Workshop on Perinatal Exposure to Dioxin-like Compounds. V. Immunologic Effects

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The immune system comprises a highly integrated network of multiple tissues and cell types with complicated interactions and effects. It is modulated by the endocrine and nervous systems and there is growing realization of its multifunctionality. The session focusing on immunologic effects of dioxin and related compounds following perinatal exposure involved a review of the immunotoxic effects that have been reported for polychlorinated aromatic hydrocarbons (PHAHs), a discussion of species differences in responses, and development of the immune system, and data from two ongoing epidemiological studies comparing the immune status of children exposed to higher-than-average concentrations of PHAHs both perinatally and lactationally. — Environ Health Perspect 103(Suppl 2):157–160 (1996)

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Introduction

Dr. Kerkvliet reviewed the role of the immune system in health and disease (1,2). Immunosuppression may result in an increased incidence of disease from bacteria, viruses, and parasites as well as an increase in tumors. Inappropriate activation of the immune system or loss of normal suppressor cell control is associated with allergies, hypersensitivity, and autoimmunity. The immune system involves a complex network of cells (macrophages, lymphocytes, other white blood cells) that communicate via soluble mediators such as the interleukins and other cytokines. The interactions are highly regulated. The macrophage initiates certain immune responses by taking up foreign materials nonspecifically and presenting antigens on its surface to T cells, which become activated. Various T-cell subtypes secrete different cytokines, which are involved in the function of other T cells as well as controlling the activity of B cells.

Studies in the 1970s demonstrated that TCDD treatment resulted in dramatic effects on the thymus (3–5). In fact, at relatively high doses of TCDD in adults, dioxin treatment resulted in atrophy of the lymphoid tissue. However, the ability of dioxin to modulate a number of immune responses has been shown to occur at doses much lower than those resulting in thymic atrophy. Nevertheless, prenatal effects on the thymus and/or T-cell-mediated immunity may be critical. The immunotoxic effects of TCDD have been shown to be mediated by binding to the Ah receptor, based on structure/activity studies and use of mice genetically different in their responsiveness to TCDD (6–9). The cellular targets of TCDD appear to be multiple, as suggested by the different assays and end points that have been assessed. While B cells are clearly targets of TCDD action, they appear less sensitive than macrophages and T cells (10). Approaches to studying the immunotoxicity of TCDD have involved in vivo, ex vivo, and in vitro studies (11–13). The in vitro experiments are very sensitive to culture conditions, and dioxin's effects appear dependent upon unknown serum factors (11).

A decrease in the ability to respond to a primary antibody challenge, often measured by responsiveness to sheep red blood cells (SRBC), is a sensitive and reproducible response to dioxin exposure in both mice and nonhuman primates (8,13,14). However, the immunotoxicity of dioxin in people is unclear. There have only been a limited number of investigations involving different study designs and parameters. Furthermore, there is an inherent difficulty in measuring the immune status of humans noninvasively, and for any assay that has been used there is a broad range of "normal" within the human population. Some of the assays that have been successfully used in animals have not been applied to humans. Often the cohort exposure is not validated and the immune status often has been examined long after exposure. The outstanding issues involve understanding the mechanism in animal models and basis for species specificity and sensitivity (15). The recent reports of alterations in lymphocyte subsets induced by TCDD require validation, both in animal models and in humans.

Dr. Smialowicz compared the immunotoxic effects of TCDD in rats vs mice. While thymic involution occurs in both species, the response to the SRBC is clearly different (16). The ED₅₀ for immunosuppression in the mouse is approximately 0.7 μg/kg. In contrast, no immunosuppression is observed in the rat; instead, an increase in response to the antigen is observed at 3 μg/kg. Examination of T-cell phenotypes revealed no apparent effect due to TCDD in the mouse, even when the ability to respond to the SRBC was completely suppressed. In contrast, in two strains of rats in which response to the antigen was enhanced by TCDD, there was a decrease in the T-suppressor population (CD4–CD8+), an increase in double negative T cells (CD4–CD8–), and an increase in IgM+ B cells. These alterations are consistent with enhanced response to a primary antigen challenge. Whether there are...
The fetal thymus also appears to be more sensitive than the adult thymus. Dioxin alters thymic differentiation, causing changes in the total number of cells in each lymphocyte subset (25). There may be an effect on immature populations of intrathymic cells due to a defect in differentiation. Alternatively, there may be changes in migration of cells from the bone marrow to the thymus (25-27). Recent studies have examined markers such as terminal deoxynucleotidyl transferase (TdT) and recombinase activating gene (RAG) that are present only in lymphoid stem cell populations and involved in gene rearrangements. TdT protein and mRNA decrease following TCDD exposure both in the fetal liver and bone marrow, while no effects are noted in the thymus. This decrease correlates with thymic atrophy as does a decrease in RAG mRNA. Studies have demonstrated that the TCDD-treated prothymocytes are unable to repopulate the thymus of irradiated mice. This suggests that a direct effect of TCDD on bone marrow stem cell populations may contribute to the elicited thymic atrophy. Future studies are needed to examine the issue of dose–response relationships, chronic low-dose effects, effects on B cells, role of cytokines, and hormonal involvement. Furthermore, it is necessary to determine if or how an effect of TCDD on bone marrow stem cell populations may affect other, more subtle, aspects of the immune system other than thymic size. It is important to note that while estrogen may also produce thymic atrophy via effects on prothymocytes, the effects of TCDD cannot be blocked by estrogen receptor antagonists.

Arctic Quebec is the site of an ambitious series of studies discussed by Dr. Dewailly (28). Infectious disease (e.g., meningitis, measles) incidence, as well as that of otitis, is 20-fold higher in the first year of life among the Inuit than in individuals living in southern Quebec. This appears to be associated with immune dysfunction as measured by a low immunization take rate, and raised issues of altered host resistance. The Inuits have elevated levels of PCBs, PCDDS, and PCDFs (29,30). Current investigations are focused on immunologic examinations of the babies at 2, 6, and 12 months, and comparison of breast-fed to nonbreast-fed infants. Is breast feeding protective against disease? Is breast feeding, due to the high level of lactational transfer of PHAhs, associated with elevated disease incidence? There is a suggestion that babies with acute otitis have been nursed by mothers with higher levels of PHAhs or have nursed longer than non-affected infants. The T-helper/T-suppressor cell ratio may also decrease with increased exposure, although it is still within the normal range. A negative correlation was detected between the decrease in CD4/CD8 ratio and the total toxic equivalency. Prenatal exposure of rodents to TCDD also appears to result in a decrease in the relative population of T-helper versus T-suppressor cells in the thymus. Further studies are clearly warranted in this population, with emphasis on lymphocyte subset information and determination of immunization-take rates. In addition, it would be helpful to have a control population whose mothers have "normal" PHA levels to examine whether the immunosuppressive effects noted are due to prenatal or lactational exposure.

Dr. Helge stressed the importance of conducting parallel studies in animals and humans. In studies of TCDD-exposed marmosets, changes in the ratio of CD4+CD29+ helper cells to CD8+CD56+ cytotoxic cells were noted following extremely low dose (10 ng/kg) treatment of adults (31). However, at even lower doses, the helper cells actually increased (32). The meaning of the biphasic nature of this response is not clear. In vitro treatment of marmoset cells results in suppression of the normal stimulatory effect of pokeweed mitogen on B cells (33).

Bottle- and breast-fed human infants are being examined and compared for lymphocyte subsets as well as their response to stimulation by pokeweed mitogen, PMA, Con A, and anti-CD3. Lower stimulation by all of these mitogens was observed in breast-fed infants. This could be associated with colostrum, which is known to inhibit stimulation because the lack of mitogen responsiveness disappeared by the time the babies were 5 months old. The T-cell subsets are being examined in cord blood and in the children to determine whether breast feeding is associated with changes similar to those observed in the dioxin-treated marmosets. This population study would also benefit from a comparison to babies whose mothers have lower levels of PHAhs than in the group currently under examination for the effects of breast feeding.

It is clear that there is a need to develop sensitive and feasible methods to examine immune responses in children exposed to TCDD prenatally, lactationally, or both. More effort must also be directed at examining the immune effects that have been noted to occur in animals, such as the suppression of the primary antibody response.
Alterations in lymphocyte subsets is clearly a promising area for future investigation, with potential for use as biomarkers. One conclusion that appears clear is that TCDD and related compounds have the ability to alter differentiation of cells in the immune system, as well as in other systems of the body. Viewing dioxin as a chemical that has the ability to disrupt cellular differentiation puts into perspective not only its effects on the immune system but also its dysregulatory role in development of the nervous and reproductive systems.

REFERENCES

1. Dean JH, Luster MI, Boorman GA. Immunotoxicology. In: Immunopharmacology (Sirois P, Rola-Pleszczynski M, eds). New York: Elsevier, 1982:349.
2. Benjamin E, Leskowitz S. Immunology. A Short Course. 2nd ed. New York: Wiley-Liss, 1991.
3. Vos JG, Moore JA, Zinkl, JG. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the immune system of laboratory animals. Environ Health Perspect 5:149–162 (1973).
4. Vos JG, Moore JA. Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Int Arch Allergy 47:777–794 (1974).
5. Vos JG, Moore, JA, Zinkl, JG. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in C57B1/6 mice. Toxicol Appl Pharmacol 29:229–241 (1974).
6. Vecchi A, Sironi M, Canegrati MA, Recchis M, Garattini S. Immunosuppressive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in strains of mice with different susceptibility to induction of aryl hydrocarbon hydroxylase. Toxicol Appl Pharmacol 84:334–441 (1983).
7. Kerkvliet NI, Brauner JA, Matlock JP. Humoral immunotoxicity of polychlorinated diphenyl ethers, phenoxynaphthoxins, dioxins and furans present as contaminants of technical grade pentachlorophenol. Toxicology 36:307–324 (1985).
8. Silkworth JB, Antrim L. Relationship between Ah receptor-mediated polychlorinated biphenyl (PCB)-induced humoral immunosuppression and thymic atrophy. J Pharmacol Exp Ther 235:606–611 (1985).
9. Morris DL, Jordan SD, Holsapple MP. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on humoral immunity: I. Similarities to Staphylococcus aureus Cowan strain I (SAC) in the in vitro T-dependent antibody response. Immunopharmacology 21:159–170 (1991).
10. Kerkvliet NI, Oughton J. Acute inflammatory response to sheep red blood cell challenge in mice treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD): phenotypic and functional analysis of peritoneal exudate cells. Toxicol Appl Pharmacol 119:268–257 (1993).
11. Vecchi A, Montovani A, Sironi M, Luini M, Cairor M, Garattini S. Effect of acute exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin on humoral antibody production in mice. Chem Biol Interact 30:337–341 (1980).
12. Kerkvliet NI, Brauner JA. Flow cytometric analysis of lymphocyte subpopulations in the spleen and thymus of mice exposed to an acute immunosuppressive dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ Res 52:146–164 (1990).
13. Kerkvliet NI, Steppan, Brauner JA, Deyo JA, Henderson MC, Tomar RS, Bühler, DR. Influence of the Ah locus on the humoral immunotoxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) immunotoxicity: evidence for Ah receptor-dependent and Ah receptor-independent mechanisms of immunosuppression. Toxicol Appl Pharmacol 105:26–36 (1990).
14. Trphyonas H, Luster MI, Schiffman G, Dawson LL, Hodgen M, Germolec D, Hayward S, Bryce F, Loo JCK, Mandy F, Arnold DL. Effect of chronic exposure of PCB (Aroclor 1254) on specific and nonspecific immune parameters in the Rhesus (Macaca mulatta) monkey. Fundam Appl Toxicol 16:773–786 (1991).
15. Kerkvliet N, Burleson GR. Immunotoxicity of TCDD and related halogenated aromatic hydrocarbons. In: Immunotoxicology Immunopharmacology, 2nd ed (Dean JH, Luster MI, Munson AE, Kimber I, ed). New York: Raven Press, 1994; 97–122.
16. Smialowicz RJ, Riddle MM, Williams WC, Diliberto J.J. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on humoral immunity and lymphocyte subpopulations: differences between mice and rats. Toxicol Appl Pharmacol 124(2): 248–256 (1994).
17. Luebke RW, Copeland CB, Diliberto JJ, Akubue PI, Andrews DL, Birnbaum LS. Assessment of host resistance to Trichinella spiralis in mice following pre-infection exposure to 2,3,7,8-TCDD. Toxicol Appl Pharmacol 125(1):7–10 (1994).
18. Luebke RW, Copeland CB, Andrews DL. Host resistance to T. spiralis infection in rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Fundam Appl Toxicol (in press).
19. Burleson GR, Lebrec H, Yang Y, Ibanes JD, Pennington KN, Birnbaum LS. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on influenza virus host resistance in mice. Fundam Appl Toxicol (submitted).
20. Yang Y, Lebrec H, Burleson GR. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on viral replication and natural killer (NK) activity in rats. Fundam Appl Toxicol 23:125–131 (1994).
21. Fowles BJ, Pardal DM. Molecular and cellular events of T-cell development. Adv Immunol 44:207 (1989).
22. Vos JG, Moore JA. Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Int Arch Allergy 47:777–794 (1974).
23. Luster MI, Boorman GA, Dean JH, Harris MW, Luebke RW, Padarath Singh ML, Moore JA. Examination of bone marrow, immunologic parameters and host susceptibility following pre- and postnatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Int J Immunopharmacol 2:301–310 (1980).
24. Thomas PT, Hinsdill RD. The effect of perinatal exposure to tetrachlorodibenzo-p-dioxin on the immune response of young mice. Drug Chem Toxicol 2:77–98 (1979).
25. Fine JS, Gasiewicz TA, Silverstone AE. Lymphocyte stem cell alterations following perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Mol Pharmacol 35:18–25 (1989).
26. Fine JS, Gasiewicz TA, Fiore NC, Silverstone AE. Prothymocyte activity is reduced by perinatal 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure. J Exp Pharmacol Ther 255:1–5 (1990).
27. Fine JS, Silverstone AE, Gasiewicz TA. Impairment of prothymocyte activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin. J Immunol 144:1169–1176 (1990).
28. Dewailly E, Brueneau S, Laliberte C, Belanger D, Gingras S, Ayotte P, Nantel A. Weight, size, head circumference and TSH of Inuit newborn prenatally exposed to high levels of organochlorines. Dioxin ‘92:10:257–259 (1992).
29. Dewailly E, Brueneau S, Laliberte C, Belles-Isles M, Weber JP Roy R. Breast milk contamination by PCBs and PCDDs/PCDFs in arctic Quebec: preliminary results on the immune status of Inuit infants. Dioxin ‘93:13:403–406 (1993).
30. Dewailly E, Nantel A, Brueneau S, Laliberte C, Ferron L, Gingras S. Breast milk contamination by PCDDs, PCDFs, and PCBs in Arctic Quebec: a preliminary assessment. Chemosphere 25:1245–1249 (1992).
31. Neubert R, Jacob-Muller U, Stahlmann R, Helge H, Neubert D. Polyhalogenated dibenzo-p-dioxins and dibenzofurans and the immune system. 1. Effects on peripheral lymphocyte subpopulations of a non-human primate (callitrichis jacchus) after treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Arch Toxicol 64:345–349 (1990).
32. Neubert R, Golor G, Stahlmann R, Helge H, Neubert D. Polyhalogenated dibenzo-p-dioxins and dibenzofurans and the immune system. 4. Effect of multiple-dose treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on peripheral lymphocyte subpopulations of a non-human (callithrix jacchus). Arch Toxicol 66:250–259 (1992).

33. Neubert R, Jacob-Muller U, Stahlmann R, Neubert D. Polyhalogenated dibenzo-p-dioxins and dibenzofurans and the immune system. 2. In vitro effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on lymphocytes of venous blood from man and a non-human primate. Arch Toxicol 65:213–219 (1991).