.

To summarize, there is some controversy associated with the role played by the *Ah*-R in TCDD-induced immunotoxicity. This controversy is particularly evident for a number of studies that have investigated the direct effects of HAH on the in vitro Ab response, although a few in vivo studies have challenged the restricted view that the mechanism of TCDD-induced changes in immunocompetence is solely *Ah*-R-dependent. The remaining sections describe possible actions by which TCDD could contribute to the mechanism of effect on immunocompetence. Whenever possible, we have included those studies that have attempted to determine the role of the *Ah*-R in these actions.

Role of Phosphorylation

Kinase activation and/or protein phosphorylation induced by 2,3,7,8-TCDD have been described in both isolated B-cells and in thymocyte membranes. Although both 2,3,7,8-TCDD and phorbol 12-myristate, 13-acetate (PMA) enhanced phosphorylation of a number of proteins of various molecular weights when added to cultures of highly purified splenic B-cells, the addition of 2,3,7,8-TCDD selectively increased nonphospholipid-dependent kinase activity (i.e. no affect on kinase activity in the presence of exogenous

phospholipid) (30). Moreover, Kramer and coworkers observed that TCDD selectively increased the phosphorylation of an approximately 45 kd protein that was not affected by PMA, suggesting that 2,3,7,8-TCDD is activating a kinase other than, or in addition to, PKC. That such phosphorylation may be involved in the mediation of 2,3,7,8-TCDD-induced suppression of the Ab response is suggested by two preliminary observations: both human and murine gamma interferon are able to reverse both 2,3,7,8-TCDD-induced splenic B-cell-specific phosphorylation and 2,3,7,8-TCDD-induced suppression of in vitro Ab response in mice (61, 62); the specific proteins that are phosphorylated in isolated splenic B-cells following 2,3,7,8-TCDD administration, are not phosphorylated in similarly treated isolated splenic T-cells. This finding suggests the existence of kinases activated in B-cells by dioxin that are either not activated or are not present in T-cells (61). In an earlier study, Kramer et al reported that 2,3,7,8-TCDD also does not appear to activate PKC in cultured EL4 thymoma cells (63). In these studies, treatment with 2,3,7,8-TCDD did not compete with ^{3}H-phorbol dibutyrate for binding to cytosolic PKC, had no effect on PKC activity in vitro, did not stimulate the translocation of PKC to the membrane, or have an effect on phorbol ester-stimulated translocation, did not inhibit growth of EL4 cells in culture, and did not stimulate IL-2 production. More recently, Germolec et al have confirmed that 2,3,7,8-TCDD induces increased kinase activity in isolated splenic B-cells and reported that this activity is associated with a tyrosine kinase (64). The increase in kinase activity was reported to occur within minutes following TCDD exposure, suggesting that nuclear translocation and de novo protein synthesis were not involved, although studies with congenic mice did suggest *Ah*-R involvement. In agreement with the reports by Kramer and coworkers, the studies by Germolec et al showed that TCDD did not appear to affect PKC. In contrast, Bombick et al demonstrated increases in both tyrosine kinases and PKC in mouse thymic membrane preparations from mice treated with 2,3,7,8-TCDD in vivo (65). cAMP-stimulated kinases and cAMP levels were not significantly affected. Additionally, quercetin, a bioflavenoid reported to inhibit certain tyrosine kinases in vitro, reversed both 2,3,7,8-TCDD-induced thymic atrophy and kinase activation, suggesting a causal relationship between phosphorylation and toxicity. In the studies by Bombick and coworkers (65), 2,3,7,8-TCDD was also shown to increase levels of mRNA bearing homology to v-*erb*-A, and the authors hypothesized that the effects of this toxicant are mediated by tyrosine kinase stimulation secondary to the activation of this oncogene, or an oncogene that is homologous to it. At the dose of 2,3,7,8-TCDD used, neither kinase activity nor c-*erb*-A expression was observed in DBA/2J mice, suggesting that the *Ah*-R is in some way involved in the observed kinase activation.

Reports of 2,3,7,8-TCDD-induced phosphorylation in immunocompetent

cells and/or lymphoid organs are consistent with effects in other tissues. The enhancement of differentiation in human epidermal cells, the human keratinocyte cell line, SCC-12F, and XB/3T3, murine teratoma cells has been associated with decreases in the binding of EGF to its receptor (28, 66–69). In XB/3T3 cells, decreases in EGF binding have also been associated with 2,3,7,8-TCDD-induced increases in kinase activity, possibly leading to activation of this receptor and stimulating EGF-like effects (68). In a number of reports of studies with hepatic plasma membranes from the rat, mouse guinea pig, and hamster, Matsumura and coworkers have also demonstrated 2,3,7,8-TCDD-induced decreases in EGF binding that precede and are believed to mediate the 2,3,7,8-TCDD-induced toxic effects (70). 2,3,7,8-TCDD and EGF produced similar profiles of developmental toxicity in the mouse fetus and the extent of reduction in EGF binding segregated with *Ah*-R functionality in responsive and nonresponsive mouse strains (71). The decline in EGF receptor number has been associated with increased phosphorylation of this receptor by 2,3,7,8-TCDD and with increases in PKC and both cAMP-dependent and cAMP-independent tyrosine kinases (71–74). Quercetin, a reported inhibitor of tyrosine kinases in vitro, appears to antagonize 2,3,7,8-TCDD-induced lethality in mice (72). Additionally, 2,3,7,8-TCDD has been reported to increase pp60src in hepatocytes from the rat and guinea pig, mouse thymus, and NIH-3T3 mouse fibroblasts (75).

More work is needed to identify the kinase systems that are sensitive to 2,3,7,8-TCDD as well as any substrates for these kinases that could be tied to the actions of 2,3,7,8-TCDD. Whether or not the binding and/or translocation of the *Ah*-R itself involves obligatory phosphorylation and/or kinase activity is not clear; but it has been suggested (8). Note too that the profile of phosphorylation within a cell can be altered by changes in phosphatases, as well as kinases. To date, no one has determined the effects of 2,3,7,8-TCDD or any HAH on a phosphatase.

Role of Calcium

Alterations in calcium homeostasis within various tissue systems have been implicated to contribute to the toxic manifestations of 2,3,7,8-TCDD exposure in animals. Al-Bayati et al have reported that exposure of guinea pigs to 40 μg/kg 2,3,7,8-TCDD produced significant increases in the Ca^{++} content of liver microsomes, mitochondria, and cytosol within 5 days posttreatment (76). These changes occurred in a dose- and time-dependent manner and were concluded to reflect either a redistribution of intracellular Ca^{++} or an increase in membrane permeability; either of which could contribute to hepatocellular damage and/or cell death. Canga et al have also reported that a single acute dose of 10 μg/kg 2,3,7,8-TCDD in the guinea pig caused a significant decrease in β-adrenergic-induced contractility of the right ventricular papil-

lary muscle within 5 days following exposure (77). In addition, responsiveness to low-frequency stimulation was enhanced, responsiveness to increases in extracellular Ca^{++} concentrations was attenuated, and isoproterenol-elicited aftercontractions in K^+-depolarized preparations were increased in magnitude. These latter findings are consistent with the conclusion that 2,3,7,8-TCDD causes an increase in the concentration of intracellular Ca^{++} in papillary muscle. However, 2,3,7,8-TCDD did not block slow Ca^{++} channels and it was further postulated that this compound may act directly or indirectly to decrease Na^+/K^+-ATPase activity in the membrane, as has been previously reported to occur in the rat liver (78). Inhibition of this pump can increase the intracellular Ca^{++} concentration by altering Na^+/Ca^{++} exchange across the membrane. Evidence for the indirect actions of 2,3,7,8-TCDD on the Na^+/K^+-ATPase comes from a previously published report in which cytochrome P-450 of renal medullary cells was shown to metabolize arachidonic acid to a product that inhibits Na^+/K^+-ATPase activity (79).

In addition to the studies outlined above, more recent work involving Ca^{++}-mediated mechanisms of toxicity has contributed additional insight into the mechanism of action of dioxin on the thymus. McConkey et al, working with isolated thymocytes from young adult rats, found that incubation in the presence of increasing concentrations of 2,3,7,8-TCDD (1–100 nM) caused a dose-dependent increase in cytosolic free Ca^{++} concentrations, followed by DNA fragmentation by the activation of a Ca^{++}-dependent endonuclease. These effects resulted in losses of cell viability that were readily apparent at a concentration of 10 nM (80). Treatment of cells with cyclohexamide or incubation in Ca^{++}-free media prevented both the increase in cytosolic Ca^{++} and the DNA fragmentation, thus demonstrating a direct correlate with the thymocyte suicide shown to occur following acute exposure to glucocorticoids.

The results by McConkey and coworkers are of obvious importance to an understanding of the mechanism of action of TCDD on the thymus, as well as from a more general perspective. The fact that 2,3,7,8-TCDD may increase intracellular Ca^{++} means that this xenobiotic may have similarities to the actions of hormones, which are independent of the classical steroid model. Two groups have recently reported that administration of progesterone caused a rapid influx of calcium ions into sperm by an action that was probably mediated by a cell-surface receptor (81, 82). It is also intriguing to speculate that a dioxin-induced increase in Ca^{++} may activate other enzymes in addition to the endonuclease studied by McConkey et al, namely Ca^{++}/calmodulin-dependent kinases. Therefore, a fruitful area of research may be to study the effects of 2,3,7,8-TCDD on calcium in isolated lymphocytes and to determine whether these changes can be correlated with the increased phophorylation. Note that previous work from Luster et al (20) indicated that

exposure to TCDD had no effect on phosphoinositide (PI) hydrolysis in anti-Ig-stimulated B-cells. Activation of B-cells with anti-Ig stimulates PI metabolism, which generates a number of products important to signal transduction, including inositol-1,4,5-trisphosphate (IP_3), a potent trigger for the release of intracellular calcium (83).

Role of Hormonal Systems

Of current concern to the toxicities in laboratory animals induced by 2,3,7,8-TCDD exposure is the relationship between dioxin and hormonal action. Recent reports have suggested that interactions between dioxin and various hormones may contribute to the complex profile of toxicities encountered in animal models. When administered to animals, many hormones demonstrate patterns of toxicity similar to 2,3,7,8-TCDD. One of the earliest questions raised about a hormonal influence in dioxin-induced immunotoxicity was of a possible interaction with corticosteroid. The adrenal axis has potent modulatory effects upon the immune system (84) and many similarities exist between the profile of action of 2,3,7,8-TCDD and corticosteroid. However, several investigators have concluded that increases in the levels of corticosteroids do not contribute to the toxicity by 2,3,7,8-TCDD (85–87). The remaining sections describe results for two other hormonal systems, estrogen and prolactin, with potent actions on immunocompetent cells and reported interactions with 2,3,7,8-TCDD.

INTERACTIONS BETWEEN TCDD AND ESTROGEN The relationship between 2,3,7,8-TCDD and estrogen has been the most thoroughly studied. Like 2,3,7,8-TCDD, administration of exogenous estrogens causes immunosuppression, thymic involution, and decreased resistance to challenge with pathogens (88–91). In addition, estrogens have been shown to modulate lymphocyte function directly, and can either enhance or suppress lymphocyte responses depending on the dose administered (92–94).

Gallo and coworkers have studied the effects of 2,3,7,8-TCDD on uterine development in the C57B1/6 mouse, and have reported an antiestrogenic effect (95). Treatment with 2,3,7,8-TCDD caused a reduction in normal uterine development and in estrogen-induced uterine swelling at low estrogen levels. Induction of AHH, epoxide hydrolase, and cytochrome P-450 by 2,3,7,8-TCDD were shown to occur independently of estrogen administration. Furthermore, coadministration of estrogen was shown to override the antiestrogen effect of dioxin and, depending on dose, decreased the amount of a protein that comigrated with epoxide hydrolase and 'P-450a', thus demonstrating a unique effect on protein synthesis via an interaction between estrogen and 2,3,7,8-TCDD in the liver. They concluded that estrogen may interact directly with 2,3,7,8-TCDD at the level of their respective receptors

and that the antiestrogenic effects of dioxin were possibly independent of the *Ah*-R and AHH induction. This same group has further hypothesized that estrogen receptor modulation and associated physiological compensations may cause many of the observed effects of 2,3,7,8-TCDD (96). This theory has been extended to include species variations in susceptibility to 2,3,7,8-TCDD-induced toxicity, in which certain species would be able to synthesize estrogen and to effectively increase tissue concentrations, thereby overcoming dioxin's antiestrogenic effects. These animals would show some toxicities, but these would relate to the elevated levels of estrogen in a given tissue system. This hypothesis is supported by the more recent report by Umbreit et al in which distinct differences in species susceptibility to dioxin correlate with the levels of steroid UDP-glucuronyl transferase (sUDPGT) activity (guinea pig > mouse > hamster; where guinea pigs are the most sensitive to toxicity and lethality) (97). However, rats and those mouse strains that differ in their degree of susceptibility to 2,3,7,8-TCDD, do not correlate with the levels of sUDPGT, which suggests that other factors may be determinants of such susceptibilities within a given species.

Additional evidence for the antiestrogenic effects of TCDD comes from a second recent report by Umbreit et al, in which they investigated the interaction between 2,3,7,8-TCDD and estrogen agonists (estradiol) and antagonists (tamoxifen) (98). The most striking finding was that tamoxifen treatment in various combinations with 2,3,7,8-TCDD dramatically increased dioxin toxicity in a synergistic manner. This effect was manifested as a decrease in time to death and an increase in percent mortality. This treatment had no effect on relative liver or uterine weight changes, as compared to dioxin alone, and it was concluded that 2,3,7,8-TCDD toxicity is at least partially manifested through interactions with estrogen and the estrogen receptor complex. Further studies are warranted to extend these interaction studies to include parameters reflecting immunocompetence.

INTERACTIONS BETWEEN TCDD AND PROLACTIN Prolactin (PRL) appears to be actively involved in the development of immunity and immune responses (99–103). PRL receptors have been found on both T- and B-lymphocytes (104), and a prolactinlike activity, which stimulates lymphocyte proliferation and which can be inhibited by anti-PRL antibodies, is reportedly produced by T-lymphocytes following Con A stimulation (101, 102, 105).

Jones et al have shown that a single i.p. administration of 50 μg/kg 2,3,7,8-TCDD alters the circadian rhythms of PRL and several other hormones in male Sprague-Dawley rats (87). PRL was the earliest to be affected, and may mediate the subsequent effects on other hormones. Serum PRL levels were significantly decreased within 4 hours of 2,3,7,8-TCDD administration, but rebounded to become significantly increased over control values

by 7 days after treatment. Additionally, PRL-induced elevation of ornithine decarboxylase (ODC) activity was decreased by 2 days after treatment in both the spleen and thymus, and remained decreased relative to control, 7 days after treatment in the spleen (the thymus was not evaluated at 7 days posttreatment), suggesting that 2,3,7,8-TCDD down-regulated PRL receptors in these organs. The reported increase in serum PRL by day 7 is a response consistent with receptor down-regulation. Jones et al speculated that the immunotoxicity associated with 2,3,7,8-TCDD exposure may be mediated by the observed modulation of serum PRL levels and PRL receptors. In a subsequent communication, Russell et al suggested that the 2,3,7,8-TCDD-induced suppression of serum PRL is secondary to a direct effect of dioxin on the hypothalamus that increases dopamine secretion (106). Direct treatment of cells from the adenohypophysis did not inhibit their ability to secrete PRL, but coadministration with pimozide, a dopamine receptor antagonist, did reverse the 2,3,7,8-TCDD-induced suppression of serum PRL. The authors concluded that a hypothalamic site of action for 2,3,7,8-TCDD and the resultant increase in dopamine may be the causative event in dioxin's suppression of serum levels of pituitary hormones and the consequent toxicity. Pohjanvirta et al have recently reported enhanced toxicity following intracerebroventricular administration in both Long-Evans and Han/Wistar rats, further demonstrating CNS susceptibility and supporting a role for the CNS in 2,3,7,8-TCDD-induced toxicity (107). In contrast, Moore et al failed to observe consistent alterations in serum PRL levels in Sprague-Dawley rats, and concluded that alterations in plasma PRL concentrations do not play a critical role in the toxicity of 2,3,7,8-TCDD (108). Moreover, Snyder et al, in the only study to date that specifically addresses the potential role of PRL in dioxin-induced immunosuppression, demonstrated in their preliminary results that coincident exposure to pimozide did not alter the in vivo suppression of the antibody response in female B6C3F1 mice exposed to a single oral dose of up to 30 μg/kg 2,3,7,8-TCDD (109). More work is needed to further characterize the nature of the complex interaction between 2,3,7,8-TCDD and PRL.

In summary, several groups have begun to consider whether interactions between 2,3,7,8-TCDD and more classical hormone systems may contribute to the complex profile of toxicity associated with this xenobiotic. Clearly, more work is needed to further characterize both the mechanism for, and the extent of, these interactions. The need for these interactive studies is particularly strong for in vivo studies where the interpretation for the observed effects by 2,3,7,8-TCDD must take into account possible indirect roles played by these hormone systems. In this regard, it is interesting to speculate that the differences between the actions of 2,3,7,8-TCDD on immunocompetence in vivo and in vitro (i.e. where the support for a role by the *Ah*-R is much stronger in the in vivo studies) may in part be due to the fact that the latter

approach assesses the direct actions by TCDD on lymphocytes in the virtual absence of hormonal influences.

SUMMARY AND FUTURE DIRECTIONS

Although effects on immunocompetence should clearly be included among the actions associated with the profile of toxicity by 2,3,7,8-TCDD, the mechanism(s) for these effects have yet to be elucidated. The material discussed in this review suggests that whatever mechanism(s) are involved may include both *Ah*-R-dependent and *Ah*-R-independent processes. The former possibility has represented the primary focus of most, if not all, previous reviews on the actions associated with 2,3,7,8-TCDD. The latter possibility may include such actions as a change in intracellular calcium or a change in the activity of a kinase/phosphatase system, and may involve interactions with classical hormone systems, such as estrogen or prolactin.

Although this review has focused primarily on TCDD-induced changes in immunocompetence and lymphocyte function, the first two recommendations for future action can serve as general statements about HAH-induced toxicity. First, with respect to the *Ah*-R-dependent processes, more work is needed to identify the protein/mRNA systems that are controlled by the DRE of the genome and that contribute to the alterations in cell growth and differentiation associated with exposure to TCDD. Second, with respect to the *Ah*-R-independent processes, the efforts must be centered around identifying possible membrane alterations that are not dependent on any nuclear events (i.e. such as an increase in calcium or the activation of a membrane-associated kinase), and that could lead to changes in signal transduction processes involved in cellular activation and subsequent differentiation.

We believe that two additional recommendations for future action need to be emphasized regarding the characterization of the specific nature of the effects of 2,3,7,8-TCDD and the HAH on immunocompetence. First, and perhaps most fundamentally, an effort is required to confirm that the *Ah*-R is present in isolated lymphocytes. This effort should include studies on how the expression of the receptor is affected by changes in the state of activation of the lymphocyte. This type of analysis would provide the most definitive evidence for, and presently represents a key missing link in a model centered around a role for the *Ah*-R in dioxin-induced immunosuppression. Second, more work should be directed toward establishing whether there is a relationship between the possible activation of lymphocytes by dioxin, as reported by this and other laboratories, including some results from exposed human populations, and the immunosuppression traditionally associated with the HAH.

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