The Biomarkers of Immunotoxicity of the Polychlorinated Dibenzodioxins / Dibenzofurans (PCDD/Fs) of the Occupationally Exposed Humans.

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Introduction

It is well known that acute and chronic exposure with 2,3,7,8 - tetrachlordibenzo-*p*-dioxine (TCDD) and related compounds caused marked immune abnormalities in experimental animals. There are thymic atrophy, abnormal lymphocytes differentiation and proliferation, disturbed humoral and cellular immune response. However in spite of a great number of the investigations the common pattern of the immune disoders after accidental or occupational exposure with PCDD/Fs in humans is not completely understood. The factor of importance is that the cohorts under research frequently include persons exposed to PCDD/Fs, however the fact of the body contamination was not really documented by the analytical methods. In this regard only the results of the parallel chemical-biological studies are of primary importance. From 1996 the donors with no known occupational exposure to dioxin and related compounds and the occupationally exposured persons (Khimprom - factory, Ufa) suffered from chloracne in 1968 - 1969 were under investigation in our laboratoty. The programme of the investigation for each person included the evaluation of the PCDD/Fs body burden level and basic clinical laboratory methods of the cellular and humoral immunity. The previous results were reported at the "DIOXIN-97" Symposium. The final results of the investigation are presented in this paper.

Material and Methods

The control group of 20 (13 men and 7 women) persons included volunteers (approx. 50 years of age), with no known occupational exposure with the PCDD/Fs . The cohort of 20 (14 men, 6 women) persons was occupational exposures (the workers of the Khimprom-factory, Ufa) of the same age. 18 persons from this group suffered from chloracne in 1968 - 1969.

The PCDD/Fs body burden level was determined in the whole blood samples (40 ml) as was previously described (1).

Peripheral blood mononuclears (PBM) were isolated by the Ficoll-Paque (Pharmacia Fine Chemicals, Sweden) gradient centrifugation. Indirect immunofluorescence method was performed for the PBM subsets determination (1).

For the mitogenic response determination the washed PBM ($1*10^6$) were resuspended in the culture medium (HEPES-modified RPMI-1640, containing 10% heat-inactivated foetal calf serum, 50 μg/ml gentamicine. PHA (10 μg/ml), ConA (5 μg/ml), PWM (10 μg/ml) were used as mitogens (all reagents were obtained from Sigma). The antiCD3 monoclonal antibodies (aCD3mAb, ICO-90, 2.5 μg/ml) was also used. The cultures were incubated for 72-h at 37° in CO_2-incubator. In some cases 2,3,7,8-TCDD (final concentration 10 nM) was added simultaneously with PHA or aCD3. The TCDD was dissolved in DMSO (final DMSO concentration 0.4%). The results were evaluated morphologically in the Gimsa-stained smears.

Parametric and non-parametric methods were used for the statistic procedures.

Results and Discussion.

The I-TEQ and TCDD levels for both groups are illustrated in Table 1. The I-TEQ for the cohort was more than 3-fold higher and the TCDD body burden level was 5 fold higher than for the control group. The structure of the contamination by the hexa- and penta - isomeres of the PCDD/Fs was the same as it had been previously reported (1). No significant differences of the I-TEQ and TCDD levels (Median) were found between the cohort men (I-TEQ = 180 ppt, TCDD = 152 ppt) and cohort women (I-TEQ = 130 ppt, TCDD = 102 ppt). So the fact of the PCDD/Fs contamination was well documented by the analytical methods for each person of the alternative groups.

Table 1. The I-TEQ PCDD/Fs and 2, 3, 7, 8 – TCDD blood levels (ppt lipids) of the control group (1) and occupationally contaminated cohort (2).

	I-TEQ PCDD/Fs Me (min – max)	2, 3,7, 8 – TCDD Me (min - max)
1	**50 (20 - 78)**	**23 (11 - 48)**
2	**166 (83 – 654)**	**110 (54 - 500)**

As it was reported (1) the 8 occupational exposures (4 men, 4 women) were characterized by the increased number of CD4+, HLA-DR+, CD10+ - lymphocytes (statistically significant) and the higher (statistically nonsignificant) percentage of the Fas/Apo-1 antigen (CD95)- bearing lymphocytes. When the number of the observations increased the pattern of abnormalities in the cohort remains, the same, Table 2. However, no signicant differences in the number of CD4+-lymphocytes and HLA-DR+-lymphocytes were observed. The reason was in the change of the sex structure of the cohort (14 men, 6 women). After the data breakdown and one-way ANOVA procedure it was shown, that the structure of the main PBM subpopulations of the cohort men was practically the same as for the control one. Contrary, in the cohort women the percentage of the CD4+- lymphocytes, HLA-DR+-lymphocytes, CD72+-B-cells (nonsignificant) was increased number and the percentage of CD16+-NK-cells was decreased versus the control one.The differences in the percentage of the CD95+-lymphocytes between the groups were also more prominent for women, rather than for men. These data agreed with the fact of the preferentially expression of Fas/Apo-1 antigen on CD4+,CD45Ro+ memory or previously activated T-cells, small number of CD8+ T-cells and IgD-,CD20+ activated B-lymphocytes, but not NK-cells (2). Since the disturbed estrogen metabolism after TCDD exposition is well documented, the alteration of the estrogen-depended regulation of PBM subpopulation structure may be a key role in the cohort women.

The main sex-independed biomarker of the PBM subpopulation structure abnormalities in the exposures was the appearence of CD10+-cells. There was a significant correlation

between the PCDD/Fs body burden level (I-TEQ) and the percentage of CD10+-lymphocytes (Spearmans R = 0.62, P<0.001). However, the morphologically immature cells in blood smears were not found. The CD10 (J5/CALLA) expressed on the normal and activated early B, pre-B cells in bone marrow (higher on reactive marrow), lymph nodes and blood and found in some T- and B-lymphomas and in the case of immune activation (3,4). Since non of cohort persons suffered from lymphoproliferative syndrom we assumed, that the appearence of CD10+-cells reflects the alterations in B-cells maturation or differentiation under PCDD/Fs long-lasting exposure. The selective effect of TCDD on the B-cells maturation was also shown by the other investigations (5,6).

Table 2. PBM subsets of the control group and and PCDD/Fs – contaminated cohort.

Cluster of differentiation	CD-positive cells, %					
	Whole group		men		women	
CD3	60.2	+0.3	62.5	-1.5	56.7	+3.2
CD4	43.0	+4*	46.0	-0.8	38.7	+11.6**
CD8	28	-1.5	28.8	-2.8	25.3	+2.5
CD72	11.5	+1.8	12.1	+1.0	10.3	+4.2
CD16	23.1	-1.1	20.6	+3.2	26.2	-5.3**
HLA-DR	21.6	+5.0	23.3	+2.3	19.1	+10.4**
CD25	13.8	0	14.8	+0.2	13.4	-1.4
CD95	35.3	+5.0	37.8	+4.4	31.0	+7.1*
CD10	3.0	+7.8**	3.3	+8.8**	3.0	8.0**

The data are illustrated as the Mean of the control group and the differences between the Mean of the cohort are given in the cross-hatched area. * - $P < 0.1$; ** - $P < 0.05$; Student's "t" test.

The considerable differences between the groups were observed when the mitogenic response of PBM was studied, Table 3. In comparison with the non-exposures, the percentage of the blast forms in the cohort PBM cultures was markedly increased. This was shown both for the control (non-stimulated) cultures (spontaneous proliferation) and for the cultures stimulated with the broad spectrum of mitogens - PHA (T-cells), ConA (subset of T-cells), PWM (B-cells, T-dependent) and aCD3 mAb (T-cells stimulation via TCR-assotiated glycoprotein). Because of the high background proliferation the stimulation index (blast forms in the mitogen-stimulated cultures/blast forms in the unstimulated cultures) was decreased in the cohort PBM culures. The presence of 2,3,7,8- TCDD (10 nM) caused significant depression of the mitogenic response in PHA- and aCD3-stimulated control group cultures, while only slight depression of the mitogenic activity of the cohort PBM was observed. So, the PBM of the exposures were more resistant to the toxicant addition in the *in vitro* conditions. As it was shown in Table 2, there was no considerable difference in the percentage of the lymphocytes, bearing IL-2 receptor (IL-2R, CD25) in the peripheral blood of both groups. The same was shown for the unstimulated 72-h PBM cultures. The exposition of the unstimulated PBM with the 2,3,7,8-TCDD (72 h, 10 nM) caused the elevation (nonsignificant) of the percentage of CD25+-cells both in the cohort and control PBM cultures. At the same time, contrary to the control group PBM, the 2,3,7,8-TCDD-exposed and non-exposed cohort PBM, were characterized by the disproportional increased PHA-induced IL-2R expression. However, the aCD3-induced IL-2R expression was practically similar in both groups. Thus, high mitogenic responsiveness, abnormal PHA-

induced (but not aCD3-induced) IL-2R expression, as well as specific TCDD-induced response of the cohort PBM was observed. No sex difference in the mitogenic response and CD25+ expression were observed. These data are confirmed by others, showing abnormal cell-cycle regulation, growth factors production and IL-2 gene transcription (direct transcriptional activation) as the biological responses to the 2,3,7,8-TCDD (7,8,9).

Table 3. Mitogenic response and CD25 expression in 72-h cultures of the peripheral blood mononuclears (PBM) of the control group and PCDD/F_S – contaminated cohort.

Parameters / Culture conditions:					Blast forms, %		Index of stimulation		CD25 Positive cells, %	
TCDD	PHA	ConA	PWM	aCD3 mAb						
-	-	-	-	-	2.5	+5.0*			13	+3
+	-	-	-	-	2.8	+4.7*			21	0
-	+	-	-	-	26	+6	7.5	-2.7*	20	+24*
-	-	+	-	-	12	+2	6.1	-2.1*		
-	-	-	+	-	12	+4	3.0	-0.7*		
-	-	-	-	+	15	+10	10	-5*	42	0
+	+	-	-	-	17	+11	6.4	-2.4	16	+24*
+	-	-	-	+	10	+12	5.0	-1.0	40	-6

The data are illustrated as the Median of the control group and the differences between the Median of the cohort are given in the cross-hatched area. *- P<0.05 Mann-Whitney "U" test.

It is tempting to speculate that TCDD interfer with the different pathways of the signalling cascade of the lymphocyte activation and the biological response of this system is different in the population and PCDD/Fs-contaminated humans.

Thus, the results of our study demonstrate that the long-lasting PCDD/Fs contamination impaired fine mechanisms of lymphocytes differentiation and cell cycle regulation. The mechanisms of these alterations are polymodal (and may be sex-depended) and some of them may be regarded as the specific adaptation to the xenotoxicant.

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