

STUDIES ON DISORDERS OF CELL - MEDIATED AND HUMORAL IMMUNITIES IN LONG - TERM DIOXIN EXPOSED VETERANS IN VIETNAM.

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Introduction:

Studies of different authors from USA, Japan, Germany, Vietnam have demonstrated that levels of TCDD and TEQ in dioxin contaminated southern people blood and fat tissue are significantly higher than those in non contaminated with dioxin people living in Hanoi although more than two decades have elapsed since the end of the spraying operations. (TCDD 2,2 ppt, TEQ 8,8ppt in Hanoi people, TCDD 17ppt, TEQ 18, 5ppt in Hochiminh city people, TCDD 17,5ppt and TEQ 31.7ppt in Song Be Province people- Schecter A.et al-1992 , Le Cao Dai et al. 1993, Le Bich Thuy et al. 1993). The dioxin levels in breast milk of southern mothers is also significantly higher than that of northern ones, 3-8 folds high compared with that of English, American, Japanese, Canadian, German mothers(Schecter A. 1992, Le Cao Dai et al.1993). The levels of TCDD and TEQ in blood and fat tissue of veterans from Tay Nguyen front during the war, living now in Hanoi have proved significantly higher than those of veterans from Northern fronts(6,1ppt TCDD and 40,3ppt TEQ versus 1,2 ppt TCDD and 12ppt TEQ- Schecter A.1994).

Diseases currently recognized as related to herbicide exposure can be originated from high dioxin levels in human bodies and also from veterans with non detectable dioxin levels in their bodies. Dioxins can promote their pathogenic effects when entering in organisms and then eliminated almost totally. Diseases can appear after exposure from 1 to 30 years. (Published by Institute of Medicine- American National Academy Press in August 1993 and by American National Academy Scientific Council in July 1995), However, till to now, researchers of all the world do not recognize yet reproduction function pathology(infertility, spontaneous abortion, stillbirths, congenital malformations, premature birth, molar pregnancies...), immunological disturbances and disorders of neurologic, psychologic, metabolism, circulatory and respiratory systems as diseases related to herbicide exposure. In our studies we aimed to continue our investigations about immunologic disorders in dioxin exposed veterans, consisting of their cell- mediated immunity as well as their specific humoral response against the recombinant hepatitis B virus vaccine Engerix.

Materials and methods:

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Two groups of exposed veterans with their control matches. Group I: 6 veterans, 48-60 years old were immunized with Engerix B vaccine (recombinant DNA hepatitis B vaccine) of Smithkline Beecham Company. Data of Vietnamese adult controls are taken from Nguyen Thu Van et al (1995).

Immunization schedule: 0,1,2,12 months. These parameters are determined: before immunization: HBsAg and anti-HBsAg in sera. After immunization the anti-HBsAg is determined dynamically, after the first immunization 2 months and a half, 4 months, 6 months and 14 days after boosting at 12th month. The HBsAg is determined by ELISA technique (with kits of Boehringer Mannheim Co.), the anti-HBsAg by PHA (passive hemagglutination assay) with kits of Korea Green Cross Co.

Group II: 27 veterans, 43-72 years old. These parameters are determined: total lymphocyte count; T lymphocyte and subpopulation count (by IIF with monoclonal antibodies from Boehringer Mannheim Co.), their TNF-beta secretory capacity when stimulating with PHA (10 µg/ml) of 48h cultured cells in RPMI 1640, 20% FCS-Difco.

Total monocyte count and their TNF-alfa secretory capacity of 48h cultured adherent cells. Anti-TNF-alfa and -beta are from Boehringer Mannheim Co. All cell types are counted in 1 µl of peripheral blood. Results are treated by statistical analysis and evaluated by t test.

Results:

1. Anti-HBsAg response before and after immunization with Engerix B vaccine:

1.1. Before immunization: all are negative (6/6 cases) in HBsAg, as the anti-HBsAg titer was negative in 2/6 cases, very low in 2/6 cases and normal in 2/6 cases (with titer of 1250 and 2500 mIU/ml).

1.2. After immunization: After the first immunization 2 months and a half the response is normal in 4/6 (1250-2500 mIU/ml), very weak in 1/6 (50 mIU/ml), negative in 1/6. After the first immunization 4 and 6 months the results are similar to that of 2 months and a half. At 12th month, the HBsAg titer decreased in 2 and increased in 2 of 4 positive cases 14 days after boosting at the 12th month no response in 2/6 veterans 4/6 are with 2-8 folds high response in comparison with that at 6th month (2500 - 10.000 mIU/ml, considered as normal).

2. The lymphocyte count: 2360 ± 2502 , significantly augmented in comparison with that of control group (1582 ± 505 , $p < 0.05$). Almost no difference between studies and control values of TCD6⁺ and TCD4⁺ cells ($p > 0.05$), but TCD8⁺ cells significantly augmented, comparing with control values (991 ± 1462 versus 316 ± 146 , $p < 0.05$). The T4/T8 ratio is 0,89 versus 1,29 of control group. The TNF-beta secretory cells 48h cultures: 493 ± 510 , significantly augmented comparing with control values ($p < 0.05$).

3. The monocyte count: 288 ± 264 , no difference between control values ($p > 0.05$). The TNF-alfa adherent secretory cells of 48h cultures: 107 ± 111 almost similar to normal values (112, n:5).

Discussion:

1. In Vietnam adults immunized after schedule 0,1,6, Nguyen Thu Van (1995) noted a positive response in 87-95% of vaccinated persons at 6th month. The percentage of responders published by Institute of Pasteur, Paris performing in Dakar with Hevac B (produced from plasma) or Genhevac B (recombinant DNA hepatitis B) is 100%. With American recombinant DNA HB vaccine performed in Singapore, 100% are

responders. 100% of children in France give response after the 3rd immunization with Genhevac(after Nguyen Thu Van et al.1995).So, we are sure that the percentage of Engerix B vaccine responders of our investigated veterans diminished significantly(66,5% versus 100%), even after repeated immunization at 12 th month the frequency of positive response is unchangeable. The antibodies titer of responders is similar to that of normal healthy persons. Our results are different to those published by Vu Trieu An et al(1993), Lundberg K.et al(1991) who demonstrated that dioxin is not toxic for the number and function of B lymphocyte. but are concordant well with those obtained by Luster M.I.et al(1991) and especially in dioxin 94, Harper N.et al. have reported that the specific humoral response of B6C3F1 mice against TNP- LPS is decreased significantly(detected by ELISA technics and plaque forming cells) if the mice are exposed with 2,3,7.8 - TCDD, particularly with 3,3',4,4',5-pentaCB (1994).

2.The cell- mediated immunity: The lymphocyte, T lymphocyte and TCD4+ cell count are almost normal in this group of veterans. The TCD8+ cells augmented significantly($p < 0.05$), the T4/T8 ratio decreased clearly(0,89 versus 1,29), but remained in the low limit of normal values. In our study performed with a group of veterans in 1994, this ratio decreased slightly(1,1 versus 1,3) because of the TCD4+ cells decrease in number and unchanged TCD8+ cells(Phan Thi Phi Phi et al.1994). The low ratios of T4/T8 noted in our investigations in comparison with respectively control groups concorded with the published results of Smoggers et al.(1993) with slightly decrease in T4 and significantly increase in T8 cells when detecting in children of 9-14 years old from dioxin intoxicated bearing-child mothers. The TNF-beta secretory cells of 48h cultures increases significantly comparing with the results obtained from control group($p < 0.05$). Our previous study in 1994 veterans also demonstrated that the TNF- beta secretory capacity decrease significantly in 24h cultures ($p < 0.025$), but increases in 48h($p: 0.25$). In combination with the results from the veterans group in 1995-1996, it is clear that the response of cultured cells with PHA and the TNF-beta secretory capacity is slower , thus the response time does not fit in with the antigenic stimulation and so retard the growth and activation of different TNF-beta-dependent cell types.Only 7/27 veterans have the monocyte count sharply decreased, significantly different to the monocyte mean value in the whole investigated group, concordingly with results of our previous study and with vechi A.et.al. Who had reported that the IL-1 production of macrophage is decreased(1987).

Conclusion:

1. The frequency of primary and secondary specific humoral response to Engerix B vaccine in dioxin exposed veterans of our study is 66.5% (4/6 cases) and their response titer is concordant with the normal value published in Vietnam.

2. The T4/T8 ratio is low. because of the increase of T8+ cell number.

3. The TNF-beta secretory cells of 48h cultures in dioxin exposed veterans augment significantly in comparison with that in normal group.

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