Immunological Effects of Background Prenatal and Postnatal Exposure to Dioxins and Polychlorinated Biphenyls in Infants.

Weisglas-Kuperus N[1], Sas TCJ[1], Koopman-Esseboom C[1], van der Zwan C[2], de Ridder MAJ[3], Beishuizen A[1], Hooijkaas H[4], Sauer PJJ[1]

[1] Department of Pediatrics, Division of Neonatology, Erasmus University and University Hospital/Sophia Children's Hospital, Dr Molewaterplein 60, 3015 GJ Rotterdam, The Netherlands

[2] National Institute of Public Health and Environmental Protection, P.O.Box 1, 3720 BA Bilthoven, The Netherlands

[3] Institute of Epidemiology and Biostatistics, Erasmus University, P.O.Box 1738 3000 DR, Rotterdam, The Netherlands

[4] Department of Immunology, Erasmus University and University Hospital Rotterdam, Dr.Molewaterplein 50, 3015 GE, Rotterdam, The Netherlands

1. Introduction

Many animal studies have shown adverse effects of PCDDs, PCDFs (summarized as dioxins) and PCBs on the immune system. The most consistent finding in these studies is thymic atrophy. In utero and lactational exposure is a more sensitive period for the immunotoxic effects than adult exposure, suggesting that in animals during maturation the immune system is particularly sensitive to these compounds [1,2,3,4,5,6].

Data regarding the potential toxic effects of PCDDs, PCDFs and PCBs on the immune system in human beings are scarce. In vitro studies of human venous blood and lymphocyte fractions incubated with low doses of TCDD demonstrated a decrease in B-cells and CD4+ (helper) T-cells and an increase in CD8+ (cytotoxic) T-cells[7]. The first indication that PCBs and dioxins might be immunotoxic in vivo came from studies in accidentally exposed humans[8,9,10,11,12,13,14,15]. In highly industrialized, densely populated Western European countries, like the Netherlands, dioxin levels in human milk samples can be especially high (10-100 pg toxic equivalent (TEQ) / g milk fat). Whether prenatal and postnatal exposure to these high background levels of PCDDs, PCDFs and PCBs can alter the immune system in human infants and whether the health of the infant is adversely affected by these pollutants is not known. In our study we explored the immunological effects of environmental prenatal and postnatal background exposure to PCDDs, PCDFs and PCBs in infants from birth to eighteen months of age. This study is part of the Dutch PCB/Dioxin Study, a larger prospective longitudinal study on possible adverse health effects of these pollutants on human infants.

2. Methods

Our total study group consisted of 207 healthy mother-infant pairs, of which105 infants were breast-fed and 102 children were bottle-fed. Prenatal PCB exposure was estimated by the PCB sum (PCB congeners 118, 138, 153 and 180) in maternal blood and the total TEQ level in human milk (17 dioxin and 8 dioxin-like PCB congeners). Postnatal PCB/dioxin exposure was calculated as a product of the total TEQ level in human milk multiplied by weeks of breast-feeding. The number of periods with rhinitis, bronchitis, tonsillitis and otitis during the first 18 months of life was used as an estimate of the health status of the infants. Humoral immunity was measured at 18 months of age by detecting antibody levels to mumps, measles and rubella. White blood cell counts (monocytes, granulocytes and lymphocytes) and immunological marker analyses CD4+ T-lymphocytes, CD8+ T-lymphocytes, activated T-lymphocytes (HLA-DR+CD3+), as well as TcR$\alpha\beta$+, TcR$\gamma\delta$+, CD4+CD45RA+ and CD4+CD45RO+ T- lymphocytes, B-lymphocytes (CD19+ and/or CD20+) and NK cells (CD16+ and/or CD56+/CD3-) in cord blood and venous blood at 3 and 18 months of age were assessed in a subgroup of 55 infants. Details are described in a previous paper[16].

3. Results

There was no relationship between pre- and postnatal PCB/dioxin exposure and upper or lower respiratory tract symptoms or humoral antibody production. A higher prenatal PCB/dioxin exposure was associated with an increase in the number of TcR$\gamma\delta$+ T-cells at birth and with an increase in the total number of T-cells and the number of CD8+ (cytotoxic), TcR$\alpha\beta$+ and TcR$\gamma\delta$+ T-cells at 18 months of age. A higher prenatal as well as postnatal PCB/dioxin exposure was associated with lower monocyte and granulocyte counts at 3 months of age (Table1).

Table 1: Spearman rank correlation coefficients of the significant correlations ($p \leq .05$) between the immunological parameters and the total TEQ: dioxin TEQ, planar, mono-ortho and di-ortho PCB congener TEQ levels in breast-milk.

	Dioxin and dioxin-like PCB TEQ levels in breast-milk Spearman rank correlation coefficients				
	Dioxin TEQ	Planar PCB TEQ	Mono-ortho PCB TEQ	Di-ortho PCB TEQ	Total TEQ
White Cell Blood Counts					
Monocytes at 3 months	-.55**	-.37	-.67**	-.51*	-.64**
Granulocytes at 3 months	-.40	-.40	-.44	-.34	-.47*
T-cell markers					
CD3+CD8+ at 18 months	.80**	.71**	.52	.68*	.65*
TcR $\alpha\beta$+at 18 months	.71**	.50	.44	.61*	.57*
TcR $\gamma\delta$+ in cordblood	.57**	.32	.40	.34	.50*

*=$p \leq .05$, **=$p \leq .01$

4. Discussion

In our study two different effects of PCB/dioxin background exposure on the developing immune system of human infants were found:

- Prenatal PCB/dioxin exposure was associated with changes in T-cell subpopulations in the blood. These changes were mainly seen at 18 months of age. At that age a higher prenatal PCB/dioxin exposure was associated with an increase in the total number of T-cells as well as with an increase in the number of CD8+ (cytotoxic), TcR$\alpha\beta$+ and TcR$\gamma\delta$+ T-cells. These prenatal effects of PCB/dioxin exposure on changes in T-cell subpopulations at a later age are consistent with findings in other human studies. In children born to mothers living in a TCDD contaminated environment in Time Beach Missouri during and after pregnancy a decrease in CD4+ (helper) T-cells and an increase of CD8+ (cytotoxic) T-cells has even been demonstrated at 9 to 14 years of age[14]. In one preliminary report from Northern Quebec, Inuit infants whose mothers have elevated levels of PCBs and dioxins in their breast-milk, the CD4+ (helper) : CD8+ (cytotoxic) T-cell ratio was decreased at 6 and 12 months of age but not at 3 months of age[15]. Our results are also in agreement with animal studies, where perinatal TCDD exposure produces an alteration in the normal thymocyte maturational process[4,5]. Moreover, in vitro studies of human venous blood and lymphocyte fractions incubated with TCDD demonstrated a decrease in CD4+ (helper) T-cells and an increase in CD8+ (cytotoxic) T-cells[7]. In contrary to the above studies, we did not find a decrease in CD4+ (helper) T-cells. All these studies were, however, conducted in vitro or in higly exposed infants whereas our study was conducted in background PCB/dioxin exposed infants.
- A higher prenatal as well as postnatal PCB/dioxin exposure was associated with lower monocyte and granulocyte counts only at 3 months of age. The effects on the lymphocyte count fell short of statistical significance. Our findings are in agreement with animal studies where a direct effect on the fetal liver and neonatal bone marrow was found after perinatal dioxin exposure[1,6]. In Taiwan, in the Yucheng incident, changes in the monocyte maturational process were found in PCB poisoned patients[9]. Our results also agree with a previous study in Dutch infants where a negative correlation between dioxin concentrations in breast-milk and the number of granulocytes was found at one week of age[17].

There was no evidence of increased upper or lower respiratory tract symptoms or altered humoral antibody production in relation to PCB/dioxin exposure. Although there were differences in the leucocyte (sub)population between high and low PCB/dioxin exposed infants, all values were within the normal range. Moreover, subtle changes in the number of blood leucocytes do not simply mirror alterations in the cell composition of lymphoid and non-lymphoid organs, nor do they simply reflect functional defects. In children born to accidentally highly exposed women, in the Yucheng incident, an increased incidence of respiratory symptoms was found[12]. The Inuit infants whose mothers have elevated levels of PCBs and dioxins in their breast-milk, experienced more episodes of acute otitis media at 3 to 6 months of age[15]. In a prospective longitudinal study of background PCB exposure in the USA, however, there was no adverse effect on the frequency of physician visits for various illnesses[18].The magnitude of the above described changes in the immune status of background exposed infants associated with prenatal PCB/dioxin exposure, as compared with accidental high exposure, might be too subtle to induce these clinical symptoms. There are,

however, some limitations to our health questionaire. The number of periods with rhinitis, bronchitis, tonsillitis and otitis was counted during the first 18 months of life. No subdivision in shorter time periods was made. This might be the reason that we did not find a relationship between the number of respiratory infections and the leucocyte (sub)populations under study. There was, however, a significant relationship between the antibody levels and the number of CD8+ (cytotoxic) and TcR$\gamma\delta$ T-lymphocytes at 18 months of age. Therefore one might speculate that the lower numbers of monocytes and granulocytes at the age of 3 months could have resulted in more (subclinical) infections during the first months of life and in an increase in the number of CD8+ (cytotoxic) T-cells therafter.

In conclusion, background levels of PCB/dioxin exposure influences the human fetal and neonatal immune system. Although there is no evidence of clinical symptoms or direct changes in the humoral immunity response in infancy and the results of the white blood cell counts and immunological marker analyses were all within the normal range, the described changes in the T-cell lymphocyte population could persist into later child- or adulthood and could presage difficulties, like immune suppression, allergy or autoimmunity [19]. Follow-up of these children to adulthood is therefore needed. In the mean time the prevention of further environmental and food chain contamination is essential, along with their monitoring in various commodities.

5. References

1. Fine JS, TA Gasiewicz, NC Fiore, AE Silverstone (1990). Prothymocyte activity is reduced by perinatal 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure. J Pharmacol Exp Ther Oct; 255, 128-132.
2. Dencker L, E Hassoun, R d'Argy, G Alm (1985). Fetal thymus organ culture as an in vitro model for the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin and its congeners. Mol Pharmacol 27, 133-140.
3. Korte M, R Stahlman, R Thiel, T Nagao, I Chaoud, H van Loveren, JG Vos, D Neubert (1990). Effects of 2,3,7,8-terachlorodibenzo-p-dioxin on hepatic monooxygenases and resistance to trichinella spiralis infection in rat ofspring after perinatalexposure. In: Huntziger O, Fiedler H (eds) Organohalogen Compounds, Eco-Informa Press, Bayreuth, Dioxin I, pp89-94.
4. Holladay SD, P Lindstrom, BL Blaylock, CE Comment, DR Germolec, JJ Heindell, MI Luster (1991). Perinatal thymocyte antigen expression and postnatal immune development altered by gestational exposure to tetrachlorodibenzo-p-dioxin (TCDD). Teratology 44, 385-393.
5. Blaylock BL, SD Holladay, CE Comment, JJ Heindel, MI Luster (1992). Exposure to tetrachlorodibenzo-p-dioxin (TCDD) alters fetal thymocyte maturation. Toxicol Appl Pharmacol 112, 207-213.
6. Fine JS, TA Gasiewicz, AE Silverstone (1989). Lymphocyte stem cell alterations following perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Mol Pharmacol 35, 18-25.

7. Neubert R, U Jacob-Muller, H Helge, R Stahlmann, D Neubert (1991). 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 (Callithrix jacchus). Arch Toxicol 65, 213-219.
8. Shigematsu N, S Ishimaru, R Saito, RT Ikeda, K Matsuba, K Sugiyama, Y Masuda (1978).
Respiratory involvement in polychlorinated biphenyls poisoning. Environmental Research 16, 92-100.
9. Chang KJ, KH Hsieh, TP Lee, SY Tang, TC Tung (1982). Immunologic ecaluation of patients with polychorinated biphenyl poisoning: determination of phagocyte Fc and complement receptors. Environmental research 28, 329-334.
10. Chang KJ, KH Hsieh, TP Lee, SY Tang, TC Tung (1981). Immunologic evaluation of patients with polychorinated biphenyl poisoning: determination of lymphocyte subpopulations. Toxicology and applied pharmacology 61, 58-63.
11. Chang KJ, KH Hsieh, SY Tang, TC Tung, TP Lee (1982). Immunologic evaluation of patients with polychorinated biphenyl poisoning: hypersensitive response and its relation to clinical studies. J of Tox Env Health 9, 217-223.
12. Rogan WJ, BC Gladen, KL Hung, SL Koong, LY Shih, JS Taylor, YC Wu, D Yang, NB Ragan, CC Hsu (1988). Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 241, 334-336.
13. Hoffman RE, PA Stehr-Green, KB Webb, RG Evans, AP Knutsen, WF Schramm, JL Staake, BB Gibson, KK Steinberg (1986). Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. JAMA 255, 2031-2038.
14. Smoger GH, PC Kahn, GC Rodgers, S Suffin, P McConnachie (1993). In utero and postnatal exposure to 2,3,7,8-TCDD in Times beach Missouri; 1. Immunological effects; Lymphocyte phenotype frequencies. Short paper In: Dioxin '93. 13th International Symposium on Clorinated Dioxins and Related compounds. Vienna, pp 345-348.
15. Dewailly E, S Bruneau, C Laliberte, M Belles Iles, JP Weber, P Ayotte, R Roy (1993). Breast milk contamination by PCBs and PCDDs/PCDFs in Arctic Quebeq: Preliminary results on the immune status of inuit infants. Short paper In: Dioxin '93. 13th International symposium on chlorinated dioxins and related compounds. Vienna, pp 403-406.
16. Weisglas-Kuperus N, TCJ Sas, C Koopman-Esseboom, C van der Zwan, MAJ de Ridder, A Beishuizen, H Hooijkaas, PJJ Sauer (1995). Immunological Effects of Background Prenatal and Postnatal Exposure to Dioxins and Polychlorinated Biphenyls in Infants. Pediatr Res pp404-410.
17. Pluim HJ 1993. Clinical laboratory manifestations of exposure to background levels of dioxins in the perinatal period. Dioxins, pre- and postnatal exposure in the human newborn, Thesis, Amsterdam University, Amsterdam, the Netherlands,pp 93-102.
18. Rogan WJ, BC Gladen, JD McKinney, N Carreras, P Hardy, J Thullen, J Tingelstad, M Tully (1987). Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) in human milk: effects on growth, morbidity, and duration of lactation. Am J Public Health 77, 1294-1297.
19. Schuurman HJ, H Van Loveren, J Rozing, JG Vos (1992). Chemicals trophic for the thymus: risk for immunodeficiency and autoimmunity. Int J Immunopharmacol 14, 369-375.