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Sub-Chronic Effect of DDT on Humoral Immune Response in Mice

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The widespread use of DDT in public health programs and in agriculture has resulted in the ultimate carry over of these persistent residues to 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 (immune surveillance system) may occur after acute or chronic DDT exposure (reviewed by Vos 1977). Although there appears to be a consensus of opinion regarding the acute effect of DDT on the immune system, chronic or sub-toxic exposure to small amounts remain largely unanswered. Moreover the effect of low doses (sub-toxic) on longer exposure to DDT on immune system has not been studied so far. 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. Keeping this in view the present study was designed to evaluate the effect of p,p'DDT, administered in sub-chronic doses over a long period, on primary and secondary humoral immune responses in albino mice.

MATERIALS AND METHODS

Technical grade DDT (95%) was received through the courtesy of M/s Hindustan Insecticide Ltd., India. Pure p,p' DDT (m.p. 108-109° C) was obtained by repeated crystallization of the technical material from 95% ethanol showing a single peak by gas liquid chromatograph (GLC).

National Institute of Communicable Diseases colony bred male albino mice (Hissar strain), weighing 18-20 grams were housed four in a cage and individually labelled. They were divided into four groups and fed a laboratory diet containing 0 (control), 20, 50 or 100 ppm of p,p' DDT per day and water ad libitum for 3 to 12 weeks.Each treatment group consisted of 25-30 mice. The appropriate amount of p,p'DDT dissolved in groundnut oil was incor-

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porated in their diet and mixed manually for at least 30 minutes to ensure even distribution of DDT. The analysis of ten random treated samples by GLC demonstrated an even distribution of DDT in various batches of test food, indicating uniform exposure of all animals to the toxicant. The control animals received equal volume of groundnut oil in an identical manner. Food consumption, general condition and any other sign and symptom were looked for daily. Weekly body weight of each animal was recorded.

Sheep red blood corpuscles (SRBC) washed thoroughly and finally suspended in normal saline (0.15 M) were injected in mice intraperitoneally in 0.1 ml doses. Approximately 25×10^6 cells were administered in the primary and 50×10^6 cells in the secondary immunization. The secondary immunization was administered 2 weeks after the primary injection. Blood samples were collected by cardiac puncture from chloroform anaesthetized mice on the 7th day after primary immunization and 5th day after secondary immunization. The serum was separated from individual samples and kept at -20° C until analysed. The liver, spleen and thymus were removed immediately and weighed. The spleen was dissected out, teased finally for spleen cells under aseptic condition and used for the estimation of antibody forming cells by splenic plaque-forming cells (PFC) assay of Jerne et al (1969). The serum antibody titre to SRBC was measured by haemagglutination technique according to Herbert (1979). Titration was also carried out with antisera pre-incubated with 0.1M 2-Mercaptoethanol (2-ME) at 37° C for 60 minutes for the estimation of 7S type of antibodies. The antibody titres are expressed as log 2 of the reciprocal of the first dilution where no visible agglutination was observed.

All the experiments were carried out in duplicate or triplicate. The average was used in a 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.

RESULTS AND DISCUSSION

The absence of any effect of p,p'DDT on body weight indicates that this compound itself did not produce any stress responsible for the observed immunosuppressive effect in the present study. Significant changes in spleen and liver weights in dose-time dependent pattern were observed (Table-1). Such an observation had been made earlier in rats fed with 200 ppm p,p'DDT for 35 days (Wassermann et al 1969).

Table 1. Body and relative organ weights of SRBC stimulated mice exposed to p,p'DDT for different concentration and durations*+

Duration of exposure (weeks)	Dietary concentration (ppm)	Body wt (gram)	Liver/Body wt. ratio x 10^{-4}	Spleen/Body wt. ratio x 10^{-4}	Thymus/Body wt. ratio x 10^{-4}
3	0	25.80±3.36	46.38±3.59	4.90±0.53	2.32±0.48
	20	24.60±3.88	45.00±4.56	4.92±1.34	2.33±0.56
	50	25.60±3.87	48.40±5.48	4.80±0.60	2.41±0.77
	100	24.90±4.15	50.40±5.07	4.60±0.66	2.38±0.62
6	0	32.50±3.40	50.03±4.20	4.38±0.40	1.88±0.60
	20	32.53±5.20	51.77±4.58	4.30±0.68	1.81±0.34
	50	30.60±3.20	52.49±3.28	4.10±0.88	1.95±0.48
	100	31.00±4.60	54.76±3.81[a]	4.21±0.50	1.95±0.48
8	0	34.28±4.81	50.77±2.28	4.70±0.32	1.58±0.41
	20	34.17±4.31	50.80±4.23	4.50±1.10	1.77±0.52
	50	33.70±3.35	53.50±4.20	4.00±0.77[a]	1.80±0.62
	100	32.40±2.50	54.80±3.20[b]	4.06±0.70[a]	1.60±0.31
12	0	37.35±3.07	49.80±2.54	4.90±0.38	1.24±0.29
	20	35.20±4.50	50.82±5.18	5.00±0.52	1.45±0.24
	50	38.35±4.43	54.66±4.20[a]	4.38±0.36[b]	1.28±0.10
	100	38.14±5.00	57.16±5.18[c]	4.00±0.46[c]	1.05±0.32

* Mice were immunized with 25×10^6 SRBC intraperitoneally 7 days before last exposure.
\+ Values represent mean ± SD, n=8-12 mice per treatment group.
[a] Significantly different from the mean of the control by student's t-test $p < 0.05$
[b] $p < 0.01$; [c] $p < 0.001$

Table 2. Effect of p,p'DDT on primary and secondary heamagglutination titres of mouse antiserum against SRBC incubated with and without 2-Mercaptoethanol (2ME)*

Duration of exposure	Group (ppm)	Primary Haemagglutinational $-\log_2$ titre**		Secondary Haemagglutination $-\log_2$ titre**	
		Without 2ME	With 2ME	Without 2ME	With 2ME
3 weeks	0	8.20±1.55	4.00±1.65	11.00±1.84	9.33±1.80
	20	8.20±1.40	3.50±1.87	10.80±1.50	9.80±1.50
	50	7.73±1.85	4.10±1.76	10.70±1.78	7.80±1.70
	100	7.70±1.64	4.00±1.53	9.13±1.50[a]	7.00±1.50[b]
6 weeks	0	8.50±1.80	4.10±1.70	10.50±1.75	9.00±1.50
	20	8.20±1.70	4.00±1.50	10.56±1.85	9.82±1.83
	50	7.46±1.74	3.70±1.75	9.50±1.80	8.00±1.25
	100	7.00±1.77	4.00±1.50	8.44±1.60[a]	7.00±1.80[a]
8 weeks	0	8.50±1.50	4.20±1.80	10.57±1.10	9.00±1.00
	20	8.40±1.52	4.50±1.67	10.00±1.25	8.80±1.40
	50	7.30±1.77	4.00±1.52	9.80±1.12	7.20±1.82
	100	7.00±1.80	4.00±1.77	8.70±1.50[b]	7.20±1.50[a]
12 weeks	0	8.66±1.60	4.50±1.70	10.60±1.58	9.50±1.87
	20	8.20±1.88	5.00±1.75	10.20±1.75	8.00±1.72
	50	7.40±1.55	3.60±1.90	9.80±1.70	6.60±1.66
	100	6.60±1.70[b]	3.40±1.89	8.40±1.68[b]	6.00±1.50[c]

* The primary antibody titre was determined on day 7 following an intraperitoneal administration of 25×10^6 SRBC. The secondary antibody titre was determined on day 5 following a secondary ip administration of 50×10^6 SRBC. The secondary immunization was administered two weeks after the primary injection.

** Values represent the mean± SD of 10 to 12 mice in each group. Experiment was repeated twice or thrice and average was considered for determination of mean values.

[a] Significantly different from control; $p<0.05$; [b] $p<0.02$; [c] $p<0.01$

Table 3. Effect of p,p'DDT on primary direct splenic-plaque cells response in mice*

Duration of exposure	Days after antigen	Distribution of PFC/10^6 spleen cells** Control	20ppm	50ppm	100ppm
3 weeks	2	142±46	242±80	120±33	151±57
	3	290±44	300±52	313±37	303±68
	4	633±60	641±78	602±101	577±112
	5	336±55	422±80	260±60	271±43[b]
	6	201±50	251±66	162±29	176±62
6 weeks	2	157±25	168±49	100±38	90±30[a]
	3	344±45	300±70	292±28	203±66[c]
	4	660±78	649±127	590±130	560±108[a]
	5	405±77	373±90	361±100	334±60[a]
	6	219±70	263±60	190±56	248±26
8 weeks	2	232±57	196±71	176±60	122±45[a]
	3	392±40	314±80	298±51[a]	293±50[b]
	4	685±80	628±106	600±128	503±56[c]
	5	420±57	400±60	312±60	308±64[c]
	6	313±68	273±52	208±48	247±58
12 weeks	2	183±40	222±75	108±35	180±40
	3	378±45	385±80	203±50[b]	203±50[c]
	4	648±85	613±65	501±101[a]	490±50[c]
	5	391±46	300±58	290±35[b]	210±46[c]
	6	298±25	290±60	203±47	178±48[c]

* The direct splenic plaque-forming cells (PFC) response was determined on days 2-6 following an intraperitoneal administration of $25x10^6$ SRBC

** All the data are presented as the mean ± SD. n=8 per group.

a Significantly different from control; $p < 0.05$

b $P < 0.02$; c $P < 0.01$.

Mice exposed to 100 ppm DDT for 3 to 8 weeks did not show any significant alteration in primary antibody titre to SRBC (Table-2). Further exposure for 12 weeks showed significant decrease in primary antibody titre without any change in 2-ME resistant antibody titre. The secondary antibody titre (with and without 2-ME) showed marked decrease throughout the experiment after 100 ppm DDT exposure. Subba Rao and Glick (1977) observed similar depression in 2-ME resistant antibody titre to SRBC in chickens exposed to 100 ppm p,p'DDT for 40 days.

Effect of p,p'DDT on direct splenic plaque-forming cells (IgM producing cells) are summarized in Table 3 and 4. Mice exposed to p,p'DDT for 3 weeks showed only reduction in secondary PFC. However, further exposure for

Table 4. Effect of p,p'DDT on the secondary direct splenic plaque forming cells response in mice*

Duration of exposure	Days after antigen	Distribution of PFC/10^6 spleen Cells** Control	20ppm	50ppm	100ppm
3 weeks	2	112±28	120±35	113±25	92±35
	3	380±50	350±53	310±50	290±48[a]
	4	178±38	160±41	200±40	220±50
	5	120±30	125±34	108±30	125±25
6 weeks	2	100±25	118±36	97±22	92±20
	3	388±48	351±75	346±50	294±40[b]
	4	188±40	146±40	210±43	181±46
	5	145±35	119±36	154±40	150±50
8 weeks	2	118±26	98±36	97±18	78±20[b]
	3	370±61	347±68	264±40[b]	255±45[b]
	4	161±31	115±38	220±90	90±35[a]
	5	112±27	112±43	124±40	112±34
12 weeks	2	110±26	131±40	108±31	58±35[b]
	3	350±55	308±38	210±45[b]	165±50[b]
	4	167±46	121±35	185±50	87±25[b]
	5	104±40	130±45	121±30	82±30

* The direct secondary splenic plaque-forming cells (PFC) response was determined on days 2-5 following a secondary intraperitoneal administration of $50x10^6$ SRBC. The secondary immunization was administered 2 weeks after the primary injection.

** All data are presented as the mean±SD. n=8-12 per group.

[a] Significantly different from control; $p<0.05$

[b] $p<0.01$

more than 6 weeks showed a time and dose dependent decrease in primary and secondary PFC response. The peak antibody formation occurred on the fourth day of primary response and third day in the secondary response in control as well as in treated animals indicating that there was no delay in antibody formation after DDT exposure. The sequential study of PFC kinetics revealed that suppression not only occurred at peak days but pre and post peak days too. The reduction in the primary PFC was not associated with decrease in serum antibody response to SRBC in DDT exposed mice. Suppressed PFC response was noticed after 6 weeks of exposure and decreased antibody titre only after 12 weeks of DDT exposure. The present study also demonstrates that p,p'DDT treatment resulted in a suppression of primary 19S type plaque-forming cells in response to SRBC in mice

at doses much lower than reported earlier (Wiltrout et al 1978). Administration of p,p'DDT resulted in decreased lymphocyte population and germinal centres of spleen (Street and Sharma 1975), possibly due to cytotoxicity (Gabliks and Friedman 1969), may be the mechanism responsible for the reduced PFC response observed in present study. DDT may also exert an indirect effect on humoral immune response by its well known interaction with steroid metabolism (Kupfer 1969). Further studies are necessary to establish the effect of DDT on discrete lymphocyte macrophage physiology and to relate cellular function to intracellular concentration of pesticide.

These results suggested a depression of primary and secondary humoral immune response in mice exposed to sub-toxic doses of p,p'DDT. This suppression was found to increase in dose-time dependent pattern. In general the immunosuppressive effect was more pronounced in secondary antibody response than primary. However, suppression of primary as well as secondary antibody response occurred at longer exposure similarly. The effects of p,p'DDT on secondary immune response are more time dependent than on dose, suggest a threshold susceptibility to exposure.

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