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Effects of Sub-Chronic DDT Exposure on Humoral and Cell-Mediated Immune Responses in Albino Rats

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The widespread use 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) in public health programs and in agriculture has led to environmental contamination and the ultimate carry-over of these persistent residues from the food chain into humans (Ramachandran et al. 1984). Human health affects of these pesticide residues are yet to be satisfactorily answered. It has become clear that important changes in host immunity may occur after acute or chronic exposure to DDT (Vos 1977; Faith et al. 1981; Banerjee 1984). However, the consequence of prolonged exposure to low dosages of DDT in the form of residues in food on the integrity of the immune system remains largely unanswered.

Our previous studies have shown that sub-chronic administration of DDT produces marked suppression of humoral immune response in mice (Banerjee et al. 1986). At present, very little is known about the sub-chronic effect of DDT on cell-mediated immune response. More extensive and systematic studies on dose-time relationship in different experimental animal models appear to be essential before evaluating the potentiality of DDT on immune system of mammalian host. For the inclusion of immunological parameters in safety evaluation of chemicals it has been considered desirable to investigate immuno-competence in experimental animals following low level dietary exposure to DDT. Keeping this in view the present study was designed to evaluate the effect of DDT, administered in sub-chronic doses over a long period, on humoral and cell-mediated immune responses in albino rats.

MATERIALS AND METHODS

The source and purity of the p,p'DDT used in the present experiments were the same as those presented in an earlier paper (Banerjee et al. 1986). Tetanus Toxoid (Tetanus Vaccine, Bio Vaccine Pvt. Ltd., Hyderabad), Freund's complete adjuvant (Difco Laboratories, Detroit, MI) and tissue culture medium RPMI-1640 (Centron Research Laboratories, Bombay) were used.

Wistar male albino rats weighing 85-90 g were obtained from the

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National Institute of Communicable Diseases animal house colony and randomly divided into four groups. The animals were maintained under similar conditions as reported earlier (Banerjee and Hussain 1986) and provided with standard laboratory diet containing o (control), 20, 50 or 100 ppm of p,p'DDT and water ad libitum for 8-22 weeks. The preparation of the diets and exposure of animals to the toxicant has been described previously (Banerjee et al. 1986; Banerjee and Hussain 1986). Food consumption, general condition and any other clinical symptom were looked for daily. Body weights were recorded weekly. Rats (10-12 animals/group/DDT level) were randomly selected from each group and sacrificed at 8,12, 18 and 22 weeks of exposure and used for each study as described below.

Animals (10-12 rats/group/DDT level) were immunized subcutaneously with tetanus toxoid (0.2 mL) mixed with an equal volume of Freund's complete adjuvant, 20 days before terminating the exposure. These immunized rats were injected intraperitoneally with 5 mL sterile liquid paraffin (0.88 g/mL BDH, England) 48 h before use. An equal number of animals in each dose group were maintained unstimulated. Unstimulated rats were treated similarly except they were not immunized with tetanus toxoid.

Blood samples were collected after termination of exposure from chloroform anesthetized rats by cardiac puncture without opening the abdomen. The serum was separated from the individual samples and kept at -20^{o}C until analysed. Heparin was used in collecting whole blood for leucocyte migration inhibition (LMI) test. Peritoneal macrophages were collected as described earlier (Banerjee and Hussain 1986) for macrophage migration inhibition (MMI) test. The liver, spleen and thymus were removed immediately, blotted and weighed.

The serum antibody titre to tetanus toxoid was estimated by indirect haemagglutination technique, quantitation of serum immunoglobulin (IgM and IgG) was carried out by single radial immuno-diffusion, serum albumin and globulin fractions were studied by zone electrophoresis, and LMI and MMI were assayed in vitro by capillary method according to the procedures as described in detail in a recent publication from this laboratory (Banerjee and Hussain 1986).

The results are expressed as mean and standard deviation (SD). The Student's t-test and one-way analysis of variance (ANOVA) were employed to assess the statistical significance of the treatment effects (Banerjee et al. 1986; Banerjee and Hussain 1986).

RESULTS AND DISCUSSION

Relatively high levels 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). Admittedly, significant amount of DDT residues have been detected in food grains (2.1 to 35 ppm), animal products (1.5 to 34.05 ppm), butter (1.24 to 26.43 ppm) and oil

Table 1. Relative spleen and thymus weights of tetanus toxoid stimulated rats exposed to DDT for different duration*

Duration of exposure (Weeks)	Level of exposure (ppm)	Spleen wt/BW ratio x 10^{-3}	Thymus wt/BW ratio x 10^{-3}
8	0	3.31±0.26	2.40±0.52
	20	3.32±0.40	2.30±0.24
	50	3.07±0.71	2.41±0.12
	100	3.10±0.40	2.30±0.18
12	0	3.34±0.16	2.00±0.31
	20	3.34±0.20	2.00±0.75
	50	3.24±0.31	2.16±0.66
	100	3.25±0.21	2.00±0.70
18	0	3.16±0.20	2.11±0.35
	20	3.12±0.25	2.08±0.80
	50	3.05±0.17	2.01±0.31
	100	2.80±0.20[a]	1.87±0.15
22	0	3.10±0.20	2.10±0.45
	20	3.00±0.43	1.87±0.22
	50	2.55±0.20[a]	1.96±0.11
	100	2.48±0.30[b]	2.05±0.15

* Data presented as the mean relative weight ± SD of 10-12 rats in each group.

a Significantly different from respective control group, $p < 0.05$ (Student's t-test), $p < 0.05$ (ANOVA);

b $p < 0.02$ (Student's t-test), $p < 0.05$ (ANOVA).

(22.14 to 25.72 ppm) from India (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). 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 widespread 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 rats to DDT in the diet at levels of 20-100 ppm for 8-22 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 rats (data not shown). The absence of any effect of DDT on body weight at these exposure levels indicates that this compound itself did not produce any stress responsible for the observed immuno-suppressive effect in the present study.

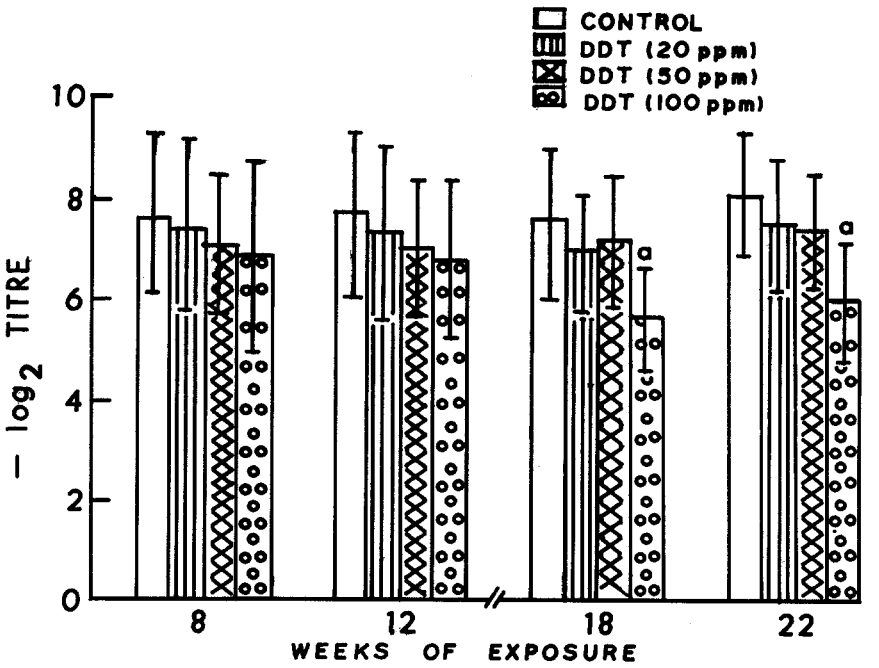

Figure 1. Antibody response to tetanus toxoid in rats exposed to various concentrations and durations of DDT. Results are expressed as $-\log_2$ antibody titre. Height of the bar represents mean $\pm$ SD of 10-12 rats in each group. a - Significantly lower than respective control by statistical comparison, $p < 0.05$ (Student's t-test), $p < 0.05$ (ANOVA).

The rats exposed to 100 ppm DDT for 8-12 weeks, 50 ppm DDT for 8-18 weeks or 20 ppm DDT for 8-22 weeks did not show any significant change in spleen weight ratio (Table 1). Further treatment for 18-22 weeks at 100 ppm level or 22 weeks at 50 ppm level showed significant decrease in spleen weight. However, these animals did not show any alteration in thymus weight throughout the course of experiment. Such an observation had been made earlier in mice exposed to 50 or 100 ppm DDT for 8-12 weeks (Banerjee et al. 1986).

Serum antibody titre, immunoglobulin levels and serum albumin versus globulin ratio were studied for the assessment of humoral immune response. Rats exposed to 100 ppm DDT for 18-22 weeks showed significant decrease in serum antibody titre to tetanus toxoid (Figure 1). No significant effect on antibody titre was observed in rats exposed to 20 or 50 ppm DDT. Similar depression in antibody titre to sheep red blood cells had been observed in mice exposed to 100 ppm DDT for 12 weeks (Banerjee et al. 1986).

The effect of DDT on albumin and globulin fractions of serum in tetanus toxoid stimulated and unstimulated rats are given in Table 2. Serum globulin values were computed as ratios to albumin. This procedure was followed for convenience in evaluating serum electrophoresis data and was considered to be interpretable for

Table 2. Effect of DDT on albumin versus globulin ratio of serum in tetanus toxoid stimulated and unstimulated rats*

Duration of exposure (Weeks)	Level of exposure (ppm)	Albumin/globulin ratio** Unstimulated	Stimulated
8	0	1.28±0.14	0.95±0.22
	20	1.25±0.12	0.96±0.11
	50	1.28±0.08	1.00±0.20
	100	1.25±0.10	1.00±0.12
12	0	1.21±0.15	0.98±0.20
	20	1.32±0.24	1.00±0.26
	50	1.28±0.25	1.00±0.22
	100	1.30±0.12	1.05±0.10
18	0	1.25±0.11	0.95±0.11
	20	1.20±0.18	0.95±0.18
	50	1.30±0.15	1.05±0.12
	100	1.20±0.15	1.12±0.12[a]
22	0	1.26±0.16	0.90±0.15
	20	1.31±0.15	0.97±0.16
	50	1.20±0.17	1.05±0.12[a]
	100	1.24±0.10	1.18±0.10[b]

* Unstimulated rats were treated similarly except immunization.

** Albumin/globulin ratios are calculated from percentage of total protein content. Values represent mean ± SD of 10-12 rats per group.

a Significantly different from the respective control, $p < 0.05$ (Student's t-test), $p < 0.05$ (ANOVA);

b $p < 0.01$ (Student's t-test), $p < 0.05$ (ANOVA).

the purpose of these experiments (Banerjee and Hussain 1986). Albumin and globulin concentrations in serum were consistent and unaffected by DDT treatments imposed. However, the effect of tetanus toxoid injection on humoral immune response can be seen readily from increased globulin level (decreased A/G ratio) in stimulated animals. Globulin level was significantly decreased (increased A/G ratio) in the stimulated 50 ppm DDT group exposed for 22 weeks and 100 ppm DDT group exposed for 18-22 weeks when compared with the stimulated control group.

The effect of DDT on serum immunoglobulin (IgM and IgG) levels is shown in Table 3. The serum immunoglobulin levels did not show any significant change after DDT exposure. The IgG and IgM fractions of serum immunoglobulin tend to increase after tetanus toxoid administration. This increase in IgG level after tetanus toxoid immunization was significantly lower in rats exposed to 50 ppm DDT for 22 weeks or 100 ppm DDT for 18-22 weeks as compared to antigen-stimulated control and found to be well correlated with decreased globulin levels in these animals (Table 2).

Table 3. Serum immunoglobulin concentrations in tetanus toxoid unstimulated and stimulated rats exposed to various levels of DDT*

Duration of exposure (Weeks)	Level of exposure (ppm)	Unstimulated		Stimulated	
		IgM	IgG	IgM	IgG
8	0	0.52±0.10	12.5±2.53	0.70±0.12	15.5±1.50
	20	0.46±0.21	12.7±2.34	0.67±0.10	15.7±3.00
	50	0.48±0.20	10.9±2.30	0.70±0.16	14.6±1.50
	100	0.46±0.11	11.1±1.30	0.63±0.12	14.1±1.75
12	0	0.51±0.13	12.4±2.15	0.72±0.11	15.1±1.20
	20	0.50±0.15	11.8±2.00	0.66±0.15	15.0±1.80
	50	0.45±0.17	12.1±1.60	0.65±0.10	14.1±3.10
	100	0.50±0.14	11.6±1.60	0.67±0.13	14.8±2.40
18	0	0.50±0.10	11.2±1.88	0.68±0.13	15.0±1.62
	20	0.50±0.11	12.1±2.00	0.70±0.10	14.7±2.50
	50	0.43±0.15	11.1±2.30	0.62±0.11	14.1±2.10
	100	0.40±0.13	10.1±1.10	0.66±0.16	12.6±1.80[a]
22	0	0.52±0.15	11.5±2.20	0.70±0.12	15.2±1.20
	20	0.45±0.15	12.2±2.50	0.65±0.15	14.7±2.10
	50	0.50±0.10	10.1±1.50	0.58±0.12	12.7±2.80[a]
	100	0.40±0.10	10.4±1.20	0.63±0.10	12.1±2.00[b]

* Immunoglobulin concentrations are presented as mean ± SD in mg/mL. Ten to twelve rats were used in each group.
a $p < 0.05$ (Student's t-test or ANOVA);
b $p < 0.01$ (Student's t-test or ANOVA).

However, DDT at these exposure levels did not affect the increase of the IgM fraction in rats after tetanus toxoid immunization. A similar decrease in serum globulin fraction and immunoglobulin levels has been reported in rats and rabbits exposed to organochlorine pesticides (Vos 1977; Banerjee and Hussain 1986). These results indicate that an important change in host immunity may occur after DDT ingestion.

The activity of lymphokines such as migration inhibitory factors produced by stimulated lymphocytes or macrophages (LMI or MMI) from DDT exposed animals which have been activated in vitro by tetanus toxoid was used as a measure of cell-mediated immunity. Figure 2 summarises the effect of DDT exposure on cell-mediated parameters. Rats exposed to 50 or 100 ppm DDT and subsequently immunized with tetanus toxoid showed marked decrease in the ability of antigen-sensitized cells to release migration inhibition factors which prevent leucocyte and macrophage migration in vitro suggesting impairment of effector mechanism. Dose-time dependent inhibition were found when both the ability to produce MMI and LMI were evaluated. Although it appears that the depression of cell-mediated immunity extends to the primary humoral response,

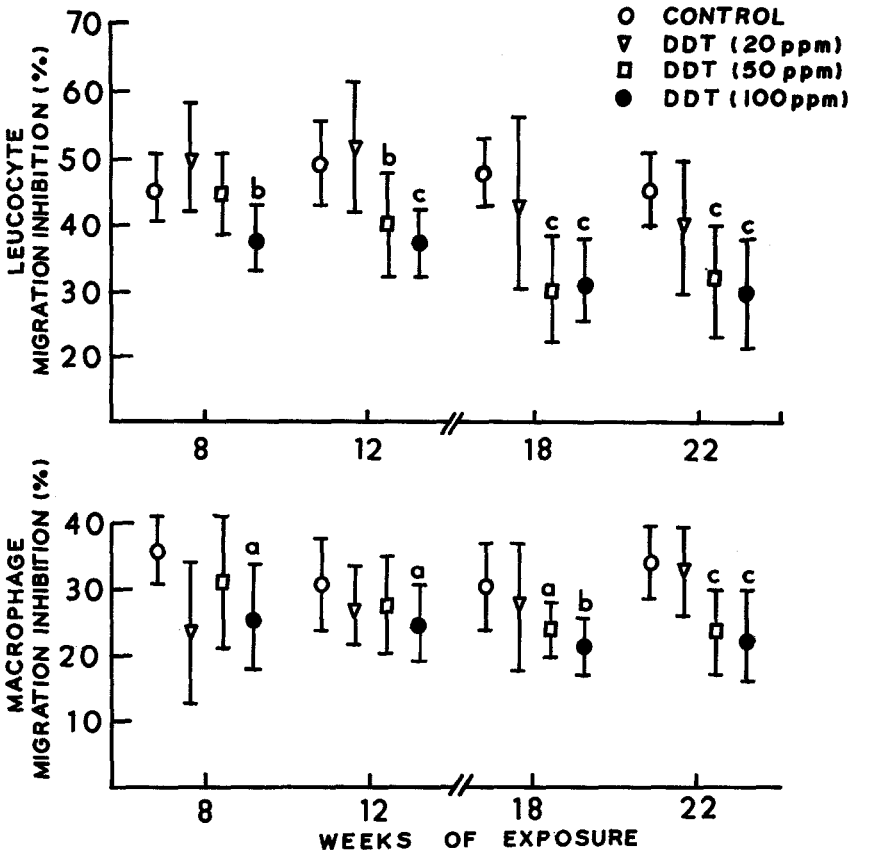

Figure 2. Leucocyte and macrophage migration inhibition tests in rats exposed to various concentrations and durations of DDT. Each value represent the mean percentage migration inhibition ± SD of 10-12 rats per group. a - $p < 0.05$ (Student's t-test or ANOVA); b - $p < 0.02$ (Student's t-test), $p < 0.05$ (ANOVA); c - $p < 0.01$ (Student's t-test or ANOVA).

more light can be thrown in this direction by studying the response to a thymus independent antigen.

The results of the present study reveal suppression of humoral and cell-mediated immune responses in rats exposed to sub-toxic doses of DDT. This suppression was found to increase in dose-time dependent pattern. The effect of DDT on immune responses are more time dependent than on dose, suggest a threshold susceptibility to exposure. Adverse effect of DDT on immune function could place the host in a more vulnerable position towards various pathogens.

Immunotoxicity of DDT in rats was observed at dose levels which did not produce overt toxicity. It is apparent that a more complete understanding of the toxicity of DDT is necessary to study human health hazards and establish guidelines for acceptable residues in the environment. It is emphasized that the threshold level of the chemical below which no effect would be seen depends on

the method of testing for immune responses, animal species, endocrine and nutritional status of the host and type of antigen against which the responses are studied (Street and Sharma 1975; Vos 1977; Banerjee et al. 1986; Banerjee and Hussain 1986). More extensive studies on dose-time relationship in different experimental animals appear to be essential in order to evaluate the effect of DDT on human immune system. Since there are numerous functions associated with the immune system, it is necessary to study multiple parameters to properly evaluate the effect of DDT on immune response and in particular the involvement in immunocompetence. Further investigations are in progress on primary and secondary immune cytokinetics, lymphocyte-mediated cytotoxicity and reticulo-endothelial system in order to understand its mechanism of immuno-suppression and the possible health hazards due to continued use of DDT.

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