LATE EFFECTS ON CELL - MEDIATED IMMUNITY IN DIOXIN-EXPOSED VETERANS

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Many studies have been dealing with dioxin and related compounds effects experimentally or in acute victims of them (3,4,5,7,11,12,13,14,21,22) especially their effects on immune system (12,13,14,21,22). It was very rare to find out studies about dioxin effect on immune system in man. Vũ Triêu An et al (23) found that in chronic dioxin-intoxicated veterans at Viet Yên, Hà Bắc, the Mantoux test with PPD, the number of ERFC and Ea decreased while the non-specific humoral responses remained unchanged. After K.Lundberg et al.(12) dioxin did not provoke any alteration on B-cell development of chicken embryos and on specific immune responses in mice. These data suggested us to deal only with the disorders in cell-mediated immunity (specific and non-specific) in veterans who had served in chronic dioxin-sprayed areas (B3 zone, determined by South Vietnam dioxin- map of Committee 10.80) as compared to the control group. We are very sorry that we had no means to measure the dioxin level in fat tissue of these investigated veterans at the time of evaluation of immunological parameters.

MATERIALS AND METHODS

- Experimental group: 30 veterans who had served 5-10 years in B3 zone, aged between 50 and 70 years.
- Control group: 20 veterans who had served in A zone, i.e. never served in South Vietnam in and after the war, age, sex, living- standard-environment being similar to the experimental group. These parameters have been used:

- Total leukocyte count
- Count of different leukocytes lineage especially the number of lymphocytes, monocytes and natural killer cells.
- Number of T pan, T helper, T suppressor and ratio Th/Ts.
- The TNF-bêta secretory capacity of Th $CD4^+$ cells and IFNgamma-of Th, Ts, NK, macrophages in cell culture with PHA.P stimulation (10 microgram/ml). Positive secretory cells were evaluated at 24h, 48h after culture of 5.10^5 mononuclear cells in 0,5 ml of RPMI 1640 medium with 20% FCS-Difco.
- Measurement of protein concentration (mg/ml) in culture supernatant.

- Detection of autoantibodies antinuclear antigen by Hep-2-cell line with sera dilution 1:80 and titration of positive sera.

These parameters were performed by indirect immunofuorescence test (the monoclonal antibodies against T and T subpopulations, TNF-bêta, IFNgamma given by Boehringer Mannheim Co., FRG, the Hep-2 cell line was a gift from Blood Transfusion Center of Amsterdam to Vietnam National Institute of Hygiene and Epidemiology). Results are treated by statistical analysis and evaluated by t test.

RESULTS

TABLE 1

Leukocyte count and count of different leukocytic subpopulations in microliter of blood of the investigated groups.

Parameters / Group	Total Leuc.	Neutro	Eo	Mono	Lympho	NK
Control (n:20)	6226,66 ± 1060,48	3966 ± 1005	263 ± 220	215 ± 114	1582,8 ± 505,38	85,9 ± 77,89
Experimental (n:30)	6240 ± 1323	3797 ± 992	251 ± 169	137 ± 81	1876 ± 504,63	126,68 ± 87,15
p	>0,4	>0,25	>0,4	:0,01	<0,025	:0,05

Remarks: *In chronic intoxicated veterans the myeloleukocytes are decreased, especially significantly with monocytes, while the total lymphocytes and natural killer cells are significantly increased.*

TABLE 2

Number of Tpan, Thelper and Tsuppressor/microliter of blood in investigated groups.

Param / Group	Tpan	Thelper	Tsuppressos	Th/Ts
Control (n:19)	890 ± 335	409 ± 215	316 ± 146	1,3
Exp. (n:30)	347 ± 331	349 ± 290	309 ± 139	1,1
p	:0,05	:0,25	>0,4	

Remarks: *The T pan number in experimental group decreased significantly while the Th one being low. The Ts number remained unchanged so the ratio Th/Ts decreased.*

TABLE 3

The TNF-bêta secretory capacity of ThCD4+ cells when stimulated by PHA.P.

Param. / Group	Number of secretory cells in culture/mcl of blood		% of non-secretory cells in culture	
Control `	At 24h	At 48h	At 24h	At 48h
	454 ± 289 (n:14)	70 ± 90 (n:9)	22	55
Experimental	263 ± 269 (n:23)	108 ± 131 (n:14)	30	48
p	< 0,025	: 0,25		

Remarks: *Normally we can detect about 40-50o/o TNF-bêta secretory cells at 24h culture with many molecules on cell surface. Almost no secretory cells at 48h. These cells decreased significantly in the experimental group with p<0,025.*

TABLE 4

The IFNgamma secretion of T lymphocytes, macrophages and NK cells when stimulated by PHA.P.

Param. / Group	Number of secretory cells in culture/mcl of blood		% of non-secretory cells in culture	
Control `	At 24h	At 48h	At 24h	At 48h
	577 ± 319 (n:15)	99 ± 73 (n:10)	6	50
Experimental	700 ± 430 (n:24)	217 ± 247 (n:18)	11	40
p	: 0,25	> 0,05		

Remarks: *The secretory cells in the experimental group is higher than the one of control group (p:0,25) and lasted longer (p>0,05)*

TABLE 5

Protein concentration (mg/ml) in culture supernatant of mononuclear cells.

Param. Group	Protein concentration in mg/ml
Control (n:18)	1,869 ± 0,71
EXP (n:28)	1,34 ± 0,62
p	< 0,005

So the secretion of cytokines including the non-measured cytokines decreased significantly ($p<0,005$).

TABLE 6

Antinuclear antibodies in sera of investigated groups.

Param. / Group	n	Age	% of positive sera at dilution 1: 80	ANA titer
Control	16	45-70	0	(-)
EXP.	24	45-70	12,5	1:1280

DISCUSSION

Orange agent, a mixture of two herbicides (2,4-D and 2,4,5 -T) which Americans sprayed in an area of 10% of South Vietnam surface from 1962-1971. It contained a very toxic substance 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). There were about 170 kg TCDD sprayed in many determined South Vietnam areas (10). The studies of the professors in Committee 10.80 et coll with American, Japanese, German experts in this fields have proved that higher dioxin lever in South Vietnam human bodies is due to orange agent exposure (10). Lê Cao Đài et al. (10) found that in the same group of exposed men there are different dioxin levels in their organisms. Many of them with very low, some with high, the highest being 103 ppt. Its effects in different individuals are also undoubtedly different.

Results of experimental studies have revealed that TCDD and its deviates are very toxic (5). low doses of dioxin are sufficient to provoke an wasting syndrome, many malignant tumours may appear in different tissues (3,7,9,11(. Pregnant women who had dioxin exposed may have congenital malformations in children (8). Alterations of skin, mucous, hematopoietic organs and deficiency of immune system have been demonstrated (4,5,13,21,22). The IL -1 production of macrophages decreased, the IL-1 responsiveness of thymocytes and of T lymphocytes are severely depressed especially when the dioxin exposure is repeated 5 times (A. Vecchi, M. Sironi et al.) (22).TCDD provokes thymic atrophy in 80% of mice with gestations at 14th day or

in young mice. (A.E.Silverstone, N.C.Fiore et al.)(21), inhibition of thymocyte differentiation (M.I.Luster et al.)(13) but enhances the Langerhans cells number in the skin of nude mice, increasing contact hypersensitivity (S.Madli)(14).E.Funseth et al. (4) have demonstrated that the NK cell activity in peripheral blood and in spleen is augmented in dioxin intoxicated mice while their T cell activity in spleen has decreased, their mitogen responsiveness is disturbed significantly, their number of Tc has decreased and become hypersensitive to infections. The authors have found that the dioxin victims have got a high NK cell activity 17 years after the exposure. What are the disorders in cell-immunity in our investigated veterans who served in South Vietnam?

First we can see clearly in the experimental group that the originated bone-marrow leukocytes is decreased, especially with monocytes (p: 0,01) although the intoxication has passed beyond 20-25 years. The total count of lymphocytes and NK cells are augmented significantly, compensated enough for the decreased myeloleukocytes, so the total leukocyte count remained unchanged. Analysis in detail the changes in lymphocyte lineage in experimental group we can see (table 2) the significant low T pan number (747 ± 331/microl. of blood in comparison with control group 890 ± 335/microl.of blood , p: 0,05). The T helper is decreased slightly (349 ± 290/mcl of blood in comparision with 409 ± 215/mcl of blood). The Ts is almost unchanged, so the Th/Ts ratio is decreased. It is clear that the augmentation of total lymphocyte count of dioxin-exposed veterans is due to the significant B lymphocyte and NK cell augmentation. This remark concorded with the conclusions of the above-mentioned authors that B cell function and number remained intact (4,12,13). After Cinader et al. (1,2) there is a negative correlation between age and T pan cell number. So we can see that there is an acceleration of ageing phenomenon in the T pan lymphocyte system as compared with the age-matched controls (747 ± 341/mcl of blood versus 890 ± 335/micl. of blood). The same acceleration of ageing phenomenon is also found with ThCD4$^+$ cells: their number and their TNF-bêta are more decreased (table 3). TNF-bêta is a lymphokine with very short half life, normally is undetectable in biologic fluid. The induction of other cytokine secretion is initiated by TNF-bêta such as the secretion of acute proteins, IL-1, INFgamma, TIN-alfa, GM-CSF, IL-6, promotion of endotoxin shock and of adhesive molecules in endothelial cells. Because of deficiency of TNF-bêta in chronic dioxin intoxicated veterans the growth and activation of many cell types are decreased in comparison with data from controls. In them, the cell reaction was slower and lasted longer when stimulated by mitogen or may be because of mitoses of secretory cells making the TNF-bêta positive cells augmented after 48h of culture. The number of INF-gamma secretory cells is augmented in experimental group, especially after 48 culture. It may be due to a limited compensative reaction ($p>0,05$) when the TNF-bêta is decreased, making a good non-specific resistance against pathogenic agents. But in table 5 we can see that the total soluble cytokines of cultured cell is diminished significantly ($p<0,005$). The diminution of membrane-associated is also detected (table 3 and 4). The number of monocytes is also significantly decreased (table 1) indirectly concorded with the results obtained by Vecchi et al. (22). NK cell number is augmented significantly in the experimental group. The high level of INF-gamma, a biomofulator in elimination of cancer cells, may be the cause of NK cell and macrophage activation (15).

Studies about late effects in immune system to individuals who had been exposed atomic-bomb radiation effect in Hiroshima and Nagasaki (Japan) the authors of RERP (Radiation effect

research foundation) demonstrated that in these victims 40-45 years ago, their non-specific immune reaction are increased, while are decreased the specific ones and the autoantibodies appear in higher frequency (RERF 1992) (20) .J.J.Oppenheim (17) emphasized that when IFN gamma appears the most important thing is a high Ia/DR expression in monocytes responding to environmental stimulation. After J.J. Oppenheim because of decrease in Th $CD4^+$ cell number, IL-2 secretion decrease, the APV (arginine vasopressing) and oxytoxin may replace the IL-2 for inducing the IFN-gamma secretion, cGMP activation for enhancing the IFN-gamma production. The leukotriens from macrophages also induce IFN gamma secretion. But with very high IFN gamma one can see undesirable autoimmune effects such as glomerulonephritis, inflammatory reactions with strong tissue-damage effects (17). Here, the frequency of positive antinuclear antibodies is higher than in control group with very high titer (1:1280). This accords with the above-mentioned data that B cell number is elevated and so the B/T lymphocyte ratio is higher. The high percentage of positive ANA in chronic intoxicated veterans also expressed an acceleration of ageing phenomenon. We must emphasize that there are 9 cases from 30 veterans with dioxin-exposed are severely immune depressed and just 3 from 9 cases are detected positive ANA.

CONCLUSION

Base on results obtained we can draw the following remarks:

1. There is an acceleration of ageing phenomenon in specific cell-mediated immune components and reactions in veterans who had been living in repeatedly dioxin-sprayed areas in comparison with controls. The antinuclear antibodies are revealed more frequently with very high titers.

2. The number of some non-specific immune factors with a large spectrum of activity (like NK cell and IFN-gamma) are higher than in controls.

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