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Suppression of Cellular Immune Responses in BALB/c Mice Following Oral Exposure to Permethrin

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Permethrin is a member of one of the newest classes of insecticides, the synthetic type I pyrethroids. Since its synthesis in 1973 (Elliott et al. 1973), permethrin has gained in popularity due to its photostability in addition to high activity against insects and relatively low mammalian toxicity compared to other insecticide classes (Elliott, 1976, Elliott et al. 1978, Casida, 1980, Papadopoulou-Mourkidou, 1983). These reports on toxicity do not, however, examine potential effects on immune responses. The immune system is exquisitely sensitive to many toxic chemicals at exposure levels that cause no overt toxicity (Koller, 1980, Dean and Murray, 1991, Luster et al., 1987). This sensitivity is most likely due to the complexity and diversity of the immune system as well as to general properties of many xenobiotics such as reactivity with macromolecules and rapidly dividing cells. Although no data are available regarding potential immunotoxic effects of permethrin, there are reports describing decreased humoral responses and altered cellular responses induced by the type II pyrethroid cypermethrin in rats and rabbits (Desi et al., 1985). In addition, Tamang et al. reported that cypermethrin suppressed both cellular and humoral immune responses in mice and goats (Tamang et al., 1988). In this report, we extend these observations to examine the immunotoxic potential of the type I pyrethroid permethrin.

MATERIALS AND METHODS

BALB/c female mice weighing 18-20 grams and C57Bl/6 female mice were purchased from Harlan Sprague Dawley, Inc.(Indianapolis, IN). All animals were housed under controlled temperature (22 $\pm$ 3°C) and lighting (12/12 hr light/dark cycle). Mice were allowed food and water *ad libitum*.

Permethrin (ChemService, West Chester, PA) was dissolved in corn oil (Hunt-Wesson Inc., Fullerton, CA) and stored at room temperature protected from light. BALB/c mice were divided into 4 groups of 5 animals each and administered either 0, 0.4, 0.04 or 0.004 mg/kg body weight/day of permethrin in 0.1-0.2 ml of corn oil over a 10-day period. These total doses corresponded to 0% (control), 1%, 0.1% or 0.01% of an

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oral LD_{50}. The route of administration was by oral gavage. After dosing, the animals were allowed to rest for 3 days after which immunological assessment was initiated.

Animals were sacrificed by cervical dislocation and total body weight determined. Spleens were then removed aseptically, weighed and placed in 5 ml of culture medium [RPMI 1640 supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 2 mM L-glutamine (Mediatech, Washington, D.C.), 1% sodium pyruvate, 1% nonessential amino acids, 50 μg gentamicin sulfate and 5×10^{-5} M 2-mercaptoethanol (all from Sigma Chemical Co., St. Louis, MO)]. A single-cell suspension of splenocytes was prepared by mechanical disruption using the plunger of a 3-ml syringefollowed by aspiration through a 25-gauge needle. The splenocytes were resuspended in culture medium and enumerated using a Coulter model Zf electronic cell counter (Coulter Electronics, Hialeah, Fl).

Lymphoproliferative assays were performed as previously described (Holladay et al, 1991, Rosenthal et al., 1987). Briefly, 2×10^5 splenocytes in 100 μl culture medium were added to triplicate wells of 96-well flat-bottomed microtiter plates. To the cells were added the T cell mitogen concanavalin A (con A; Sigma) or the B cell mitogen lipopolysaccharide (LPS; E. coli O55:B5; Sigma) at 1 μg/ml and 40 μg/ml, respectively. The cells were pulsed with 1 μCi [^{3}H] thymidine ([^{3}H]TdR; NEN, Boston, MA) over the last 6 hours of a 72 hour incubation at 37° C and 5% CO_2. Proliferation was assessed by harvesting the cells onto glass fiber filters using a Skatron cell harvester(Skatron, Sterling, VA) and measuring [^{3}H] TdR incorporation into splenocytes. Also, unidirectional mixed lymphocyte responses (MLR) to allogeneic lymphocytes were performed using a 4:1 stimulator (mitomycin C-treated splenocytes from C57Bl/6 mice) to responder BALB/c splenocytes. The cultures were incubated for 5 days, pulsed with 1 μCi [^{3}H] TdR per well over the last 18 hours of incubation and harvested as described for mitogen assays.

Cytotoxic T lymphocyte (CTL) activity was measured in splenocytes sensitized *in vitro* with mitomycin C-treated EL-4 thymoma cells (Holladay et al., 1991). Briefly, freshly prepared splenocytes from permethrin-treated or control BALB/c mice were cocultured with mitomycin C-treated EL-4 cells at a 100:1 responder:sensitizer ratio for 5 days at 37° C and 5% CO_2. After 5 days, aliquots of 5×10^5 sensitized splenocytes were added to triplicate wells of a 96-well round-bottomed microtiter plate containing $Na_2{}^{51}CrO_4$ (NEN) - labeled EL-4 target cells at effector:target ratios of 25:1 and 12:1. The plates were incubated for 5 hours at 37° C and the supernatants harvested. The percent target cell lysis was determined using the formula:

% cytotoxicity = $[(cpm_e - cpm_{sr})/(cpm_t - cpm_{sr})] \times 100$

where cpm_e = cpm in experimental wells, cpm_{sr} = spontaneous release, and cpm_t = total release upon addition of 0.5% Triton X-100. Natural killer (NK) cell cytotoxicity against a tumor cell target was performed as previously described (13). Briefly, 1×10^4 YAC-1 tumor cells, labeled with $Na_2{}^{51}CrO_4$ (NEN), were added to triplicate wells of a round-bottomed 96-well microtiter plate. Splenocytes from permethrin-treated or control mice were added to the wells at 100:1 and 50:1 effector:target cell

ratios. The supernatants were collected after 5 hours incubation at 37° C. Percent cytotoxicity was determined as described for the CTL assay.

All results were expressed as mean ± SEM. Comparisons between means of the treatment groups was by a two-way analysis of variance followed by Duncan's Multiple Range Test using the SAS statistical software package (SAS Institute, Cary, NC). The default alpha value of 0.05 was used as the standard of statistical significance.

RESULTS AND DISCUSSION

Data indicate that the immune system appears to be a sensitive target for permethrin activity. Permethrin exposure significantly reduced the unidirectional MLR in the highest dose group (1% of LD_{50}). The data in Figure 1 show reductions of 3.3%, 19.3% and 40.5% in blastogenesis at the 0.01%, 0.1% and 1.0% permethrin dosages, respectively. These data indicate an alteration in the ability of T lymphocytes to recognize and respond to foreign antigen.

Cytotoxicity is an important effector function of lymphocytes requiring both foreign antigen recognition and cytotoxic effector activity. Splenocytes from permethrin-exposed mice yielded a statistically significant reduction in cytotoxic activity of T lymphocytes compared to splenocytes from vehicle-exposed animals. As shown in Figure 2, there was a small but statistically significant decrease in CTL activity in the 1% dose group compared to vehicle control.

NK activity was also reduced by permethrin exposure. The results in Figure 3 show a reduction in cytotoxicity produced by NK cells with statistical significance in 1% dose group. The reduction in ^{51}Cr release was 15%, 32% and 42% for the 0.01%, 0.1% and 1.0% dose groups, respectively, compared to control.

Body and spleen weights were not affected by exposure to permethrin. The data (Figure 4) are presented as spleen wt.:body wt. ratios to account for animal size variation. These data indicate that gross observable toxicity such as body weight loss or specific organ weight reduction due to permethrin exposure did not occur at the dosages used in this study.

The ability of splenic lymphocytes to proliferate in response to the T cell mitogen Con A or the B cell mitogen LPS was not altered by permethrin. No significant differences were noted comparing mice exposed to vehicle only to mice receiving any of the 3 dosage levels of permethrin (Figure 5).

To summarize, this study extends the data suggesting potential immunotoxic effects of pyrethroid insecticides by defining for the first time specific cellular immune alterations in mice after oral exposure to the type I pyrethroid insecticide permethrin. Functions requiring specific antigen recognition and/or effector function (MLR, CTL, NK) were decreased while nonspecific mitogen stimulations and body and organ weights were not affected by the dosages used. Although pyrethroids possess a high selectivity factor (insect:mammal ratio), the

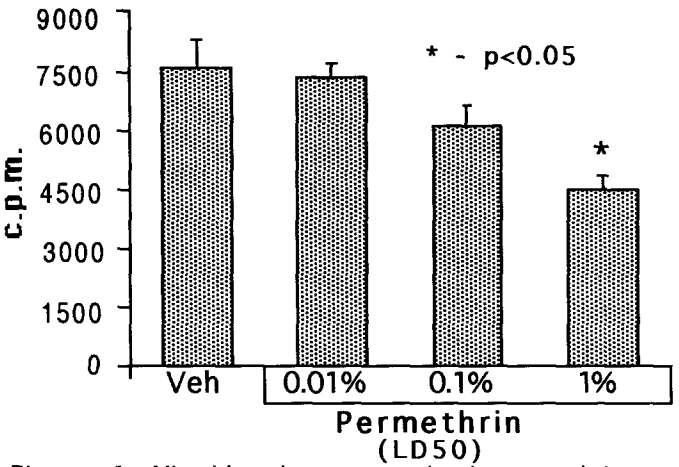

Figure 1. Mixed lymphocyte reaction in permethrin-treated BALB/c mice. Splenocytes from permethrin- and vehicle-treated mice were stimulated with mitomycin C-treated C57Bl/6 splenocytes as described. Data expressed as the mean + SEM of a representative experiment. N = 5.

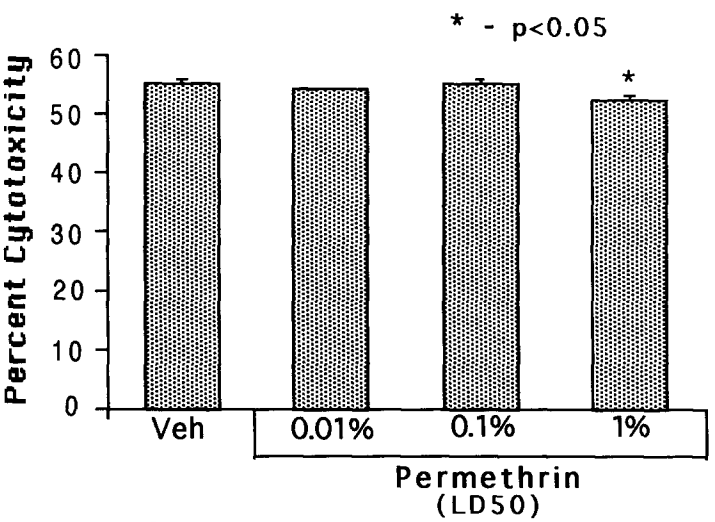

Figure 2. Cytotoxic T lymphocyte activity in permethrin-treated BALB/c mice. Splenocytes from permethrin- and vehicle-treated mice were sensitized in vitro with EL-4 thymoma cells for 5 days as described. Results of the ^{51}Chromium release assay are expressed as the mean + SEM of a representative experiment. N = 5.

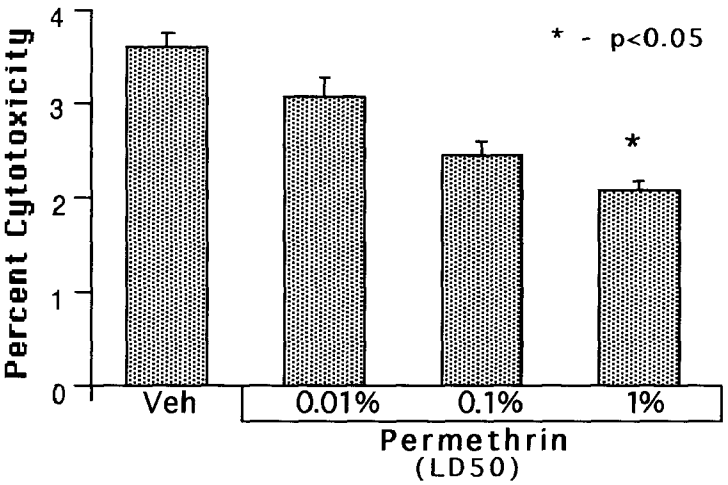

Figure 3. Natural killer cell activity in permethrin-treated BALB/c mice. Splenocytes from permethrin- and vehicle-treated mice were incubated with ^{51} Chromium labeled Yac-1 lymphoma cells as described. Data expressed as the mean + SEM of a representative experiment. N = 5.

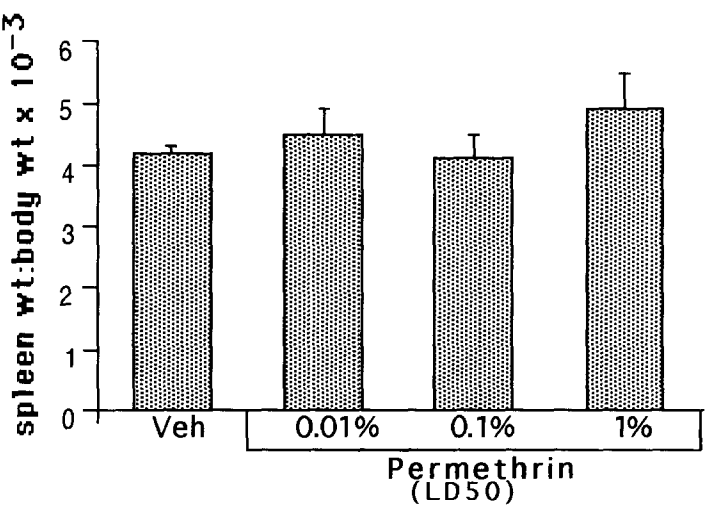

Figure 4. Spleen weight:body weight ratios in permethrin-treated BALB/c mice. Female BALB/c mice were weighed before and after permethrin treatment as described. Spleens were weighed prior to splenocyte preparation. Data expressed as the mean + SEM of a representative experiment. N = 5.

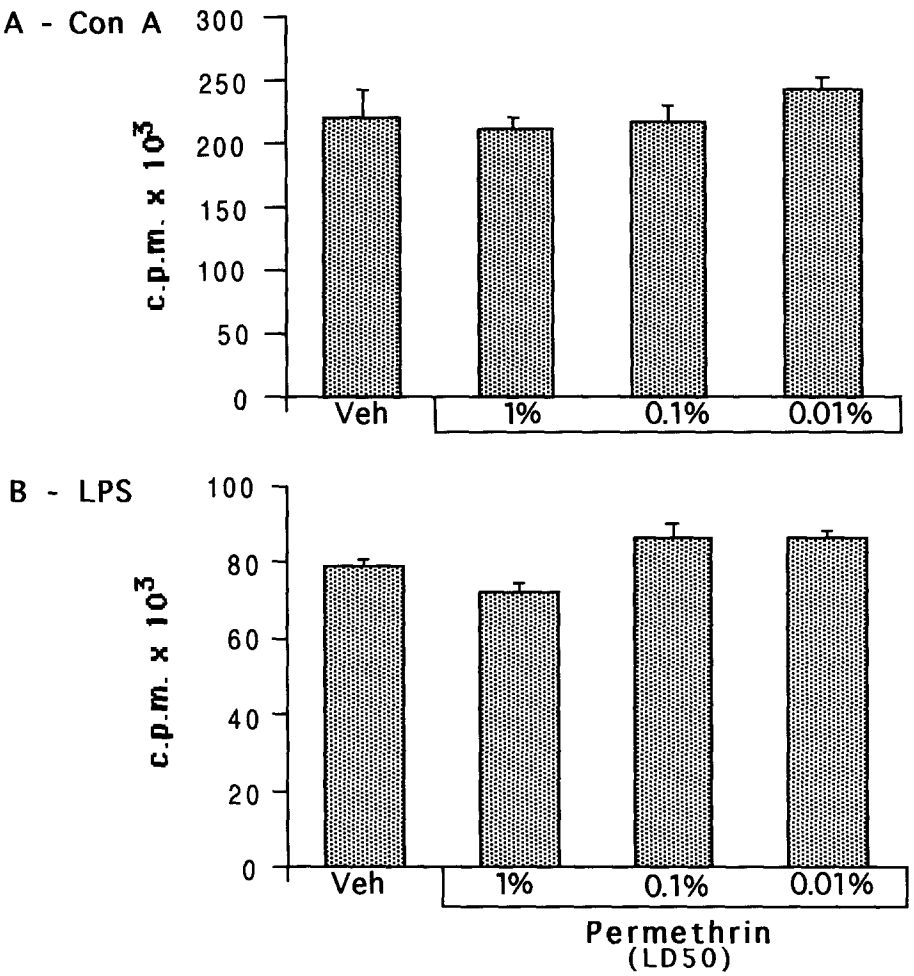

Figure 5. Mitogen stimulation in permethrin-treated BALB/c mice. Splenocytes from permethrin- and vehicle-treated mice were stimulated with A) concanavalin A or B) lipopolysaccharide as described. Data expressed as the mean + SEM of a representative experiment. N = 5.

present data indicate a potential for alterations in immune function in mammalian systems. These alterations could adversely affect the health status of an exposed individual and, therefore, should be considered when safety standard and/or exposure limits are developed.

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