Peripheral Blood Lymphocyte Subpopulations of Persons Highly Contaminated with Polychlorinated Dibenzodioxins/Dibenzofurans (PCDD/PCDFs)

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1. Abstract

Parallel chemical-biological investigations were performed to evaluate significant exposure-related immunotoxic effects of PCDD/Fs in humans. Body concentrations of PCDD/Fs and peripheral blood lymphocyte CD-phenotype were determined for individuals with no known exposure to PCDD/Fs (group 1) and occupational exposures (group 2). Blood level of dioxin and related compounds were 2 - 4 fold higher for the 2-nd group. There was no difference in percentage of CD3+, CD8+, CD72+, CD16+, CD25+, CD45RA+ lymphocytes between the observed groups. PCDD/Fs -contaminated persons were characterized by the increased number of CD4+, HLA-DR+, CD10+ - lymphocytes, low CD45 antigen expression and the increased (statistically insignificant) number of CD95+ - lymphocytes.

2. Introduction

In spite of the intensive investigations there is no clear pattern of immunotoxic action of polychlorinated dibenzodioxins/dibenzofurans (PCDD/Fs) in humans. There are many reasons, which make it difficult to analyze the accumulated and newly received knowledge.

The factor of importance is considerable differences in the individual toxicity and the rate of elimination of PCDD/Fs, depended on the genetic variability of Ah-receptor expression (including lymphocytes) and inductive capacity of P4501A1 in humans [1-4]. The majority of the clinically estimating immunological parameters are of a broad range of normal values. Thus the subclinical immunomodulating effect of ecotoxicants is hardly evaluated. It is of no small importance that the cohorts under research frequently include persons exposed to PCDD/Fs, however the fact of body contamination was not really documented by chemical analytical methods.

For the evaluation of the significant exposure-related effects the joint chemical-

biological investigations are of the special importance, where the parallel determination of body burden PCDD/Fs content and immunoreactivity state are performed.

In the present investigation the PCDD/Fs blood concentration and peripheral blood lymphocyte CD-phenotype were studied for 9 donors with no known occupational exposure to dioxin and related compounds (control group 1) and 8 occupational exposures (Khimprom, Ufa) - group 2. 6 of the 8 subjects reported a history of chloracne in 1968 - 1969. No specific health problems were correlated with PCDD/Fs exposure in group2.

3. Experimental Methods

3.1. Analytical methods

The PCDD/Fs content in the whole blood samples (40 ml) was determined according to [5]. The sample was spiked with 15 $^{13}C_{12}$ - 2,3,7,8 - PCDD/Fs. The sample extract clean up was perfomed by use of the standard method on the multilayer silica, carbon and alumina columns. The $^{13}C_{12}$-1234-TCDD and $^{13}C_{12}$ - 123789-HxCDD were added as the internal standards to the final extract in 5 µl of dodecane. All the criteria of US EPA Method 1613 were fulfilled. The measuring system consisted of VG Autospec-Ultima HRMS and Carlo Erba 8035 Gc (MS:SIR, 10000, El+, 36 ev, reg. 2 ions; GC:I&W Scientific DB-5, 60 m, 0.25 um, injection volume -1 ul).

3.2. Immunological methods

Mononuclears from the heparinized blood samples were obtained after «Lymphorep» (Pharmacia Fine Chemicals) gradient centrifugation. Cells were washed for two times (150g) with 0.15M phospate-buffered salt solution (pH 7.2), contained 3 mM sodium azide and 0.2% BSA (Serva) and resuspended in the same medium. Immunophenotyping was performed by indirect immunofluorescent technique using mice anti-human monoclonal antibodies of clones LT (Sorbent, Russia) and ICO (Diagnotex, Russia): LT3 (CD3), LT4(CD4), LT8(CD8), LNK 16 (CD16), 3F3 (CD72), ICO 46 (CD45), ICO 166 (CD45Ra), LT HLA-DR, ICO 124 (CD10), ICO 105 (CD25), ICO 160 (CD95). Sheep F(ab»)2-FITC labled fragments against mouse IgG were used as «second» antibodies. MBU-15 Lomo Fluorescent Microscope System was used for evaluation of CD-positive cells.

Nonparametric Mann-Whitney «U» test was used for statistic procedure.

4. Results and Discussion

The TCDD level in the first observed group accounted for (X ± SD): 20.78 ± 8.09 pg/g lipid (Figure 1). The TCDD level in the second group was of the oder of 89.70 ± 57.10 pg/g lipid (p level = 0.005). The total equivalent contamination factor (I -TEQ) for the 1-st group was 41.90 ± 18.8 pg/g lipid, for the 2-nd one - 129.72 ± 70.9 pg/g lipid (p level = 0.001). In this case the 1,2,3,4,7,8- and 1,2,3,7,8,9-HxCDD level in the 2-nd observed group was more than three times as high as in the 1-st group (p level = 0.05); the levels of PeCDD, 1,2,3,6,7,8-HxCDD, OCDD and that of TCDF, PeCDF, HxCDF in the 2-nd observed group were 20 - 40% higher in comparing with the 1-st group (the differences for each derivative are statistically insignificant).

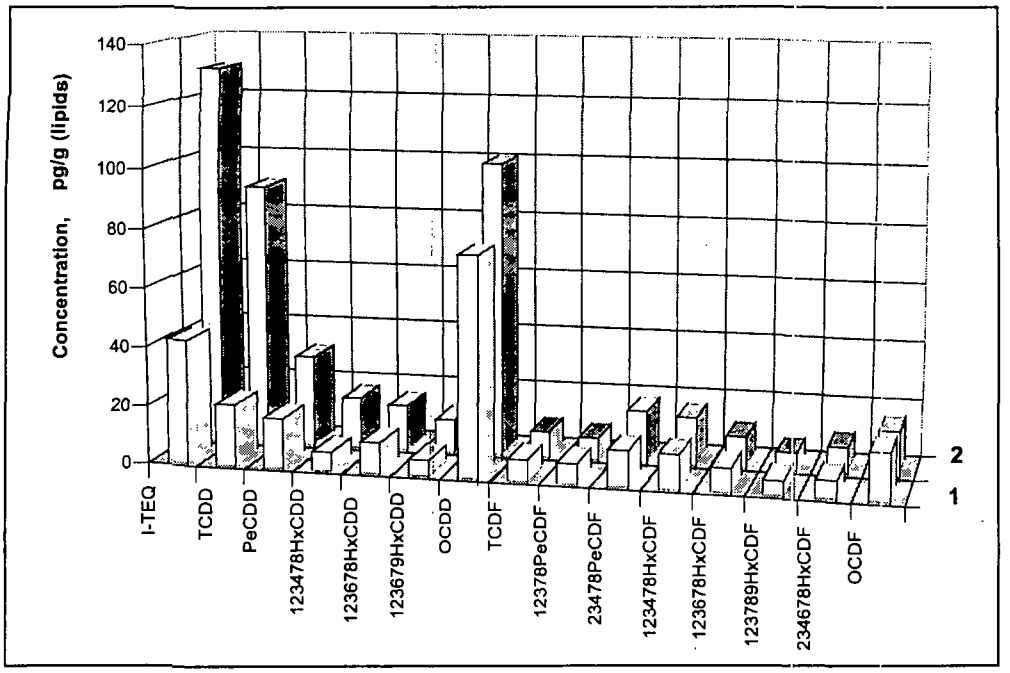

Figure 1. Levels of PCDD/Fs in blood samples (average means, pg/g lipids) of non-exposed (control group 1) donors and occupationally exposed (group 2) individuals.

There was no statistically significant difference (Table 1) in the percentage of pan T-cells (CD3+), supressor/cytotoxic lymphocytes (CD8+), B- cells (CD72+), natural killer T-cells (CD16+), and interleukin-2 receptor expression (CD25 positive lymphocytes) and CD45RA - isoform bearing lymphocytes between control (1) and PCDD/Fs-contaminated (2) cohorts. At the same time the number of helper/inducer T-cells was increased in group 2-persons. These findings differ from those described for Yucheng patients [6] in which the decreased T-cells and T-helper/inducer cells number were reported. For the occupational exposures with well documented adipose tissue level of TCDD positive the correlation between TCDD level and the percentage of total T-cells, CD8+, T11(CD2+) was found, while the percentage of CD4+ -cells did not changed[7]. However the positive correlation between PCDD/Fs and co-PCBs, in breast milk and the percentage of CD4+ cells in the babies blood was recently found [8]. The increased number of CD4+ - cells was also found in Yucheng children [9].

Considerable difference in the percentage of HLA-DR+ cells, CD10+ cells (T-, B-lymphocyte precusores) and CD45 (common leucocyte antigen) expression between the observed groups were determined. The group 2-individuals were characterized by the increased percentage of HLA-DR+ lymphocytes, CD10+ lymphocytes, while CD45 expression decreased.

Table 1

Peripheral blood lymphocyte subpopulations of control (1) and PCDD/Fs- contaminated (2) groups of persons

Cluster of differentiation	CD - positive cells % (X ± SD)		p level*
	1 (n = 9)	2 (n = 8)	
CD 3	60.2 ± 7.0	63.0 ± 6.0	n.s.
CD 4	42.6 ± 8.4	52.0 ± 9.9	0.043
CD 8	30.8 ± 11.1	30.3 ± 10.0	n.s.
CD 72	10.2 ± 4.0	12.8 ± 6.4	n.s.
CD 16	21.2 ± 7.5	20.4 ± 7.3	n.s.
CD 45	81.4 ± 5.6	73.7 ± 8.5	0.048
CD 45RA	43.7 ± 14.7	47.0 ± 7.5	n.s.
HLA-DR+	21.1 ± 4.9	28.0 ± 2.9	0.012
CD 25	14.0 ± 6.4	13.8 ± 5.4	n.s.
CD 10	2.4 ± 1.2	10.9 ± 5.5	0.003
CD 95	30.9 ± 8.6	37.0 ± 8.2	0.100

* - Mann-Whitney «U» Test

The tendency (p level = 0.100) of increasing number of cells bearing the apoptose-assotiated protein Apo/Fas-1 (CD95) was also observed in PCDD/Fs contaminated persons.

The toxic effect of TCDD on lymphocyte maturation and cell cycle, as well as the genotoxicity of organochlorine compounds for human lymphocytes are well known [10,11]. Probably, the increased number of the phenotypically immature cells with low CD45 expression in PCDD/Fs contaminated person reflects the abnormalities in lymphocyte

differentiation, especially B-cells because of the simultaneous expression of CD10 and HLA-DR on B-precusores [12]. In this case the increased number of CD4+ and CD95+ lymphocytes may be an adaptative reaction to the long-lasting toxicant exposition.

5. References

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