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Sub-Chronic Effect of DDT on Humoral Immune Response to a Thymus-Independent Antigen (Bacterial Lipopolysaccharide) in Mice

B. D. Banerjee

Immunotoxicology and Eco-Biology Laboratory, Department of Biochemistry, National Institute of Communicable Diseases, 22-Sham Nath Marg, Delhi 110 054, India

The widespread use of 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) over number of years, coupled with its extreme stability and slow metabolism, has led to environmental contamination and the ultimate carry-over from the food chain into humans (Ramachandran et al. 1984). This has necessitated accurate identification of the possible health hazards due to continued use of DDT.

Human health effects of these pesticide residues are yet to be satisfactorily answered. It is becoming increasingly clear that important changes in host immunity may occur after DDT exposure (Vos 1977; Faith et al. 1980; Banerjee 1984). Furthermore, recent studies in our laboratory have suggested that suppression of primary and secondary humoral immune responses to sheep red blood cells (SRBC), a thymus dependent (T-dependent) antigen, in mice may occur after sub-chronic DDT exposure (Banerjee et al. 1986). While the decrease in humoral immune response to SRBC indicated a functional impairment in the ability to produce antibodies following antigenic challenge in DDT exposed mice, it did not provide information as to which cells (i.e T cells and/or B cells) might be impaired. Since the antibody response to a T-dependent antigen is effected, it would be more desirable to investigate the response to a thymus independent (T-independent) antigen. This will ascertain whether the altered immune response was due to the impairment of T-cells or the B-cells ability to produce antibody and insight can be obtained into the mode of immunosuppression of DDT that interfere with humoral immune response.

In continuation of our previous work, the present study was undertaken to investigate the effect of sub-chronic doses of DDT on humoral immune response to Escherichia coli lipopolysaccharide (LPS), a thymus independent antigen, in mice.

MATERIALS AND METHODS

The source and purity of the p,p'DDT used in the present experiment were the same as those presented in an earlier paper (Banerjee

Send reprint requests to B D Banerjee at the above address.

et al. 1986). Escherichia coli lipopolysaccharide (0127 : B8), goat anti-mouse IgM-horseradish peroxidase conjugate, o-phenylenediamine and Tween-20 were purchased from Sigma Chemical Co., St. Louis, MO. Tissue culture medium RPMI-1640 was obtained from Centron Research Laboratories, Bombay. The polystyrene microtitre plates (Titertek) was received from Flow Laboratories, Irvine.

National Institute of Communicable Diseases colony bred male albino mice (Hissar strain), weighing 18-20 grams were maintained under similar conditions as reported earlier (Banerjee et al. 1986; Banerjee and Hussain 1986) and provided with laboratory diet containing 0 (control), 20, 50 or 100 ppm of p,p'DDT and water ad libitum for 3 to 12 weeks. Each treatment group consisted of 12-15 mice. The preparation of the diets has been described previously (Banerjee et al. 1986).

Mice were immunized intraperitoneally with 100 µg of LPS, dissolved in phosphate buffered saline (PBS), 0.15 M, pH 7.2, five days before terminating the exposure. Blood samples were collected after termination of exposure from chloroform anesthetized mice by cardiac puncture. The serum was separated from individual samples and kept at -20^{o}C until analyzed. The spleen was removed immediately, teased finally for spleen cells under aseptic condition for the estimation of LPS-specific antibody forming cells.

The splenic plaque-forming cells (PFC) assay for LPS was done largely according to the method of Jerne et al. (1969) using SRBC coated with LPS. An aliquot containing 0.1 mL packed SRBC was incubated with 0.5 mL LPS (1 mg/mL dissolved in PBS) at 37^{o}C for 60 min. and cells were than washed five times with the medium RPMI-1640. The LPS-coated SRBC were resuspended at a dilution of 1:15 for the plaque assay. Each spleen cell preparation was assayed on SRBC and LPS-coated SRBC and the difference being the LPS-specific PFC. Results are expressed as PFC per million spleen cells.

The serum IgM-antibody titre to LPS was measured by enzyme-linked immunosorbent assay (ELISA) according to Vos et al. (1979). The antibody titres are expressed as $\log_2$ of the reciprocal of the highest dilution giving a positive reaction.

All the experiments were carried out in duplicate. The average was used in the determination of mean $\pm$ standard deviation (SD). Results of DDT exposed group were compared with respective control group. Significance of the differences was assessed by student t-test. A "p" value of 0.05 or less was considered to be significantly different from control. The data from each group were also analyzed by one-way analysis of variance (ANOVA) according to Goldstein (1964). F ratio was calculated by two-sample comparison of variance arising within-samples (error) and between-samples. F values were considered significant when exceeds the critical values of the variance ratio for p = 0.05.

RESULTS AND DISCUSSION

The persistence and extreme stability of DDT in the environment is the ultimate source of contamination at dietary level. Significant amount of DDT residues have been reported in food grains (2.10 to 35.00 ppm), animal products (1.50 to 34.05 ppm), butter (1.24 to 26.43 ppm) and oil (22.14 to 25.72 ppm) from India (Krishnamurti 1984). Further, relatively high level of DDT and its metabolites have been reported in body fat (0.32 to 380 ppm), blood (0.02 to 4.61 ppm) and milk (0.04 to 2.35 ppm) of Indian population (Ramachandran et al. 1984; Krishnamurti 1984). Food is thus considered to be the main source of DDT residues in the human body, accounting for 80 to 90 per cent of their total dietary intake (Ramachandran et al. 1984; Krishnamurti 1984). The assessment of the hazards to human arising from very small quantities of DDT residues in food and the environment is an indispensable pre-requisite of the safety evaluation of this insecticide. Hence, for the inclusion of immunological parameters in safety evaluation of chemicals it has been considered desirable to investigate immunocompetence in experimental animals following low level dietary exposure to DDT (Vos 1977; Faith et al. 1980; Banerjee et al. 1986). Keeping in view the DDT residue levels in food commodities, it was considered appropriate to incorporate DDT 20-100 ppm in the diet of experimental animals for the purpose of sub-chronic toxic study. Testing of sub-toxic effects upon immune responses is important in relation to health aspects of pesticides particularly due to wide-spread use of DDT and its persistence in the environment (Vos 1977; Faith et al. 1980; Ramachandran et al. 1984; Banerjee et al. 1986).

Exposure of mice to DDT in the diet at the levels of 20-100 ppm for 3-12 weeks produced no overt toxicity signs and symptoms. No significant differences were noted in mortality rate, body growth rates and food intake between controls and treated mice (data not shown) and falls in line with those reported earlier (Banerjee et al. 1986). The absence of any effect of DDT on body weight indicates that this compound itself did not produce any stress responsible for the observed immunosuppressive effect in the present study.

The effect of DDT on serum antibody titre and splenic plaque-forming cells in response to a T-independent antigen, LPS, are summarized in Table 1. Mice exposed to 100 ppm DDT for 6 to 12 weeks showed significant alteration in IgM-antibody titre to LPS. Similar depression in primary IgM-antibody titre to SRBC in mice exposed to 100 ppm DDT for 12 weeks has been reported earlier (Banerjee et al. 1986). Mice exposed to DDT showed a time and dose dependent decrease in LPS-specific primary PFC response. The reduction in the primary IgM-PFC was not well correlated with decrease in serum IgM-antibody response to LPS in DDT exposed mice. Suppressed PFC response was noticed after 3 weeks of exposure in a dose-time dependent pattern whereas decreased anti-body titre was observed after six weeks only in 100 ppm DDT exposed mice. The present study also demonstrates that DDT treatment resulted in a suppression of primary IgM-PFC in response to a T-independent antigen in mice at similar dose levels reported

Table 1. Effect of DDT on humoral immune response to LPS in mice*

Duration of exposure (Weeks)	Level of exposure (ppm)	Antibody titre $(-\log)_2$	PFC/10^6 cells
3	0	6.20±1.50	128±26
	20	6.20±1.20	120±30
	50	5.70±1.58	110±25
	100	5.73±1.46	80±18[a]
6	0	6.00±1.50	130±25
	20	6.40±1.07	128±34
	50	6.80±1.37	78±22[a]
	100	4.00±1.50[a]	72±20[b]
8	0	6.00±1.00	125±25
	20	5.80±1.40	105±30
	50	4.80±1.40	77±20[a]
	100	4.20±1.20[a]	74±20[b]
12	0	6.60±1.30	122±20
	20	6.20±1.80	130±30
	50	5.25±1.50	70±28[a]
	100	4.10±1.20[b]	58±24[b]

* Data presented as the mean ± SD of 10-12 mice in each group.
a Significantly lower than respective control by statistical comparisons; $p < 0.05$ (Student's t-test); $p < 0.05$ (ANOVA).
b $p < 0.01$ (Student's t-test); $p < 0.05$ (ANOVA).

earlier for a T-dependent antigen (Banerjee et al. 1986).

In the present study, mice exposed to the DDT at exposure concentration which produced no mortality or other obvious clinical signs of general toxicity, had a significant suppression in the IgM response to T-independent antigen suggesting that DDT may have had a direct effect on B-lymphocytes. The suppression of antibody-mediated immunity in DDT exposed mice could occur at any of the several stages in B-lymphocyte development. It could be due to decreased lymphocyte population and germinal centers of spleen (Street and Sharma, 1975), cytotoxicity (Gabliks and Friedman 1969) and/or interaction of DDT with steroid metabolism (Kupfer 1969).

Thus the present study reveals suppression of the humoral immune response to a T-independent antigen in mice exposed to sub-toxic doses of DDT. This suppression was found to increase in dose-time dependent pattern. The effects of DDT on humoral immune response to a T-independent antigen are more time dependent than on dose, suggest a threshold susceptibility to exposure. Adverse effect of DDT on primary humoral immune function could place the host in a more vulnerable position towards various pathogens. It is apparent that a more complete understanding of the immunotoxicity of DDT is necessary to study human health hazards and

establish guidelines for acceptable residues in the environment. Further investigations are in progress on primary and secondary immune cytokinetics, lymphocyte-mediated cytotoxicity, lymphoid cell distribution and reticuloendothelial system to elucidate the phenomenon in order to understand its mechanism of immunosuppression and the possible health hazards due to continued use of DDT.

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